# GENOME-WIDE QTL MAPPING FOR POST-FLOWERING DROUGHT TOLERANCE AND VALIDATION OF CHARCOAL ROT RESISTANCE QTLs IN NILs OF SORGHUM

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**BY**

**SUVARNA PATIL**

**DEPARTMENT OF BIOTECHNOLOGY**
**COLLEGE OF AGRICULTURE, DHARWAD**
**UNIVERSITY OF AGRICULTURAL SCIENCES,**
**DHARWAD – 580 005**

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# 1. INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop worldwide, major food and/or feed crop in the semi-arid and drought prone areas of the world. Sorghum is a versatile grain and it can be used in making unleavened breads, boiled porridge, malted beverages such as beer and popped grains. Sweet sorghum is also used in ethanol production and considered as an important candidate species in bio-fuel research. Starch obtained from the waxy sorghum is useful for sizing in the textile industry. Some sorghum varieties are rich in antioxidants and all are glutin-free, although there are several research programmes globally that are attempting to transform sorghum with wheat gluten genes in order to improve the suitability of sorghum flour for making leavened bread.

Sorghum is grown both during southwest monsoon (*kharif*) and post-monsoon season (*rabi*) in India. Generally, *rabi* sorghum crop produces high quality grains as they mature during winter season which is a clear, dry and rain free climate all over India. Drought stress is one of the major agronomic problems that limit the attainment of maximum yield more than any other environmental parameters (Krieg, 1975 and Abdula *et al.,* 2004). Stalk rot or charcoal rot caused by *Macrophamina phaseolina* is a prominent *rabi* disease which affects crop during post-flowering stage.

Drought resistance by plants can be divided into two groups; drought avoidance and drought tolerance. Drought avoidance is a mechanism for avoiding lower water status in tissues by maintaining cell turgor and cell volume either through aggressive water uptake by an extensive root system or through reduction in water loss from transpiration and other non-stomatal pathways. Drought tolerance is a mechanism by which plant maintains metabolism even at low water potential. Drought stress generally changes plant growth and development pattern by suppressing cell division, organ growth, net photosynthesis, protein synthesis and alters hormonal balance in major plant tissue, hence, causes severe effects on yield. Drought stress has also been shown to cause alterations in the chemical composition and physical properties of the cell wall such as, lignification which begin after the cell elongation stops (Peleman *et al.,* 1989).

A common biochemical adaptation to drought is osmotic adjustment, which results from synthesis of certain metabolites and osmolytes (Yancey *et al.,* 1982 and Bartels and Sunkar, 2005). These osmolytes are hydrophilic, highly soluble molecules, able to produce a salvation surface that capture water molecules and make it available during water limitation. Examples of these osmolytes include amino acids, glycine-betaine, sugars and sugar alcohols, which are non-toxic at their higher concentrations and thus do not interfere with cellular metabolism. One of the major signals operating during drought stress and other abiotic stresses is the plant hormone abscisic acid (ABA) (Furini, 1996). Drought induced ABA in turn is known to induce various early and later responsive genes involved in a signaling cascade for the regulation of downstream biochemical protective mechanisms besides playing other important roles (Shinozaki and Yamaguchi-Schinozaki, 1997). ABA accumulated during water-stress conditions in leaf tissues around the guard cells is known to promote stomatal closure (Little and Eidt, 1968), which dramatically reduces foliar transpiration, in many cases by two orders of magnitude or more, and is a primary defense response of the plant against dehydration.

Improving the drought tolerance of sorghum is one of the most important objectives of plant breeders focusing on this crop. Minimizing the yield losses resulting from moisture stress, which is a regular feature of most sorghum growing environments requires concentrated efforts. Three growth stages (GS2) have been identified in sorghum that are critical in understanding drought tolerance. GS1 seedling establishment (early vegetative stage), GS2, pre-flowering (panicle differentiation to flowering) and GS3, is post-flowering (grain fill to physiological maturity of grain). The most damaging drought stress the one that occurs during the post-flowering stage of crop growth (referred as terminal drought). Genotypes sensitive to this type of drought are characterized by pre-mature leaf and plant senescence, stalk collapse, lodging, charcoal rot and reduced grain number and size.

In sorghum, the best characterized form of tolerance to stress during post-flowering stage of crop growth is 'stay-green' trait. Stay-green is the ability of plant to withstand premature leaf and plant death, develop grain normally and resist charcoal rot and lodging when exposed to moisture stress during the late stages of grain development (Woodfin *et al.,* 1988; Xu *et al.,* 2000 and Mahalakshmi and Bidinger, 2002). Delayed leaf senescence in sorghum has been linked to higher grain yields, particularly in environments in which available water during grain filling is not adequate to support potential transpiration (Borrell *et al.,* 1999, 2000b).

Stay-green genotypes have been found to contain higher cytokinin levels (McBee, 1984 and Ambler *et al.,* 1987) and more stem sugars (Duncan *et al*., 1981; McBee and Miller, 1982 and Dahlberg, 1992) than senescent genotypes under certain conditions. In addition, stay-green hybrids assimilate more nitrogen and have higher specific leaf nitrogen than senescent hybrids suggesting a link between nitrogen status and the stay-green trait (Borrell and Hammer, 2000 and Borrell *et al.,* 2001). Increased accumulation of soluble sugars in stay-green types appeared to be associated with a great functional leaf area during grain filling, thereby reducing their dependence on stored assimilate from the stem to fill the grain (Duncan *et al*., 1981 and McBee, 1984). A higher concentration of stem sugars improves the digestible energy content of the stover, making stay-green a valuable trait for both grain and fodder production in dual purpose sorghum (Oosterom *et al*., 1996).

Water Use Efficiency (WUE) determined by using Carbon Isotope Discrimination (CID) has been used as an indirect selection strategy for drought tolerance. Breeding for higher WUE is considered as an important trait from physiological view point. Crop genotypes that produce higher plant biomass per unit of water consumed are breeders choice especially in areas where moisture is the constraining factor. CID provides an integrated measurement of transpiration efficiency (TE), the ratio of dry matter produced to water transpired of C3 crop species (Ehdaie *et al.,* 1991). During photosynthesis, plants favor incorporation of the light carbon isotope ($^{12}C$) over the heavy isotope ($^{13}C$), with the result that CID is positively correlated with the ratio of internal leaf $CO_2$ concentration to ambient $CO_2$ concentration (Ci/Ca) and negatively associated with TE (Ehdaie *et al*., 1991). Thus, a high Ci/Ca leads to a higher CID and a lower TE. The major advantage of using CID in selection is its high heritability, which is primarily due to small G x E interactions in dry land areas (Merah *et al*., 2001). It is positively correlated with grain yield in cereals within and across contrasting environments (Teulat *et al.,* 2001b). Markers diagnostic of individual QTLs represent an important surrogate for physiological trait measurements (Price and Courtois 1999), and may ultimately improve selection efficiency through marker-assisted breeding.

Drought tolerance in sorghum through stay-green route is known to impart characters of resistance. This disease is caused by a weak pathogen which manifests when plant experience drought stress during grain filling stage. In *rabi* sorghum, this disease is of economic importance and very little process has been made due to complex nature of inheritance and possible heterogenous nature of pathogen. Host plant resistance is only practical solution as the pathogen is soil borne and chemical control is hardly possible. Identification of QTLs and their deployment through marker assisted selection (MAS) would be a royal route to manage this disease.

Molecular markers have been useful in understanding genetic nature of complex traits. Molecular markers have been used to develop high density genetic linkage map and to determine the location of QTLs that control the expression of the complex polygene trait (Harris *et al*., 2007 and Srinivas *et al*., 2009). Different kinds of genomic-DNA based markers have been developed in sorghum and used to construct linkage maps (Varshney *et al*., 2007). A number of genetic linkage maps of sorghum have been published in the last decade using microsatellites, several are purely based on Restriction Fragment Length Polymorphism (RFLP) markers (Crusta *et al.,* 2000), others include Amplified Fragment Length Polymorphism (AFLP) markers (Klein *et al.,* 2001), Simple Sequence Repeats (SSR) markers (Bennetzen *et al.,* 2001 and Klein *et al.,* 2001), Randomly Amplified Polymorphism DNA (RAPD) markers (Tuinstra *et al.,* 1996,) and morphological markers (Bennetzen *et al.,* 2001).

Using molecular linkage maps and quantitative trait loci mapping technology, it is possible to estimate the number of loci governing a particular trait of agronomic importance and to determine their map positions in the genome (Tanksley, 1993).

The identification of such genomic regions/QTLs governing traits of agronomic importance can create a base for rapid, detailed, and direct genetic manipulation of them through MAS. QTLs have been identified using these genetic linkage maps predominantly containing anonymous molecular markers for many agronomically important traits including plant early development, yield and its component traits, plant height and other growth characters (Feltus *et al.,* 2006), pre- and post-flowering drought stress (stay-green) tolerance (Xu *et al.,* 2000; Haussmann *et al.,* 2002 and Srinivas *et al.,* 2009) and for important biotic stresses like striga (Haussmann *et al.,*, 2004) and charcoal rot (Reddy *et al*., 2008). The QTL mapping approach identifies specific parental alleles that make a positive contribution and to transfer better alleles from one genetic background to another using MAS strategies (Tanksley and Nelson, 1996).

Backcross breeding programme is aimed at gene introgression from a donor line into the genomic background of a recipient line. The basis of a marker-assisted backcrossing (MAB) strategy is to transfer a specific allele at the target locus from a donor line to a recipient line while selecting against donor introgressions across the rest of the genome. The molecular markers in such programs has received considerable attention in the recent past and markers could be used to assess the presence of the introgressed gene (foreground selection) when direct phenotypic evaluation is not possible or too expensive or only possible late in the development (Tankslit, 1983). Molecular markers have potential to genetically dissect the progeny at each generation, increase the speed of the selection process, thus increasing genetic gain per unit time (Tanksley *et al.,* 1989 and Hospital, 2003).

Backcrossing is often the chosen method to introduce a new trait into a breeding programme and molecular methods can very efficiently increase the efficiency of backcrossing by facilitating early selection of donor allele through foreground selection and background selection. The main advantages of MAB are: (1) efficient foreground selection for the target locus, (2) efficient background selection for the recurrent parent genome, (3) minimization of linkage drag surrounding the locus being introgressed, and (4) rapid breeding of new genotypes with favorable traits. The effectiveness of MAB depends on the availability of closely linked markers and/or flanking markers for the target locus, the size of the population, the number of backcrosses and the position and number of markers for background selection (Frisch and Melchinger, 2005).

A reasonably saturated linkage map and rigorous field evaluations for phenotyping the target traits are essential for mapping stable QTLs for complex traits such as post-flowering drought tolerance and components traits of yield, this effort can also greatly aid in pyramiding such QTLs into chosen elite sorghum varieties for elevated drought / charcoal rot resistance. Keeping these points in view following objectives were undertaken in the present study.

1. Molecular mapping of gene based markers in two recombinant inbred line (RIL) populations and development of genetic linkage maps.
2. Phenotyping and QTL mapping of component traits of drought tolerance (stay-green and delta $^{13}C$) in RIL populations.
3. Marker assisted introgression and field evaluation of lines introgressed with charcoal rot resistant QTLs.

# 2. REVIEW OF LITERATURE

Drought is a major limiting factor to agriculture and considered as the most important cause of yield reduction in crop plants all over world (Boyer, 1982). In agriculture, drought tolerance refers to the ability of a crop plant to produce its economic product with minimum loss in a water deficit environment relative to a water-constraint free production environment. Sorghum is an important crop worldwide for its unique ability to produce satisfactory yield under a wide range of harsh environmental conditions. Improving sorghum's tolerance to various abiotic stresses would further improve yield and yield stability in marginal growing areas with increase in production efficiency.

Drought is the availability of inadequate water including precipitation and soil water storage capacity (Mitra, 2001). Drought resistance is a complex phenomenon which involves drought escape, drought (dehydration) avoidance and desiccation tolerance mechanisms (Blum, 1988 and Zhang *et al.,* 1999). Drought escape is the ability of a plant to escape periods of drought, especially during the most sensitive periods of its development, early flowering and/or short growth duration. It is advantageous in environments with terminal drought stress and where physical or chemical barriers inhibit root growth. Drought avoidance is a mechanism which avoids lower water status in tissues during drought by maintaining cell turgor and cell volume either through aggressive water uptake by an extensive root system or through reduction of water loss from transpiration and other non-stomatal pathway such as through the plant cuticle and epicuticular wax (Ludlow and Muchow, 1990).

Drought tolerance being a complex trait, the advent and use of DNA markers has facilitated a benefit for understanding and handling of this trait through analysis and mapping of Quantitative Trait Loci (QTL). Advancement in DNA marker technology and marker assisted selection procedures has enabled precise introgression of QTLs in to desired genetic background. Review of literature pertaining to various aspects of present study is presented here.

## 2.1 Plant response to drought stress

### 2.1.1 Physiological adaptations to drought stress

Drought tolerance involves complex mechanisms working in combination to avoid or tolerate water deficits. A number of physiological responses have been observed against drought (Ludlow and Muchow, 1990; Fukai and Cooper, 1995; Ma *et al*., 2007 and Vasquez-Robinet, 2008). One of the most important adaptation consists of accumulation of water to delay or escape from the stress by adopting some anatomical changes. Drought-tolerant plants are able to cope up with stress by diminishing their metabolic functions, which can be recovered once water potential increases (Chandler and Bartels, 1999 and Bartels, 2005). Relevant examples of physiological adaptations to drought stress are reviewed in Table 1.

Plant minimise water loss principally through stomatal closure, the accumulation of osmotically active metabolites and retention of water by proteins or altered resistance to water flow. In some species decrease leaf water content leads to consequent stomatal closure which results in reduced $CO_2$ availability and production of reactive oxygen species such as superoxide radicals (Sgherri *et al.,* 1993). Stomata, highly specialized cells involved in gas exchange, can account for a high water loss through leaf transpiration. Plants adaptation to drought involves stomatal closure during stress (Blum, 1996). This adaptation implies the accumulation of gases such as, $CO_2$ which diminish photosynthesis (Bohnert and Sheveleva, 1998). In order to detoxify $H_2O_2$ and intermediates produced during oxygenic metabolism, enzymes like glutathione reductase and superoxide dismutase increases in response to drought stress and are probably very important in development of drought tolerance.

Further, drought stress has also been shown to cause alterations in the chemical composition and physical properties of the cell wall. Such changes may involve the expression of genes encoding *S*-adenosylmethionine synthetase. Under non-stressful conditions, increased expression of *S*-adenosyl- L-methionine synthetase has been correlated with areas where lignification occurs.

**Table 1: Physiological traits relevant for response to drought conditions**

| Plant traits | Effects relevant to yield | Modulation under stress | References |
|---|---|---|---|
| Stomatal conductance/ leaf temperature | More/less rapid water consumption | Stomatal resistance increases under stress | Jones, 1999; Lawlor and Cornic, 2002 |
| Photosynthetic capacity | Modulation of concentration of Calvin cycle enzymes and elements of the light reactions | Reduction under stress | Lawlor and Cornic, 2002 |
| Timing of phonological phases | Early/late flowering, maturity and growth duration, synchrony of silk emergence and anthesis, reduced grain number | Wheat and barley advanced flowering, rice delayed, maize asynchrony | Slafer *et al.,* 2005; Richards, 2006 |
| Anthesis-silking interval ASI in maize | ASI is negatively associated with yield in drought conditions | Drought stress at flowering causes a delay in silk emergence relative to anthesis | Bolanos and Edmeades, 1993; Edmeades *et al.,* 2000 |
| Starch availability during ovary/embryo development | A reduced starch availability leads to abortion, reduced grain number | Inhibition of photosynthetic activity reduces starch availability | Boyer and Westgate, 2004 |
| Partitioning and stem reserve utilization | Lower/higher remobilization of reserves from stems for grain filling, effecting kernel weight | Compensation of reduced current leaf photosynthesis by increased remobilization | Blum, 1988; Slafer *et al.,* 2005 |
| Stay-green | Delayed senescence | | Rajcan and Tollenaar, 1999 |
| Single plant leaf area | Plant size and related productivity | Reduced under stress wilting, senescence, abscission | Walter and Shurr, 2005 |
| Rooting depth | Higher/lower tapping of soil water resources | Reduced total mass but increased root/shoot ratio, growth into wet soil layers, re-grows on stress release | Hoad *et al.,* 2001 |

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| Plant traits | Effects relevant for yield | Modulation under stress | References |
|---|---|---|---|
| Cuticular resistance and surface roughness | Higher or lower water loss, modification of boundary layer and reflectance | | Sharp *et al.,* 2004 |
| Photosynthetic pathway | C3/C4/CAM, higher WUE and greater heat tolerance of C4 and CAM | | Cushman, 2001 |
| Osmotic adjustment | Accumulation of solutes, ions, sugars, poly-sugars, amino acids, glycinebetaine | Slow response to water potential | Serraj and Sinclair, 2002 |
| Membrane composition | Increased membrane stability and changes in aquaporine function | Regulation in response to water potential changes | Tyerman *et al.,* 2002 |
| Antioxidative defense | Protection against active oxygen species | Acclimation of defence systems | Reddy *et al.,* 2004 |
| Accumulation of stress/related proteins | Involved in the protection of cellular structure and protein activities | Accumulated under stress | Ramanjulu and Bartels, 2002; Cattivelli *et al,,* 2002 |

Thus, the increased expression of some gene in drought-stressed tissue could also be thus due to lignification in the cell wall. Lignification processes seem to begin after the cell elongation stops under prolonged drought stress (Peleman *et al.,* 1989).

Anatomical adaptations observed in tolerant plants consist of spongy tissues which act as water reservoirs; growth is also impaired and plants reduce their foliar area to limit evaporation (Passioura, 1996). Minimized radiation absorption through increased epicuticular wax load, leaf orientation, leaf senescence, shedding of leaves, leaves and stem pubescence, increasing reflectance of the leaf surface and reduced chlorophyll and carotenoid content leads to poor light absorption and conversion to energy. It results in inhibition of photosynthetic activity and reduced starch availability (Boyer and Westgate, 2004 and Colom and Vazzona, 2003). Similar other strategies for anatomical adaptations are the rolling of the leaves, floral abscission and alteration in cuticle permeability (Taiz and Zeiger, 1998), delayed senescence (Rajcan and Tollenaar, 1999), single plant leaf area reduction under stress which affects plant growth and productivity (Walter and Shurr, 2005), delay in silk emergence relative to anthesis in case of maize (Bolanos and Edmeades, 1993 and Edmeades *et al.,* 2000) and increased membrane stability (Tyerman *et al,.* 2002). Roots detect a water gradient and redirect its growth towards moisture source, which leads to reduction in total mass with increased root/shoot ratio, growth into wet soil layers and regrowth on stress release (Lambers *et al.,* 2000 and Hoad *et al.,* 2001).

### 2.1.2 Biochemical responses to drought stress

The plants respond to stress condition through complex biochemical changes to cope with stress. It mainly focused on maintaining water potential. At the cellular level, the cell membrane as well as the endomembrane systems dramatically change their disposition and limit organelle function as well as cell integrity in response to the stress (Gigon *et al.,* 2004). Drought has been known to cause alterations in the chemical composition and physical properties of the cell wall and the changes such as lignifications of cell wall, which are associated with the expression of genes encoding S-adenosyle methionine syntheses (Espartero *et al*., 1994). Genes encoding proteins with sequence similarity to proteases has been reported for getting induced by drought in pea (Gurrero *et al*., 1990) and *Arabidopsis* (Koizumi *et al.,* 1993). It has been reported that these enzymes are functionally active in degradation of irreparably damaged proteins produced during drought condition in plant (Gurrero *et al*., 1990 and Singla *et al*., 1997).

During drought stress, protein residues might be modified by chemical processes such as deamination, isomerization or oxidation and it thus likely that enzymes with functions in protein repair are up-regulated or induced during drought. Mudgett and Clark (1994) has reported increased levels of L-isoaspartly methyl transferases, which play important role in converting the drought damaged L-isoaspatly residues back to L-aspartly residues. During dehydration conditions, the cell wall rigidity can provides mechanical protection (Verslues *et al.,* 2006). A common biochemical adaptation to drought is osmotic adjustment, which results from synthesis of certain new metabolites (Yancey *et al.,* 1982 and Bartels and Sunkar, 2005). These osmolytes are hydrophilic highly soluble molecules, able to produce a salvation surface that capture water molecules and make it available during water limitation. Examples of these osmolytes are amino acids, glycine-betaine, sugars and sugar alcohols, which are non-toxic at their higher concentrations and thus do not interfere with cellular metabolism.

Previous studies have demonstrated that genetic manipulation for accumulation of low molecular weight osmolytes results in increased tolerance to water stress in transgenic plants (Rathinasabapathi *et al*., 1994 and Saneok *et al*., 1995). Osmolytes have some additional additional functions besides turgidity maintenance, such as coping with oxidative stress by producing reactive oxygen species (Chen and Murata, 2002 and Bartels and Sunkar, 2005). Being ubiquitous in nature, Proline posses osmolyte properties expressed by P5CS TF. It also play role in protection of plasma membrane integrity (Mansour, 2000), as a source for carbon and nitrogen (Peng *et al.,* 1996) and a reactive-oxygen scavenger (Smirnoff and Cumbes, 1989 and Hong *et al.,* 2000). Glycine betaine has also been described as osmoprotectant, maintaining water equilibrium in plant organs (Chen and Murata, 2002). Various soluble sugars and carbohydrates serve as solutes for osmotic adjustment. Sugar such as sucrose, maltose and trehalose are also known to stabilize the activity of enzymes during senescence (Carpenter *et al*., 1987).

Decreasing leaf water content and consequent stomata closure resulted in reduced $CO_2$ availability, active oxygen species and increased photo-respiratory activity, which resulted in the production of $H_2O_2$ toxins (Mitler and Zilinskas, 1994). To scavenge these toxins, genes encoding detoxifying enzymes such as ascrobate peroxide are found to be up-regulated (Mittler and Zilinskas, 1994).

Aquaporin (AQP) protein family is another example of stress-protecting proteins, facilitating water uptake and allocation by forming cellular water pores. During drought stress, protein residues might be modified by chemical processes such as deamination, isomerization or oxidation and thus enzymes that functions in protein repair are up-regulated or get induced during drought. Heat Shock Proteins (HSP), also known as molecular chaperones, are widely distributed in nature; known to get highly accumulated during stress. They are involved in proper protein folding and assembly and prevent protein thermal aggregation (Lee *et al*., 1995). Thus, facilitating the recovery of cell functions after abiotic stress. These proteins are classified according to their molecular weight: the Hsp70 family (family DnaK), the chaperonins (GroEL and Hsp60), the Hsp90 family, the HSP 100 family (Clp) and the family of small HSP (sHsp) (Wang *et al*., 2004). It has been found that cyclophilin, a chaperon protein with systemic properties and involved in protein folding, get highly induced during drought stress conferring multiple abiotic stress tolerance in plants.

### 2.1.3 Drought responses at the molecular level

Drought tolerance is considered as a quantitative trait, which involves the participation of a complex set of genes, (Montalvo-Hernandez *et al.,* 2008). The plant perceiving drought stress, changes in the gene expression pattern have been monitored (Ramanjulu and Bartels, 2002, Shinozaki and Yamaguchi-Shinozaki, 2000; Knight and Knight, 2001 and Zhu, 2001). Results of these experiments reported the expression of genes ranging from those whose products are involved in early response such as signal transduction, transcription and translation factors; to late response genes, such as water transport, osmotic balance, oxidative stress and damage repair. Some adaptive responses like early flowering, growth inhibition are also observed as a consequence of such changes (Bray, 2002).

Relevant examples of genes conferring drought tolerance, their functions and mechanism of action are reviewed in Table 2.

#### 2.1.3.1 Drought sensing and signal transduction

Molecular studies have identified many genes that are induced or up regulated by osmotic stress (Ingram and Bartlet, 1996). The plant regulator like abscisic acid (ABA) is a key endogenous messenger mediating drought stress response. and hence also thought to be involved in the signal transduction (Pla *et al.,* 1991 and Raghavendra *et al.,* 2010). Experiments using mutants where ABA biosynthesis pathway were blocked, in the demonstrated that specific genes require elevated levels of ABA for expression during water and low-temperature stress (Cohen and Bray, 1990 and Pla *et al.,* 1991). Drought induces high levels of ABA, which ultimately lead to the major changes in gene expression and adaptive physiological responses (Christmann *et al.,* 2007). Hence, it is considered that ABA has a central role in early plant response to drought.

Four signal-transduction pathways have been reported to be involved in plant responses to osmotic stress: among them two are ABA-dependent (I and II) and two are ABA independent (III and IV). These pathways lead to activation or synthesis of transcription factors such as MYB/MYC and bZIP. ABA-dependent pathway I require protein synthesis to activate transcription factors MYC/MYB and/or bZIP, which bind to DNA regions other than ABA-responsive elements (ABREs). ABA-dependent pathway II activates bZIP, a transcription factor that turns on gene expression through binding to ABREs. ABA-independent pathway IV induces gene expression through activation of DREBP (drought response- element-binding protein), which binds to the DRE (drought response element) motif and leads to induction of cold and drought-induced genes. Though ABA independent pathway III is not yet well understood, these four signaling pathways are known to cross-talk and converge to activate stress-response gene expression. This type of stress induced gene expression is further broadly categorized in to three groups: genes encoding proteins with known enzymatic or structural functions, regulatory proteins and proteins with as yet unknown function(s).

**Table 2: Transcription factors and other genes conferring drought tolerance in plants**

| Genes | Function | Mechanism of action | References |
|---|---|---|---|
| DREBs/CBFs; ABF3 | Stress induced transcription factors | Enhanced expression of downstream stress related genes confers drought/cold/salt tolerance. Constituently over expression can lead to stunting growth. | Oh *et al.,* 2005; Ito *et al.,* 2006 ; Ashraf, 2010 |
| SNAC1 | Stress induced transcription factor | SNAC1 expression reduces water loss increasing stomatal sensitivity to ABA. | Hu *et al.,* 2006 |
| *OsCDPK7* | Stress induced Ca-dependent protein kinase | Enhanced expression of stress responsive genes. | Saijo *et al.,* 2000 |
| Farnesy;/transferase ERA1 | Negative-regulator of ABA sensing | Down-regulation of farnesyl transferase enhances the plant's response to ABA and drought tolerance reducing stomatal conductance. | Wang *et al.,* 2005 |
| Mn-SOD | Mn-superoxide dismutase | Over expression improves stress tolerance also in field conditions. | McKersie *et al.,* 1996 |
| *AVP1* | Vacuolar H+- pyrophsphatase | Over expression facilitate auxin fluxes leading to increased root growth. | Gaxiola *et al.,* 2001; Park *et al.,* 2005 |
| *HVA1; OsLEA3* | Stress induced LEA proteins | Over-accumulation of LEA increases drought tolerance also in field conditions. | Bahieldin *et al.,* 2005; Xiao *et al.,* 2007 |
| *ERECTA* | A putative leucine-rich repeat receptor-like kinaseis a major contributor to a locus for Δ on Arabidopsis chromosome 2 | ERECTA acts as a regulator of transpiration efficiency with effects on stomatal density, epidermal cell expansion, mesophyll cell proliferation and cell-cell contact. | Masle *et al.,* 2005 |

*Contd…..*

| Genes | Function | Mechanism of action | References |
|---|---|---|---|
| *otsA* and *otsB* | *Escherichia coli* trehalose biosynthetic genes | Increased trehalose accumulartion correlates with higher soluble cabohydrate levels, elevated photosynthetic capacity and increased tolerance to photo-oxidative damage. | Garg *et al.,* 2002 |
| P5CS | δ-Pyrroline 5-carboxylate synthetase | Enhanced accumulation of praline leads to increased osmotolerance. | Kavi Kishor *et al.,* 1995; Zhu *et al.,* 1998 |
| MtlD | Mannitol-1-phosphate dehydrogenase | Mannitol accumulation leads to increased osmotolerance. | Abebe *et al.,* 2003 |
| GF14λ | 14-3-3 protein | Lines overexpressing GF14λ have a 'stay-green' phenotype, improved water stress tolerance and higher photosynthetic rates under water deficit conditions. | Yan *et al.,* 2004 |
| NADP-Me | NADP-malic enzyme | The over expression decreased stomatal conductance and improves WUE. | Laporte *et al.,* 2002 |
| *WXP1* | | Increasing cuticular wax. | Zhang *et al.,* 2005 |
| *HARDY* gene | | Deep green color, more bundle. | Karaba *et al.,* 2007 |
| *Rd29A* | | Sheath cells. | Chen *et al.,* 2006 |

Stress-induced proteins with known functions include water channel proteins, key enzymes for osmolyte (proline, betaine, sugars, and polyamines) biosynthesis, detoxification enzymes and transport proteins. It has been demonstrated that most of the regulatory proteins are involved in signal transduction (Nakashima *et al*., 2009).

ABA is synthesized through the carotenoid biosynthesis pathway. ABA concentration is altered when there are changes in cellular dehydration. Reduction in turgor results in rapid synthesis of this phytohormone (Quarrie and Lister, 1984 and Guerrero and Mullet, 1986). Increased levels of ABA can, in turn, induce changes in gene expression resulting in stomatal closure in leaves, inhibition of photosynthesis and the growth of leaves, stems and hairy roots. Transgenic plants overproducing MYC and MYB showed higher sensitivity to ABA and revealed osmotic stress tolerance (Abe *et al*., 2003). Studies reported that *SPK1* get induced expression by ABA, dehydration, salinity and low temperature, while its repression decreases sensitivity to ABA (Osakabe *et al.,* 2005).

2.1.3.2 Drought induced genes at transcriptional level

Plant gene expression is controlled at different levels and a significant number of drought-induced genes appear to be controlled at the transcriptional level. Transcription, the first step in the expression of any gene, plays a central role in the regulation of the gene expression. Transcription is controlled by numerous transcription factors that mediate the effects of intercellular and extracellular signals. Transcription factors (TFs) are the master regulators that control gene clusters. A single TF can control the expression of many target genes through a specific binding of the TF to the *cis*-acting element in the promoters of respective target genes (Nakashima and Shinozaki, 2006).

Bioinformatics analyses have identified induction of several TF under drought stress (Bartels and Sunkar, 2005; Marcotte *et al.,* 1989; Abe *et al.,* 1997; Ashraf *et al.,* 2008 and Ashraf, 2010). TF are classified in to six families: AP2/ERF (APETALA2/ethylene-response factor) (Marcotte *et al.,* 1989 and Abe *et al.,* 1997), bZIP (Basic leucin-zipper protein); MYB/MYC, Zinc-finger protein (Rodríguez-Uribe and D'Connell, 2006 and Abe *et al.,* 1997); CDT-1 (Furini *et al.,* 1997) and NAC families (Yamaguchi-Shinozaki and Shinozaki, 1994; Bartels and Sunkar, 2005 and Umezawa *et al.,* 2006); DREB TF leading to the enhanced expression of downstream stress related genes and conferring drought/cold/salt tolerance. Constituent over expression of these TF genes can lead to stunted growth in plants (Oh *et al.,* 2005 and Ito *et al.,* 2006). SNAC1 is a stress induced transcription factor, whose expression reduces water loss by increasing stomatal sensitivity to ABA (Hu *et al.,* 2006).

Well-studied group of TFs involved in drought and cold tolerance are the CBF (C-repeat binding factor) genes also known as *DREB1* (dehydration-responsive element binding protein) genes. DREB protein potentially bind to DRE element specifically and regulates the expression of downstream target genes. Several genes encoding DREB-like transcription factors have already been cloned from *Arabidopsis*, which includes *DREB2A*, *DREB2B* and *CBF4*. They were all significantly induced under drought, cold and high salinity stress conditions (Liu *et al.,* 1998). Expression of *GmDREB2*, a soybean DRE-binding transcription factor gene, induced the expression of *Rd29A* and *cor15a* genes that already have been identified as downstream genes of *AtDREB1A* in *Arabidopsis*, and therefore enhanced their tolerance to drought and high-salt stresses (Ming Chen *et al.,* 2006). The induction of many genes responsive to drought or cold conditions such as *kin1*, *cor6.6*, *rd17* and *rd29A* are related to the presence of a DRE *cis*-element in their promoter regions. Kasuga *et al.* (1999) reported significant stress tolerance without strong growth retardation by expressing *DREB1A/CBF3* in *Arabidopsis* under the control the promoter from the stress-inducible *rd29a* gene. Oh *et al.* (2005) also reported enhanced drought tolerance in rice plants that constitutively over expressed either *CBF3* or *ABF3* (a bZIP TF from *Arabidopsis*).

WXP1, another AP2/EREBP TF from alfalfa (*Medicago truncatula*), has been found to be responsible for enhanced drought tolerance in transgenic alfalfa plants by increasing cuticular wax, presumably by improving the water retaining capacity of the plant (Zhang *et al.,* 2005). The *Arabidopsis HARDY* gene (*HRD*) is yet another example of an AP2/EREBP TF that was recently found to provide enhanced drought tolerance in transgenic *Arabidopsis* and rice plants (Karaba *et al.,* 2007).

Rice lines transformed with the *HRD* gene shows a deep green color, more bundle sheath cells and more tillers with better water use efficiency and better drought tolerance, these parameters are correlated with a lower transpiration rate and a higher net carbon assimilation rate.

2.1.3.3 Drought-induced proteins

Translational control is another mechanism regulating plant responses to abiotic stress. Synthesized proteins have direct functions in membrane and protein protection. Late Embryogenesis Abundant (LEA) proteins are generally divided into five groups, and they appear to protect cellular structures from dehydration stress; however, the exact functional role of these hydrophilic proteins remains poorly understood. Proposed roles of drought induced proteins involves water binding or replacement, hydration buffers, ion sequestration, osmotic adjustment or reverse chaperones and transport of nuclear-targeted proteins during stress (Ingram and Bartels, 1996). LEA family proteins fall in group of proteins and also described as highly accumulated in plant embryos (Dure *et al.,* 1981 and Galau *et al.,* 1986). LEA proteins expressing at basal levels during normal conditions can get induced to high levels during osmotic and drought stress (Ingram and Bartels, 1996; Barrera-Figueroa *et al.,* 2007). HVA1; OsLEA3 are hydrophilic LEA proteins consistently expressed with increased level during drought (Bahieldin *et al.,* 2005 and Xiao *et al.,* 2007). HVA1 a group 3LEA protein from barley and LEA 25, group 4 LEA protein from tomato have been shown to have a functional role in stress tolerance. Reports indicated that the level of *HVA1* gene expression is much higher than that of *HVA22.* Interestingly, expression of the maize *AVP1* transcription factor in barley aleurone layers activates the ABRC in the *HVA1* gene but not in the *HVA22* gene. The presence of *AVP1* and ABA treatment has a synergistic effect on the ABRC of the *HVA1* gene but not on that of *HVA22.* However, ABRC1 and ABRC3 do not display tissue specificity as both of them function in aleurone tissue as well as in vegetative tissues (Qingxi Shen, 1996). The *HVA1*gene has been used successfully to confer stable tolerance to osmotic stress in rice (Xu *et al.,* 1996) and wheat (Patnaik and Khurana, 2003).

Lines overexpressing GF14λ a 14-3-3 protein, have a 'stay-green' phenotype with improved water stress tolerance and higher photosynthetic rates under water deficit conditions (Yan *et al.,* 2004). The over expression of NADP-malic enzyme, NADP-Me decreases stomatal conductance and improves WUE (Laporte *et al.,* 2002). ERECTA, a putative leucine-rich repeat receptor-like kinaseis acts as a regulator of transpiration efficiency with effects on stomatal density, epidermal cell expansion, mesophyll cell proliferation and cell-cell contact (Masle *et al.,* 2005). WRKY proteins which are unique to plants contain either one or two WRKY domains, a 60-amino acid region highly conserved among the family members. They play a key role in regulation of the pathogen induced defense responses (Wu *et al*., 2005), abiotic stress responses (Fowler and Thomashow, 2002; Seki *et al*., 2002 and Mare *et al*., 2004) and also involved in various physiological processes including senescence, trichome development and biosynthesis of secondary metabolites (Eulgem *et al*., 2000). Increased trehalose accumulartion by *otsA* and *otsB* genes correlates with higher soluble cabohydrate levels, elevated photosynthetic capacity and increased tolerance to photo-oxidative damage (Garg *et al.,* 2002). Plant *NF-Y* function appears to be important in plants response to drought stress. Over-expression of *NF-YA5* reduces drought susceptibility, anthocyanin production, and stomatal aperture (Nelson *et al*., 2007). The over expression of NADP-malic enzyme by NADP-Me decreases stomatal conductance and improves WUE (Laporte *et al.,* 2002).

## 2.2 Response to drought stress in sorghum

Sorghum has a wide range of adaptability and can be grown in wide series of environments. Drought stress has diverse effects on yield depending on the development stage at which it occurs (Agboma *et al.,* 1997). Incidence of drought stress at seedling stage may lead to higher dry root weights, longer roots, coleptiles and higher root: shoot ratios (Zekri, 1991; Matsuura *et al.,* 1996; Pace *et al.,* 1999; Takele, 2000; Dhanda *et al.,* 2004; Kashiwagi *et al.,* 2004). Although sorghum has ability to cope with many types of stresses, including heat, drought, salinity and flooding (Ejeta and Knoll, 2007) but still in arid and semi-arid regions, this crop is usually affected by water stress at the reproductive stage and grain filling stage (Tuinstra *et al.,* 1997 and Kebede *et al.,* 2001).

The growth stage at which moisture stress occurs, is very important in determining the response or reaction of sorghum to water stress. In sorghum three growth stages have been identified that are critical in understanding drought tolerance: GS1, seedling establishment (early vegetative stage); GS2, pre-flowering (panicle differentiation to flowering); and GS3, post-flowering (grain fill to physiological maturity of grain). Two distinct and important types of drought stress responses have been identified and described in sorghum and are related to GS2 and GS3 *viz*., pre-flowering and post-flowering drought responses respectively (Rosenow *et al.,* 1983; Rosenow, 1987).

### 2.2.1 Pre-flowering drought resistance

Sorghum lines with distinct phenotypic response to pre-flowering and post-flowering drought stress have been characterized and excellent sources of resistance to each type of stress have been identified (Rosenow, 1993). The pre-flowering response occurs when the plants are under significant moisture stress before flowering, especially from panicle differentiation to flowering (GS-2). This type of stress directly affects panicle size, grain number and grain yield. Tuinstra *et al*. (1996) found six regions in the sorghum genome associated with pre-flowering drought tolerance and eight additional regions generally associated with yield or yield components under fully irrigated conditions. Four major QTLs responsible for pre-flowering drought have also been identified in linkage map C, E and F using SC56 × Tx7000 crosses in sorghum (Kebede *et al.,* 2001).

### 2.2.2 Post-flowering drought resistance

Post-flowering drought response in sorghum is expressed when moisture stress occurs during the grain development stage (GS-3). Drought that occurs during post-flowering stage of crop growth and is not relieved until maturity is referred to as terminal drought and is such that genotypes are grown on stored soil moisture. Rapid premature leaf death generally occurs when water is limited during the grain filling period. Premature leaf senescence in turn leads to charcoal rot, stalk lodging and significant yield loss. Drought stress during the post-flowering period accelerates senescence in many plant species by driving many physiological processes in the same direction as normal senescence (Nooden *et al.,* 1997). In sorghum, the best characterized form of tolerance to stress during this post-flowering stage of crop growth is the so called ‘stay-green’ trait.

## 2.3 Stay-green trait in sorghum

Stay-green is a general term given to a variant in which the relative senescence (compared to control) is delayed compared with a standard genotype, characterised by the maintenance of green stem and upper leaves when water is limiting during grain filling. The term stay-green has been used to describe an important component of post-flowering drought response in sorghum (Rosenow and Clark, 1981). The stay-green trait results in greater functional photosynthetic leaf area during grain filling and ever after physiological maturity. The differences in onset and rate of senescence can be explained by differences in the nitrogen dynamics of the plant at leaf and whole plant levels (Borrell *et al.,* 2000a, 2000b and Borrell and Hammer, 2000). Stay-green is the ability of plant to withstand premature leaf and plant death, develop grain normally and resist charcoal rot and lodging when exposed to moisture stress during the late stages of grain development (Rosenow, 1984; Rosenow and Clark, 1981; Rosenow *et al.,* 1983; Tenkouano *et al.,* 1993; Walulu *et al.,* 1994 and Woodfin *et al.,* 1988).

Thomas and Smart (1993) classified stay-green into four types : A, B, C and D. In type A, senescence is initiated late but then proceeds at a normal rate. Type B stay-green initiates senescence on schedule, but thereafter senescence comparatively slow down and in type C chlorophyll may be retained more or less indefinitely but physiological functions such as photosynthesis are affected *i.e*., senescence is proceeding internally although the surface pigmentation is retained. The type D is unlimited colour retention which may be related to premature death seen in *Herbacium* species or achieved by rapid killing as in the case of frozen foods. Therefore, greenness is retained though the physiological functions have been fully lost. Types C and D are therefore non-functional forms of stay-green.

Genotypic variation for the stay-green trait was observed by Borrell *et al*. (1998) in grain sorghum hybrids when water was limiting during grain filling stage. Green leaf area at maturity (GLAM) is an excellent indicator of stay-green. The key components determining GLAM are total plant leaf area (TPLA), duration of leaf senescence and rate of leaf senescence. Duration of leaf senescence is a function of the timing of the senescence and the timing of physiological maturity. Two factors affecting the components of GLAM are water deficit and genotype. It has also been indicated that time and severity of drought is critical in determining both leaf area development and subsequent senescence. Environmental conditions resulting in high leaf area production at anthesis followed by severe post anthesis water deficit and are most conductive to the expression of stay-green. They examined nine hybrids from crosses of three females varying in rate of leaf senescence (AQL 39, senescent, AQL41, intermediate, B35, stay-green and three males similarly varying R69264, senescent, RQL36, intermediate, RQL12, stay-green). They observed genotypic variation for TPLA onset of leaf senescence, duration of leaf senescence and rate of leaf senescence and they concluded that the mechanism of leaf area maintenance also varied between the B35 and RQL12 sources of stay-green.

Stay-green in sorghum has been associated with higher yield under water limited conditions. Yield increases in stay-green types have been attributed directly to the maintenance of photosynthetic capacity during grain filling period. During post-anthesis, drought tolerant hybrids containing the stay-green trait maintain more photosynthetically active leaves compared with other hybrids (Rosenow *et al.,* 1983; McBee, 1984; Wolfe *et al.,* 1988b and Borrell *et al.,* 2000a, 2000b). Sorghum genotypes with the stay-green trait continue to fill their grain normally under drought (Rosenow and Clark, 1981) and exhibit resistance to charcoal rot (Rosenow, 1984) and lodging (Henzell *et al.,* 1984). The stay-green trait does not appear to reduce the grain yield of hybrids while at the same time it improves lodging resistance and resistance to diseases (Borrell and Hammer, 2000). Such genotypes also contain increased levels of basal stem sugars (Duncan, 1984) and cytokinins (McBee, 1984) than senescent genotypes, which may reduce the rate of drought-induced senescence (Thomas and Smart, 1993). Increased accumulation of soluble sugars found in stay-green genotypes may reduce the dependence on stored assimilates from the stem to fill the grains (McBee *et al*., 1983).

Expression of stay-green has also been reported in other crops including maize (Tollenar and Daynard, 1978), tomato (Akhtar *et al*., 1999) and oat (Helsel and Frey, 1978). A stay-green mutant of the pasture grass *Festuca pratensis* Huds, has also been identified and subsequently studied, which has led to a better understanding of the biochemistry of senescence (Kingston-Smith *et al*., 1997; Hauck *et al*., 1997; and Thomas *et al*., 1999).

### 2.3.1 Inheritance of stay-green in sorghum

E36-1 and B35 stay-green types, are well adapted to tropical and temperate climates respectively. B35 (PI 534133) is a selection from a converted (dwarf, early flowering) version of IS 12555, an Ethiopian land race (Rosenow *et al.,* 1983, 1996). Stay-green trait in B35 is influenced by a major gene that exhibits varied levels of dominance gene action depending on the environment. Reports also indicate that the gene(s) controlling stay-green are additive or partially dominant depending on the specific environment. Studies of back cross progenies showed the dominance nature of the gene(s) for stay-green trait in B35 as per the report of Richard *et al*., (1994).

It was suggested that environment has a strong influence on the mode of expression of those gene(s) controlling stay-green trait in B35. Under field environment, the behaviour of this character was more dominant than under the extreme water stress environment of the rainout shelter, where the gene action was described as additive to partial dominance. Walulu *et al*. (1994) concluded that stay-green trait is a quantitative trait controlled by nuclear gene/s.

## 2.4 Water use efficiency

WUE has different meaning in different disciplines. In agronomic context WUE is defined as the economic yield of a crop per unit of water applied or rainfall received in the growing season (Turner *et al.,* 1986). In leaf $\Delta$ was found to vary in the range 4.90-5.10% in maize (Heng *et al.,* 2005), 3.10-4.15 in sorghum (Hammer *et al.,* 1997), 2.75-4.5% in pearl

$$\frac{\text{mmol of } CO_2 \text{ fixed}}{\text{mmol of water transpired}}$$

millet (Bruck *et al.,* 2000) and 4.2-5.2% in sugarcane (Meinzer *et al.,* 1994). WUE is defined as mmoles of $CO_2$ fixed per moles of water transpired (Morgan and LeCain, 1991).

WUE =

At a whole plant level, WUE is expressed as the ratio of total dry matter to the total amount of water transpired. WUE also referred evapo-transpiration efficiency (Tanner and Sinclair, 1983).

$$\text{WUE (whole plant level)} = \frac{\text{Dry matter production (g)}}{\text{Water lost in transpiration (kg)}}$$

Stable isotope of carbon

Carbon Isotope Discrimination (CID or $\Delta^{13}C$ ) was established to be correlated with WUE in many crop species . There are two naturally occurring stable isotopes of carbon, $\Delta^{12}C$ and $\Delta^{13}C$. Most of the carbon is $\Delta^{12}C$ (98.9%) with 1.1% being $\Delta^{13}C$. The overall abundance of $^{13}C$ relative to $^{12}C$ in plant tissue is commonly less compared to its proportion in atmosphere, indicating that CID occurs during the fixation of $CO_2$ into plant biomass. Because the isotopes are stable and non-radioactive in nature, the information is inherent in the ratio of abundance of carbon isotopes, presented by convention as $\Delta^{13}C/\Delta^{12}C$, is invariant as long as carbon is not lost. The $\Delta^{13}C$ in plant samples is generally determined using a sophisticated analytical instrument called Isotope Ratio Mass Spectrometer (IRMS) specially designed for high precision measurements of the molar ratio (R), defined as:

$$R = \Delta^{13}CO_2/\Delta^{12}CO_2$$

Hubick *et al* (1990) has reported that there is significant variation for $\Delta$ in sorghum genotypes and $\Delta$ has significantly correlated with yield. The correlation may be because of differences in photosynthetic efficiency, transpiration efficiency or both. Henderson *et al.,* (1998) has found that variation in $\Delta$ reflected WUE of 30 *Sorghum bicolor* genotypes. Selection for high WUE results in higher yield levels if high WUE is because of photosynthetic efficiency (Hubick and Gibson, 1993).

The existence of genotype by environment (G × E) interactions for grain yield in crops has complicated selection and breeding strategies for many years. G × E interactions are noticeable when genotypes being evaluated rank differently among trials conducted in different locations and seasons (Wright *et al.,* 1993). For any trait to be successfully exploited in breeding program, in addition to significant genetic variability, a low G × E interaction is preferred. Low G × E for TE (Transpiration Efficiency), however, has been reported in legume such as peanut (Wright *et al.,* 1998). Significant variation among peanut cultivars in WUE under well watered and water limited conditions has been shown in isolated plants in the glasshouse and in small canopies in the field. Further, G × E interaction for WUE and $\Delta^{13}C$ was shown to be low, while heritability of $\Delta^{13}C$ was high indicating that these traits could be used for selecting high WUE in peanut breeding programs (Wright *et al.,* 1993). However, relatively low heritability of WUE and large G × E was reported in sorghum indicating that selections will only be effective when evaluated in uniform environment (Henderson *et al.,* 1998).

## 2.5 Genetic variability, heritability and correlation studies in drought associated traits in sorghum

### 2.5.1 Genetic variability parameters

The success of a crop breeding program is based on the availability of genetic variability and the efficient utilization of such variability. It is largely based on the ability of the breeder to identify superior genotypes within segregating populations.

Phenotypic variability expressed by a genotype or a group of genotypes in any species can be partitioned into genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV). Being the heritable part of the total variability, the magnitude of genotypic component on yield and its component characters influences the selection strategies to be adopted by the breeder. It has been observed that the estimates of genetic variance were smaller than their respective phenotypic variances (Khan *et al*., 2005).

Galton (1889) enunciated that only a part of a continuous variation was due to heredity (H). Trait heritability estimation is a first step towards assessing the amount of genetic variation present in a breeding population. Regardless of the type of heritability estimate, heritability is broadly defined, as the proportion of observable field variation that is due to genetic factors (Nyquist, 1991). Lush (1949) defined heritability in broad sense as the ratio between the genotypic variance as a whole that is due to phenotype. Lush (1949) further again defined heritability in narrow sense as the ratio of additive genetic variance to the phenotypic variance. Heritability values can be used as a measuring scale to determine genetic relationship between parents and progeny (Memon *et al*., 2007). Heritability is also on the higher side for all the traits indicating that these traits are controlled by additive genes and it can be assumed that they will remain stable under varied environmental conditions. Heritability studies provide valid information about the traits that are transmitted from parents to offspring and also to the successive generations. Several studies have been conducted on sorghum populations to estimate trait heritabilities and phenotypic and genotypic correlations among those traits. (Liang *et al*., 1969; Chung and Liang *et al*., 1970; Lothrop *et al*., 1985a and Sanchez-Gomez, 2002). .

The genetic gain (GA) that can be expected for a particular character through selection is the product of its heritability, phenotypic standard deviation and selection differential as proposed by Burton and Devane (1953). High heritability coupled with genetic advance indicates that additive gene effects are operating and selection for superior genotype is possible (Arunkumar *et al*., 2004). Such studies help plant breeders to predict a successful cross with high heritability transmission to the progeny and thus are useful in the incorporation of characters into the offspring. High value of heritability and predicted genetic advance clarifies that the selection among genotype would be effective for yield and yield components. High heritability (broad sense) associated with high genetic advance reveals strong contribution of additive genetic variance for expression of the traits and the selection based on these traits could play a vital role in improving grain yield.

The studies conducted by various researchers have shown that high heritability alone is not enough for selection in advance generations; and hence it must be accompanied with substantial amount of genetic advance (Memon *et al*., 2007). However, if a character or trait is controlled by non additive gene action, it gives high heritability but low genetic advance. While for the character governed by additive gene action, the heritability and genetic advance would be high. Heritability and genetic advance enables the breeders to use best genetic stock for improving the crop. A character which have higher range of genetic variability, high heritability and high genetic advance would be an effective tool to improve seed yield. It has been reported that high value of GCV, PCV, broad sense heritability and genetic advance were stated for oil yield and protein yield. Summary of reviews on variability, heritability and genetic advance for various traits in sorghum are briefed in Table 3.

### 2.5.2 Correlation studies

Knowledge of the relationship among plant characters is useful while selecting traits for yield improvement. Correlation analysis provides the information of interrelationship of important plant characters and hence, leads to a directional model for direct and/or indirect improvement in grain yield (Khan *et al*., 2004). The correlation coefficient analysis helps to determine the nature and degree of relationship between any two measurable characters. It resolves the complex relationships between events into simple forms of association. The association of characters can be measured as the coefficient of correlation (Galton, 1889). The basic concept of correlation was elaborated and discussed by Fischer (1918) and Wright (1921) for plant breeding programmes. The main purpose of correlation in crop plants is the detailed understanding of complex characters, such as yield per plant.

**Table 3: Summary of review on variability, heritability and genetic advance for various traits in sorghum**

| Sl. No. | Characters | Class | References | | |
|---|---|---|---|---|---|
| | | | Variability | Heritability $h^2$ | Genetic advance over per cent mean |
| 1 | Days to 50 per cent flowering | Moderate | Prabhakaran, 2001; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Chavan *et al.,* 2010 | Liang and Walter, 1968; Finker *et al.,* 1976; Raja and Parikh, 1980; Dinakar, 1985 | Mukri *et al.,* 2007; Chavan *et al.,* 2010 |
| | | Low | Pate,l *et al.,* 1980; Raja and Parikh, 1980; Nimbalkar *et al.,* 1988; Mukri *et al.,* 2007 | Kanaka and Goud, 1982; Kulkarni and Shinde , 1987 | Naphade, 1973; Khanure, 1993; Rajkumar *et al.,* 2007 |
| | | High | Sindagi *et al.,* 1970; Basu, 1971; Khanure, 1993; Reddy *et al.,* 1996 | Liang *et al.,* 1969; Chung and Liang, 1970; Basu, 1971; Phul *et al.,* 1972; Naphade, 1973; Singh and Singh, 1973; Bhat, 1975; Eckebil *et al.,* 1977; Dhimer and Desai, 1978; Singh and Makne, 1980; Nagabasaih *et al.,* 1981; Kukadia *et al.,* 1983; Bello and Obilana, 1985; Pauli, 1986; Phul and Allahrang, 1986; Worthman *et al.,* 1987; Nimbalkar *et al.,* 1988; Khanure, 1993; Reddy *et al.,* 1996; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Bello *et al.,* 2007; Mukri *et al.,* 2007; Chavan *et al.,* 2010 | Basu, 1971; Phul *et al.,* 1972; Bhat, 1975 ; Reddy *et al.,* 1996 ; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004 |

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| Sl. No. | Characters | Class | References | | |
|---|---|---|---|---|---|
| | | | Variability | Heritability $h^2$ | Genetic advance over per cent mean |
| 2 | Plant height | Moderate | Dhimer and Desai, 1978; Chavan *et al.,* 2010 | Vasudeva Rao, 1973; Janom, 1974; Eckebil *et al.,* 1977 | Arunkumar *et al.,* 2004 |
| | | Low | Lonc , 1969 | - | Singh and Singh, 1973; Amirthadvarathinam *et al.,* 1994; Basu, 1971; Naphade, 1973; Shinde and Nayeem, 1979; Patel *et al.,* 1980a; Singh and Makne, 1980; Salil Kumar and Singhania, 1984; Khanure, 1993; Biradar, 1996; Sankarapandian *et al.,* 1996; Nguyen *et al.,* 1998; Mallinath *et al.,* 2004; Mukri *et al.,* 2007; Chavan *et al.,* 2010 |
| | | High | Kranti Kumar *et al.,* 1970; Basu, 1971; Aristarkova, 1976; Bello *et al.,* 2007; Naphade and Ailwar, 1977; Patel *et al.* 1980; Singh and Makne, 1980; Berenji, 1990; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Mukri *et al.,* 2007 | Naphade, 1973; Singh and Singh, 1973; Shinde and Nayeem, 1979; Singh and Makne, 1980; Salil Kumar and Singhania, 1984; Kumar and Singh 1986; Worthman *et al.* 1987; Biradar, 1996; Rao and Patil, 1996; Nguyen *et al.,* 1998; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Bello *et al.,* 2007; Mukri *et al.,* 2007; Chavan *et al.,* 2010 | |
| 3 | Earhead length | Moderate | Mukri *et al.,* 2007; Rajkumar *et al.,* 2007; Kachapur and Salimath, 2009 | Basu, 1971; Nagabasaih, 1981; Dinakar, 1985; Rajkumar *et al.* 2007; Kachapur and Salimath, 2009 | Rajkumar *et al.,* 2007; Kachapur and Salimath, 2009 |
| | | Low | Lonc, 1969; Aristarkova, 1976; Naphade and Ailwar 1977 | Rao and Goud, 1979 | Khunure, 1993 |
| | | High | Swarup and Chaugale 1962; Khanure, 1993; Biradar, 1996, Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004 | Swarup and Chaugale, 1962; Fanous *et al.,* 1971; Singh and Singh, 1973; Bhat, 1975; Patel, 1980a; Khanure, 1993; Biradar, 1996; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Bello *et al.,* 2007 | Bhat, 1975; Shinde, 1981; Patil and Thombre, 1986 ; Biradar, 1996; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004 |

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| Sl. No. | Characters | Class | References | | |
|---|---|---|---|---|---|
| | | | Variability | Heritability $h^2$ | Genetic advance over per cent mean |
| 4 | Spicklets per head | Moderate | Chavan *et al.,* 2010 | Mukri *et al.,* 2007 | - |
| | | Low | | | |
| | | High | Bereji, 1990; Khanure, 1993; Biradar, 1996; Mukri *et al.,* 2007 | Giriraj and Goud, 1981; Desai *et al.,* 1983; Kukodia *et al.,* 1983; Patil and Thombre, 1986; Dinakar, 1985; Nimbalkar *et al,.* 1988; Khanure, 1993; Biradar, 1996; Bello *et al.,* 2007; Chavan *et al.,* 2010 | Desai *et al.,* 1983; Patil and Thombre, 1985; Nimbalkar *et al.,* 1988; Khanure, 1993; Biradar, 1996; Mukri *et al.,* 2007; Chavan *et al.,* 2010 |
| 5 | Number of leaves | Moderate | Mukri *et al.,* 2007 | | Mukri *et al.,* 2007 |
| | | Low | | | |
| | | High | Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004 | Bello *et al.,* 2007; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Mukri *et al.,* 2007 | Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004 |
| 6 | 100 grain weight | Moderate | Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Mukri *et al.,* 2007; Chavan *et al.,*2010 | Mukri *et al.,* 2007 | Mukri *et al.,* 2007; Kachapur and Salimath, 2009 |
| | | Low | Kachapur and Salimath, 2009 | Liang *et al.* 1969; Liang and Walter, 1968; Fanous *et al.* 1971 | Fanous *et al.* 1971 ; Patel *et al.*, 1980 |
| | | High | Sindagi *et al.,* 1970; Abu-El-Gasim, 1975; Shinde and Nayeem, 1979; Singh and Makne, 1980; Prabhakar, 2001 | Shinde and Nayeem, 1979; Obilana and Okoh, 1984; Kumar and Singh, 1986; Phul and Allah Rang, 1986; Nimbalkar *et al.,* 1988; Nguyen *et al.,* 1999; Prabhakar, 2001; Arunkumar *et al.,* 2004; Bello *et al.,* 2007; | Singh and Makhne 1980; Shinde, 1981; Nguyen *et al.,* 1990; Arunkumar *et al.,* 2004; Chavan *et al.,* 2010 |

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| | | | | | |
|---|---|---|---|---|---|
| | | | Chavan *et al.,* 2010 | | |
| **Sl. No.** | **Characters** | **Class** | **References** | | |
| | | | **Variability** | **Heritability $h^2$** | **Genetic advance over per cent mean** |
| 7 | Panicle exertion | Moderate | Mukri *et al.,* 2007; Chavan *et al.,* 2010 | Mallinath *et al.,* 2004 | |
| | | Low | | | |
| | | High | Mallinath *et al.,* 2004, | Bello *et al.,* 2007; Mukri *et al.,* 2007; Naphade, 1973; | Mukri *et al.,* 2007; Chavan *et al.,* 2010 |
| 8 | Grain yield per plant | Moderate | Chavan *et al.,* 2010, | Chavan *et al.,* 2010 | Naphade, 1973; Shinde, 1979; Phul and Allah Rang, 1986 |
| | | Low | Wenzel *et al.,* 1998; Bello *et al.,* 2007 | Liang and Walter, 1968; Ciobanu, 1968; Singh and Singh 1973; Jan-Orn 1974; Miller 1975; Obilana and Okoh, 1984 | |
| | | High | Abu-El-Gasim, 1975; Shinde *et al.,* 1978; Kumar and Singh, 1986; Nimbalkar and Bapat, 1988; Biradar, 1996; Rao and Patil, 1996; Reddy *et al.,* 1996; Can and Yoshida, 1999; Prabhakar, 2001; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Mukri *et al.,* 2007; Rajkumar *et al.,* 2007; Kachapur and Salimath, 2009 | Shinde and Nayeem, 1979; Salilkumar and Singhania, 1984a; Desai *et al.,* 198; Kumar and Singh, 1986; Phul and Allah Rang, 1986; Rao and Patil, 1996; Prabhakar, 2001; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Mukri, *et al.,* 2007; Rajkumar *et al.,* 2007; Kachapur and Salimath, 2009; Mukri *et al.,* 2007; Chavan *et al.,* 2010 | Singh and Makne, 1980; Phul and Allah Rang, 1980; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Mukri *et al.,* 2007; Rajkumar *et al.,* 2007; Kachapur and Salimath, 2009; Chavan *et al.,* 2010 |
| 13 | Stem girth | Moderate | Mukri *et al.,* 2007 | | Mukri *et al.,* 2007 |
| | | Low | | | |
| | | High | Punnuri, 2004; Reddy, 2006; Rajkumar *et al.,* 2007; Kachapur and Salimath, 2009 | Punnuri, 2004; Reddy, 2006; Rajkumar, *et al.,* 2007; Kachapur and Salimath, 2009 | Punnuri, 2004; Reddy, 2006; Rajkumar, *et al.,* 2007; Kachapur and Salimath, 2009 |

Correlation coefficients, although very useful in quantifying the size and direction of trait associations, can be misleading if the high correlation between two traits is a consequence of the indirect effect of other traits (Dewey and Lu, 1959). High and positive correlations were observed between all the pairs of characters evaluated in the field, indicating the possibility of obtaining indirect gains through the selection of high heritability characters. Being an extremely complex trait, grain yield is the result of the expression and association of several plant growth components and the multiplicative end product of many factors (Grafius, 1959). Yield being dependent on physico-morphological features of the plant, it is desirable to know the individual contribution of these characters for developing effective selection strategies. Reviews on correlation studies in sorghum are presented under Table 4

## 2.6 DNA markers and molecular mapping

One of the main objectives of plant breeder is to improve the existing cultivars, which are deficient in one or more traits. It is done by crossing such cultivars with lines that possess the desired trait. A conventional breeding programme thus involves crossing whole genomes followed by selection of the superior recombinants from the several segregation products. Indeed, such a procedure is laborious and time consuming, involving several crosses, several generations careful phenotypic selection and the linkage drag (tight linkage of the undesired loci with the desired loci) may make it further difficult to achieve the desired objective. Advent of DNA marker technology, development of several types of molecular markers and molecular breeding strategies offered possibilities to plant breeders and geneticists to overcome many of the problems faced during conventional breeding.

DNA markers are simply inherited, phenotypically neutral, polymorphic, abundant and ideal for the analysis of the complex traits. Molecular markers are useful in genetics, plant breeding programmes and construction of genetic linkage maps. Molecular markers have been used to identify and characterize QTLs associated with several complex traits in sorghum, including plant height and maturity (Pereira *et al.,* 1994), characters associated with plant domestication (Patterson *et al.,* 1995), disease resistance (Gowda *et al.,* 1995), drought tolerance (Tuinstra *et al.,* 1996, 1997 and 1998), striga tolerance (Haussmann *et al.,* 2004).

Molecular markers are now widely used to track loci and genome regions in several crop-breeding programmes, as molecular markers tightly linked with a large number of agronomic and disease resistance traits are available in major crop species (Phillips and Vasil, 2001; Jain *et al*., 2002 and Gupta and Varshney, 2004). These molecular markers include: (i) hybridization-based markers such as restriction fragment length polymorphism (RFLP) (Botstein *et al.,* 1980), (ii) PCR-based markers: random amplification of polymorphic DNA (RAPD) (Williams *et al.,* 1990), amplified fragment length polymorphism (AFLP) and microsatellite or simple sequence repeat (SSR), (Litt and Luty, 1989; Yu *et al.,* 2004 and Varshney *et al.,* 2005) and (iii) sequence-based markers: single nucleotide polymorphism (SNP) (Wang *et al.,* 1998; Kanazin *et al.,* 2002 and Varshney *et al.,* 2007). The majority of these molecular markers has been developed either from genomic DNA libraries (e.g. RFLPs and SSRs) or from random PCR amplification of genomic DNA (e.g. RAPDs) or both (e.g. AFLPs). DNA markers can be generated in large numbers and can prove to be very useful for a variety of purposes relevant to crop improvement. For instance, these markers have been utilized extensively for the preparation of saturated molecular maps (genetical and physical).

A DNA marker is considered good or powerful, if it is easy to detect, highly polymorphic and distributed across genome at random. The earliest DNA marker system, the RFLP have proved to be very useful, but their development and utilization is laborious, time consuming, expensive and not suitable for high throughput automation. For these reasons, PCR based markers such as RAPD, AFLP, SSRs, SNPs and their derivatives have become popular for molecular genetic studies (Paterson, 1996). Among PCR based markers, SSR markers quickly became the markers of choice for plant and animal genomes during the last decade because of the small sample size (genomic DNA) requirement for their analysis and their suitability for automation and high throughout (Hearne *et al.,* 1992 and Gutierrez *et al.,* 2005). Other uses of molecular markers include gene introgression through backcrossing, germplasm characterization, genetic diagnostics, characterization of transformants, study of genome organization and phylogenetic analysis (Jain *et al.,* 2002 and Gupta and Varshney, 2000), QTL mapping for charcoal rot disease resistance (Patil, 2009), mapping QTL for bioenergy traits in sweet sorghum (Lekgari, 2010).

**Table 4: Review of literature on correlation of different characters with grain yield in sorghum**

| Characters | Correlation with yield | | References |
|---|---|---|---|
| | Direction | Significant / Non-significant | |
| Per cent GLA 15 DAF | Positive | Significant | Borrell *et al.,* 2000; Coulibaly, 2002; Haussman *et al.,* 2002; Kassahun *et al.,* 2009; Reddy *et al.,* 2010 |
| Per cent GLA 30 DAF | Positive | Significant | Borrell *et al.,* 2000; Coulibaly , 2002; Haussman *et al.,* 2002; Kassahun *et al.,* 2009; Reddy *et al.,* 2010 |
| Per cent GLA 45 DAF | Positive | Significant | Borrell *et al.,* 2000; Coulibaly, 2002; Haussman *et al.,* 2002; Kassahun *et al.,* 2009; Reddy *et al.,* 2010 |
| Total green leaf area | Positive | Significant | Henzell *et al.,* 1992; Borrell *et al.,* 2000; Coulibaly, 2002; Haussman *et al.,* 2002; Kassahun *et al.,* 2009; Reddy *et al.,* 2010 |
| Days to 50% flowering | Positive | Significant | Liang *et al.* 1969; Crook and Casady, 1974; Sindagi *et al,*. 1970; Patel *et al.,* 1980b; Jadhav *et al.,* 1994; Veerabadhiran *et al.,* 1994; Mallinath *et al.,* 2004 |
| | Negative | Non-significant | Bueno, 1980; Mukri *et al.,* 2007 |
| Stem girth | Negative | Significant | Aba *et al.,* 2001 |
| Plant height | Negative | Significant | Jeewad, 1993 |
| | | Non-significant | Crook and Casady, 1974; Patel *et al.,* 1980; Pokle *et al.,* 1973 |
| | Positive | Significant | Nimbalkar *et al.,* 1988; Thombre and Patil, 1985; El-Hifney *et al.,* 1972; Potdukhe *et al.,* 1994; Patil *et al.,* 1995; Taurchi and Rezai, 1997; Setimala *et al.,* 1998; Desai *et al.,* 1999; Aba *et al.,* 2001; Sunku *et al.,* 2002; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Kenga *et al.,* 2006 |

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| Characters | Correlation with yield | | References |
|---|---|---|---|
| | Direction | Significant / Non-significant | |
| | | Non-significant | Kottura and Chawan, 1927; Crook and Casady, 1974; Thombre and Patil, 1985; Bakheit, 1990; Yang and Yang, 1995; Setimela *et al.,* 1998 |
| Earhead length | Positive | Significant | Chavan and Singh, 1975; Goud and Shastri, 1974; Jeyaprakash, 1997; Taurchi and Rezai, 1997; Iyanar *et al.,* 2001; Tiwari *et al.,* 2003; Kenga *et al.,* 2006; Mukri *et al.,* 2007 |
| | Negetive | | Arunkumar *et al.,* 2004; Ezeaku *et al.,* 2006 |
| Panicle exertion | Positive | Significant | Iyanar *et al.,*2001 |
| | | Negetive | Mallinath *et al.,* 2004; Aba *et al.,* 2001 |
| Spicklets per head | Positive | Significant | Nimbalkar *et al.,* 1988; Tourchi and Rezai, 1997; Mahammad, 2000; Aba *et al.,* 2001; Mallinath *et al.,* 2004; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Mukri *et al.,* 2007 |
| | | Non-significant | Bakheti, 1990 |
| | Negative | Significant | Tiwari *et al.,* 2003 |
| 100 grain weight | Positive | Significant | Aba *et al.,* 2001; Veerabadhiran and Kennedy, 1994; Ezeaku *et al.,* 2006; Mukri *et al.,* 2007; Mallinath *et al.,* 2004 |
| | Negetive | | |
| Number of leaves | Positive | Significant | Sunku *et al.,* 2002; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Mukri *et al.,* 2007 |

### 2.6.1 Simple sequence repeats (SSR)

SSRs or microsatellites are ubiquitous in eukaryotes. These are the class of molecular markers which depends on the availability of short oligonucleotide repeat sequences in the plants genome, is the simple sequence repeat polymorphism or microsatellites (Tautz and Rentz, 1984 and Hearne *et al.,* 1992). Microsatellite contain 1 to 6 bp DNA sequence motifs repeated several times (Tautz and Renz, 1994). Popularly described as simple sequence repeats (SSRs) in plants (Morgente and Oliveri, 1993) and as short tandem repeats (STRs) in animal systems, these SSRs are wide-spread in genomes (Edwards *et al.,* 1991). These motifs known as di, tri, tetranucleotide repeats *etc.* accordingly. They appear scattered randomly throughout the genome. Earlier, Jeffreys *et al*. (1995) used the term minisatellites for microsatellites. Previously, Litt and Lutty (1989) had introduced the term 'microsatellite' to characterize simple sequence stretches amplified by the polymerase chain reaction (PCR). They were also described as Simple Sequence Length Polymorphisms (SSLPs) by Tautz and Rentz (1984), Short Tandem Repeats (STRs) by Edwards *et al*. (1991) and Variable Number Repeats (VTRs) by Nahamura (1987). SSRs are thought to arise and develop due to replication slippage and a mutation that could expand or contract them. In humans about three per cent of the genome is occupied by SSRs, distributed throughout the genome in both coding and non-coding regions (Tothet *et al.,* 2000), respectively termed as genic SSRs and genomic SSRs (Varshney *et al.,* 2005).

SSR polymorphism reflects polymorphisms based on the number of nucleotide sequence flanking the repeat is used to design primers to amplify the different number of repeat units in different varieties. This type of polymorphism is highly reproducible. These primers are very useful for rapid and accurate detection of polymorphic loci and the information could be used for developing a physical map based on these sequence tags. SSR markers are fairly cheap and no sequence information is required for their detection, gives good polymorphism as well as requiring only a small quantity of DNA to start with (Varshney *et al.,* 2007). There is an evidence to suggest that SSRs might as well regulate gene expression (Kunzler *et al.,* 1995; Moxon and Wills, 1999). Genic SSRs have high proportion of high quality markers than genomic SSRs and they showed prominent bands and distinct allelic peaks (Thiel *et al.,* 2003; Kota *et al.,* 2001; Yu *et al.,* 2004; Saha *et al.,* 2004; Nicot *et al.,* 2004; Cho *et al.,* 2000 and Eujayl *et al.,* 2001).

The National Centre for Biotechnology Information (NCBI) EST Database (dbEST) contain an ever increasing number of these 'single pass' cDNA sequence, meaning that the resources necessary for the efficient development of large number of so called EST-SSRs. The de-novo generated nuclear SSRs are referred to as genomic SSRs (Varshney *et al.,* 2005) or anonymous SSRs (Ellies and Burke, 2007). These also form a class of functional markers as a putative function can be deduced for an EST-SSR based on corresponding ESTs. Thus, EST-SSR markers have advantages of both functional markers such as complete linkage with trait locus (Anderson and Lufferstedt, 2003) end of SSRs such as highly polymorphic multi-allelic, codominant and random distribution (Powell *et al.,* 1996). Genic SSRs show a characteristic distribution throughout the genome and get concentrated in gene rich regions, unlike genomic SSRs, which are clustered around the centromere (Thiel *et al.,* 2003; Yu *et al.,* 2004 and Gao *et al.,* 2004). Majority of SSRs contain $(AG/TC)_2$ and (AC/TG)n repeats at 52 per cent and 91 per cent respectively (Bhattramakki *et al.,* 2000). AG/TC repeats were predominant in SSRs isolated by Brown *et al*. (1996). It was also found that about 57 per cent of SSRs contained triplets of GC rich regions located in coding regions of the DNA (Wang *et al.,* 1994).

Distribution of genic SSRs on the genetic map will show the distribution of genes in the genome. Genic SSRs have the potential of being functional markers in cases where polymorphism in the repeat motifs affect the function of the gene (Anderson and Lubberstedt, 2003), permitting direct allele selection if they are shown to be associated or responsible for the target trait (Sorrells and Wilson, 1997). Yu *et al*. (2004) has identified two EST-SSR markers linked to photoperiod response gene (ppd) in wheat. Mapping candidate genes and genic SSRs also facilitates genomic alignment across related species (Yu *et al.,* 2004 and Varshney *et al.,* 2005). EST-SSRs have been integrated and genome wide genetic maps have been prepared in many crops *viz*., wheat (Yu *et al.,* 2004 and Gao *et al.,* 2004); barley (Thiel *et al.,* 2003); rye (Khlestkina *et al.,* 2004); soybean (Zhang *et al.,* 2004), potato (Feingol *et al.,* 2005); kiwi fruit (Fraser *et al.,* 2004) and raspberry (Gragam *et al.,* 2004) *etc.*

EST-SSR markers have been reported to be less polymorphic compared to nuclear SSRs in crop plants because of greater DNA sequence conservation in transcribed regions (Scott *et al.,* 2000 and Rungis *et al.,* 2004). Genic SSRs, like any genomic markers, are useful in the construction of linkage maps, to know functional diversity, transferability and comparative mapping (Varshney *et al.,* 2005). A large number of genic SSRs have been assigned to genetic linkage map of wheat (Yu *et al.,* 2004), cotton (Han *et al.,* 2004) and rice (Temnykh *et al.,* 2000). Map location of 46 SSR loci (Taramino *et al.,* 1997 and Kong *et al.,* 2000) and 113 novel SSR loci, including SSR containing gene loci, (Bhattramakki *et al.,* 2000) have been reported in sorghum. SSR makers have been integrated with their existing RFLP based map (Xu *et al*.,1994,; Kong *et al*.,1997; Peng *et al*., 1999 and Bhattramakki *et al*., 2000). EST-SSR marker has also been mapped on sorghum linkage map (Nagaraj *et al.,* 2005 and Patil, 2009),

### 2.6.2 Non-genic SSRs markers

Microsatellites are highly reproducible co-dominant markers that offer the potential for automation, facilitating the rapid screening of plant lines (Gupta *et al.,* 1996). Nuclear SSR markers have been used in a variety of plant species and in genetic mapping initiatives for cereals including wheat, barley (Roder *et al.,* 1998), rice (Temnykh *et al.,* 2000) and sorghum (Sanchez *et al.,* 2002).

SSR containing clones isolated from BAC (Bacterial Artificial Chromosome), enriched gDNA libraries and database sequences that contain SSRs were the sources for the sorghum SSRs mapped by Bhattaramakki *et al*. (2000). Targeted isolation of SSR loci using BAC clones as proposed by Cregon *et al*. (1999) is likely to be the most efficient method for placing SSR loci in the segments.

BTx623 is the reference genotype used for sorghum molecular marker genotyping. It was the source of DNA used to construct the enriched libraries and the two sorghum BAC libraries that are currently available (Bhattramakki *et al.,* 2000).

Map localization of 46 SSR loci (Turomino *et al.,* 1997) and 113 novel SSR loci (Kong *et al.,* 2000) have been reported. SSR markers have been incorporated into an existing RFLP based map of sorghum (Xu *et al.,* 1994; Kong *et al.,* 1997; Peng *et al.,* 1999 and Bhattramakki *et al.,* 2000).

### 2.6.3 Single nucleotide polymorphism

SNPs marker is just a single base change in a DNA sequence, with a usual alternative of two possible nucleotides at a given position. For such a base position with sequence alternatives in genomic DNA to be considered as an SNP, should occur in a population scale. SNP is a DNA sequence variation occurring when a single nucleotide- A-T-C or G- in the genome differs between members of a species (or between paired chromosomes in an individual). It may occur in the coding, non-coding and intergenic regions of the genome, thus enabling the discovery of genes as a result of the differences in the nucleotide sequences. SNPs are excellent markers for association mapping of genes controlling complex traits and provide the highest map resolution (Botstein and Risch, 2003; Brookes, 1999; Bhattramakki *et al.,* 2002 and Varshney *et al.,* 2007). SNPs are the most frequent type of variation found in DNA and their discovery together with insertions/deletions has formed the basis of most differences between alleles. SNPs can thus be explained as any polymorphism between two genomes that is based on a single nucleotide exchange. In plants, studies on the occurrence and nature of SNPs are beginning to receive considerable attention, particularly in *Arabidopsis* where over 37, 000 SNPs have been identified through the comparison of two accessions (Jander *et al.,* 2002).

Improvements in sequencing technology and availability of an increasing number of EST sequences have made direct analysis of genetic variation at the DNA sequence level possible (Buestow *et al.,* 1999 and Soleimani*et al.,* 2003). Majority of SNP genotyping assays are based on one or two of the following molecular mechanisms: allele specific hybridization, primer extension, oligonucleotide ligation, Single strand Confirmation Polymorphism (SSCP) and invasive cleavage (Sobrino *et al.,* 2005).

The single-strand conformation polymorphism analysis is one of the methods used for SNPs detection (Orita *et al.,* 1989, Shi, 2001 and Varshney *et al.,* 2007). SSCP is the mobility shift analysis of single-stranded DNA sequences on neutral polyacrylamide gel electrophoresis, to detect polymorphism produced by differential folding of single stranded DNA due to subtle difference in sequence of single base pair to 300 bp (Orita *et al.,* 1989). In single-strand conformation polymorphism, portions of the DNA with expected polymorphisms are first amplified by PCR. PCR products are denatured and this creates single-stranded DNAs, which are separated on a non denaturing polyacrylamide gel. Fragments are generated with a single base modification and form a different conformer which migrates differently (Varshney *et al.,* 2007).

SSCP can identify the heterozygosity of the DNA fragment in DNAs of same molecular weight and can even detect the changes of a few nucleotide bases. The PCR based SSCP analysis is a rapid, simple and sensitive technique for detection of various mutations, including single nucleotide substitutions, insertion and deletions; in PCR amplified DNA fragments (Hayashi, 1993). The SSCP gels have been used to increase throughput and reliability of scoring during mapping by PCR fingerprinting in plants (Li *et al.,* 2005 and Varshney *et al.,* 2007).

## 2.7 QTL analysis and mapping for drought stress tolerance

Most agronomically important traits of crop plants have complex inheritance patterns and are under the control of many genes. The genetic loci associated with complex traits are called quantitative trait loci (QTLs). Traits controlled by these QTLs are often strongly influenced by the environment. Because of this, the segregation patterns observed for such polygenic traits appear to deviate from the relatively simple patterns of Mendelian inheritance and hence the underlying genes controlling these traits are hard to trace. This limitation has been overcome by the construction of highly saturated molecular maps in many crop plants. The theoretical basis of interpreting the association of marker loci with QTLs was provided by Mather and Jinks, 1971; Jayakar, 1970; McMillan and Robertson, 1994; Tanksley *et al*., 1982; Soller and Beckmann, 1983; Edwards *et al*., 1987 and Cowen, 1988. Likewise, the use of flanking marker loci for QTL identification was suggested by Lander and Botstein (1989) and Knapp *et al*. (1989).

QTL analysis is to look for association between the phenotypic variation in quantitative traits and the marker alleles segregating in the mapping population. QTL analysis has three essential stages: mapping of markers (genotyping), scoring of the trait (phenotyping) and association of the trait with the marker (QTL mapping).

### 2.7.1 Mapping population

Mapping population is the prerequisite for QTL analysis, developed by crossing two parents which differ contrastingly for trait under study. Different types of populations may be developed for mapping purposes. The type chosen depends on the available resources, such as infrastructure, funds and labor as well as timeline to project completion. An $F_2$ population undergoes just one cycle of meiosis, has all possible combination of parental alleles, is easy to construct, can be developed quickly, is recombinant along each pair of homologous chromosomes, provides finite supply of seed tissue and has as much as twice the information generated by a backcross individual.

The RIL population is among the most widely used mapping population due to its many advantages. A RIL population may be propagated indefinitely, which allows for multilocation testing, possibly decreasing error variance while increasing phenotypic variance. Precision is increased up to two times that of $F_2$'s. The development of RIL populations is time consuming. Double haploid (DH) populations may be propagated indefinitely, perfect homozygosity and faster to develop than RILs. Finally, near isogenic lines (NILs) are developed by either backcrossing the progeny to one of the parents or by selfing and selecting families segregating for the trait of interest.

### 2.7.2 Molecular mapping in sorghum

Genetic diversity in sorghum has been estimated utilizing several types of molecular markers (Tao *et al.,* 1993; Vieling *et al.,* 1994; Brown *et al.,* 1996; Taramino *et al.,* 1997 and Uptmoor *et al.,* 2003).

Brown *et al*. (1996) developed 15 SSR markers and were able to identify polymorphic loci among 17 temperately and tropically adopted lines of sorghum. Fifteen SSR marker loci are widely spread on the sorghum genome and 14 of them have been mapped to nine of ten sorghum linkage groups.

A set of 104 RFLP probes were used to evaluate the genetic diversity among a large set of elite proprietary sorghum inbred lines by Ahnert *et al*. (1996). SSR loci give good discrimination between closely related individuals in some cases even when only a few loci were employed (Powell *et al.,* 1996 and Kong *et al.,* 2000). Pereira *et al*. (1994) performed segregation analysis on $F_2$ population using seven SSR loci in order to verify the reliability of SSR derived polymorphism for sorghum genetic mapping.

Bhattramakki *et al*. (2000) reported primer sequences for 147 sorghum SSR loci and genetic linkage map locations for 113 of these. Schloss *et al*. (2002) reported 70 additional sorghum SSR primer sequences derived from sorghum cDNA clones that had previously been mapped as RFLP markers. Ghbru *et al*. (2003) carried out an analysis to assess genetic diversity of 28 Eritrean landraces of sorghum, using a high throughout SSR based strategy developed by Kresovich and Coworkers (Dean *et al.,* 1999 and Smith *et al.,* 2000).

Comparative analysis was carried out by Uptmoor *et al.* (2003) on the genetic relatedness of 46 sorghum accessions from Southern Africa by using molecular markers like AFLP, RAPDs, SSRs and they concluded that all these sorghum accessions were uniquely fingerprinted by all three marker systems. Folkertsmon *et al*. (2005) reported use of 21 SSR markers to assess genetic diversity in the Guinea-race of sorghum in support of a breeding program aiming to develop $F_1$ hybrid cultivar within this race as a means of increasing food security in the more humid regions of Western and Central Africa.

Casa *et al*. (2005) assayed 98 simple sequence repeat (SSR) loci distributed throughout the genome in a panel of 104 accessions comprising 73 landraces and 31 wild sorghums. Evaluation of SSR polymorphisms indicated that landraces retained 86 per cent of the diversity observed in the wild sorghums. Reddy *et al*. (2008) genotyped with 62 nuclear, 11 genic SSR and 19 RAPD markers in 93 RILs derived from a cross between inbred lines IS22380 (susceptible) $\times$ E36-1 (resistance). Nagaraj *et al*. (2005) mapped thirteen linkage groups containing 60 simple SSR loci by using a set of sorghum RILs obtained from the cross 96-4121 (green bug-tolerant) x Redlan (green bug susceptible). The linkage group spanned a distance of 603.5 cM with the number of loci per LG varying from 2 to 14. Seventeen additional SSR loci were unlinked at a log of odds value of 3.0.

In $F_2$ population derived from a cross of B2V4 x 1383-2, Duan *et al*. (2009) mapped 122 Methylation Sensitive Amplified Polymorphism (MSAP) and SSR markers, which spanned a length of 483.6cM with 11 linkage groups.

### 2.7.3 Sorghum genetic linkage maps

Genetic linkage maps are fundamental for the localization of genes conferring biotic and abiotic stress tolerance. Linkage maps of organisms are constructed to map genomic regions controlling qualitative and quantitative traits to exercise indirect selection for several agronomic traits and to isolate the genes involved based on their map position. Sorghum genome mapping based on DNA markers began in early 1990s and since then, many genetic maps have been developed with a large number of DNA-based markers including RFLPs, AFLPs and SSRs (Botstein *et al*., 1980; Vos *et al*., 1995; Litt and Luty, 1989; Haussmann *et al*., 2004; Punnuri, 2004; Reddy *et al*., 2008 and Patil, 2009). An overview of published sorghum genetic linkage maps is reviewed in Table 5.

Genetic maps based on molecular markers have several advantages over classical maps (Subudhi and Nguyen, 2000). Neutrality, abundance and codominant nature of some type of DNA markers allow for thorough coverage of entire species genomes, addressing questions of evolution, genetic diversity, and phylogeny relevant to germplasm selection and improvement.

Hulbert *et al.* (1990) developed the first sorghum genome map using DNA probes that were previously mapped in the maize genome. Pereira *et al.* (1994) developed a sorghum linkage map with 10 complete linkage groups using maize and sorghum probes.

To increase the effectiveness of mapping information and facilitate efforts to map agronomically important traits, Tao *et al.* (1993), by utilizing a variety of probes, including sorghum, maize and sugarcane genomic DNA, maize and sugarcane cDNA, cereal anchor probes and eight SSR loci, reviewed and compared their sorghum map with other published maps.

Two recombinant inbred populations developed from crosses B35 x Tx700 and B35 x Tx430 have been extensively used for QTLs associated with the stay-green trait in sorghum (post flowering drought tolerance) (Xu *et al.,* 1994 and 2000).

Map developed by Chittenden *et al*. (1994) from an interspecific cross combination (*Sorghum bicolor* × *Sorghum propinquum*), all other maps including that of Xu *et al*. (1994) were established using intra specific cross combinations. Dufour *et al*. (1997) published a map based on maize, sugarcane and cereal anchor probes. In most cases, $F_2$ mapping populations were used in map development with the exception of Dufour *et al*. (1997) who used RIL population for development of linkage groups. Tao *et al*. (1998) and Peng *et al*. (1999), used RILs population for construction of a composite linkage map.

Construction of an integrated sorghum linkage map with RFLP and SSR markers using the recombinant inbred lines derived from the cross between BTx623 and IS3620C by Kong *et al*. (2000). The markers were distributed across the 10 linkage groups (LG); covering 187.2 cM of the genome based on LOD score >5.0.

An integrated SSR (113 loci) and RFLP (323 loci) marker based genetic linkage map of sorghum was constructed using a mapping population of 137 $F_8$ RILs derived from the cross between BTx623 and JS3620C (Bhattramakki *et al.,* 2000). Most of the SSR primer sequences reported was developed from clones isolated from two sorghum BAC libraries and three enriched sorghum genomic DNA (gDNA) libraries. Very few of the sorghum SSRs primer sequences reported were developed from the sorghum DNA sequences present in the public databases. Loci detected by 323 RFLP prove enzyme combinations and 313 SSR primer pairs were mapped (LOD score $\geq$3.0), of the SSR primer developed, 165 (53%) were found to detect polymorphism in a population composed of 18 diverse sorghum lines.

High density genetic map of the sorghum genome by using AFLP technology and a recombinant inbred line population derived from the cross between BTx623 and IS3620C was constructed by Menz *et al*. (2002). The 1713 cM map encompassed 2926 loci (2454 AFLP, 136 SSRs and 203 cDNA and genomic clones from rice, barley, oat and maize) distributed on ten linkage groups. Of these markers mapped, 692 comprised a LOD $\geq$3.0 framework map on which the remaining markers were placed with lower resolution (LOD $\leq$3.0).

Sorghum-rice comparative map developed by BLASTing sequences from 2509 genetically mapped sorghum loci against the rice genome assembly was examined by Paterson *et al*. (2002). The position of 1626 corresponding loci could be plotted based on the rice physical locations and sorghum genetic location. This revealed much colinearity with eight sorghum linkage groups (A, D, E, F, G, H, I and J) corresponding to single rice chromosomes (1, 4, 12, 2, 5, 11, 6 and 8) and two sorghum linkage groups (B and C) differing from rice by translocations between chromosomes 7/a and 3/a, respectively.

Bowers *et al*. (2003) constructed a high density genetic recombination map of sequence tagged sites for sorghum, which will be framed for comparative mapping, structural and evolutionary genomics of tropical grains and grasses. Also they reported a genetic recombination map for sorghum of 2512 loci spaced at an average 0.4 cM interval based on 2050 RFLP probes, including 865 heterologous probes. Mapped loci identify 61.5 per cent of the recombination events in their progeny set and reveal strong positive cross interference acting across intervals of $\leq$50 cM.

A comprehensive reference map of sorghum genome would be an essential research tool for functional and comparative genomics. In this direction, a combined sorghum linkage map from two recombinant inbred populations was constructed using SSR, AFLP and RAPD markers. The two recombinant inbred populations analyzed consisted of 225 (RIP1) and 226 (RIP2) of $F_{3-5}$ lines developed from the crosses IS9830 × E36-1 (RIP1) and N13 × E36-1 (RIP2) respectively. The genetic map of RIP1 and RIP2 spanned 1498 cM and 1549 cM respectively with 137 and 157 markers distributed over 11 linkage groups (Haussman *et al.,* 2002a).

Hamblin *et al*. (2004) population genetic parameters in sorghum were estimated by surveying 27 diverse sorghum accessions for sequence variations at a total of 29,186 bp in 95 short regions derived from RFLP map. Nagaraj *et al*. (2005) mapped thirteen linkage groups containing 60 simple SSR loci by using a set of sorghum recombinant inbred lines (RILs) obtained from the cross 96-4121 (green bug – tolerant) × Redian (green bug susceptible). The linkage group (LG) spanned a distance of 603.5 cM, with the number of loci per LG varying from 2 to 14. Seventeen additional SSR loci were unlinked at a log of odds value of 3.0.

Kim *et al*. (2005a, 2005b, 2005c) integrated genetic, physical and cytological perspective of the *Sorghum bicolor* genome by Fluorescent *In situ* Hybridization (FISH) of landed BACs and relative length of metaphase chromosomes were estimated. They used elite inbred line BT × 623 to estimate the molecular size of each chromosome and established the size based nomenclature for sorghum chromosomes (SBI-01 to SBI-10) and linkage groups (LG-01 and LG-10), which represents a reasonable choice for standard unified chromosome nomenclature.

NILs developed for the same four stay-green QTL (*Stg1-Stg4*) using BTx642 (formerly B35) as the stay-green donor, has shown that these stay-green QTL individually reduce the post-flowering drought-induced leaf senescence in the recipient senescent genetic background of RTx7000 (Harris *et al.,* 2007).

QTLs associated with stalk rot resistance in sorghum using 93 RILs derived from a cross between inbred lines IS22380 (susceptible) x E36-1 (resistance). This population was genotyped with 62 nuclear and 11 genic SSR and 19 RAPD markers and linkage group was conducted using Mapmaker/QTL software by Reddy *et al*. (2008). They identify for three parameters percentage lodging, length of infection and number of internodes crossed, five QTL at Dharwad and 4 QTL at Bijapur locations on the linkage group A, B, D and I. Reddy *et al*., (2008) reported the linkage map with segregating markers on 10 linkage groups with total length of the genetic map of 650 cM.

Duan *et al*. (2009) developed methylation genetic linkage map of sorghum, constructed using a $F_2$ population derived from a cross of B2V4 × 1383-2 based on Methylation Sensitive Amplified Polymorphism (MSAP) and SSR markers. The map includes 11 linkage groups was constructed with 151 loci covering 483.6 cM, 122 of them were MSAP markers, of which 89 loci were produced from *EcoRI/MspI* enzyme digestion and 33 loci were produced from *EcoRI/HpaII* enzyme digestion. The average and maximal distances between adjacent two individual markers were 3.2 and 22.7 cM respectively.

Construction of sorghum consensus map consisted of a total of 1997 markers mapped to unique loci (1190 DArT loci and 839 other loci) spanning 1603.5 cM and with an average marker density of 1 marker/0.79 cM (Mace *et al,.* 2009).

### 2.7.4 QTL analysis and mapping

The theoretical basis of interpreting the association of marker loci with QTLs was provided by Muthur and Jinks (1971); Jayakar (1970); McMillan and Robertson (1994); Tanskley *et al*. (1982); Soller and Beckmann (1983); Edwards *et al*. (1987) and Cowen (1988). Like-wise the use of flanking marker loci for QTL identification was suggested by Londer and Botstein (1989) and Knapp *et al*. (1989). QTL analysis can be done by using statistical procedures like maximum likelihood ratio (Lander and Botstein, 1989), non-linear regression (Knapp *et al*., 1990), linear regression (Bridges *et al*., 1991) or a combination of maximum likelihood and multiple regressions (Jansen and Stam, 1994; Zeng, 1994). Software packages to carry out the data analysis include: MAPMAKER/QTL (Lander *et al*., 1987), QGENE (Tanksley and Nelson, 1996), QTL Cartographer (Basten *et al*., 1994), and *PLAB* (Utz, 1995 and Utz and Melchinger, 1996).

QTL analysis looks for association between a quantitative trait and a marker alleles segregating in a population. For simple interval mapping the most widely used software is MAPMAKER (Lander *et al.,* 1987). MAPMAKER is based on the concept of the LOD score (Morton, 1955). MAPMAKER/EXP is an interactive linkage analysis package initially developed by Lander *et al*. (1987) for constructing primary linkage maps using segregating population derived from experimental crosses.

**Table 5: Overview of published sorghum genetic linkage maps**

| Sl. No. | Reference | Size and type of mapping population | Parent lines | Mapped markers | Linkage groups | Genome length (cM)[a] |
|---|---|---|---|---|---|---|
| 1. | Hulbert *et al.,* , 1990 | 55 ($F_2$ plants) | Shanqui Red kaoliang from China | 37 RFLP maize probes or cloned genes | 8 | 283 R |
| | | | M91051 zera zera from East Africa | | | |
| 2. | Binelli *et al.,* 1992 | 149 ($F_2$ plants) | IS18729 caudatum-*bicolor* from Texas | 21 RFLP maize probes | 5 | 440 U |
| | | | IS24756 durra-caudatum from Nigeria | | | |
| 3. | Whitkus *et al.,* 1992 | 81 ($F_2$ plants) | IS2482C *S. bicolor* ssp. *bicolor* | 85 RFLP maize probes | 13 | 949 H |
| | | | IS18809 *S. bicolor* ssp. *arundinaceum* | 7 Isoenzymes | | |
| 4. | Berhan *et al.,* 1993 | 55 ($F_2$ plants) | Same parents as Hulbert *et al.,* 1990 | 96 RFLP maize probes or cloned genes | 15 | 709 R |
| 5. | Chittenden *et al.,* 1994 | 56 ($F_2$ plants) | BTx 623 *S. bicolor* | 256 RFLP sorghum probes | 10 | 1445 U |
| | | | Unnamed accession of *S. propinquum* | 20 RFLP probes of maize, rice or oat | | |
| 6. | Pereira *et al.,* 1994 | 78 ($F_2$ plants) | CK60 *S. bicolor* ssp. *bicolor* | 191 RFLP maize probes | 10 | 1530 U |
| | | | PI229828 *S. bicolor* ssp. *drummondii* | 10 RFLP sorghum probes | | |
| 7. | Xu *et al.,* 1994 | 50 ($F_2$ plants) | BTx 623 zea zera × kafir | 179 RFLP sorghum probes | 14 | 1789 K |
| | | | IS3620C guinea line | 11 RFLP maize probes | | |
| 8. | Ragab *et al.,* 1994 | 93 ($F_{2:3}$ families) | BSC 35 | 38 RFLP sorghum probes | 15 | 633 H |
| | | | BTx 631 | 33 RFLP maize probes | | |
| 9. | Lin *et al.,* 1995 | 370 ($F_2$ plants) | Same parents as Chittenden *et al.,* 1994 | 78 RFLP sorghum probes | 11 | 935 K |
| 10. | Tuinstra *et al.,* 1996, 1997 | 98 (RIL $F_{5:7-8}$) | TX 7078 | 150 RAPD | 17 | Ca 1580 R |
| | | | B35 durra sorghum from Ethiopia | 20 RFLP probes from maize or sorghum | | |
| 11. | Dufour *et al.,* 1997 | 110 (RIL $F_5$) | IS 2807 *Caudatum* from Zimbabwe | 126 RFLP maize probes | 13 | 977 H |
| | | | 379 guinea from South Africa | 19 RFLP sugarcane probes | | |
| | | | | 4 clones genes, 2 morphological markers | | |
| | | 91 (RIL $F_5$) | IS 2807 *Caudatum* from Zimbabwe | 115 RFLP maize probes | 12 | 878 H |
| | | | 249 guinea from Burkina Faso | 8 RFLP sugarcane probes | | |

*Contd…..*

| Sl. No. | Reference | Size and type of mapping population | Parent lines | Mapped markers | Linkage groups | Genome length (cM)[a] |
|---|---|---|---|---|---|---|
| | | | | 4 cloned genes, 1 morphological marker<br>164 RFLP maize probes<br>19 RFLP sugarcane probes<br>3 cloned genes, 2 morphological markers | 13 | 1095 H |
| 12. | Taramino *et al.,* 1997 | 68 ($F_2$ plants) | Same parents as Pereira *et al.,* 1994 | 7 SSR added to map of Pereira *et al.,* 1994) | 10 | 1575 U |
| 13. | Ming *et al.,* 1998 | 56 ($F_2$ plants) | Ssame parents as Chittenden *et al.,* 1994 | 328 RFLP probes of sorghum and other cereals | 10 | 1750 K |
| 14. | Tao *et al.,* 1998 | 120 (RIL $F_5$) | QL39<br>QL41 both elite lines from Australia | 68 RFLP sorghum probes<br>87 RFLP of maize, sugarcane, rice, oat, barley<br>8 SSR, 3 morphological traits | 21 | 1400 U |
| 15. | Boivin *et al.,* 1990 | | | 343 RFLP and morphological markers | 11 | 1352 H |
| | | 110 (RIL $F_5$) | First population of Dufour *et al.,* 1997 | 128 RFLP sorghum, rice, oat or barley probes<br>176 AFLP | 11 | 1899 H |
| 16. | Boivin *et al.,* 1999 | | | 151 RFLP loci mapped by Dufour *et al.,* 1997) | | |
| 17. | Crasta *et al.,* 1999 | 96 (RIL $F_{6:7}$) | B35 durra from Ethiopia | 142 RFLP sorghum, maize, rice or wheat clones | 14 | 1602 K |
| 18. | Peng *et al.,* 1999 | 137 (RIL $F_{6-8}$) | Same parents as Xu *et al.,* 1994 | 323 RFLP from sorghum, maize, rice, oat, barley | 10 | 1347 K |
| 19. | Bowers *et al.,* 2000 | 65 ($F_2$ plants) | Same parents as Chittenden *et al.,* 1994 | 2399 Loci based on 1925 RFLP probes | 10 | 1200 U |
| 20. | Kong *et al.,* 2000 | 137 (RIL $F_{6-8}$) | Same parents as Xu *et al.,* 1994 | 33 SSR | 10 | 1287 K |
| 21. | Tao *et al.,* 2000 | 152 (RIL $F_5$) | Same parents as Tao *et al.,* 1998 | 101 RFLP<br>17 SSR<br>166 markers mapped by Tao *et al.,* 1998) | 14 | 1871 U |

*Contd…..*

| Sl. No. | Reference | Size and type of mapping population | Parent lines | Mapped markers | Linkage groups | Genome length (cM)[a] |
|---|---|---|---|---|---|---|
| 22. | Xu *et al.,* 2000 | 98 RIL( $F_7$) | B35 durra from Ethiopia<br>Tx 7000 elite public line used in USA | 162 RFLP sorghum and maize probes, cloned genes or sequenced DNA probes | 10 | 837 H |
| 23. | Bhattramakki *et al.,* 2000 | 137 RIL ($F_{6-8}$) | Same parents as Xu *et al.,* 1994 | 116 SSR<br>354 RFLP or SSR markers mapped by Peng *et al.,* 1999 or Kong *et al.,* 2000 | 10 | 1406 K |
| 24. | Bennetzen *et al.,* 2001 | 125 RIL ($F_5$) | Framework map derived from comparison of the maps of Kong *et al.,* 2000; Peng *et al.,* 1999; Pereira *et al.,* 1994 and Berhan *et al.,* 1993 | 154 RFLP<br>34 SSR, 10 morphological markers | 10 | 1450 U |
| 25. | Klein *et al.,* 2001 | 125 RIL ($F_5$) | RTx 430 elite line from USA<br>Sureno | 44 SSR<br>85 AFLP, 1 morphological trait | 10 | 970 K |
| 26. | Haussmann *et al.* 2002 | 225 RIL( $F_{3:5}$) | IS9830 *Caudatum* line from Sudan<br>E36-1 Ethiopian guinea-*Caudatum* | 125 AFLP, 1 morphological trait<br>45 SSR, 14 RFLP, 3 RAPD | | |
| | | 226 RIL ($F_{3:5}$) | N 13 Indian durra<br>E 36-1 Ethiopian guinea-*Caudatum* | 158 AFLP<br>54 SSR, 16 RFLP | 12 | 1410 H |
| | | | Composite map of the two populations | 339 AFLP, SSR, RFLP and RAPD markers | 11 | 1424 H |
| 27. | Menz *et al.,* 2002 | | BTx623 x IS3620C | 2454 AFLP, 136 SSRs | 10 | 1713 cM |
| 28. | Bowers *et al.,* 2003 | | | 2050 RFLP | 10 | |
| 29. | Nagaraj *et al.,* 2005 | | RIL 96-4121 green bug – tolerant) × 60 SSR | | 13 | 603.5 cM |
| 30. | Harris *et al.,*2007 | NIL | BTx642 x RTx7000 | | | |
| 31. | Duan *et al.,* 2009 | $F_2$ population | B2V4 × 1383-2 | 122 MSAP | 11 | 483.6 cM |
| 32. | Mace *et al.,* 2009 | | | 1190 DarT, 839 other loci | 10 | 1603.5 cM |

[a]H, K = Map distances estimated using the mapping functions of Haldane 1919 and Kosambi 1944, respectively;

U = Mapping function not specified; R = Recombination frequency %

MAPMAKER/EXP performs full multipoint linkage analysis for dominant, recessive (such as qualitative phenotypic marker) and co-dominant (such as RFLP) markers in BC, $F_2$ and $F_3$ (self) intercrosses and recombinant inbred (RI) lines.

MAPMAKER/QTL is a companion program to MAPMAKER/EXP. To apply a linkage map to QTL analysis, MAPMAKER has been modified to carry out QTL analysis using mathematical models and interfaces very much like the original program (Lander and Botstein, 1989). It uses the technique of interval mapping and simultaneous search. Interval mapping uses the power of a complete genetic map to discern weak effects from genetic distance between marker locus and putative QTL. While simultaneous search allows mapping multiple QTL across the genome at once, thus, reducing overall non-genetic variance in the trait. Intervals between adjacent pairs of markers along a chromosome are scanned and the likelihood profile of a QTL being at any particular point in each interval is determined, to be more precise, the log of the ratio of the likelihood (LOD) of there being one QTL vs. no QTL at a particular point (Lander and Botstein, 1989).

QTL Cartographer is a suite of programs for mapping quantitative trait loci onto a genetic linkage map. The general experiment begins with a pair of inbred parental lines that differ in the trait of interest and for a set of marker genotypes. The programs use linear regression, interval mapping (Lander and Botstein, 1989) composite interval mapping (CIM) (Zeng 1993; Zeng 1994) or multiple interval mapping (Zeng, and Kao *et al.,* 1999) methods to dissect the underlying genetics of the quantitative traits. Mapping is done using a set of linked genetic markers with known recombination frequencies. Genetic linkage maps and data files can be imported from MAPMAKER. The mapping program uses a dynamic algorithm that allows a host of statistical models to be fitted and compared, including type of gene action, QTL-environment interactions, and close linkage. QTL Cartographer mapping programs can handle data from backcrosses, intercrosses, double haploids and recombinant inbreds as well as a few other experimental designs. It is a powerful package that is customizable for its various mapping functions (such as centiMorgan, Morgan and recombination distances) and statistical thresholds (such as permutation times and significance levels). The inclusion of appropriate markers as cofactors in multiple regression models removes the confounding effects of QTL placed outside the intervals being tested (Jansen 1993 and Zeng 1994). CIM has a better power to detect linked QTL (Zeng 1994). Dao-hua he *et al*. (2005) did QTL analysis by QTL Cartographer software v2.0, using composite interval mapping method (LOD 3.0). A total of 21 QTLs were identified for fiber yield and yield components in cotton, which were located on 15 linkage groups.

### 2.7.5 QTL stability

A key step in cultivar improvement is the accurate selection of superior genotypes with improved performance for at least one specific trait (Paterson *et al*., 1991b). Breeders must deal with several environmental factors that cause differential cultivar performance. This differential performance is known as the genotype x environment (GE) interaction (Fehr, 1991). The success of any breeding program will depend upon identifying the factors which influence this GE interaction and taking appropriate steps to isolate with controlling their effects through adequate experimental design, appropriate cultural practices, and multilocation testing.

QTL that show consistent expression across diverse environments are ideal candidates for MAS (Velboom and Lee, 1996). QTL analysis across environments has been widely studied in rice. Xu *et al*. (2002) reported that QTL sharing frequencies between two environments varied from 9.5% for drought avoidance to 52.9% for 1000-grain weight. In average, 30% of the QTL under study were shared between both environments. Generally, QTL that explained a large portion of the phenotypic variance (flooding tolerance and paste viscosity) had the highest sharing frequency between environments. As the number of environments under study increased, the QTL sharing frequencies decreased. Paterson *et al.* (1991a) reported that only four of the 29 QTL identified for different traits of tomato were significant across environments. Freyer and Douches (1994) reported that only 20% of the QTL identified were consistent across environments.

## 2.7.6 QTL mapping in sorghum

Utilization of molecular markers in germplasm improvement presents many advantages to plant breeders. They are commonly used for proprietary control of elite germplasm through molecular fingerprinting. Also, with the aid of genetic maps, researchers have found useful linkages between molecular markers and qualitative/quantitative agronomic traits. In addition to providing important information on the genetic inheritance of these traits, these linkages may be utilized in marker assisted selection programs to facilitate germplasm advancement, especially during offseason growing, pyramiding or stacking of several "resistance" genes, and introgression of "exotic" genes into elite germplasm.

Due to its wide adaptation to harsh environments, sorghum has been widely studied to identify genomic regions related to drought tolerance. Selection based on markers would assist in evaluating breeding lines for the presence of genes conditioning drought and charcoal rot tolerance and other quantitative traits in sorghum (Tuinstra *et al.,* 1997). Genomic regions responsible for variation in tiller number, rhizomatousness, and ratooning ability have been identified in intra and interspecific crosses of *Sorghum* (Lin *et al.,* 1995 and Hart *et al.,* 2001). Grain quality-related traits such as dehulling yield, kernel flouriness, friability, hardness and weight as well as amylose, protein and lipid content were studied by Rami *et al*. (1998). At least one QTL was identified for each of the traits studied, with phenotypic variation explained percentages ranging from 13.7% for kernel friability, to 57.1% for kernel flouriness. Klein *et al*. (2001) identified QTL for foliar disease and grain mold resistance in a sorghum RIL population. Five QTL affected grain-mould incidence, each accounting for 10 to 23% of the phenotypic variation observed. Agrama *et al*. (2002) identified several QTL affecting both resistance and tolerance to two greenbug (*Schizaphids graminum* Rondani) biotypes. Due to the ability of this pest to change biotypes, markers linked to the QTL might be utilized for marker-assisted selection to effectively deploy genetic resistance genes and extend the useful life of elite germplasm. The first reports of genomic regions associated with grain yield per se and grain yield components (per cent seed set and height) were done by Tuinstra *et al*. (1996), who measured grain yield under drought and fully irrigated conditions to test the pre-flowering drought tolerance of the germplasm.

Paterson *et al*. (1998) located nine and four QTLs correlated to phenotypic variation of seed size and number respectively. A major chromosomal region involved in grain yield component was identified on LG A in a *Sorghum caudatum* x *S. guinea* RIL population (Rami *et al.,* 1998). Hart *et al*. (2001) mapped several QTL that control various morphological and physiological traits directly related with grain yield variation in a BTx623 x IS3620C sorghum RIL population. These QTLs were located in linkage groups A, E, G, and I, explaining as much as 85.9% of the phenotypic variation of the trait. Molecular markers for resistance of sorghum to hemiparasitic weed, *Striga hermonthica* were mapped in two recombinant inbred populations (RIP 1 and RIP 2, $F_{(3:5)}$ lines) developed from the crosses IS9830 x E36-1 (RIP 1) and N13 x E36-1 (RIP 2). The genetic maps of RIP1 and RIP2 spanned 1498 cM and 1599 cM, respectively, with 137 and 157 markers distributed over 11 linkage groups. Composite interval mapping detected 11 and 9 QTLs in set 1 and 2 of RIP1 explaining 77.6 and 80 per cent of genetic variance, respectively (Haussmann *et al.,* 2004).

The QTL, *QPhe-sbi06-1*, explaining 21.6% of phenotypic variance identified QTLs reported for plant height on LG 6 by Brown *et al.* (2006) and LG I by Hart *et al*., (2001). This QTL corresponds to the *Dw2* gene conditioning plant height QTL on LG D by Lin *et al.* (1995). The detection of all the major plant height QTL on SBI-06 and SBI-07 observed in study done by Feltus *et al*. (2006) suggesting that these regions play an important role in the control of plant height variation in sorghum.

Tadmor and Er/Apm, QTLs associated with grain Delta have been detected in barley grown in three Mediterranean field environments. Eight QTLs for Delta co-located with QTLs for physiological traits related to plant water status and/or osmotic adjustment, and/or for agronomic traits previously measured on the same population, and perspectives for characterising drought tolerance were discussed (Teulat *et al*., 2002). Putative QTLs for $^{13}C$ were identified on chromosomes 2, 4, 8, 9, 11, and 12 across plant parts, stages, and years. Differential expression of QTL for $^{13}C$ among suggested that each QTL a stage specific role. Some QTLs for 13C were associated with or colocated with QTLs for leaf traits, which indicating that their physiological role may be complex.

Values of $^{13}C$ at maturity were negatively correlated with harvest index and grain yield. However, genetic association of these traits could not be clarified due to the absence of co-located QTLs. Further examination would be useful to elucidate the physiological and morphological functions of QTLs for $^{13}C$ found in this study in irrigated land in rice ( Laza *et al*., 2006).

Three QTLs have been identified for charcoal rot resistance across locations and seasons on chromosome 2 and chromosome 9 using the RIL population of the cross IS2238 x E36-1 (Patil, 2009). Fourteen QTLs were mapped which are associated with bioenergy traits in sweet sorghum by Lekgari, 2010, in 165 RIL population at four locations.

Winn *et al*. (2009) study focuses on mapping the QTLs linked to high lysin digestibility trait. This trait has been defined as a single recessive gene, our results uncovered that two major QTLs on chromosome 1 are associated with protein digestibility one QTL (locus 1 from the HD parent) unfavorably affects digestibility and one QTL (locus 2 from the HD parent) only 20 cM. Srinivas *et al*. (2009) identified 49 QTLs for 12 agronomic traits using the RIL population of the cross 296B x IS18551.

### 2.7.7 QTL mapping for stay-green in sorghum

QTL studies for the stay-green trait resulted in the identification of several genomic regions associated with post-flowering resistance. Tuinstra *et al*., (1997a) identified components of grain development and post-flowering drought tolerance in sorghum. They identified 13 genomic regions associated with one or more measures of post-flowering drought tolerance. Two QTLs were identified with major effects on grain yield and stay-green under post-flowering drought stress condition several different QTLs were associated with expression of the stay-green trait. Two stay-green QTLs, one on linkage group F (SBI-09) and another one on linkage group G (SBI-10) were positively associated with grain yield under fully irrigated conditions when the differences in stay-green were not expressed. QTL analysis indicated an association between the stay-green trait and the rate of grain development at a locus on linkage group H (SBI-08). The stay-green trait was associated with a lower rate of grain filling.

Crasta *et al*. (1999) identified seven QTLs for the stay-green trait using RILs derived from B35 × Tx430. Three major stay-green QTLs (*Stg*A, *Stg*D and *Stg*G) contributed to 42 per cent of the phenotypic variability (LOD 9.0) and the remaining four minor QTLs (*Stg*B, *Stg*1.1, *Stg*1.2 and *Stg*J) contributed an additional 25 per cent of the phenotypic variability in stay-green ratings. Xu *et al*. (2000) identified four stay-green QTLs located on three linkage groups. The QTL (*Stg*1 and *Stg*2) were on linkage group A with other two (*Stg*3 and *Stg*4) on linkage groups D and J, respectively. *Stg*1 and *Stg*2 accounted for 13 to 20 per cent and 20 to 30 per cent of the phenotypic variability respectively were consistently identified in all trials at different locations in two years.

They also identified three QTL, for leaf chlorophyll content (*chl1, chl2 and chl3*) that together explained 25 to 30 per cent of the observed phenotypic variability. The QTLs for chlorophyll content were observed to overlap the above three stay-green QTLs present on the C and B linkage groups. The genomic regions corresponding to *Stg*1 and *Stg*2 contain an ABA-responding gene and genes to key photosynthetic enzymes and heat shock proteins.

Assessment of the consistency of QTLs controlling the stay-green trait in sorghum across several environments and genetic backgrounds was done by Subudhi *et al*. (2000). They evaluated the B35 × RTx7000 derived RIL population. The earlier map (Xu *et al.,* 2000b) was expanded by the addition of 91 markers. They mapped four stay-green QTLs and identified that there are partial similarities in their position in case of the QTLs detected on LGC (SBI-03) and LGB (SBI-02) of the B35 × RTx7000 derived population and the B35 × RTx7078 derived population previously reported by Tuinstra *et al*. (1997a).

Identification of QTLs associated with post-flowering drought tolerance in sorghum using an $F_7$ RIL population derived from cross SC56 × RTx7000 by Kebede *et al*. (2001). Nine QTLs distributed across seven of the ten linkage groups were detected for the stay-green trait in several environments using the composite interval mapping method. They also identified three stay-green QTLs present on sorghum LGA (SBI-01), LGG (SBI-10) and LGJ (SBI-05) that were consistently detected across different terminal drought stress environments.

Comparative mapping studies showed that two of the sorghum stay-green QTLs identified in their study corresponds to stay-green QTLs detected in maize. In addition to this, QTLs responsible for sorghum lodging tolerance and pre-flowering drought tolerance were detected.

Sanchez *et al*. (2002) reported four QTLs associated with the stay-green trait in sorghum using the RIL population derived from cross B35 × RTx7000, which were reported previously by Xu *et al*. (2000b) and Subudhi *et al*. (2000) and linkage maps were constructed using with RAPD, SSRs and RFLP markers. Therefore, major QTLs reported by Crasta *et al*. (1999), Xu *et al*. (2000b) and Subudhi *et al*. (2000) were consistently identified in all field trials and accounted for 53.5 per cent of the observed phenotypic variance for the stay-green trait.

A set of 72 diverse genotypes of sorghum for their pattern of post-flowering leaf senescence under terminal drought condition to identify superior sources of the stay-green trait was evaluated (Mahalakshmi and Bidinger, 2002). Leaf senescence patterns were determined by fitting logistics or linear functions to the percentage of green leaf area (% GLA). They identified several tropically adopted lines with stay-green expression equivalent to those of the best temperate adapted lines with stay-green expression equivalent to those of the best temperate adapted lines *viz*., B35 and KS19.

Using two recombinant inbred population (RIPs); IS9830 × E36-1 (RIP1) and N13 × E36-1 (RIP2) identified QTLs explaining 31 to 42 per cent of the phenotypic variation for green leaf area percentage at 15, 30 and 45 days after flowering (E36-1 is the stay-green source) (Haussmann *et al*., 2002b). Three QTLs on LGA (SBI-01), LGE (SBI-07) and LGG (SBI-10) were common to both RIP1 and RIP2. These three QTLs from donor parent E36-1 along with the QTLs from donor parent B35 are potential candidates for transfer of the stay-green trait into locally adapted elite sorghum materials.

A study by Harris *et al*. (2007) which reported on near-isogenic lines (NILs) developed for the same four stay-green QTL (*Stg1-Stg4*) using BTx642 (formerly B35) as the stay-green donor, has shown that these stay-green QTL individually reduce the post-flowering drought-induced leaf senescence in the recipient senescent genetic background of RTx7000.

## 2.8 Marker assisted introgression, theory, practical considerations for charcoal rot resistance

### 2.8.1 Charcoal rot disease

Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goid, is the most common and probably also the most important root and stalk rot disease of sorghum. Major cultivated hosts of this fungus include: sorghum, groundnut, cabbage, pepper, chickpea, soybean, sunflower, sweet, potato, alfalfa, sesame, potato, bean, and corn. Charcoal rot is an important disease during hot, dry weather or when unfavorable environmental conditions prevail. Dhingra and Sinclair (1977, 1978), and Sinclair (these proceedings) provide comprehensive information on the biology of *M. phaseolina* and the epidemiology and control of the diseases it causes in many plant species. In sorghum, the fungus causes stalk rot during hot, dry conditions. The causal organism of charcoal is a common soil borne fungus often known by its imperfect state.

### 2.8.2 Symptoms of disease

A variety of symptoms are associated with charcoal rot. These include root rot, soft stalks, lodging of plants, premature drying of stalks, and poorly developed panicles with small inferior-quality grain (His, 1956 and Uppal *et al.,* 1936). In susceptible genotypes fungus crosses as many internodes as possible and brings about stalk lodging. The stalk will appear gray to black in colour. The pith region disintegrates into suspended fibres with charcoal powder like appearance resulting in weakened stalk and there by lodging of plants.

Marker assisted backcross breeding (MAB) is a well-known procedure for the introgression of a target gene from a donor line into the genomic background of a recipient line. The objective is to reduce the donor genome content of the progenies by repeated back-crosses to the recipient line. Marker-assisted backcross is of great practical interest in applied breeding schemes either to manipulate ‘classical’ genes between elite lines or from genetic resources, or to manipulate transgenic constructions.

From a theoretical standpoint, it is a 'simple' example of marker-based selection: in general, only two alleles are segregating, and the gametic phase is known because only one chromosome of each pair is issued from effective recombination (the chromosome from the gamete produced by the backcrossed parent). Molecular markers have become a powerful tool to use in studying the inheritance of complex traits (Paterson *et al.,* 1988 and William *et al.,* 1992). Concibido *et al*. (1996) defined MAS as an indirect selection method based on genotype rather than plant phenotype. Plant breeders and molecular geneticists can then integrate molecular biology and traditional plant breeding methodology to identify QTLs and use the probes to conduct marker-assisted selection (MAS). They reported that MAS can be used to accelerate the rate at which new cultivars are developed. MAS may be especially useful in the early generations of pedigree selection or in selection of characters that are difficult or expensive to measure such as drought and pest resistance (Lande, 1992).

Marker-assisted selection in introgression of favourable alleles at quantitative trait loci (QTL) usually comprises selection for (1) Presence of the donor allele at two markers delimiting the interval in which the putative QTL was detected (foreground selection) (2) the recurrent parent allele at markers outside the QTL interval on carrier chromosome(limited background/recombinant selection) and (3) Recover of recurrent parent allele on carrier and non carrier chromosome (Background selection).

Specific breeding programmes in which marker-assisted selection has been already put to use are: 1) gene introgression and elimination of linkage drag, 2) gene pyramiding, and 3) development of heterotic hybrids. Some of the assumptions associated with MAS are: 1) linkage relationships between markers and QTLs are consistent for progeny within the same population, 2) linkage relationships between markers and QTLs are repeatable between populations, 3) linkage relationships between markers and QTLs are real and not spurious relationships due to Type I error, 4) associations between markers and QTLs can be identified with about 100 progeny, 5) MAS will eliminate the problem of genotype x environment interactions, thus making MAS more effective than phenotypic selection.

Toojinda *et al*. (1998) reported that molecular markers could increase the efficiency of the backcrossing process in several ways. First, flanking markers can be used to identify heterozygous backcross progenies that are heterozygous for target genome regions. Advancing only these selected progenies will also have the effect of reducing linkage drag. Secondly, molecular markers increase the efficiency of backcrossing by allowing the selection of genotypes with the maximum percentage of the recurrent parent genome. Frisch *et al*. (1999) reported that marker-assisted selection could accelerate the recovery of the recurrent parent genome in backcross breeding. Hospital and Charcosset, (1997) indicated that marker assays could be advantageous in backcross breeding for both foreground and background selection. In foreground selection, the presence of the target allele in an individual is diagnosed by monitoring the genotype at flanking markers for alleles of the donor parent.

For the background genotype, using markers gives a direct estimate of the proportion of the donor genome that is still present in each backcross generation. This may be preferred over phenotypic selection, in particular for traits with low heritabilities that are difficult to measure eg., age and sex-limited traits. In several studies it has been shown that genetic markers can be used to introgress genes from one line to another (Smith *et al.,* 1987; Hillel *et al.,* 1992; Groen and Timmerman, 1992 and Groen and Smith, 1995). Selection was applied on two flanking markers, one on each side of the introgressed gene, and the selection objective was to obtain a backcross progeny that carries the donor allele at the locus of interest and recipient alleles at both flanking markers (such an individual genotype was called "double recombinant").

### 2.8.3 Efficiency of MAS

Efficiency of MAS over the expected efficiency of purely phenotypic selection generally increases with 1) larger population size, 2) length of the QTL and 3) lower heritability values of the trait. For MAS to be used in plant breeding, the marker has to be closely linked with the desired trait (Mohan *et al.,* 1997), and the selection method should be reproducible across environments with little environmental variation (Gianfranceschi *et al.,* 1996).

Many studies have shown that molecular markers can improve the efficiency of selection of quantitative trait loci in plant breeding provided large population sizes are used (Moreau *et al.,* 1999).The minimum number of individuals that should be genotyped to obtain a double recombinant in $BC_1$ is about 24,000. The same result can be obtained over two generations ($BC_2$ strategy) by genotyping 290 individuals in $BC_1$, and 500 in $BC_2$. Finally, over three generations ($BC_3$ strategy), the optimal population sizes are 120 individuals in $BC_1$, 170 in $BC_2$, and 270 in $BC_3$. Study was carried by Bouchez *et al.* (2002), where they screened 175 individuals in $BC_2$ and $BC_3$, on the basis of the computations of Hospital and Charcosset, (1997), with this population size, the risk of not obtaining at least one individual carrying donor alleles at all foreground selection markers controlling the QTL segments was below 1% (minimum population size at 1%: 115 individuals), within each $BC_2$ and $BC_3$ population, eight progenies were found heterozygous for all markers on controlled segments, which was consistent with expectations.

The efficiency of MAS was generally reduced with increasing distance between the markers flanking the target QTL. The optimal distance recommended between two flanking markers is about 5–10 cM (Hospital *et al.,* 1992). According to Visscher *et al*. (1996) for practical situations, a marker distance of 10-20 cM seems appropriate. The contribution of donor parent is reduced by half with each generation of backcrossing assuming no linkages. Molecular markers can increase the efficiency of the process in several ways. Flanking markers can be used to identify the backcross lines that are heterozygous for target genome regions. Advancing only these selected linesx will also have the effect of reducing Linkage drag (Young and Tanksley, 1989 and Tanksley and Nelson, 1996).

MAS could be more effective for plant breeding programs than purely phenotypic selection in large populations and for traits with relatively low heritability of 0.05 to 0.50 (Moreau *et al.,* 1998).

Dreher *et al*. (2003) at CIMMYT came to some preliminary conclusions on, when phenotypic screening is simple (in other words, when it is relatively easy to determine whether a given plant variety possesses a given trait, such as a certain grain color), conventional breeding is, will continue to be extremely cost-effective. Conversely, when phenotypic screening is expensive, technically difficult, or even impossible, MAS will often be advantageous. MAS offer an alternative that is simple, direct, and very reliable. Often effective selection for resistance to diseases like maize streak virus, which are strictly quarantined, can be carried out using molecular markers even at locations where the disease is not present (but with the breeding program targeting regions where the disease is currently present or is considered a significant future threat). Marker-assisted selection often allows breeders to cut down on the number of seasons needed to produce a desired product. With conventional backcrossing, it takes a minimum of five to six generations to recover the recurrent parent. Data from simulation studies suggests that at least two but possibly three or even four backcross generations can be saved by using markers (Mebrouk *et al.,* 2005).

Even a high-end MAS scheme that might run a few thousand dollars more than a conventional scheme is to the additional benefit to farmers when a variety becomes available sooner. Podlich *et al*. (2005), developed an effective approach for marker assisted selection of complex traits*, i.e.* Mapping As You Go (MAYG) approach, which continually revises estimates of QTL allele effects by remapping new elite germplasm generated over cycles of selection, thus ensuring that QTL estimates remain relevant to the current set of germplasm in the breeding program. They used simulation to investigate the effectiveness of the MAYG approach applied to complex traits.

### 2.8.4 Successful reports on MAS

Pyramiding of several major resistance genes in rice, from near-isogenic lines that each carry only one gene, into a common background successfully introgressed two QTL for stripe rust resistance in barley into a genetic background different from the one used to map the QTL (Huang *et al.,* 1997 and Hittalmani *et al.,* 2000). Toojinda *et al*. (1998). Chee *et al*. (2001) also report the successful transfer of a QTL for grain protein concentration in wheat into a different genetic background. Ahmadi *et al*. (2001) successfully introgressed two QTL for resistance to yellow mottle virus in rice. Yousef and Juvik (2002) successfully selected on three markers linked to QTL that enhanced seedling emergence in sweet corn.

Lawson *et al.* (1997) introgressed four target chromosomal regions containing five QTL for pest resistance (acylsugar accumulation) from wild tomato into cultivated tomato. The introgression of the four regions was successful at the genomic level. Ribaut *et al*. (2002a, b) introgressed five target regions containing QTL for drought tolerance reduction of anthesis-silking interval (ASI) in maize. i.e. reducing yield.

Hash *et al*. (2003), suggested the use of marker-assisted selection (MAS) as a route for the backcross transfer of previously identified stover quality traits to elite genetic backgrounds. Traits associated with improved ruminant nutritional quality of stover that were suggested for manipulation in this manner included foliar disease resistance, the stay-green component of terminal drought tolerance, and *in vivo*, *in vitro*, or NIRS-estimated dry matter digestibility.

Lecomte *et al*. (2004) introgressed five QTL strongly involved in tomato fruit quality into three different recipient lines through MAS. Thabuis *et al*. (2004) transferred resistance to *Phytophthora capsici* alleles at four QTL from a smallfruited pepper into a bell pepper recipient by three cycles of marker-assisted backcrossing.

MAS has been employed to improve the efficiency and speed up breeding programs, specifically in selection (Jonaliza *et al*., 2007). A major use of MAS is in assisting backcrossing of genes/QTLs to elite cultivars. Markers aid in the selection of target alleles and in the assessment of a recipient's parent genome. Introgression and selection of QTLs using molecular markers in foreground selection may have additional problems, since the exact position of the target is often not known. The backcrossing process with target selection resulted in 103 KMDL105 introgression lines carrying 1, 2 or 3 target combinations, where 79, 20 and 4 lines were derived from KDML105 x IR68586-F2-CA-143 (DH212) (cross 1), KDML105 x IR68586-F2-CA-31 (DH103) (cross 2) and KDML105 x IR68586-F2-CA-54 (DH126) (cross 3) crosses, respectively. The results proved that MAS aids in the transfer of target segments and may improve the recovery of the recipient genome if background selection is employed.

The *Phs* marker linked with a QTL derived from landrace G122 on linkage group B7 was backcrossed into 'Winchester' pinto, forming two $BC_3F_{4:6}$ inbred line populations. The AW9.1200 and SS18.1650 markers linked with a QTL from snap bean breeding line NY6020-4 on B8 were backcrossed into 'Maverick' pinto and 'Matterhorn' great northern, forming $BC_2F_{4:6}$ inbred line populations. The B7 QTL in the $BC_3F_{4:6}$ populations on average explained 52% of the phenotypic variation for disease reaction in the greenhouse test and 10% across four field tests. The B8 QTL explained 30% in the greenhouse and 10% in the field. Averaged across tests and populations the B7 and B8 QTL conditioned 15 and 17% reduction in disease severity score, respectively. MAS for the B7 and B8 QTL was an effective breeding tool for introgressing partial resistance to white mold into susceptible pinto and great northern dry bean market classes, but further selection for agronomic performance may be required to obtain lines worthy of commercial production Miklas *et al*. (2007).

A more rigorous evaluation of the putative drought tolerance QTL is currently underway using near-isogenic versions of the more drought-sensitive parent H 77/833-2, bred by marker-assisted introgression of various segments of LG2 from donor parent PRLT 2/89-33 in the region of the putative drought tolerance QTL. $BC_4F_3$ progenies from selected $BC_4F_2$ plants homozygous for various portions of the LG2 target region were crossed to each of five related, early-maturing seed parents, and the resulting hybrids were evaluated under a range of terminal stress environments (Serraj *et al.,* 2005).

Selected ISIAP Dorado and R 16 $BC_3F_1$ genotypes with donor segments flanked by SSR markers for single and multiple stay-green QTLs from donor B35 have been selfed to $BC_3F_2$ and individuals homozygous for QTL introgressions identified and selfed again. The resulting $BC_3F_3$ progenies were sown during the 2003/2004 post-rainy season at Patancheru, India (in which crop growth is dependant mainly on stored soil moisture), to phenotypically assess the effectiveness of the transfer of stay-green QTLs. The near-isogenic products of this backcrossing program should prove useful for determining the mechanisms involved in these drought tolerance-related traits, and helping breeders identify minimum complements of genes that are required for satisfactory drought tolerance expression (Hash *et al.,* 2003).

A set of 34 RTX7000 NILs were developed by crossing BTX642 with the senescent genotype RTX7000 followed by subsequent introgression of one or more of the BTX642 stay-green QTL regions into the RTX7000 background by (Harris *et al.,* 2007). NILs containing *Stg*1, 2, 3, and 4 were identified and found to have enhanced stay-green related phenotypes relative to RTX7000, Selection was continued until the $BC_4$ or $BC_6$ generation where the lines were selfed to create $BC_4F_{2-4}$ or $BC_6F_{2-4}$ lines. Developed 18 different RT37000 $BC_{4-6}F_{2-4}$ NILs that contain introgressed regions of BT3642 DNA. Detailed genetic analysis of the RT37000 NILs showed that these lines contain BT3642 DNA spanning all or a portion of the four major stay-green loci, *Stg*1–*Stg*4, previously identified in a cross of RT37000 and BT3642 (Subudhi *et al.,* 2000; Xu *et al.,* 2000). Several of the RT37000 NILs contained blocks of BT3642 DNA that partially or completely spanned a stay-green locus plus a variable amount of DNA flanking the target locus.

Stay-green QTLs from the donor parent have been successfully introgressed into the backcross progenies by (Kassahun *et al.* 2009), and were expressing in the genetic background of the recurrent parent R 16. The majority of the QTL introgression lines had higher leaf chlorophyll content both before and during leaf senescence. They also had either a delayed initiation of leaf senescence (supplementory irrigated environment) or slower rate of loss of % GLA (post-flowering moisture deficit environments). Reduced leaf senescence (relative % GLA in the stress/irrigated conditions) was related to higher relative grain yield in two of the three post-flowering stress environments.

# 3. MATERIAL AND METHODS

The present investigations were carried out at the Institute of Agri-Biotechnology (IABT), Dharwad. The details of material used and the methodologies adopted in the present study are as follows.

## 3.1 Phenotyping of recombinant inbred populations for post-flowering drought tolerance and yield related traits

### 3.1.1 Mapping populations

The two recombinant inbred lines (RILs) populations each consisting of 226 $F_{10-11}$ lines, were developed from the crosses IS9830 x E36-1 (RIP1) and N13 x E36-1 (RIP2). The $F_5$ seeds of mapping populations were obtained from the International *cr*ops Research Institute for Semi Arid Tropics (ICRISAT), Patancheru, Hyderabad, India and were further advanced and maintained at IABT. The E36-1, a donor for stay-green trait in these RIPs, is a high yielding line from guinea-caudatum hybrid race with Ethiopian origin, well adopted to tropical environment and also resistant to charcoal rot disease and lodging. The lines IS9830 and N13 are non stay-green types. IS9830 line is a tall Sudanese feteria belonging to the caudatum race whereas line N13 is a durra sorghum from India. Further, N13 and E36-1 were found to be significantly differing for water use efficiency as reflected by carbon isotope discrimination ($^{13}C$) and the RIP2 based on these lines were segregating for this trait. Detailed features of various sorghum genotypes used in the study are presented in Table 6.

### 3.1.2 Location, season and experimental design

The RIPs were evaluated at Main Agricultural Research Station (MARS), Dharwad (Plate 1), during *rabi* season of 2007, 2008 and 2009 and at Regional Agricultural Research Station (RARS), Bheemarayanagudi, during *rabi* seasons of 2008 and 2009 for post flowering drought tolerance and yield related component traits. The MARS, Dharwad is situated at 15°12' N latitude and 76°34' E longitude with an altitude of 678 meters above the mean sea level and RARS, Bheemarayanagudi is situated at 17°40' N latitude and 75°54' E longitude with an altitude of 523.33 meters above the mean sea level. The rainfall statistics for experimental years at both locations is presented in Table 7.

A set of 228 entries comprising of 226 RILs, two parents and checks were taken up for phenotyping. Simple lattice design was adopted on an area of 50 x 55 $m^2$ with four replications. Each replication consisted of 19 blocks, each with 12 entries planted randomly to accommodate 15 plants in a single row. The trial was laid out with a spacing of 45 x 15 $cm^2$ and all recommended package of practices were followed. In order to eliminate the border effects, three rows of M35-1 was sown on all sides of the experimental plot. The plots were thinned at 10 days after seedling emergence (DAE) to a spacing of 15 cm between plants within rows when the seedlings were at 4-leaf stage.

### 3.1.3 Phenotypic observations for stay-green and other yield related traits

Field evaluation of RIP1 and RIP2 for post-flowering drought tolerance related traits such as stay-green, water use efficiency and yield related traits was done during 2007-08, 2008-09 and 2009-10 at two locations.

**Table 6: Information on pedigree or source and distinguishing characters of the *rabi* sorghum genotypes used in the present study**

| Genotypes | Pedigree or source | Distinguishing characters |
|---|---|---|
| **E36-1** | Guinea- caudatum hybrid race with Ethiopian origin | Stay-green, resistant to charcoal rot and drought, striga susceptible , bold and white colour seed, leaves dark green, covered with blume, are elongated and more wide. Medium height with compact ear head. |
| **IS9830** | Sudanese feterita belong to the caudatum race | Senescent type, leaves light green, thin stem with medium panicle exertion. semicompact ear head with small grey colour seeds. Resistant to striga. |
| **N13** | Durra sorghum from India | Senescent type, leaves light green, narrow, not covered with wax, thin and lanky stem, loose ear head, small oval golden colour seeds. |
| **M35-1** | Selection from local Maldandi bulk population, Karnataka | A long standing variety, good quality grain, tolerant to drought and shoot fly. Susceptible to charcoal rot,180-200 cm tall, less leaf sheath waxyness, Elongated peduncle with semi compact to loose, oval ear, glumes light brown, large with white pearly, bold and round seeds, grain texture of endosperm is fully vitreous, |
| **SPV86** | R 24 x R 16<br>National Research Centre Sorghum, Hyderabad | High yielding hybrid variety, highly susceptible to charcoal rot, moderately tolerant to shootfly, leaves yellowish green, medium drooping, more leaf sheath waxyness, 150-180 cm tall, semicompact cylindrical ear, seeds round, bold and creamy, grain texture of endosperm is half vitreous, |
| **SPV570** | 5-4-1 x SB 40<br>Parabhani, Maharashtra | Hybrid variety has good fodder quality, semicompact, oval shaped panicle straw coloured glumes, creamy and lustrus seed with pink spot, high yielding lines with 120 days maturity and promising restorer line on Milo cytoplasm drought susceptible |

**Plate 1: Field evaluation of RILs for different phenotypic traits at Dharwad location during rabi 2009**

**Table 7: Monthly rainfall (mm) data for the experimental years (2007-2010), as recorded at meteorological observatory, MARS, Dharwad and RARS, Bheemarayanagudi**

| Month | Dharwad | | | | Bheemarayanagudi | |
|---|---|---|---|---|---|---|
| | 2007 | 2008 | 2009 | 2010 | 2008 | 2009 |
| January | - | - | - | 0.8 | - | - |
| February | - | - | - | 0.4 | - | - |
| March | 12.8 | 111 | 29.8 | - | 147 | - |
| April | 86.4 | 29.2 | 52.8 | 43.8 | - | - |
| May | 65 | 55.8 | 91.6 | 63.1 | 8 | 47 |
| June | 220.1 | 101.6 | 144.8 | 63.4 | 35 | 52.5 |
| July | 211.2 | 121 | 256.8 | 155 | 77 | 3.5 |
| August | 176 | 213.2 | 72.2 | 190.7 | 86 | 83 |
| September | 180.8 | 162.4 | 229 | 164.9 | 164.7 | 286 |
| October | 74.8 | 60.4 | 141 | 177 | 46.5 | 268.5 |
| November | 54 | 72.2 | 46 | 92.8 | 9 | 70 |
| December | - | - | 76.4 | 0.6 | 14 | 5.5 |
| Total (mm) | 1081.1 | 926.8 | 1140.4 | 952.5 | 587.2 | 816 |

Following observations were recorded on RIP1 and RIP2 at both locations across seasons.

1. Stay-green : Stay-green refers to genotypes with delayed leaf senescence during grain ripening under drought conditions. This was measured through an estimation of senescence. At the time of emergence of flag leaf, three representative plants in each replication were tagged, the length and width of the upper six leaves were measured and the area of each leaf was estimated as : leaf length x leaf width x 0.7 (the factor 0.7 was determined by measuring the leaf length, leaf width and actual area of 50 randomly chosen leaves). Beginning of flag leaf emergence, the percentage of each of the upper six leaves of each tagged plant remaining green was visually estimated at weekly intervals according to Mahalakshmi and Bidinger (2002). The green leaf area of each tagged plant was computed by multiplying the per cent green leaf area by measured area of each leaf and summing across the six measured leaves. The percentage of green leaf area (per cent GLA) for each plant, for each week, was calculated by dividing the estimated GLA for that week by its measured leaf area at flowering. Individual entry value for per cent GLA was derived by averaging the three individual plant values for each plot. The weekly per cent GLA data were used to fit an appropriate equation to describe the pattern of leaf senescence during the period of observations. The fitted equations for each individual entry were used to estimate the per cent GLA at 15, 30, 45 days after flowering of the individual entries as per cent GLA15 DAF, per cent GLA30 DAF, per cent GLA45 DAF, respectively (Mahalakshmi and Bidinger, 2002).

    a. Per cent GLA 15 DAF : mean green leaf area after 1$^{st}$ and 2$^{nd}$ week of flowering.

    b. Per cent GLA 30 DAF : mean green leaf area after 3$^{rd}$ and 4$^{th}$ week of flowering.

    c. Per cent GLA 45 DAF : mean green leaf area after 5$^{th}$ and 6$^{th}$ week of flowering.

2. Water use efficiency (Carbon isotope discrimination): Water use efficiency (WUE) estimation was approached through quantification of $\Delta^{13}C$ in leaf samples: Flag leaf at post flowering stage of crop was collected (5-6 for each genotype). The samples were dried at 80 ºC for 3 days and were powdered using a mortar and pestle. Care was taken to prevent mixing of different samples by washing the pestle and mortar with alcohol after grinding each sample. About 1 g of powdered leaf sample was put in glass vial and properly labeled. $\Delta^{13}C$ was measured at the National Facility for quantification of stable isotopes at Department of *cr*op Physiology, UAS, Bangalore. Mass spectrometric analysis: Carbon isotope ratios ($^{13}C/^{12}C$) in comparison with the Pee Dee Belemnite (PDB), from North Carolina standard were measured using Isotopic Mass Spectrophotometer (IRMS). The IRMS facility consists of Flash Elemental Analyzer (CE-EA 1112), for sequential combustion of biomass samples and open split interface. Powdered dry leaf samples were accurately weighed in the range of 0.8 to 1.2 mg into tin capsules. The crimped capsules with the sample were placed sequentially in the carrier of the Autosampler. The samples were dropped at precise times along with an injection of pure $O_2$ into the oxidation reactor.

The combustion (oxidation) reactor contains chromium oxide and silvered cobaltouscobaltic- oxide heated to 1050 ºC. The biomass is completely oxidised to produce $CO_2$, $N_2O$ and $H_2O$. These gases were swept into the reduction furnace using helium carrier gas. The reduction furnace contains reduced copper in quartz tubes heated to 680 ºC. In this reaction, the $N_2O$ was reduced to $N_2$ and the excess $O_2$ was absorbed. The resultant gases were then flushed through scrubbers to trap $CO_2$ and water. The pure $CO_2$ and $N_2$ gas poses through a GC column (5ºA molecular sieve) and a thermal conductivity detector (TCD) into the ion source of the IRMS. At this source, $CO_2$ was ionized by electron impacts ionization to produce molecular radicals ($CO^+$). When accelerated $CO^+$ radicals passed through a strong magnetic field, they were deflected with the radius of deflection being proportional to the molecular mass of the radicals. These deflecting $^{12}CO_2$ and $^{13}CO_2$ were collected in Faraday cups and the amplified signal was transmitted to the computer.

Based on the fractionation (isotopic composition with respect to PDB), the $\Delta^{13}C$ discrimination ($\Delta^{13}C$) in the plant sample was computed as follows:

$$\Delta^{13}C\ (\%) = (\Delta^{13}C - \delta p^{13}C\ /\ 1 + \delta^{13}C) \times 100$$

3. Total green leaf area: Total green leaf area at the time of flowering, the green leaf area of each tagged plant was computed by multiplying the per cent green leaf area with measured area (length x bredth) of each leaf and summing across the six measured leaves expressed in $cm^2$.
4. Number of leaves: Total number of leaves at the time of flowering was counted and expressed in numbers.
5. Spicklets per head: Total number of spicklets per head at the time of maturity were counted and expressed in numbers.
6. Plant height: Height at maturity was recorded from the soil surface to the ear head tip of each plant and expressed in cm.
7. Earhead length: Length from the basal whorl of the inflorescence to the tip of the panicle was measured and expressed in cm.
8. 100 grain weight: One hundred seeds collected randomly from threshed grain of each ear were counted, weighed and expressed in grams.
9. Days to 50 per cent flowering: Time interval from sowing to anthesis of at least 50 per cent of the plants was recorded as days to 50 per cent flowering and expressed in days.
10. Panicle exertion: Length between base of ear head and start of $1^{st}$ leaf at time of maturity was measured and expressed in cm.
11. Stem girth: This trait was measured at the middle of the first internode just above the ground level at the time of anthesis with the help of a thread and expressed in cm.
12. Grain yield per plant: Weight of seed from each plant recorded at the time of harvest and expressed in grams.

## 3.2 Construction of molecular genetic linkage maps for RIP1 and RIP2

The details on preparation of solutions and buffers used in the study are presented in Appendix I.

### 3.2.1 Genomic DNA extraction from sorghum leaves

DNA was extracted from a bulk of 6-7 individual plants from 226 RILs and parents from both populations. Leaves of three to four week old seedlings were taken from field and frozen in liquid nitrogen. The leaf samples were stored at –80 $^0$C until further use. Genomic DNA was prepared following Krishna and Jawali (1997) method with a few minor modifications as follows

1. Frozen tissue sample (2 g) was ground into fine powder in liquid nitrogen, using autoclaved mortar and pestle.
2. The ground tissue was transferred to 2.0 ml Eppendorf tube containing 900 µl of extraction buffer and 90 µl of 20 per cent sodium dodesyl sulphate (SDS) was added to each tube.
3. The contents were mixed well and incubated at 65 $^0$C for 10 min.
4. The contents were then cooled on ice for 10 min.
5. Potassium acetate (300 µl) was added and mixed thoroughly.
6. The contents were spun for 20 min at 13000 rpm at 4 $^0$C.
7. About 600 µl of supernatant was transferred to fresh tube and the remaining was discarded.
8. About 600 µl of isopropanol – ammonium acetate mixture was added to supernatant to precipitate nucleic acids.
9. The contents were mixed thoroughly and centrifuged for 20 min at 13000 rpm to pellet the nucleic acids.

10. The supernatant was discarded and the pellet was washed with 70 per cent alcohol, tubes were inverted on blotting paper to dry the pellet.
11. Pellet was dissolved in 200 µl of $T_{10}E_1$.
12. RNAase (5 µl) (10 mg/ml) was added to each tube. DNA was redissolved by tapping the pellet suspended in RNAase and incubated at room temperature (37 $^0$C) for 30 min.
13. The DNA was precipitated by adding 1/10$^{th}$ volume of 3 M sodium acetate and 2.5 volumes of absolute ethanol.
14. The contents were mixed gently and incubated at 4 $^0$C for 30 min.
15. The contents were centrifuged for 1 min at 3000 rpm.
16. DNA pellet was rinsed with 70 per cent ethanol twice and centrifuged at 3000 rpm for 1 min.
17. Supernatant was discarded and the tubes were put upside down on paper towel to get rid of excess ethanol.
18. Pellet was dissolved in 100 µl of $T_{10}E_1$.

Purification and quantification of extracted genomic DNA

1. Equal volume (100 µl) of phenol: chloroform: isoamylalcohol (25:24:1) mixture was added to each tube and the contents were mixed by inverting.
2. The contents were spun at 2500 rpm for 10 min and supernatant was transferred to fresh tubes.
3. Step 1 and 2 were repeated.
4. To the above supernatant, 10 µl of 3 M sodium acetate (1/10 volume of aqueous layer) and 2.5 volumes of chilled absolute ethanol was added, mixed gently and incubated at –20 $^0$C for 15-20 min.
5. The DNA was spooled in 1.5 ml Eppendorf tubes using a glass hook, washed with 70 per cent alcohol and dried.
6. DNA was dissolved in100 µl $T_{10}E_1$ and kept at –20 $^o$C till its further use.

The amount of DNA in each sample was quantified by taking the readings at 260 nm and 280 nm in the Nano Drop (UV technologies, USA).

1. Initialization of the instrument was done with nanopure water.
2. The instrument was set blank with help of 2µl $T_{10}E_1$
3. The quantity of DNA was measured by loading 2µl DNA sample on Nanodrop spectrophotometer pedestal.
4. The DNA quantity in ng/µl and OD value for each sample was noted.

The ratio between the readings at 260 and 280 nm (OD 260/OD 280) was used as an estimate of the purity of the DNA samples. Pure preparations of DNA have 260 nm/ 280 nm OD ratio between 1.7 and 1.8 (Sambrook *et al*., 2001). Computed OD values were used to dilute the DNA samples to the working concentrations of 5 ng/µl. The stock DNA solutions were diluted to 5 ng/µl of 100 ng/µl working solution for PCR. Then the amount of stock DNA solution to be taken for dilution was calculated using the following formula, where $M_1$ is the stock DNA concentration (for example, 100 ng/µl), $V_1$ is the volume of stock to be diluted, $M_2$ is the concentration of working solution (5 ng/µl) and $V_2$ is the volume of working solution to be prepared (for example, 100 µl).

$M_1V_1 = M_2V_2$

(100 ng/µl) $V_1 = (5 \text{ ng/µl}) (100 \text{ µl})$

$V_1 = (5 \text{ ng/µl}) (100 \text{ µl}) / (100 \text{ ng/µl})$

$V_1 = 5 \text{ µl}$

Then the appropriate volume from the stock was transferred to 0.5 ml microcentrifuge tube and the volume was made to 100 µl using distilled water. The DNA working solutions were stored at -20 $^0$C till further use.

### 3.2.2 PCR conditions for amplifying of genic and nuclear SSR alleles

Nuclear SSRs and EST-SSR markers developed at IABT (Arun, 2006), UAS, Dharwad were used in present study. The primer pairs were synthesized from Sigma-Aldrich, Pvt. Ltd, USA. Primers were diluted by giving a brief spin to collect the amorphous primer stock at bottom of tubes supplied by the company and then nano pure water was added to prepare stock solution of 100 pM. The tubes were incubated at 37 $^0$C for 30 mins. Then the working solution of 5 pM concentration was done using the formula mentioned in section DNA dilution.

PCR optimization

Three concentrations of each of primer pair (0.2, 0.3, 0.5 pM), $Mg^{2+}$ (1.0, 2.0, 5.0 mM), *Taq* polymerase enzyme (In-house), two concentrations each of DNA (5, 10 ng) and dNTP (0.1, 0.2 mM, Eppendorf, USA) were varied in different combinations and the combination that gave good amplification was selected. Amplifications were performed in a final volume of 25 µl with 1x assay buffer. Three different programs of "Touchdown" PCR (Don *et al*., 1991) with base annealing temperature ranges of 55 $^0$C–50 $^0$C, 60 $^0$C–55 $^0$C, and 65 $^0$C–60 $^0$C were used for standardization of annealing temperatures and based on annealing temperature range required by them to produce sharp bands without much of spurious products primer pairs were classified into three groups. In the initial annealing steps, the annealing temperature was decreased by 1$^0$C after two subsequent cycles for first 10 cycles. Products were thereafter amplified for 30 cycles at the appropriate optimum annealing temperature with a final extension of 20 min.

Amplification reaction mixture for genic and nuclear SSR markers was prepared in 96 well PCR plate tubes (Tarson Pvt. Ltd, India) containing following components in a total volume of 13 µl.

| Components | Quantity (µl/reaction) |
|---|---|
| Genomic DNA (5 ng/ml) | 1.0 |
| dNTPs mix (2.5 mM each) | 0.5 |
| Forward primer (5 pM/µl) | 0.5 |
| Reverse primer (5 pM/µl) | 0.5 |
| $MgCl_2$ (25 mM) | 1.0 |
| 10x assay buffer | 1.2 |
| *Taq* DNA polymerase (in-house) | 0.3 |
| Sterile water | 8.0 |

PCR reaction was carried out using Mastercycler gradient 5331-Eppendorf version 2.30. 31-09, Germany. The cycler was programmed as follows;

| Reaction step | Temperature range ($^0$C) | | | Time | Cycles |
|---|---|---|---|---|---|
| | 65-60 | 60-55 | 55-50 | | |
| Initial denaturation | 94 | 94 | 94 | 3 min | 1 cycle |
| Denaturation | 94 | 94 | 94 | 20 sec | |
| Annealing | 65-61 | 60-56 | 55-51 | 20 sec | 1 $^0$C/ 2 cycles |
| Primer extension | 72 | 72 | 72 | 30 sec | for 10 cycles |
| Denaturation | 94 | 94 | 94 | 20 sec | |
| Annealing | 60 | 55 | 50 | 20 sec | 30 cycles |
| Primer extension | 72 | 72 | 72 | 30 sec | |
| Final extension | 72 | 72 | 72 | 20 min | |
| Store at | 4 | 4 | 4 | Till end | |

### 3.2.3 Separation of genic and nuclear SSR markers

Parental polymorphism for genic and nuclear SSR markers

Screening of parents was carried out for identification of polymorphism before genotyping all the RILs. For this, parental DNA from IS9830/N13 ($P_1$) and E36-1 ($P_2$) were subjected to PCR amplification for the polymorphism if any with a total of 530 genic SSR and 270 nuclear SSR markers.

Separation and visualization of PCR products was done on both agarose (2.5%) as well as polyacrylamide gels (6 per cent). Agarose gels were used to check amplification and polymorphism between the two parents, if any could be detected than we carried out the genotyping for all the 226 RILs using 2.5 per cent agarose gels. The markers which did not show any detectable polymorphism between parents on 2.5 per cent agarose gels with ethidium bromide staining were carried forward to 6 per cent polyacylamide gels with silver staining procedure.

Agarose gel electrophoresis

Agarose gel electrophoresis was done for SSR markers. Agarose was casted in 2.5 per cent gels in TAE buffer (1X). Slabs were casted in a horizontal gel frame (Hoefer HE99X 18 x 30 cm Amersham Bioscience Pvt. Ltd. USA), products were visualized by incorporating 1 µl (10 mg/ml) ethidium bromide per 10 ml of gel solution and viewed in a gel documentation system (Syngene Pvt. Ltd. USA). The procedure followed for gel electrophoresis was as follows

1. The perspex tray and comb were thoroughly cleaned with 70 per cent alcohol using tissue paper.
2. The ends of perspex tray were sealed with spacers and comb was inserted.
3. Agarose gel (2.5 per cent) was prepared by adding 2.5 g agarose to 100 ml of TAE (1×) buffer (EDTA 0.5 M at pH 8).
4. The solution was boiled by putting the flask in microwave oven and allowed to cool to 60 $^0$C.
5. Ethidium bromide (10 µl of conc. 10 mg/ml) was added to the gel and mixed gently.
6. The gel was poured into the tray and air bubbles were removed by using pipette. When the gel was completely set, tape was removed and the gel was placed into the electrophoresis tank.

7. Approximately 500 ml of TAE (1×) buffer was poured into the electrophoresis tank, enough to cover the gel to a depth of 5 mm.
8. Comb was removed carefully.
9. About 1/10$^{th}$ volume of loading dye (6×) bromophenol blue dye was added to DNA samples and mixed by gentle tapping and spinning for 2-3 sec in a microcentrifuge.
10. DNA samples were loaded onto the wells and the power supply of about 80 V was provided to run the gel.
11. The power supply was switched off when loading dye was about 2 cm from positive end, and the gel was removed from the gel apparatus.
12. The gel was viewed and photographed by using gel documentation system (UVI Tech England).

Polyacrylamide gel electrophoresis (PAGE)

Six per cent polyacrylamide gel was used for separation and visualization of PCR amplified products. All these genic and nuclear SSR alleles which failed to show polymorphism, if any, in 2.5 per cent agarose gel were tried in PAGE gel. Products denaturing gel was casted in Sequi-Gen GT nucleic acid electrophoresis cell (Bio-Rad Pvt. Ltd, USA ) as per the protocol in manual published by Mahyco Research Foundation (MRF), Hyderabad. Several modifications to the original protocol was done to suit the bench level operations.

Glass plates were prepared before making the gel solution. Both outer (large) glass plate (IPC unit) and inner (small) glass plate were cleaned thoroughly with warm water and detergent. Second wash was given with deionised water. Fresh binding solution was prepared by adding 4 µl of bind silane (Sigma-Aldrich, Pvt. Ltd, USA) to 1 ml of 0.5 per cent acetic acid in 95 per cent ethanol in a 1.5 ml micro-centrifuge tube. Mixture was poured to notched plate (inner glass plate) and spread using tissue paper over the entire surface of the plate. Treated side was marked. Repelsilane (Sigma-Aldrich, Pvt. Ltd, USA) of about 250 µl was added to 750 µl of 0.5 acetic acid in 95 per cent ethanol in a 1.5 ml microcentrifuge tube. Mixture was poured on to large glass plate and spread using tissue paper.

Spacers of 0.4 mm thickness were placed along the side edges of the bind silane–treated surface (small glass plates). Large plate was put on small plate so that treated surfaces face each other (in a sandwich – like fusion). Care was taken to fit the spacers well against each other before clamping. The assembly was put in precision caster base for sealing both sides to ensure no leakage from bottom or sides.

For casting each gel, 80 ml of 6 per cent (SSRs) acrylamide gel was prepared. Just prior to pouring, 60 µl of N, N, N'N'-tetramethylethylenediamine (TEMED) and 600 µl of 10 per cent ammonium per sulphate (APS) was added to initiate polymerization. The contents were mixed gently by swirling and the bubbles were avoided. Before pouring of freshly prepared gel solution, the assembly was kept on the bench top so that it made 45-degree angle with the bench top. Assembly was tilted such that one of the bottom corners is raised above the other (off the bench top) and then carefully the solution was poured into the space between the glass plates starting at the lower corner. As the acrylamide solution filled the space, gel assembly was lowered parallel to the bench top so that both bottom corners were on the bench.

Shark toothcomb of 0.4 mm thickness (96 wells) was inserted with straight side facing the gel at the top of the gel. Comb was put straight across the top moving not more than 5 mm of notched plate. Any bubbles formed during the pouring, they were dislodged by tapping. Gel was left for 20 to 40 min for complete polymerization.

Electrophoresis of polyacrylamide

1. After the polymerization process, the assembly was detached from the clamp and precision caster base, and placed in universal base against the back wall. IPC was locked to the base in vertical position using fitting stabilizer bar.
2. Required quantity of TBE free base, boric acid and EDTA buffer (5x) was poured in upper tank with IPC unit and in the bottom chamber (1.8 L of buffer was prepared fresh each time).

3. Comb was removed and then excess polyacrylamide gel was removed with razor blade.
4. Air bubbles and unpolymerised acrylamide on the top of gel were removed by squirting with 5× TBE.
5. For SSRs, pre-run was given to achieve gel surface temperature of approximately 45 to 50 $^0$C with following conditions. Temperature 50 $^0$C, power 2000 V, 50 mA, constant watt of 75 W.
6. SSR loading dye (3x STR dye) was added to PCR products to a final of 1×, samples were denatured by heating to 95 $^0$C for 4 min and immediately cooled on ice.
7. After the pre-run, the urea was flushed from the well area using a transfer pipette and the shark tooth was carefully inserted comb into the gel, in such a way that it just touches the surface of the gel. Care was taken to avoid the piercing of gel too deeply.
8. Six µl of each samples were loaded into the wells for facilitating the sizing of the various alleles. DNA ladder of 100 bp size was loaded in the first or last well after denaturing.
9. Gel was run using the same conditions as in pre-run. It was electrophoresed till the dye reached bottom of the gel.

Visualization of SSR alleles

Staining procedure remains same for SSRs alleles. After electrophoresis, clamps were loosened and buffer was removed. Glass plates were separated using plastic wedge at the right corner. The gel was affixed to the small glass plate.

Separated DNA fragments were detected by following silver staining procedure. Each solution was prepared in separate containers. The same solutions were used four times over a period of 48 h except for developer, which was freshly prepared each time during the staining process. All steps were done in constant shaking conditions.

Silver staining for genic and nuclear SSR

1. Gel was rinsed with distilled water for 3 to 5 min and placed in a shallow plastic tray.
2. Gel was soaked in 2 L of 2 per cent acetic acid (fix solution) for 20 min.
3. The gel was rinsed with water for 2 times, each with 2 min.
4. The gel was kept for staining with 2 L of 1 per cent silver nitrate for 20 min.
5. A quick water wash was given for 10-15 sec.
6. Developer solution was added to the tray and agitated until the bands appeared.
7. Developer was removed and the plate was placed in fixer or the stop solution for 5 min.
8. Gel was placed in 2 L of impregnate solution for 15 min.
9. Lastly, gel was given water wash for 5 min and kept for drying overnight.

### 3.2.4 Genotyping the RILs for polymorphic markers

Mapping population consisting of 226 RILs and parents were screened with polymorphic genic and nuclear SSR markers. The polymorphic primer sequence information of both forward and reverse primer of genic and nuclear SSR markers genotyped in present investigation is given in Appendix II and Appendix III for both RIP1 and RIP2 population separately. By convention markers were notated as in the following example; Xiabt, Xtxp, Xsnp, Xumc, Xisp.

Marker segregation

The banding pattern obtained after genotyping were scored as follows.

1. Homozygote for the allele from parental strain $P_1$ (IS 9830/N13) at the locus as A=1
2. Homozygote for the allele for parental strain $P_2$ (E 36-1) at the locus as B=3

3. Heterozygote carrying the alleles from both $P_1$ and $P_2$ strains H=2
4. Off types showing banding pattern different from the parents and missing data for the individual at the locus as - = 0

After scoring the individual progeny the data was entered in Microsoft Excel spreadsheet and checked for goodness of fit (chi-square test). Using chi-square test, the goodness of fit for segregation of marker loci was checked. The calculated $\chi^2$ values were compared with table values at one degree of freedom for each marker locus. The marker status of any RIL or parents for a locus was scored either as $P_1$ or $P_2$ allele.

$$\chi^2 = \Sigma (O-E)/E$$

Where,

'O'-Observed frequency of allele

'E'-Expected frequency of the allele

and then saved in a format suitable for linkage analysis using MAPMAKER/EXP (3.0b) and MAPMAKER/QTL (1.1b) software.

### 3.2.5 Construction of genetic linkage maps for RIP1 and RIP2

MAPMAKER/EXP version 3.0 b (Lander and Bostein, 1989) was used for construction of linkage frame. The 'group' command with a minimum LOD score of 2.0 and a maximum distance of 30 per cent recombination was used. Multipoint linkage analysis on loci within groups was subsequently performed. For each group, a three-point linkage analysis was conducted followed by 'order' command. After all scores were checked, the "framework" command for each declared linkage group was used to construct the final map.

A set of 45 marker data for RIP1 and RIP2, which was kindly provided by Dr. Tom Hash, ICRISAT, was used as reference for assigning nuclear SSR markers to defined linkage groups. Together a total of 134 and 160 markers on RIP1 and RIP2 respectively were assigned to linkage groups using MAPMAKER/EXP version 3.0 b, to construct the linkage map which included 20 genic SSRs markers genotyped by Gebre in RIP1 and 26 genic SSRs genotyped by Sundresha in RIP2.

Linkage distance in terms of centimorgan (cM) values was calculated using Kosambi's mapping function. The SSR markers used in this study were chosen from the molecular maps, involving RIL populations N13 x E36-1, IS9830 x E36-1 published by Haussmann *et al*. (2002) and Bhattramakki *et al*. (2000). Accordingly, composite interval-mapping technique was adopted for the construction of linkage groups and QTL analysis.

### 3.2.6 QTL analysis and mapping

MAPMAKER/EXP performs full multipoint linkage analysis for dominant and co-dominant markers in a mapping population. The output of MAPMAKER/EXP contain information on linkage groups and linkage distances between the markers on each linkage group was included as input file for QTL Cartographer (2.5b) (Basten *et al*. 1997). QTL Cartographer is a suite of programs for mapping quantitative trait loci (QTLs) onto a genetic linkage map. The software QTL Cartographer (2.5b) was used to analyse the data by Composite Interval Mapping (CIM), implemented with a LOD score of 2.0 as threshold value for QTL significance. The QTLs identified for stay-green were assigned to the linkage groups based on linkage positions of markers on respective linkage maps. Threshold values with 300 times permutation and significance at 0.05 per cent was set as the cutoff for QTL detection.

QTL analysis was carried out following multiple algorithms to ensure robustness, cross validation of the data. Stable QTLs were noted by using phenotypic data from two different environments. QTLs that were stable across environments may be identified by two methodologies: QTL mapping data from different environments was combined and only QTLs that were statistically significant on average across environments were selected. QTL analyses were performed for each environment separately and environment specific QTLs were identified. QTL network (2.0) was run to study interations among the QTLs. The identification of QTLs expressed across environments would be a primary objective in any

molecular breeding program, because they establish a set of basic genomic blocks to introgress into elite genotypes.

## 3.3 Marker assisted introgression of charcoal rot resistance QTLs

Charcoal rot/stalk rot caused by the fungus *Macrophomina phaseolina* Tassi (Goid) is a devastating disease of sorghum worldwide. High yielding lines with partial levels of resistance to charcoal rot resistance have been identified through traditional breeding approaches. Identification of genomic regions harbouring the gene cascades responsible for such genetically complex traits is made possible through QTL mapping approach. Identification for a marker pairs conditioning such QTLs can facilitate specific transfer of QTLs into desired background through marker assisted backcrossing method. Three stable QTLs, with major effect and stable across three environments *viz*., Dharwad, Bijapur and Solapur were identified at IABT (Reddy *et al*., 2008; Fakrudin, 2008; Patil, 2009). One QTL each for lodging per cent (*cr1*, LG-I), number of internodes crossed by fungus (*cr2*, LG-B) and length of infection (*cr3*, LG-B) accounting for 43 per cent of phenotypic variation for charcoal rot resistance were introgressed into the recurrent backgrounds.

### 3.3.1 Plant material

Donor parent

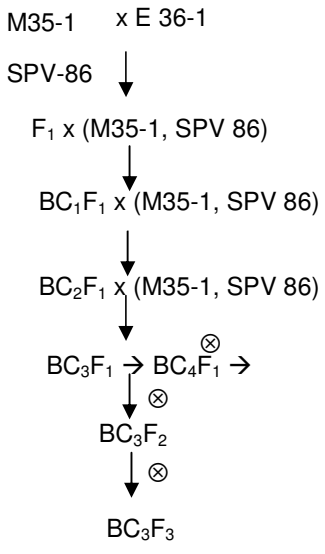
E 36-1 was used as donor parent which also has all the desirable allele for charcoal rot resistance with additive effect. It is an Ethiopian exotic line adapted to tropical conditions with charcoal rot resistance coupled with stay green trait.

Recurrent parents

SPV 86, its leaves are yellowish, drooping with dull green midrib. Grains are round and bold. Moderately tolerant to shoot fly and highly susceptible to charcoal rot disease. It is high yielding variety and popular in North Karnataka and Maharashtra states.

M35-1 : widely cultivated popular variety of Karnataka region for *rabi* growing situation. It is known for its agronomic eliteness and good grain quality with lustrous and bold seeds. Though this variety is agronomically superior, it is susceptible to charcoal rot disease and tolerant to shoot fly. Also refer to Table 6.

Marker assisted backcross scheme followed




Staggered sowing of parents, emasculation of recurrent parents and dusting pollens from donor parent.

Heterozygote identification, backcrossing it with recurrent parent.

Foreground and limited background selection for all twelve markers and backcrossing it with recurrent parent.

Foreground and limited background selection for all twelve markers and backcrossing it with recurrent parent

Foreground and limited background selection for all twelve markers in $BC_3F_1$and backcrossing it with recurrent parent.

Identification of homozygotes for all the six markers in $BC_4F_1$and slfing them.

Identification of homozygotes for all the six markers, whole genome background selection and selfing them.

Confirmation of homozygosity and field evaluation in replicated trial in sick plot with artificial infection.

### 3.3.2 Marker assisted back crossing to introgress charcoal rot resistant QTLs

The present investigation started from $BC_3F_1$ progenies. Donor and recurrent parents were sown in a staggered manner to ensure pollen availability and successful hybridization. Seeds of backcross progenies from selected individuals were sown individually in 12" earthen pots and grown in the glass house. Staggered sowing was employed to ensure synchronization in flowering of the recurrent parent and backcross progenies. Progenies from both the crosses were tagged and traced to marker status at each generation.

The list of markers used for foreground and limited background selection

| QTL | Foreground marker | | | | | Limited background marker | |
|---|---|---|---|---|---|---|---|
| | Spanning markers | Distance (cM) | LG | $R^2$ | $a_ib^2$ | Spanning markers | Distance (cM) |
| *cr1* | Xtxp176- Xtxp312 | 8.1 | I | 10.7 | 3.4 | Xtxp96-Xtxp176 | 6.7 |
| | | | | | | Xtxp312-Xtxp274 | 3.7 |
| *cr2* | Xtxp297- Xiabt73 | 18.23 | B | 13.6 | 3.8 | Xtxp48-Xtxp297 | 10.09 |
| | | | | | | Xiabt73-Xiabt82 | 0.56 |
| *cr3* | Xiabt275- Xiabt241 | 17.23 | B | 10.1 | 2.7 | Xiabt60-Xiabt275 | 34.65 |
| | | | | | | Xiabt241-Xiabt87 | 18.53 |

(Fakrudin *et al.*, 2008 and Patil, 2009)

### 3.3.3 DNA isolation

For isolation of six-inch long leaf strips from two week old seedlings were collected from individual progeny of the $BC_3F_1$, $BC_3F_2$ and $BC_3F_3$ populations derived from the crosses, SPV 86 × E36-1 and M35-1 × E36-1. DNA isolation method followed was as in 3.2.1. $BC_3F_1$ were screened for foreground and limited background selection. Selected plants were selfed to get $BC_3F_2$ then to $BC_3F_3$, which were evaluated under charcoal rot sick plot.

Scoring nuclear SSRs and genic SSRs alleles in the Backcross progenies

The banding pattern obtained after ethidium bromide staining and silver staining were scored as follows.

1. "A"-Homozygote at marker locus as that of donor parent (E36-1)
2. "B"- Homozygote at marker locus as that of recurrent parent (M35-1 / SPV86).
3. "H"-Herterozygote at marker locus as that of E36-1 and M35-1/ SPV86.
4. "O" - Off types showing banding pattern different from the parents and
5. "_" missing data for the individual at the locus

### 3.3.4 Field evaluation of $BC_3F_3$ charcoal rot resistant QTL introgressed near isogenic lines

Location, season and experimental design

The $BC_3F_3$ progenies homozygous for various QTL combinations into background of M35-1 and SPV 86 were evaluated in a charcoal rot sick plot during *rabi* 2010 at MARS, Dharwad. Random Block Design (RBD) was adopted on an area of 30 x 60 $m^2$ with five replications. The trial was laid out with length of 2 m and breadth of 2 m blocks.

Each block had four lines with ten plants in each line, a spacing of 45 × 15 cm. In order to eliminate the border effects, three rows of M35-1 was sown on all sides of the field and other recommended package of practices were followed. Plots were thinned at 10 days after seedling emergence (DAE) to a spacing of 15 cm between plants within rows when the seedlings were at 4-leaf stage. A set of 16 lines carrying various combinations of charcoal rot resistant QTLs and three parents were sown in five replications. Artificial inoculation of the fungus at 75 days after sowing (DAS) was done to each and every plant (Plate 2). Lines selection was based upon percentage of recurrent parent genome recovery. Selected lines include, all three QTLs (*cr1, cr2, cr3*), double QTLs in combinations (*cr1* and *cr2*, *cr2* and *cr3*, *cr1* and *cr3*), single QTL (*cr1*, *cr2*, *cr3*) and null QTLs in both backgrounds along with two recurrent parents and a donor parent. Set of lines include T1 to T16. T1, M35-1 (*cr1+cr2+cr3*); T2, SPV86 (*cr1+cr2+cr3*); T3, M35-1 (*cr1+cr2*); T4, M35-1 (*cr2+cr3*); T5, M35-1 (*cr1+cr3*); T6, SPV86 (*cr1+cr2*); T7, SPV86 (*cr2+cr3*); T8, SPV86 (*cr1+cr3*); T9, M35-1 (*cr1*); T10, M35-1 (*cr2*); T11, M35-1 (*cr3*); T12, SPV86 (*cr1*); T13, SPV86 (*cr2*); T14, SPV86 (*cr3*); T15, M35-1 (Null QTL); T16, SPV86 (Null QTL); T17, M35-1, recurrent parent; T18, SPV86, recurrent parent and T19, E36-1, donor parent

Artificial inoculation of the fungus at 75 days after sowing (DAS) was done. The methodology involved multiplication of the causal organism on sorghum grain and placing the sclerotia inoculum loaded grains directly on non-injured second internode from ground. A thin needle is pierced followed by an insertion of inoculums. Three parameters *viz*., per cent lodging, number of internodes crossed by the fungus and length of infection were used for assessing the reaction of individual genotype to the disease.


**Plate 2: Field evaluation of $BC_3F_3$ NILs for different combination of QTLs in charcoal rot sick plot along with artificial inoculation**

Observations

Charcoal rot component traits

Per cent lodging: The scoring of disease was done by taking per cent lodging as per the procedure described in sorghum descriptor and the same was converted to 1→ 5 scale as follows (Sorghum Descriptor, ICRISAT).

| Score | Phenotype |
|---|---|
| 1. | No damage |
| 2. | 1-10% plants damaged |
| 3. | 11-25% plants damaged |
| 4. | 26-40% plants damaged |
| 5. | More than 40% plants damaged |

Length of infection: At the time of harvest, the stem of individual plants were split opened and the spread of the fungus in terms of discolouration or fruiting bodies was measured in cm.

Number of internodes crossed by the fungus: At harvest the stem of individual plants was split opened and the number of internodes crossed by the fungus was measured.

Plant height: Plant height (cm) at maturity was recorded from the soil surface to the ear head tip of plant.

Earhead length : Length from the basal whorl of the inflorescence to the tip of the panicle was measured and expressed in cm.

Stem girth: This trait was measured at the middle of the first internode just above the ground level at the time of anthesis with the help of a thread and expressed in cm.

Number of leaves: Total number of leaves at the time of flowering was counted and expressed in numbers.

Spicklets per head: Total number of spicklets per head at the time of maturity were counted and expressed in numbers.

Panicle exertion: Length between base of ear head and start of $1^{st}$ leaf at time of maturity was measured and expressed in cm.

## 3.4 Statistical analysis

### 3.4.1 Phenotypic characters

Statistical analysis was carried out by pooling of the phenotypic data for all the three years at Dharwad and two years at Bheemarayanagudi location, rather than pooling of across different season based on Spearman's rank correlation (Spearman, 1906).

Spearman's ranked correlation : Spearman's rank correlation is a statistical technique used to test the direction and strength of the relationship between two variables.

1. This technique ranks both sets of data from the highest to the lowest. Made sure to check the tied ranks.
2. Subtracted the two sets of ranks to get the difference 'd'.
3. Squared the values of 'd'.
4. Added the squared values of d to get $\Sigma d^2$.
5. Used the formula $Rs = 1-(6\Sigma d^2/n^3-n)$ where n was the number of ranks.

If the Rs value -1, there was a perfect negative correlation.

If the Rs value falls between -1 and -0.5, there was a strong negative correlation.

If the Rs value falls between -0.5 and 0, there was a weak negative correlation.

If the Rs value was 0, there is no correlation

If the Rs value fell between 0 and 0.5, there was a weak positive correlation.

If the Rs value fell between 0.5 and 1, there was a strong positive correlation.

Analysis of variance (ANOVA)

The analysis of variance for different characters was performed by applying the formula suggested by Burton and Devane (1953).

Phenotypic and genotypic coefficients of variation

The estimates of phenotypic and genotypic coefficient of variation were obtained by using the following formulae (Singh and Chaudhary, 1977).

$$PCV\ (\%) = \frac{\sqrt{Vp}}{\bar{X}} \times 100$$

$$GCV\ (\%) = \frac{\sqrt{Vg}}{\bar{X}} \times 100$$

Where,

PCV : Phenotypic coefficient of variation

Vp : Phenotypic variance

GCV : Genotypic coefficient of variation

Vg : Genotypic variance

X : Mean of RILs

GCV and PCV values were categorized as low, moderate and high values as indicated by Sivasubramanian and Menon (1973), as follows.

0-10 % : Low

10-20 % : Moderate

20 % and above : High

Phenotypic correlation

Phenotypic correlation coefficients were estimated by using the following formula (Singh and Choudhary, 1977).

$$rp = \frac{Cov_p\ (x, y)}{\sqrt{Varp\ (x)\ Varp\ (y)}}$$

Where,

rp : Phenotypic correlation coefficient

$Cov_p$ (×, y) : Phenotypic covariance between characters x, y

Varp (×) : Phenotypic variance in character x

Varp (y) : Phenotypic variance in character y

The observed value of correlation coefficient was compared with the tabulated value for (n-2) degrees of freedom for test of significance.

Heritability

Heritability was estimated in RILs for all resistance components as the ratio of total genotypic variance to the phenotypic variance (Falconer, 1989).

$$h^2 = \frac{Vg}{Vp}$$

Where,

$h^2$ = Heritability

Vg = Genotypic variance

Vp = Phenotypic variance

The heritability percentage was categorized as low, moderate and high as given by Robinson *et al*., (1949).

0-30% : Low

30-60% : Moderate

60% and above : High

Genetic advance (GA)

The extent of genetic advance expected through selection for each of the character was calculated as in Johnson *et al*. (1955)

$$GA = h \times \rho \times K$$

h = Heritability

ρ = Phenotypic standard deviation

K = Selection differential which is equal to 2.06 at selection intensity of 5 per cent (Lush, 1949).

Genetic advance as percent mean

GA as per cent mean = $GA / \bar{X} \times 100$

Where,

GA = Genetic advance

$\bar{X}$ = General mean

Genetic advance as per cent of mean was categorized as low, moderate and high by following Johnson *et al.* (1955) as follows.

0-10% : Low

10-20% : Moderate

Above 20% : High

# 4. EXPERIMENTAL RESULTS

The results of the present investigation on "Genome-wide QTL mapping for post-flowering drought tolerance and validation of charcoal rot resistance QTLs in NILs of sorghum" are presented under the following headings.

1. Phenotyping of two recombinant inbred populations (RIPs) for post-flowering drought tolerance and yield related traits.
2. Molecular mapping of genic, nuclear SSR markers.
3. Construction of genetic linkage map, QTL analysis and mapping.
4. Introgression of stable charcoal rot resistant QTLs and field evaluation.

## 4.1 Phenotyping of two recombinant inbred populations (RIPs) for post-flowering drought tolerance and yield related traits

The recombinant inbred line population derived from IS9830 x E36-1 (RIP1) and N13 x E36-1 (RIP2) crosses were evaluated along with their parental lines during rabi season of 2007, 2008 and 2009 at Main Agricultural Research Station (MARS), Dharwad and Regional Agricultural Research Station (RARS), Bheemarayanagudi. Results of the field experiments carried out in two locations for three seasons in Dharwad and two seasons in Bheemarayanagudi for post-flowering drought tolerance related traits such as stay-green, CID and yield related traits are as follows.

The phenotypic data sets were compared for their consistency both over seasons and locations through a ranked correlation approach to determine the data pooling strategy. It was found that the data sets were more consistent within a location over season as compared to within a season across locations in both populations (Table 8a, 8b). Hence, pooling of data over season within each location was done for statistical analysis separately for each population. The data for all the parameters were checked for their normal distribution across all the seasons in both locations of field evaluation and then were subjected to statistical analysis and mapping was done on untransformed data.

### 4.1.1 Analysis of variance, variability parameters and character association estimates

The appropriate degrees of freedom (DF) and mean sum of squares (MSS) along with other genetic parameters *viz.,* range, mean, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability estimates and predicted genetic advance as per cent of mean were worked out for all the traits for both populations across locations. The analysis of variance revealed highly significant difference among RILs for all the 13 and 14 traits in RIP1 and RIP2 respectively at Dharwad and Bheemarayanagudi locations.

#### 4.1.1.1 Pooled analysis of component traits of post-flowering drought tolerance and yield related traits in IS9830 × E36-1 derived RIP at Dharwad location

Pooled analysis of variance was carried out and the estimates of variability parameters for stay-green, CID, yield and yield related traits are presented in Table 9a to 9d. The frequency distribution patterns for all the traits are presented in Figure 1 and graphical presentation of PCV and GCV are shown in Figure 2. Analysis of variance and variability parameters for Dharwad location are presented in Appendices IVa-IVl.

Per cent GLA 15 DAF

A significant difference among the RILs and GxE interaction over seasons for per cent GLA 15 DAF was recorded with a range of 67.75 to 95.00 per cent. The mean per cent GLA 15 DAF of 87.36 with 6.02 per cent of PCV and 5.39 per cent of GCV was recorded. A per cent heritability of 80.20 and GA over mean of 9.50 per cent was recorded for GLA 15 DAF in this population at Dharwad location.

**Table 8a: Pearson ranked correlations for component traits of stay-green across locations and seasons of field experimentation in recombinant inbred lines derived from the cross IS9830 x E36-1**

**a) % green leaf area 15 days after flowering**

| Season/Location | Dharwad | Bheemarayanagudi |
|---|---|---|
| 2007 on 2008 | 0.955 | |
| 2007 on 2009 | 0.950 | |
| 2008 on 2009 | 0.953 | 0.956 |

| | 2007 | 2008 | 2009 |
|---|---|---|---|
| Dharwad on Bheemarayanagudi | | 0.904 | 0.902 |

**b) % green leaf area 30 days after flowering**

| Season/Location | Dharwad | Bheemarayanagudi |
|---|---|---|
| 2007 on 2008 | 0.956 | |
| 2007 on 2009 | 0.968 | |
| 2008 on 2009 | 0.948 | 0.932 |

| | 2007 | 2008 | 2009 |
|---|---|---|---|
| Dharwad on Bheemarayanagudi | | 0.914 | 0.901 |

**c) % green leaf area 45 days after flowering**

| Season/Location | Dharwad | Bheemarayanagudi |
|---|---|---|
| 2007 on 2008 | 0.972 | |
| 2007 on 2009 | 0.958 | |
| 2008 on 2009 | 0.967 | 0.970 |

| | 2007 | 2008 | 2009 |
|---|---|---|---|
| Dharwad on Bheemarayanagudi | | 0.895 | 0.902 |

**d) Total green leaf area**

| Season/Location | Dharwad | Bheemarayanagudi |
|---|---|---|
| 2007 on 2008 | 0.969 | |
| 2007 on 2009 | 0.961 | |
| 2008 on 2009 | 0.968 | 0.979 |

| | 2007 | 2008 | 2009 |
|---|---|---|---|
| Dharwad on Bheemarayanagudi | | 0.909 | 0.880 |

**Table 8b: Pearson ranked correlations for component traits of stay-green across locations and seasons of field experimentation in recombinant inbred lines derived from the cross N13 x E36-1**

**a) % green leaf area 15 days after flowering**

| Season/Location | Dharwad | Bheemarayanagudi |
|---|---|---|
| 2007 on 2008 | 0.953 | |
| 2007 on 2009 | 0.979 | |
| 2008 on 2009 | 0.953 | 0.958 |

| | 2007 | 2008 | 2009 |
|---|---|---|---|
| Dharwad on Bheemarayanagudi | | 0.915 | 0.880 |

**b) % green leaf area 30 days after flowering**

| Season/Location | Dharwad | Bheemarayanagudi |
|---|---|---|
| 2007 on 2008 | 0.955 | |
| 2007 on 2009 | 0.956 | |
| 2008 on 2009 | 0.956 | 0.969 |

| | 2007 | 2008 | 2009 |
|---|---|---|---|
| Dharwad on Bheemarayanagudi | | 0.902 | 0.909 |

**c) % green leaf area 45 days after flowering**

| Season/Location | Dharwad | Bheemarayanagudi |
|---|---|---|
| 2007 on 2008 | 0.961 | |
| 2007 on 2009 | 0.967 | |
| 2008 on 2009 | 0.968 | 0.969 |

| | 2007 | 2008 | 2009 |
|---|---|---|---|
| Dharwad on Bheemarayanagudi | | 0.901 | 0.904 |

**d) Total green leaf area**

| Season/Location | Dharwad | Bheemarayanagudi |
|---|---|---|
| 2007 on 2008 | 0.959 | |
| 2007 on 2009 | 0.972 | |
| 2008 on 2009 | 0.965 | 0.970 |

| | 2007 | 2008 | 2009 |
|---|---|---|---|
| Dharwad on Bheemarayanagudi | | 0.902 | 0.901 |

**e) Carbon isotope discrimination**

| Season/Location | Dharwad | Bheemarayanagudi |
|---|---|---|
| 2007 on 2008 | 0.956 | |

| | 2007 | 2008 |
|---|---|---|
| Dharwad on Bheemarayanagudi | | 0.904 |

Per cent GLA 15 DAF was positively and significantly correlated with per cent GLA 30 DAF, per cent 45 DAF, total green leaf area, number of leaves, stem girth, plant height, spicklets per head, 100 grain weight and grain yield per plant. However, this trait recorded, negative correlation with days to 50 per cent flowering, earhead length and panicle exertion.

Per cent GLA 30 DAF

RILs of IS9830 x E36-1 cross differed significantly for per cent GLA 30 DAF with significant G x E interaction with a wide range of 12.54 to 89.50 per cent and mean of 63.32 per cent. The per cent PCV and GCV were 21.75 and 21.17, respectively with high heritability estimate of 94.73 with GA over mean of 42.43 per cent.

Per cent GLA 30 DAF was positively and significantly correlated with per cent GLA 15 DAF, per cent 45 DAF, total green leaf area, number of leaves, stem girth, plant height, spicklets per head, 100 grain weight, grain yield per plant but has negative correlation with days to 50 per cent flowering, earhead length and panicle exertion.

Per cent GLA 45 DAF

There was a significant G x E interaction and mean difference among the RILs for per cent GLA 45 DAF with mean of 35.80 and range of 12.00 to 81.93 per cent. The PCV and GCV for this trait were 32.48 and 31.00 per cent respectively with 91.14 per cent heritability and a GA of 73.57 per cent over mean was recorded.

Per cent GLA 45 DAF was positively and significantly correlated with per cent GLA 15 DAF, per cent 30 DAF, total green leaf area, number of leaves, stem girth, plant height, spicklets per head, 100 grain weight, grain yield per plant but has negative correlation with days to 50 per cent flowering, earhead length and panicle exertion.

Days to 50 per cent flowering

RILs differed significantly in their values with significant G x E interaction for this trait with mean days to 50 per cent flowering of 56.12 and ranging between 45.75 and 73.00 days. The per cent PCV and GCV recorded were 11.34 and 11.29 respectively with high heritability estimate of 99.05 per cent. This trait recorded 24.96 per cent genetic advance over mean.

Days to 50 per cent flowering was positively correlated with earhead length, number of leaves, panicle exertion, plant height and spicklets per head, whereas it was found to be negatively correlating with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area and grain yield per plant.

Total green leaf area

Significant difference among the RILs was recorded with significant G x E interaction for total green leaf area. The range of variation was between 111.37 and 1913.68 $cm^2$ with a mean area of 1184.23 $cm^2$. The per cent PCV and GCV value were 24.21 and 23.01 respectively with high heritability estimate of 90.29 per cent. The GA over mean was 41.96 per cent for this trait.

Total green leaf area has been found to be negative correlating with days to 50 per cent flowering, panicle exertion, stem girth and 100 grain weight, but had positive correlation with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, earhead length, number of leaves, plant height, spicklets per head and grain yield per plant.

Earhead length

RILs differed significantly in their values with significant G x E interaction with mean length of 17.79 cm and range of 5.97 and 29.90 cm. The PCV and GCV recorded were 22.65 and 16.17 per cent with heritability of 50.92 per cent and per cent GA over mean of 23.80 was recorded.

Earhead length recorded positive correlation with days to 50 per cent flowering, total green leaf area, number of leaves, stem girth, plant height and grain yield per plant. However, it had negative correlation with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, panicle exertion, spicklets per head and 100 grain weight.

**Table 9a: Pooled analysis of variance of stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2007, 2008 and 2009 at Dharwad location**

| Sl. No. | Source of variation | Mean sum of squares | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
| 1. | Replication | 3 | 548.92 | 3100.60 | 1288.49 | 748.18 | 3653248.51 | 198.52 | 19.58 | 359.42 | 57.80 | 29228.68 | 1571.81 | 40.31 | 1032.38 |
| 2. | Environment (E) | 2 | 12478.39** | 4091.01** | 26966.34** | 13515.65** | 28796554.29** | 114.74** | 313.31** | 482.45** | 18262.08** | 95189.66** | 2193.83** | 300.57** | 11286.76** |
| 3. | Genotypes (G) | 225 | 182.05** | 908.59** | 2044.96** | 285.89** | 544525.24** | 49.44** | 5.62** | 291.66** | 4.05** | 6720.06** | 753.40** | 3.32** | 941.65** |
| 4. | E x G | 450 | 46.90** | 381.89** | 369.58** | 97.48** | 124000.27** | 28.91** | 2.52** | 63.38** | 1.91** | 1284.82** | 60.39** | 0.66** | 114.04** |
| 5. | Error | 2034 | 16.52 | 7.18 | 45.77 | 8.11 | 52295.03 | 14.45 | 1.10 | 16.47 | 1.93 | 603.34 | 2.23 | 0.31 | 48.01 |
| 6. | SEm± | | 1.12 | 1.58 | 2.09 | 0.33 | 41.63 | 1.42 | 0.49 | 1.65 | 0.91 | 6.25 | 0.93 | 0.27 | 5.29 |
| 7. | CD (1%) | | 4.07 | 5.76 | 7.61 | 1.22 | 151.66 | 5.17 | 1.80 | 6.01 | 3.32 | 22.77 | 3.38 | 0.98 | 19.25 |
| 8. | CD (5%) | | 3.10 | 4.38 | 5.79 | 0.93 | 115.39 | 3.93 | 1.37 | 4.57 | 2.53 | 17.33 | 2.57 | 0.75 | 14.65 |
| 9. | CV (%) | | 4.51 | 4.20 | 18.28 | 4.87 | 19.35 | 21.22 | 14.87 | 25.20 | 21.20 | 13.54 | 4.21 | 13.01 | 20.13 |

* - Significance at 5% probability level ** - Significance at 1% probability level

$X_1$: % GLA 15 DAF $X_2$: % GLA 30 DAF $X_3$: % GLA 45 DAF $X_4$: Days to 50 % flowering $X_5$: Total GLA

$X_6$: Earhead length $X_7$: Number of leaves $X_8$: Panicle exertion $X_9$: Stem girth $X_{10}$: Plant height

$X_{11}$: Spicklets per head $X_{12}$: 100 grain weight $X_{13}$: Grain yield per plant

**Table 9b: Magnitude of genetic variability parameters of stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 × E36-1 during 2007, 2008 and 2009 at Dharwad location**

| Sl. No. | Traits | Range | | Grand mean | PCV (%) | GCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Min | Max | | | | | |
| 1. | % GLA 15 DAF | 67.75 | 95.00 | 87.36 | 6.02 | 5.39 | 80.20 | 9.50 |
| 2. | % GLA 30 DAF | 12.54 | 89.50 | 63.32 | 21.75 | 21.17 | 94.73 | 42.43 |
| 3. | % GLA 45 DAF | 12.00 | 81.93 | 35.80 | 32.48 | 31.00 | 91.14 | 73.57 |
| 4. | Days to 50 % flowering | 45.75 | 73.00 | 56.12 | 11.34 | 11.29 | 99.05 | 24.96 |
| 5. | Total green leaf area ($cm^2$) | 111.37 | 1913.68 | 1184.23 | 24.21 | 23.01 | 90.29 | 41.96 |
| 6. | Earhead length (cm) | 5.97 | 29.90 | 17.79 | 22.65 | 16.17 | 50.92 | 23.89 |
| 7. | Number of leaves | 4.50 | 10.00 | 7.05 | 20.02 | 13.27 | 43.96 | 17.02 |
| 8. | Panicle exertion (cm) | 0.69 | 31.92 | 15.92 | 39.42 | 33.43 | 71.93 | 57.97 |
| 9. | Stem girth (cm) | 2.20 | 7.20 | 6.70 | 28.14 | 22.36 | 63.15 | 58.35 |
| 10. | Plant height (cm) | 117.67 | 257.00 | 181.53 | 18.36 | 16.90 | 84.73 | 30.76 |
| 11. | Spicklets per head | 12.00 | 60.00 | 35.54 | 25.87 | 25.32 | 95.77 | 50.02 |
| 12. | 100 grain weight (g) | 2.60 | 7.07 | 3.93 | 19.74 | 15.65 | 62.88 | 29.26 |
| 13. | Grain yield per plant (g/plant) | 28.72 | 158.20 | 93.46 | 40.49 | 26.72 | 43.56 | 13.51 |

**Table 9c: Phenotypic correlation coefficients (pooled) for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2007 2008 and 2009 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.135* | 0.362** | -0.015 | 0.089 | -0.049 | 0.069 | -0.057 | 0.126 | 0.032 | 0.109 | 0.124 | 0.004 |
| $X_2$ | | 1 | 0.035 | -0.03 | 0.067 | -0.022 | 0.015 | -0.082 | 0.018 | 0.086 | 0.067 | 0.083 | 0.044 |
| $X_3$ | | | 1 | -0.026 | 0.225** | -0.024 | 0.031 | -0.201** | 0.393** | 0.11 | 0.181* | 0.115 | 0.073 |
| $X_4$ | | | | 1 | -0.248** | 0.003 | 0.077 | 0.059 | 0.205** | 0.083 | 0.036 | -0.207** | -0.008 |
| $X_5$ | | | | | 1 | 0.053 | 0.012 | -0.024 | -0.165* | 0.22** | 0.279** | -0.039 | 0.061 |
| $X_6$ | | | | | | 1 | 0.053 | -0.04 | 0.107 | 0.054 | -0.032 | -0.01 | 0.038 |
| $X_7$ | | | | | | | 1 | 0.009 | -0.045 | -0.042 | 0.009 | 0.024 | 0.029 |
| $X_8$ | | | | | | | | 1 | -0.241** | 0.177* | -0.005 | -0.127 | -0.009 |
| $X_9$ | | | | | | | | | 1 | 0.059 | 0.337** | 0.226** | 0.01 |
| $X_{10}$ | | | | | | | | | | 1 | -0.165* | -0.076 | -0.001 |
| $X_{11}$ | | | | | | | | | | | 1 | 0.117 | 0.017 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.035 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

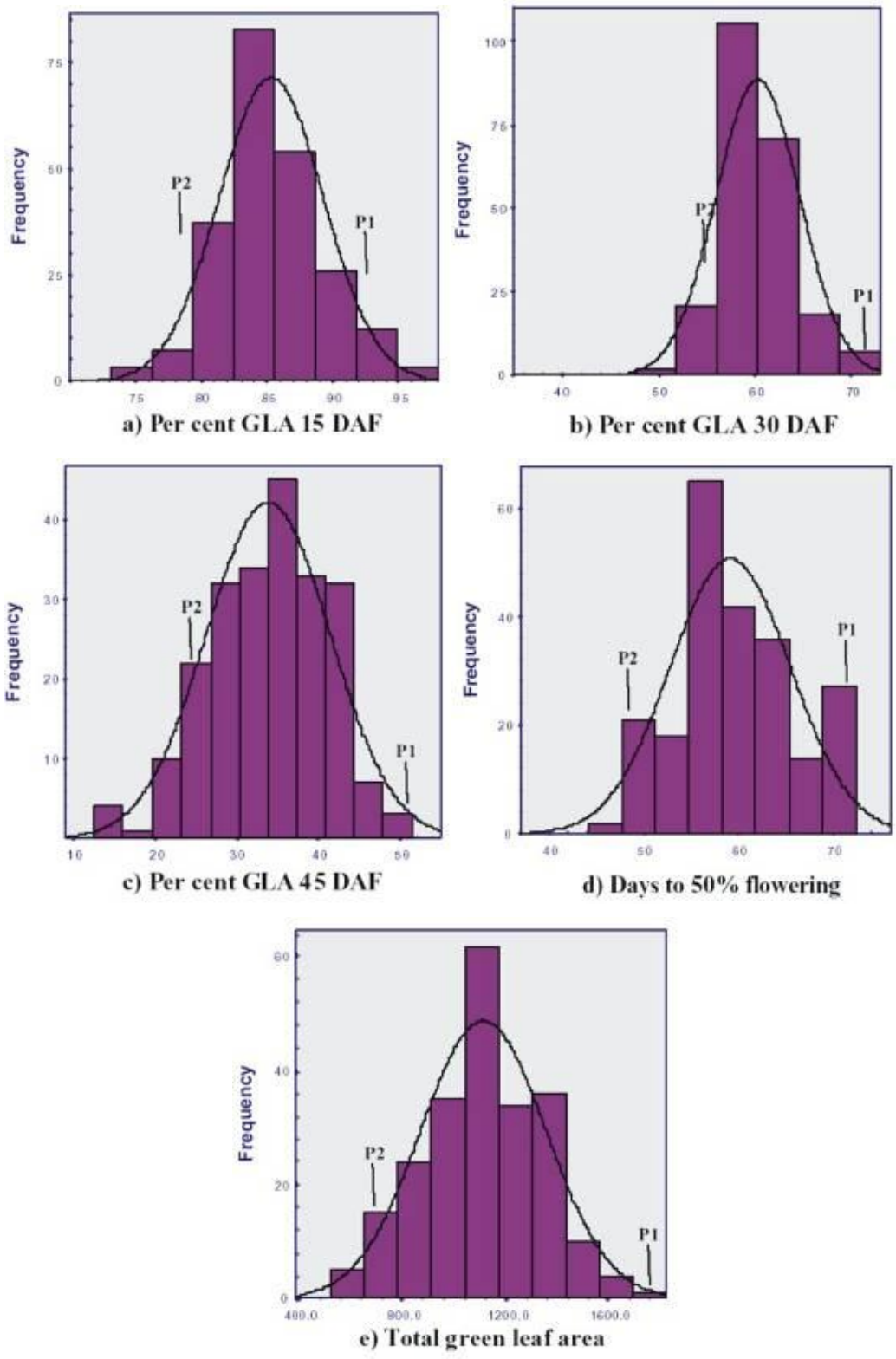
$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Table 9d: Genotypic correlation coefficients (pooled) for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2007, 2008 and 2009 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.158* | 0.408** | -0.015 | 0.106 | -0.078 | 0.126 | -0.08 | 0.172* | 0.043 | 0.124 | 0.186** | 0.012 |
| $X_2$ | | 1 | 0.037 | -0.031 | 0.077 | -0.029 | 0.033 | -0.099 | 0.029 | 0.094 | 0.07 | 0.119 | 0.06 |
| $X_3$ | | | 1 | -0.028 | 0.248** | -0.049 | 0.073 | -0.257** | 0.497** | 0.006 | 0.192 | 0.158* | 0.113 |
| $X_4$ | | | | 1 | -0.262** | 0.007 | 0.123 | 0.067 | 0.255** | 0.088 | 0.04 | -0.271** | -0.006 |
| $X_5$ | | | | | 1 | 0.062 | 0.009 | -0.027 | -0.219* | 0.244* | 0.297** | -0.055 | 0.085 |
| $X_6$ | | | | | | 1 | 0.117 | -0.065 | 0.150* | 0.095 | -0.031 | -0.032 | 0.052 |
| $X_7$ | | | | | | | 1 | 0.005 | -0.075 | -0.037 | 0.016 | 0.019 | 0.071 |
| $X_8$ | | | | | | | | 1 | -0.285* | 0.221** | -0.006 | -0.169* | -0.034 |
| $X_9$ | | | | | | | | | 1 | 0.06 | 0.429** | 0.381** | 0.046 |
| $X_{10}$ | | | | | | | | | | 1 | -0.184** | -0.113 | -0.026 |
| $X_{11}$ | | | | | | | | | | | 1 | 0.155* | 0.031 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.078 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

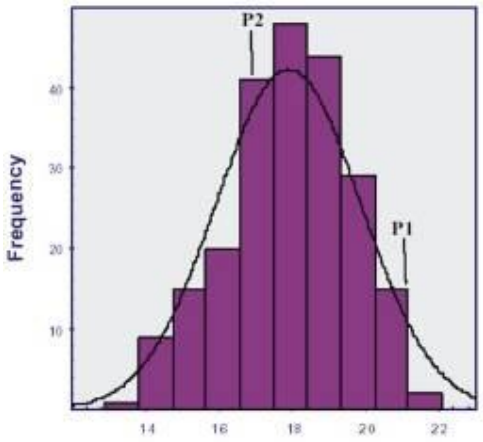
$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant



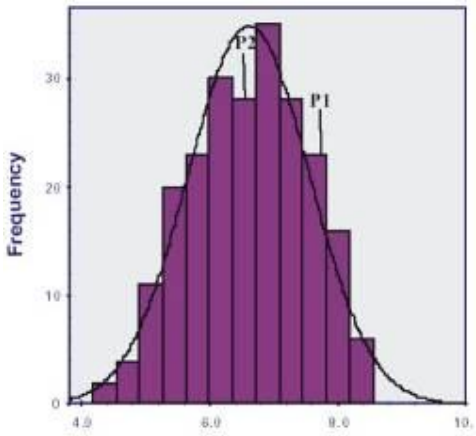

Legend : P1 – E36-1 P2 – IS9830

**Fig. 1: Frequency distribution (pooled) of stay-green and yield related traits in recombinant inbred lines derived from cross IS9830 × E36-1 evaluated at Dharwad location**

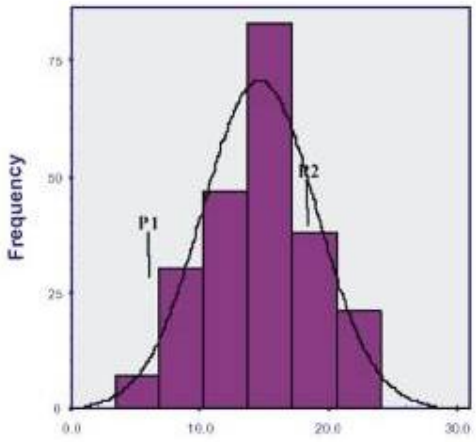
**Fig. 1 Contd…..**



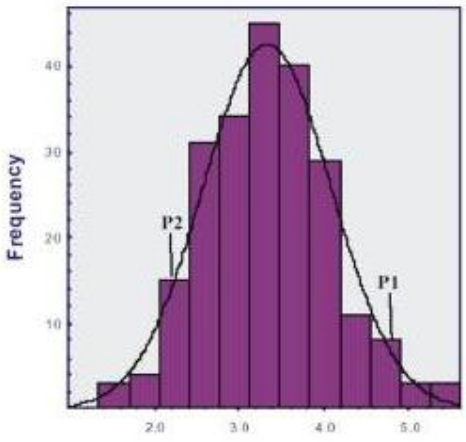

f) Earhead length




g) Number of leaves/plant

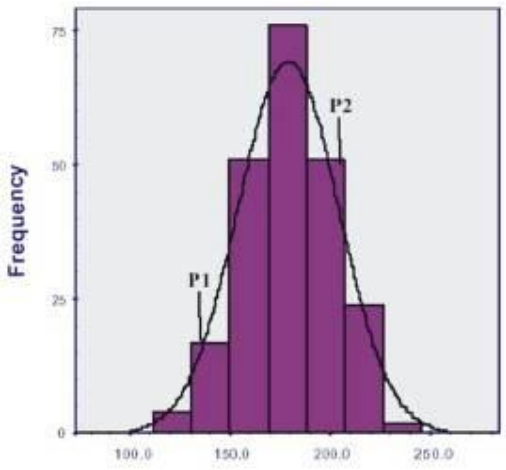



h) Panicle exertion

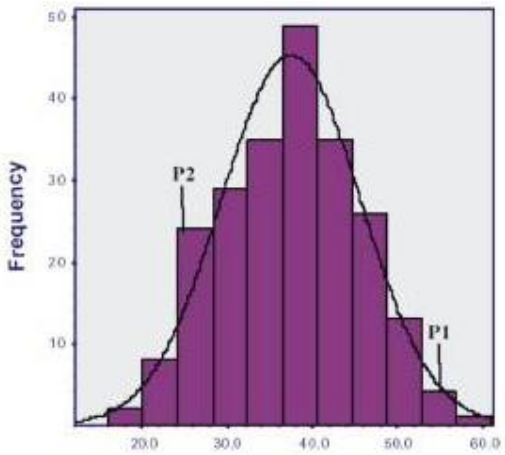



i) Stem girth

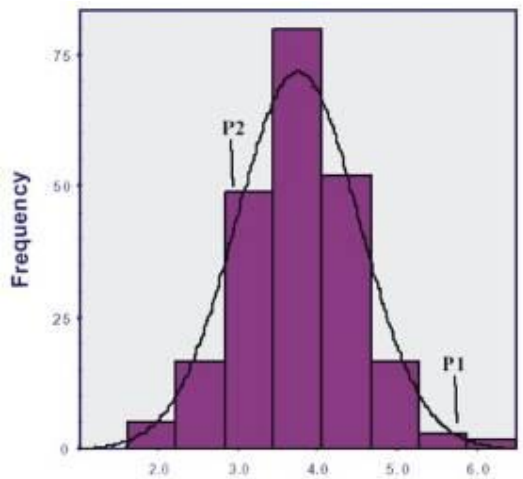
**Fig. 1 Contd…..**



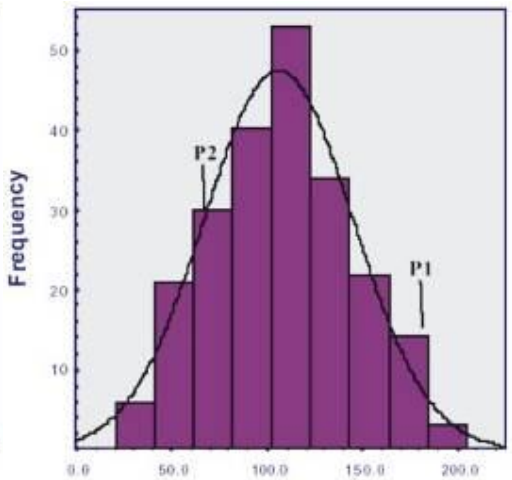

j) Plant height




k) Spicklets per head




l) 100 grain weight




m) Grain yield per plant

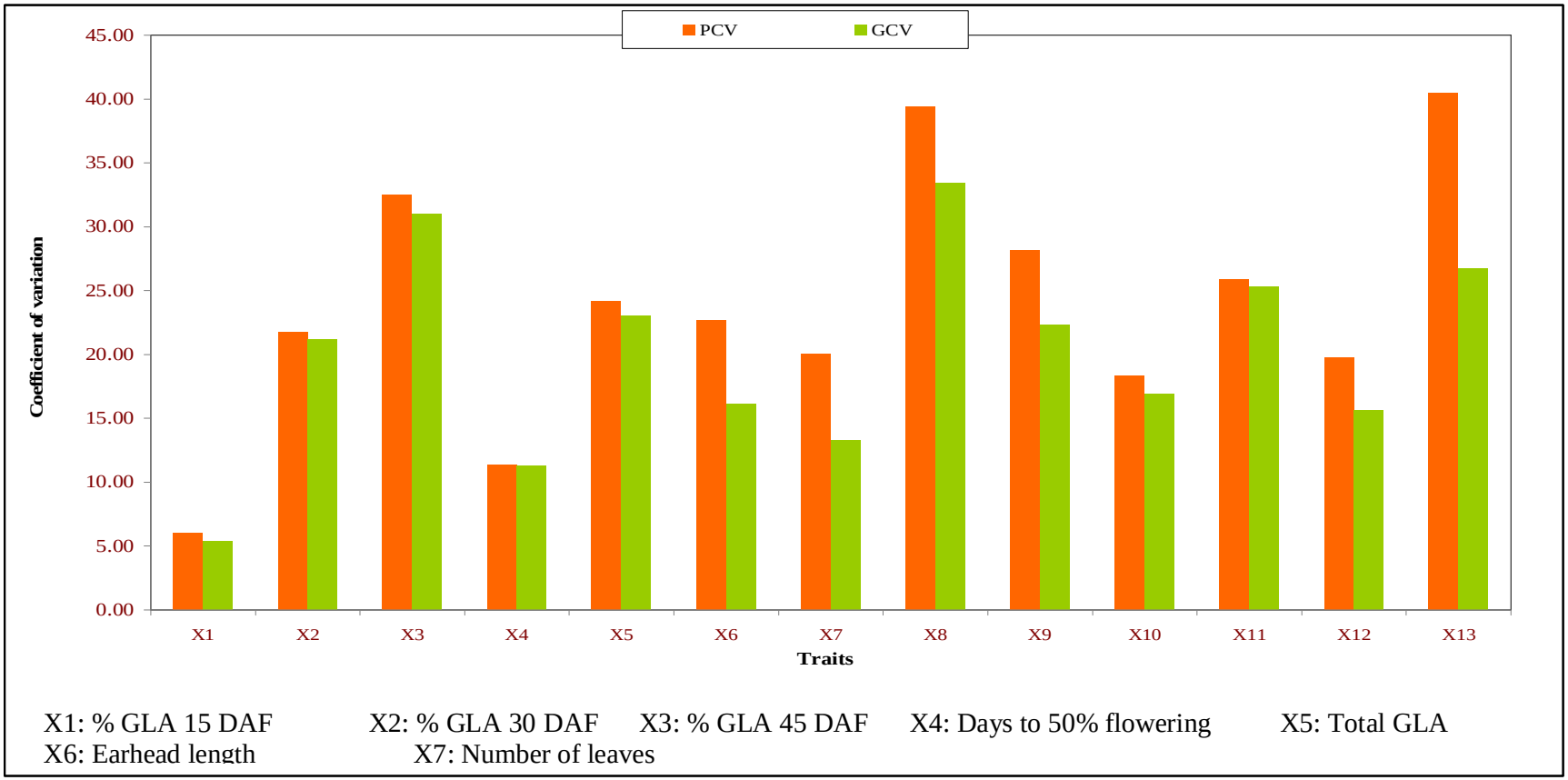



**Fig. 2: Pooled PCV and GCV for stay-green and yield related traits of recombinent inbred lines derived from cross IS9830 x E36-1 at Dharwad location**

Number of leaves

There was significant G x E interaction and significant difference among the RILs for number of leaves. The mean number of leaves recorded was 7.05 with the range of 4.50 to 10.00 leaves. The per cent GCV and PCV recorded were 13.27 and 20.02 respectively. Heritability and GA over mean recorded were 43.96 per cent and 17.02 per cent, respectively.

A positive correlation was found with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 30, per cent 45 DAF, days to 50 per cent flowering, earhead length, panicle exertion, spicklets per head, 100 grain weight and grain yield per plant, while on the other hand, it recorded negative correlation with stem girth and plant height.

Panicle exertion

Significant difference between the RILs was recorded along with significant G x E interaction for panicle exertion, the mean exertion length of 15.92 cm with a range of 0.69 to 31.92 cm. The PCV and GCV per cent recorded were 39.42 and 33.43 with heritability of 71.93. The Per cent GA over mean was 57.97 recorded for this trait.

Panicle exertion was negatively correlated with per cent GLA 15 DAF, 30, 45 DAF, total green leaf area, earhead length, stem girth, 100 grain weight, spicklets per head and grain yield per plant. On the other hand, this trait was positively correlated with days to 50 per cent flowering, number of leaves and plant height.

Stem girth

The RILs differed significantly for stem girth with a mean girth of 6.70 cm and range of 2.20 to 7.20 cm. GA over mean of 58.35 was recorded. The per cent PCV and GCV recorded were 28.14 and 22.36, respectively with heritability estimate of 63.15 per cent.

Stem girth was found positively correlating with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, days to 50 per cent flowering, total green leaf area, grain yield per plant and 100 grain weight and negatively correlating with number of leaves, panicle exertion, plant height and spicklets per head.

Plant height

High G x E interaction and significant difference between the RILs was recorded for plant height with range of variation from 117.67 to 257.00 cm and mean height of 181.53 cm. The per cent PCV and GCV recorded were 18.36 and 16.90, respectively with heritability estimate of 84.73 per cent and a GA over mean of 30.76 per cent.

Plant height was negatively correlated with number of leaves, stem girth, 100 grain weight, spicklets per head and grain yield per plant but was found to be positively correlated with number of leaves, panicle exertion, earhead length, per cent GLA 15 DAF, per cent GLA 30 DAF and per cent GLA 45 DAF.

Spicklets per head

RILs differed significantly with significant G x E interaction for number of spicklets per head. The variation ranged from 12.00 to 60.00 spicklets with a mean of 35.54 spicklets per head. The per cent GCV and PCV recorded were 25.32 and 25.87 respectively with a heritability estimate of 95.77 per cent. This trait recorded GA of 50.02 per cent over its mean

Spicklets per head positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, stem girth, 100 grain weight and grain yield per plant, but was found to be negatively correlating with panicle exertion, days to 50 per cent flowering and earhead length.

100 grain weight

A significant G x E interaction and significant difference among the RILs was recorded with mean 100 grain weight of 3.93 g, which ranged from 2.60 to 7.07 g. Per cent GA over mean of 29.36 was recorded. The per cent GCV and PCV recorded were 15.65 and 19.74 respectively with heritability estimate of 62.88 per cent.

This has positive correlation with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, number of leaves, stem girth, spicklets per head, grain yield per plant and had negative correlating with panicle exertion, days to 50 per cent flowering, earhead length and plant height.

Grain yield per plant

RILs differed significantly forgrain yield per plant with a mean yield of 93.46 g. It also recorded a significant G x E interaction. The range varied from 28.72 to 158.20 g. The PCV and GCV per cent of 40.49 and 26.72 was recorded respectively. The grain yield per plant recorded a heritability of 43.56 per cent and GA over mean of 13.51 per cent.

Grain yield per plant was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, earhead length, spicklets per head, 100 grain weight, but negatively correlated with days to 50 per cent flowering, panicle exertion and plant height.

4.1.1.2 Pooled analysis of component traits of post-flowering drought tolerance and yield related traits in N13 × E36-1 derived RILs at Dharwad location

Pooled analysis of variance was carried out and the estimates of variability parameters of stay-green, CID, yield and yield related traits are presented in Table 10a to 10d. The frequency distribution patterns for all the traits are presented in Figure 3 and graphical presentation of PCV and GCV are shown in Figure 4. Analysis of variance and variability parameters computed for each season at Dharwad location are presented in Appendices Va-VI.

Per cent GLA 15 DAF

Both mean performance and G x E interaction among RILs were differed significantly for per cent GLA 15 DAF with mean of 83.50 and range from 22.03 and 99.00 per cent. The per cent GCV and PCV recorded were 5.28 and 8.79 respectively. Heritability of 37.01 and GA over mean of 6.62 per cent were recorded for per cent GLA at 15 DAF.

Per cent GLA 15 DAF was positively and significantly correlated with per cent GLA 30 DAF, per cent GLA 45 DAF, CID, total green leaf area, number of leaves, stem girth, plant height, spicklets per head, 100 grain weight, grain yield per plant, but correlated negatively with days to 50 per cent flowering, earhead length, panicle exertion.

Per cent GLA 30 DAF

Significant difference among the RILs with significant G x E interaction was recorded for this trait. The range varied from 11.03 to 90.00 with mean of 64.51 per cent. The PCV and GCV recorded were 17.28 and 17.24 per cent respectively. The heritability estimate and GA over mean of 97.54 and 29.60 per cent were recorded respectively.

Per cent GLA 30 DAF was positively and significantly correlated with per cent GLA 15 DAF, per cent GLA 45 DAF, CID, total green leaf area, number of leaves, stem girth, plant height, spicklets per head, 100 grain weight, grain yield per plant, but correlated negatively with days to 50 per cent flowering, earhead length, panicle exertion.

Per cent GLA 45 DAF

Significant differences among the RILs for mean performance and G x E interaction were recorded. The mean per cent GLA of 41.82 and range 7.70 and 81.50 per cent was recorded with per cent GCV and PCV values of 38.82 and 41.36 respectively. The heritability estimate of 82.32 per cent and a per cent GA over mean of 54.06 was recorded.

Per cent GLA 45 DAF was positively and significantly correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, CID, total green leaf area, number of leaves, stem girth, plant height, spicklets per head, 100 grain weight, grain yield per plant, but correlated negatively with days to 50 per cent flowering, earhead length and panicle exertion.

Days to 50 per cent flowering

Analysis of variance revealed significant G x E interaction and significant difference among the RILs for days to 50 per cent flowering.

**Table 10a: Pooled analysis of variance of stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2007, 2008 and 2009 at Dharwad location**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
| 1. | Replication | 3 | 10545.65 | 1426.60 | 969.04 | 5247.24 | 3993215.86 | 835.90 | 50.57 | 77.73 | 123.85 | 21376.66 | 5696.84 | 81.62 | 365.77 | 3.29 |
| 2. | Environment (E) | 2 | 35326.40** | 126842.15** | 278069.05** | 1703.93** | 12126719.05** | 340.82** | 154.31** | 682.80** | 255.64** | 91997.77** | 3500.22** | 64.92** | 7358.34** | 0.01* |
| 3. | Genotypes (G) | 225 | 709.15** | 1552.97** | 2139.75** | 223.46** | 363227.71** | 75.31** | 4.51** | 224.51** | 2.32** | 6866.50** | 611.70** | 2.30** | 1059.09** | 0.14* |
| 4. | E x G | 450 | 158.49** | 414.38** | 749.44** | 82.76** | 176518.76** | 31.35** | 2.34** | 42.10** | 0.72** | 1874.57** | 237.36** | 0.43** | 92.90** | 0.01* |
| 5. | Error | 2034 | 98.14 | 9.11 | 31.35 | 3.58 | 47806.85 | 10.69 | 1.00 | 5.14 | 1.03 | 1493.00 | 71.58 | 0.56 | 50.62 | 0.13 |
| 6. | SEm± | | 2.98 | 0.43 | 2.89 | 1.05 | 99.10 | 1.30 | 0.35 | 1.57 | 0.30 | 17.58 | 1.15 | 0.18 | 1.00 | 0.25 |
| 7. | CD (1%) | | 10.84 | 1.56 | 10.53 | 3.83 | 361.03 | 4.73 | 1.26 | 5.72 | 1.10 | 64.05 | 4.19 | 0.65 | 3.64 | 0.93 |
| | CD (5%) | | 8.25 | 1.19 | 8.02 | 2.91 | 274.70 | 3.60 | 0.96 | 4.35 | 0.84 | 48.73 | 3.19 | 0.49 | 2.77 | 0.71 |
| | CV (%) | | 11.86 | 4.61 | 13.06 | 2.98 | 16.29 | 18.02 | 12.82 | 18.37 | 23.17 | 19.39 | 22.37 | 18.17 | 17.82 | 8.60 |

** - Significance at 1% probability level

$X_1$: % GLA 15 DAF
$X_2$: % GLA 30 DAF
$X_3$: % GLA 45 DAF
$X_4$: Days to 50 % flowering
$X_5$: Total GLA
$X_6$: Earhead length
$X_7$: Number of leaves
$X_8$: Panicle exertion
$X_9$: Stem girth
$X_{10}$: Plant height
$X_{11}$: Spicklets per head
$X_{12}$: 100 grain weight
$X_{13}$: Grain yield per plant
$X_{14}$: Carbon isotope discrimination

**Table 10b: Magnitude of genetic variability parameters of stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 × E36-1 during 2007, 2008 and 2009 at Dharwad location**

| Sl. No. | Traits | Range | | Grand mean | PCV (%) | GCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Minimum | Maximum | | | | | |
| 1. | % GLA 15 DAF | 22.03 | 99.00 | 83.50 | 8.79 | 5.28 | 37.01 | 6.62 |
| 2. | % GLA 30 DAF | 11.03 | 90.00 | 64.51 | 17.28 | 17.24 | 97.54 | 29.60 |
| 3. | % GLA 45 DAF | 7.70 | 81.50 | 41.82 | 41.36 | 38.82 | 82.32 | 54.06 |
| 4. | Days to 50 % flowering | 50.16 | 76.00 | 63.23 | 11.64 | 11.63 | 86.98 | 18.20 |
| 5. | Total green leaf area ($cm^2$) | 578.75 | 1993.40 | 1347.38 | 17.54 | 10.23 | 34.12 | 12.36 |
| 6. | Earhead length (cm) | 5.81 | 27.60 | 18.51 | 18.45 | 11.86 | 45.10 | 17.12 |
| 7. | Number of leaves | 5.15 | 10.58 | 7.74 | 10.78 | 6.27 | 26.14 | 5.94 |
| 8. | Panicle exertion (cm) | 0.68 | 23.40 | 12.58 | 31.73 | 22.53 | 55.40 | 43.48 |
| 9. | Stem girth (cm) | 1.71 | 6.53 | 4.45 | 16.66 | 5.24 | 10.31 | 2.92 |
| 10. | Plant height (cm) | 118.75 | 267.25 | 185.63 | 18.17 | 11.04 | 21.13 | 8.80 |
| 11. | Spicklets per head | 12.87 | 53.00 | 37.13 | 18.80 | 17.72 | 89.34 | 33.90 |
| 12. | 100 grain weight (g) | 2.00 | 5.75 | 4.07 | 11.34 | 7.40 | 43.23 | 10.07 |
| 13. | Grain yield per plant (g/plant) | 24.02 | 161.40 | 92.71 | 25.68 | 24.38 | 94.44 | 20.05 |
| 14. | Carbon isotope discrimination | 3.26 | 5.20 | 4.21 | 7.73 | 1.59 | 5.98 | 67.45 |

**Table 10c: Phenotypic correlation coefficients (pooled) for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2007, 2008 and 2009 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.558** | 0.463** | 0.004 | -0.005 | 0.021 | -0.016 | 0.049 | -0.077 | 0.003 | 0.089 | 0.057 | 0.048 | 0.064 |
| $X_2$ | | 1 | 0.667** | 0.003 | -0.021 | 0.051 | -0.006 | 0.104 | -0.038 | 0.043 | 0.127 | 0.054 | 0.013 | 0.158* |
| $X_3$ | | | 1 | 0.05 | -0.055 | 0.077 | -0.013 | 0.045 | -0.071 | 0.007 | 0.064 | 0.141* | 0.012 | 0.148* |
| $X_4$ | | | | 1 | -0.028 | 0.026 | 0.032 | 0.026 | 0.07 | -0.048 | -0.021 | 0.011 | 0.009 | 0.043 |
| $X_5$ | | | | | 1 | -0.116 | 0.015 | 0.093 | 0.027 | 0.053 | 0.026 | -0.049 | -0.002 | -0.014 |
| $X_6$ | | | | | | 1 | 0.018 | 0.059 | -0.028 | 0.105 | 0.121 | 0.117 | 0.028 | 0.056 |
| $X_7$ | | | | | | | 1 | 0.027 | -0.002 | 0.036 | 0.011 | -0.014 | -0.011 | 0.013 |
| $X_8$ | | | | | | | | 1 | -0.047 | -0.081 | 0.001 | 0.007 | 0.025 | 0.026 |
| $X_9$ | | | | | | | | | 1 | -0.123 | 0.304** | -0.038 | -0.048 | -0.069 |
| $X_{10}$ | | | | | | | | | | 1 | -0.06 | -0.064 | -0.02 | 0.173* |
| $X_{11}$ | | | | | | | | | | | 1 | -0.112 | -0.049 | -0.035 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.023 | 0.291** |
| $X_{13}$ | | | | | | | | | | | | | 1 | 0.039 |
| $X_{14}$ | | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability * - Significant at 5% level of probability

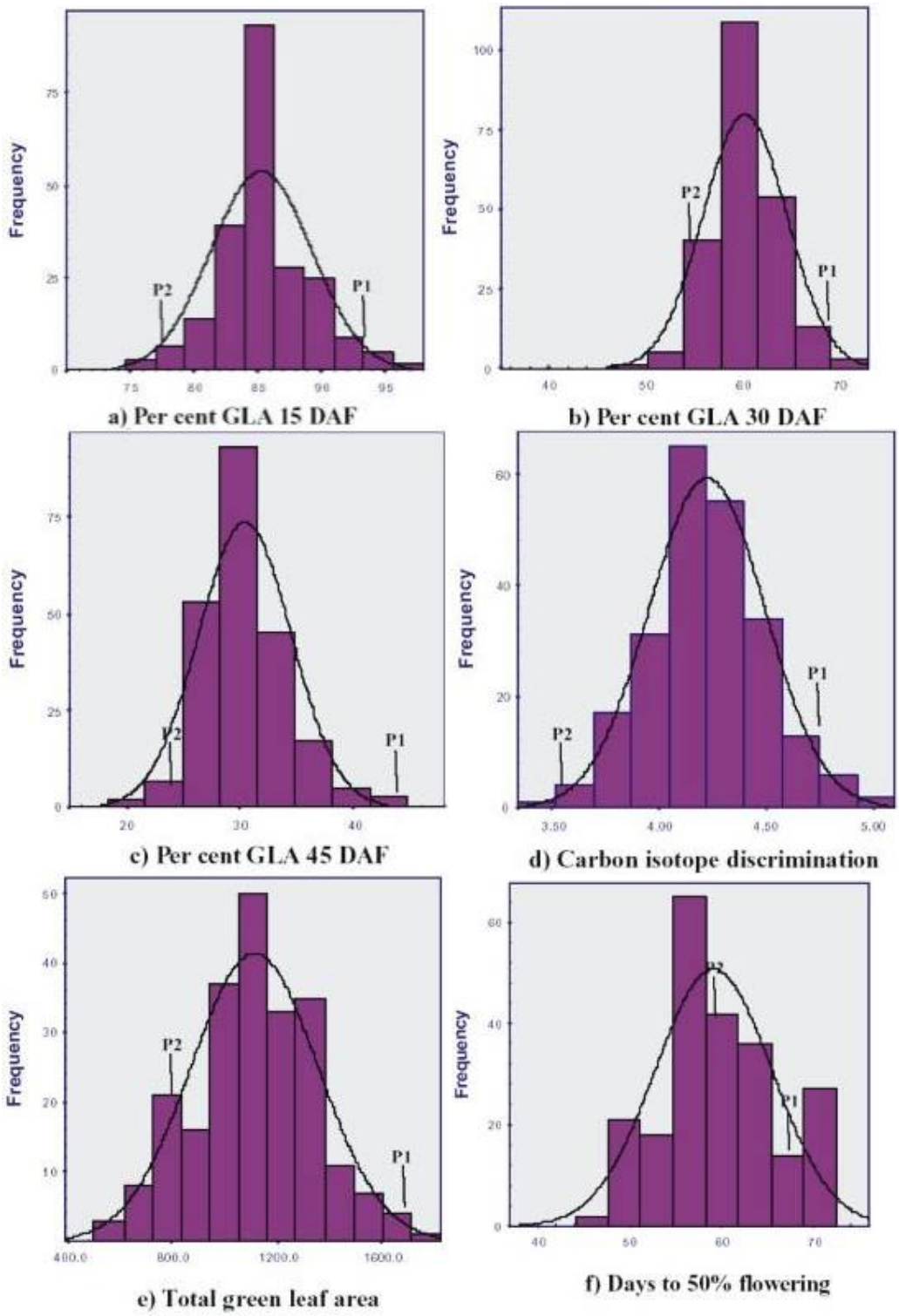
$X_1$: % GLA 15 DAF
$X_2$: % GLA 30 DAF
$X_3$: % GLA 45 DAF
$X_4$: Carbon isotope discrimination
$X_5$: Days to 50 % flowering
$X_6$: Total GLA
$X_7$: Earhead length
$X_8$: Number of leaves
$X_9$: Panicle exertion
$X_{10}$: Stem girth
$X_{11}$: Plant height
$X_{12}$: Spicklets per head
$X_{13}$: 100 grain weight
$X_{14}$: Grain yield per plant

**Table 10d: Genotypic correlation coefficients (pooled) for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2007,2008 and 2009 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.628** | 0.507** | 0.153* | -0.006 | 0.023 | -0.006 | 0.059 | -0.084 | 0.029 | 0.118 | 0.103 | 0.065 | 0.162* |
| $X_2$ | | 1 | 0.733** | 0.103 | -0.029 | 0.062 | -0.021 | 0.13 | -0.043 | 0.084 | 0.181* | 0.096 | 0.034 | 0.329** |
| $X_3$ | | | 1 | 0.429** | -0.06 | 0.081 | -0.007 | 0.061 | -0.083 | 0.005 | 0.096 | 0.281** | 0.03 | 0.308** |
| $X_4$ | | | | 1 | -0.151* | 0.149* | 0.225** | 0.281** | 0.504** | -0.81** | -0.000 | 0.334** | 0.247* | 0.329** |
| $X_5$ | | | | | 1 | -0.127 | 0.024 | 0.132 | 0.031 | 0.123 | 0.015 | -0.049 | -0.074 | -0.007 |
| $X_6$ | | | | | | 1 | 0.033 | 0.083 | -0.027 | 0.199* | 0.186** | 0.202** | 0.05 | 0.098 |
| $X_7$ | | | | | | | 1 | 0.071 | -0.015 | 0.157* | 0.012 | -0.05 | -0.116 | 0.05 |
| $X_8$ | | | | | | | | 1 | -0.08 | -0.145* | 0.016 | 0.01 | 0.017 | 0.066 |
| $X_9$ | | | | | | | | | 1 | -0.23** | 0.442** | -0.085 | -0.093 | -0.107 |
| $X_{10}$ | | | | | | | | | | 1 | -0.25** | -0.007 | -0.031 | 0.188* |
| $X_{11}$ | | | | | | | | | | | 1 | -0.22** | -0.16 | -0.11 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.132 | 0.334** |
| $X_{13}$ | | | | | | | | | | | | | 1 | 0.005 |
| $X_{14}$ | | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

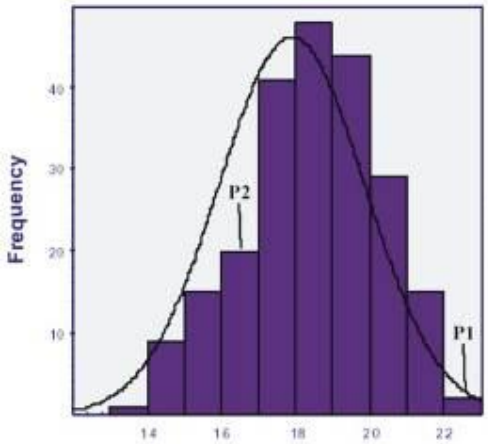
$X_1$: % GLA 15 DAF
$X_2$: % GLA 30 DAF
$X_3$: % GLA 45 DAF
$X_4$: Carbon isotope discrimination
$X_5$: Days to 50 % flowering
$X_6$: Total GLA
$X_7$: Earhead length
$X_8$: Number of leaves
$X_9$: Panicle exertion
$X_{10}$: Stem girth
$X_{11}$: Plant height
$X_{12}$: Spicklets per head
$X_{13}$: 100 grain weight
$X_{14}$: Grain yield per plant



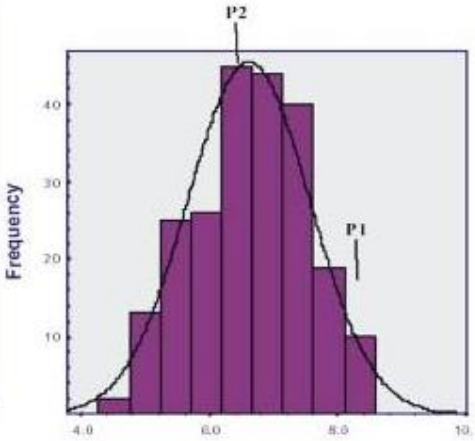

Legend : P1 – E36-1 P2-N13

**Fig. 3 : Frequency distribution (pooled) of stay-green and yield related traits in recombinant inbred lines derived from cross N13 × E36-1 evaluated at Dharwad location**

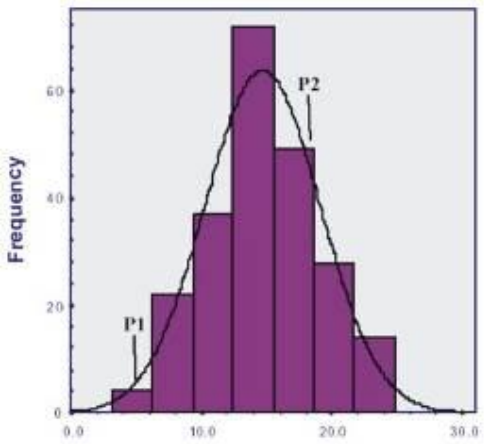
**Fig. 3 Contd….**



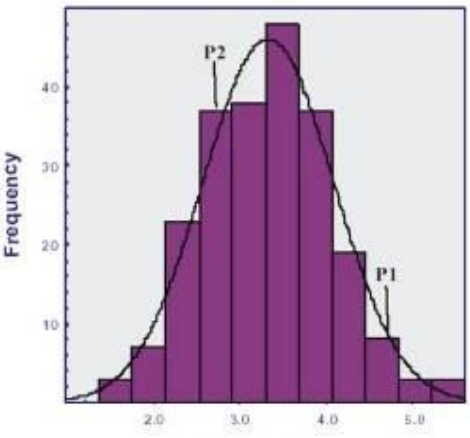

g) Earhead length




h) Number of leaves/plant

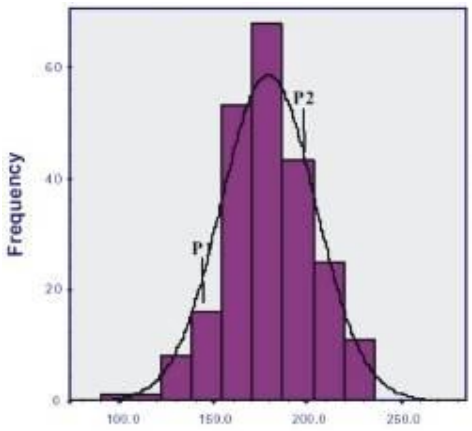



i) Panicle exertion

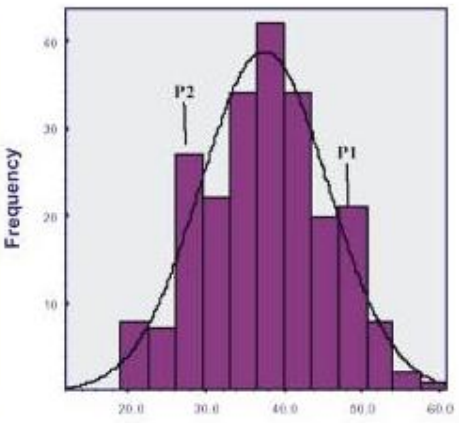



j) Stemgirth

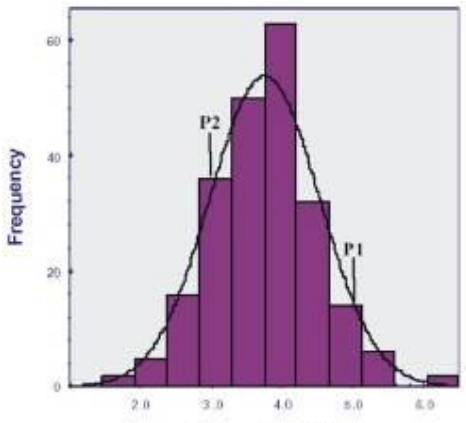
**Fig. 3 Contd….**



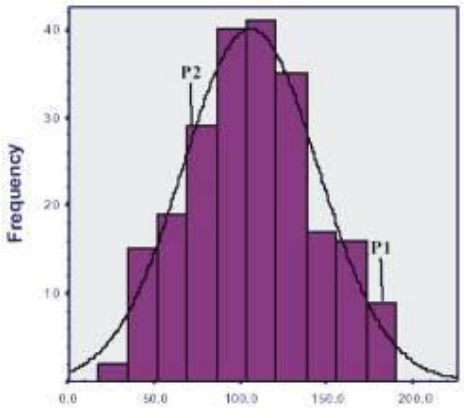

k) Plant height




l) Spicklets per head




m) 100 grain weight




n) Grain yield per plant

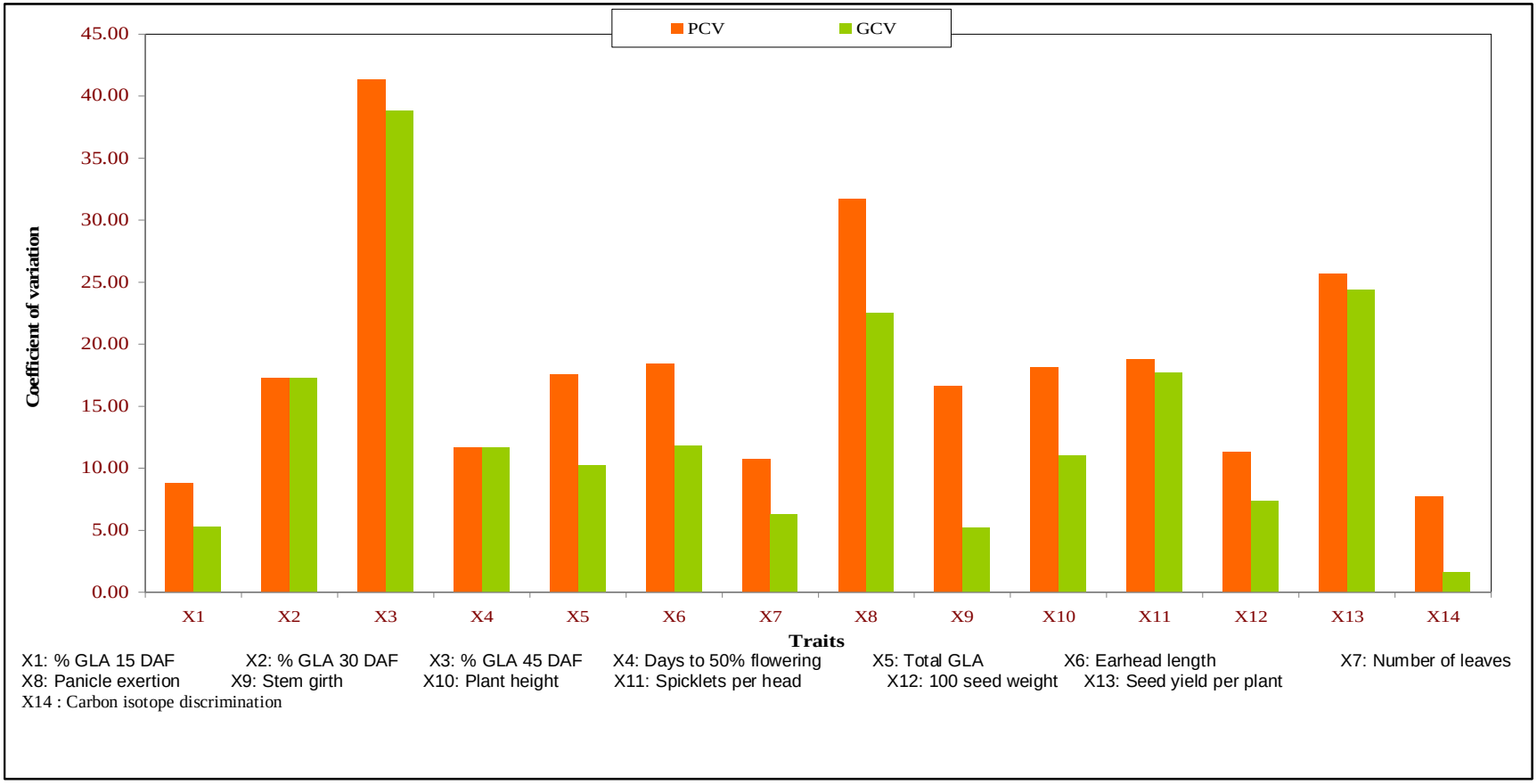



**Fig. 4: Pooled PCV and GCV for stay-green and yield related traits of recombinent inbred lines derived from cross N13 x E36-1 at Dharwad location**

The mean value of 63.23 days with range from 50.16 to 76.00 days was recorded whereas, the GA over mean of 18.20 per cent and the high heritability of 86.98 per cent with PCV and GCV of 11.69 and 11.63 per cent respectively was recorded.

Days to 50 per cent flowering was positively correlated with total green leaf area, earhead length, number of leaves, stem girth, plant height, 100 grain weight, grain yield per plant but correlated negatively with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF CID and spicklets per head.

Total green leaf area

RILs differed significantly for their mean total green leaf area with significant G x E interaction over season. The mean leaf area of 1347.38 $cm^2$ and a wide range of 578.75 to 1993.40 $cm^2$ was recorded. The per cent PCV and GCV recorded were 17.54 and 10.23 respectively with 34.12 per cent of heritability estimate and GA over mean of 12.36 per cent was recorded.

Total green leaf area was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, earhead length, number of leaves, stem girth, plant height, spicklets per head, grain yield per plant, 100 grain weight, but correlated negatively with days to 50 per cent flowering and panicle exertion.

Earhead length

There was a significant G x E interaction with significant differences between RILs at Dharwad location. The variation ranged from 5.81 to 27.60 cm with a mean length of 18.51 cm. The GCV and PCV recorded were 11.86 and 18.45 per cent respectively with per cent heritability of 45.10 and GA over mean of 17.12 per cent was recorded.

Earhead length was positively correlated with total green leaf area, number of leaves, stem girth, plant height, and grain yield per plant but correlated negatively with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, panicle exertion, spicklets per head and 100 grain weight.

Number of leaves

RILs differed significantly in their mean values with significant G x E interaction. The leaves number ranged from 5.15 to 10.58 and a mean of 7.74. The per cent PCV and GCV of 10.78 and 6.27 respectively were recorded. The per cent heritability and GA over per cent mean of 26.14 and 5.90 were recorded.

Number of leaves was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, days to 50 per cent flowering, total green leaf area, earhead length, plant height, spicklets per head, 100 grain weight and grain yield per plant, but correlated negatively with panicle exertion and stem girth.

Panicle exertion

A significant G x E interaction and significant difference among the RILs was recorded for panicle exertion length. The range of variation from 0.68 to 23.00 cm with mean of 12.58 cm was recorded and per cent genetic advance of 43.48 was recorded. The GCV and PCV per cent of 22.53 and 31.73 respectively were recorded with mean per cent heritability of 55.40.

Panicle exertion was negatively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, total green leaf area, earhead length, number of leaves, stem girth, spicklets per head, grain yield per plant but positive correlation was found with days to 50 per cent flowering and plant height.

Stem girth

High G x E interaction along with significant difference between the RILs for stem girth was recorded. The mean girth of 4.45 cm which ranged from 1.71 to 6.53 cm was recorded. The per cent PCV and GCV of 16.66 and 5.24 were recorded respectively with per cent heritability of 10.31. Per cent GA over mean of 2.92 was recorded.

Stem girth has positive correlation with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, days to 50 per cent flowering, total green leaf area, earhead length, grain yield per plant and 100 grain weight and correlated negatively with number of leaves, panicle exertion, plant height and spicklets per head.

Plant height

RILs differed significantly with significant G x E interaction for height of the plant with mean height of 185.63 cm ranged from 118.75 to 267.25 cm. The per cent PCV and GCV recorded were 18.17 to 11.04, respectively with heritability estimates of 21.13 per cent. Per cent GA over mean of 8.80 was recorded.

Plant height was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, earhead length, number of leaves, panicle exertion and correlated negatively with stem girth, spicklets per head, grain yield per plant.

Spicklets per head

Significant differences among the RILs were observed for number of spicklets per head and G x E interaction. The range of variation from 12.87 to 53.00 spicklets per head with mean of 37.13 was recorded. The per cent PCV and GCV of 18.80 and 17.72 was recorded respectively with heritability estimate of 89.34 per cent. The GA over mean of 35.90 per cent was recorded.

Spicklets per head was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, total green leaf area, number of leaves, 100 grain weight, grain yield per plant, but correlated negatively with plant height, stem girth, panicle exertion and days to 50 per cent flowering.

100 grain weight

Both mean performance and G x E interaction among RILs differed significantly for 100 grain weight with GA over mean of 10.07 was recorded. The mean weight of 4.07 g ranged from 2.00 to 5.75 g was recorded. The per cent GCV and PCV of 7.40 and 11.34 was recorded, respectively with per cent heritability estimate of 43.23.

Trait was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, total green leaf area, grain yield per plant but negatively with stem girth, panicle exertion and days to 50 per cent flowering.

Grain yield per plant

There was a significant difference among the RILs and G x E interaction with a meangrain yield per plant of 92.71 g. Variation ranged from 24.02 to 161.40 g was recorded. The per cent PCV and GCV of 25.68 and 24.38, respectively was recorded with heritability estimate of 94.44 per cent. The GA over mean of 20.05 per cent was recorded.

It was correlated positively with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, total green leaf area, 100 grain weight and negatively correlated with plant height, panicle exertion and days to 50 per cent flowering.

Carbon isotope discrimination (CID)

Significant difference among the RILs was recorded for CID which recorded a mean value of 4.21 with variation values ranged from 3.26 to 5.20. The per cent PCV and GCV of 7.73 and 1.59 was recorded respectively. Heritability estimate of 5.98 per cent and GA over mean of 67.45 per cent were recorded.

Trait was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, spicklets per head, 100 grain weight, grain yield per plant contrary negatively correlated with days to 50 per cent flowering, earhead length, panicle exertion and stem girth.

4.1.1.3 Pooled analysis of component traits of post-flowering drought tolerance and yield related traits in IS9830 × E36-1 derived RIL at Bheemarayanagudi location

Pooled analysis of variance was carried out and the estimates of variability parameters *viz.,* stay-green, CID, yield and yield related traits are presented in Table 11a to 11d. The frequency distribution patterns for all the traits are presented in Figure 5 and graphical presentation of PCV and GCV are shown in Figure 6. Analysis of variance and variability parameters were computed for Bheemarayanagudi location and presented in appendices VIa-VIh.

Per cent GLA 15 DAF

RILs differed significantly for per cent GLA 15 DAF with significant G x E interaction. A mean of 89.51 and variation range of 69.50 to 100.00 per cent was recorded. The per cent GCV and PCV of 4.74 and 7.01, respectively with heritability estimates of 45.75 per cent. The genetic advance as per cent mean of 6.61 per cent was recorded.

This trait has significant and positive correlation with per cent GLA 30 DAF, per cent 45 DAF, total green leaf area, earhead length, number of leaves, plant height, spicklets per head, 100 grain weight, grain yield per plant and negatively correlated with days to 50 per cent flowering, panicle exertion and stem girth.

Per cent GLA 30 DAF

Significant variation among the RILs and significant G x E interaction was observed. The variation ranged from 25.00 to 89.00 per cent with mean of 63.30 per cent. The per cent PCV and GCV of 17.51 and 17.45 were recorded, respectively with heritability estimate of 99.30 per cent and 35.79 per cent of GA over mean were recorded.

Per cent GLA 30 DAF has positive and significant correlation with per cent GLA 15 DAF, per cent 45 DAF, total green leaf area, earhead length, number of leaves, stem girth, plant height, spicklets per head, 100 grain weight, grain yield per plant but correlated respectively with days to 50 per cent flowering and panicle exertion.

Per cent GLA 45 DAF

Significant variation among the RILs was observed with significant G x E interaction. The mean per cent GLA 45 DAF of 33.91 with variation ranged from 12.60 to 76.00 per cent was recorded. The PCV and GCV of 48.01 and 44.93 per cent was recorded respectively with heritability estimate of 87.57 per cent and GA over mean of 87.02 per cent.

Per cent GLA 45 DAF found positively and significantly correlated with per cent GLA 15 DAF, per cent 30 DAF, total green leaf area, earhead length, number of leaves, stem girth, spicklets per head, 100 grain weight and grain yield per plant, but negatively correlated with days to 50 per cent flowering, panicle exertion and plant height.

Days to 50 per cent flowering

There was a significant difference for G x E interaction and significant difference in mean performance of RILs for days to 50 per cent flowering. The mean days to 50 per cent flowering of 53.93 with range from 38.67 to 73.00 days was recorded. The per cent PCV and GCV of 12.03 and 11.01, respectively with heritability estimate of 83.69 per cent were recorded. The GA over mean of 20.74 per cent was recorded for this trait.

Days to 50 per cent flowering has positive correlation with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, earhead length, panicle exertion and was negatively correlated with total green leaf area, number of leaves, stem girth, plant height, spicklets per head, 100 grain weight and grain yield per plant.

Total green leaf area

RILs differed significantly in their values with significant G x E interaction for total green leaf area with mean area of 1219.89 $cm^2$ ranged from 254.39 to 2951.00 $cm^2$. The per cent GCV and PCV of 17.32 and 21.57 were recorded respectively with per cent heritability of 64.43 and 28.63 per cent of GA over mean.

**Table 11a: Pooled analysis of variance of stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2008 and 2009 at Bheemarayanagudi location**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
| 1. | Replication | 3 | 802.51 | 4282.81 | 1052.32 | 1497.33 | 6655837.58 | 323.48 | 26.79 | 795.82 | 0.23 | 38082.36 | 753.81 | 37.21 | 43.61 |
| 2. | Environment (E) | 1 | 0.00** | 6773.43** | 249.18** | 179.27** | 49262234.37** | 82.33** | 333.05** | 474.53** | 228.23** | 119296.78** | 0.20** | 109.78** | 1363.17** |
| 3. | Genotypes (G) | 225 | 184.30** | 962.21** | 1882.53** | 292.70** | 391606.06** | 59.10** | 5.85** | 262.45** | 1.70** | 4806.66** | 572.56** | 2.35** | 886.79** |
| 4. | E x G | 225 | 0.02** | 0.71** | 165.77** | 2.10** | 139135.33** | 6.67** | 0.76** | 37.95** | 0.40** | 802.40** | 1.11** | 0.11** | 0.12** |
| 5. | Error | 1353 | 21.24 | 0.95 | 59.78 | 8.38 | 63245.92 | 16.02 | 0.72 | 17.74 | 0.57 | 805.33 | 1.77 | 0.29 | 8.09 |
| 6. | SEm± | | 2.31 | 0.46 | 2.88 | 1.31 | 78.46 | 1.18 | 0.23 | 1.40 | 0.30 | 9.18 | 0.41 | 0.21 | 1.41 |
| 7. | CD (1%) | | 8.42 | 1.68 | 10.51 | 4.78 | 285.82 | 4.30 | 0.85 | 5.11 | 1.08 | 33.44 | 1.49 | 0.77 | 5.13 |
| 8. | CD (5%) | | 6.41 | 1.28 | 8.00 | 3.63 | 217.47 | 3.27 | 0.64 | 3.88 | 0.82 | 25.44 | 1.13 | 0.59 | 3.90 |
| 9. | CV (%) | | 5.15 | 1.54 | 22.80 | 5.37 | 20.62 | 22.60 | 11.65 | 25.99 | 18.38 | 15.34 | 3.67 | 14.67 | 9.14 |

* - Significance at 5% probability level  ** - Significance at 1% probability level

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Table 11b: Magnitude of genetic variability parameters of stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 × E36-1 during 2008 and 2009 at Bheemarayanagudi location**

| Sl. No. | Traits | Range | | Grand mean | PCV (%) | GCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Minimum | Maximum | | | | | |
| 1. | % GLA 15 DAF | 69.50 | 100.00 | 89.51 | 7.01 | 4.74 | 45.75 | 6.61 |
| 2. | % GLA 30 DAF | 25.00 | 89.00 | 63.30 | 17.51 | 17.45 | 99.30 | 35.79 |
| 3. | % GLA 45 DAF | 12.60 | 76.00 | 33.91 | 48.01 | 44.93 | 87.57 | 87.02 |
| 4. | Days to 50 % flowering | 38.67 | 73.00 | 53.93 | 12.03 | 11.01 | 83.69 | 20.74 |
| 5. | Total green leaf area ($cm^2$) | 254.39 | 2951.00 | 1219.89 | 21.57 | 17.32 | 64.43 | 28.63 |
| 6. | Earhead length (cm) | 12.81 | 34.00 | 17.70 | 19.22 | 13.87 | 52.06 | 20.62 |
| 7. | Number of leaves | 5.17 | 13.17 | 7.27 | 13.02 | 11.34 | 75.90 | 20.35 |
| 8. | Panicle exertion (cm) | 0.75 | 32.02 | 16.20 | 38.64 | 34.54 | 79.90 | 63.51 |
| 9. | Stem girth (cm) | 2.91 | 6.78 | 4.11 | 16.96 | 9.03 | 28.37 | 9.97 |
| 10. | Plant height (cm) | 118.33 | 278.58 | 184.93 | 15.81 | 12.31 | 60.64 | 19.76 |
| 11. | Spicklets per head | 18.50 | 62.58 | 36.26 | 23.72 | 23.61 | 99.10 | 48.51 |
| 12. | 100 grain weight (g) | 1.32 | 4.47 | 3.63 | 18.13 | 13.84 | 58.33 | 21.76 |
| 13. | Grain yield per plant (g/plant) | 23.38 | 165.89 | 94.63 | 35.24 | 34.07 | 93.47 | 22.42 |

**Table 11c: Phenotypic correlation coefficients (pooled) for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2008 and 2009 at Bheemarayanagudi location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.307** | 0.172* | -0.165* | 0.178* | 0.024 | 0.136 | -0.066 | -0.03 | 0.097 | 0.145* | 0.138* | 0.036 |
| $X_2$ | | 1 | 0.445** | -0.022 | 0.189** | 0.044 | 0.217** | -0.056 | 0.014 | 0.028 | 0.122 | 0.041 | 0.059 |
| $X_3$ | | | 1 | -0.036 | 0.176* | 0.076 | 0.199** | -0.116 | 0.083 | -0.006 | 0.068 | 0.004 | 0.129 |
| $X_4$ | | | | 1 | -0.08 | 0.012 | -0.007 | 0.093 | -0.084 | -0.024 | -0.073 | -0.145* | -0.004 |
| $X_5$ | | | | | 1 | 0.143* | 0.384** | -0.168* | 0.274** | 0.213** | 0.235** | 0.021 | 0.057 |
| $X_6$ | | | | | | 1 | 0.071 | 0.049 | -0.013 | 0.098 | 0.052 | -0.105 | -0.009 |
| $X_7$ | | | | | | | 1 | -0.151* | 0.138* | 0.142* | 0.171* | -0.006 | 0.06 |
| $X_8$ | | | | | | | | 1 | -0.128 | 0.325** | -0.077 | 0.009 | -0.045 |
| $X_9$ | | | | | | | | | 1 | 0.053 | 0.085 | -0.009 | 0.055 |
| $X_{10}$ | | | | | | | | | | 1 | 0.120 | 0.044 | 0.018 |
| $X_{11}$ | | | | | | | | | | | 1 | -0.046 | 0.028 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.134 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

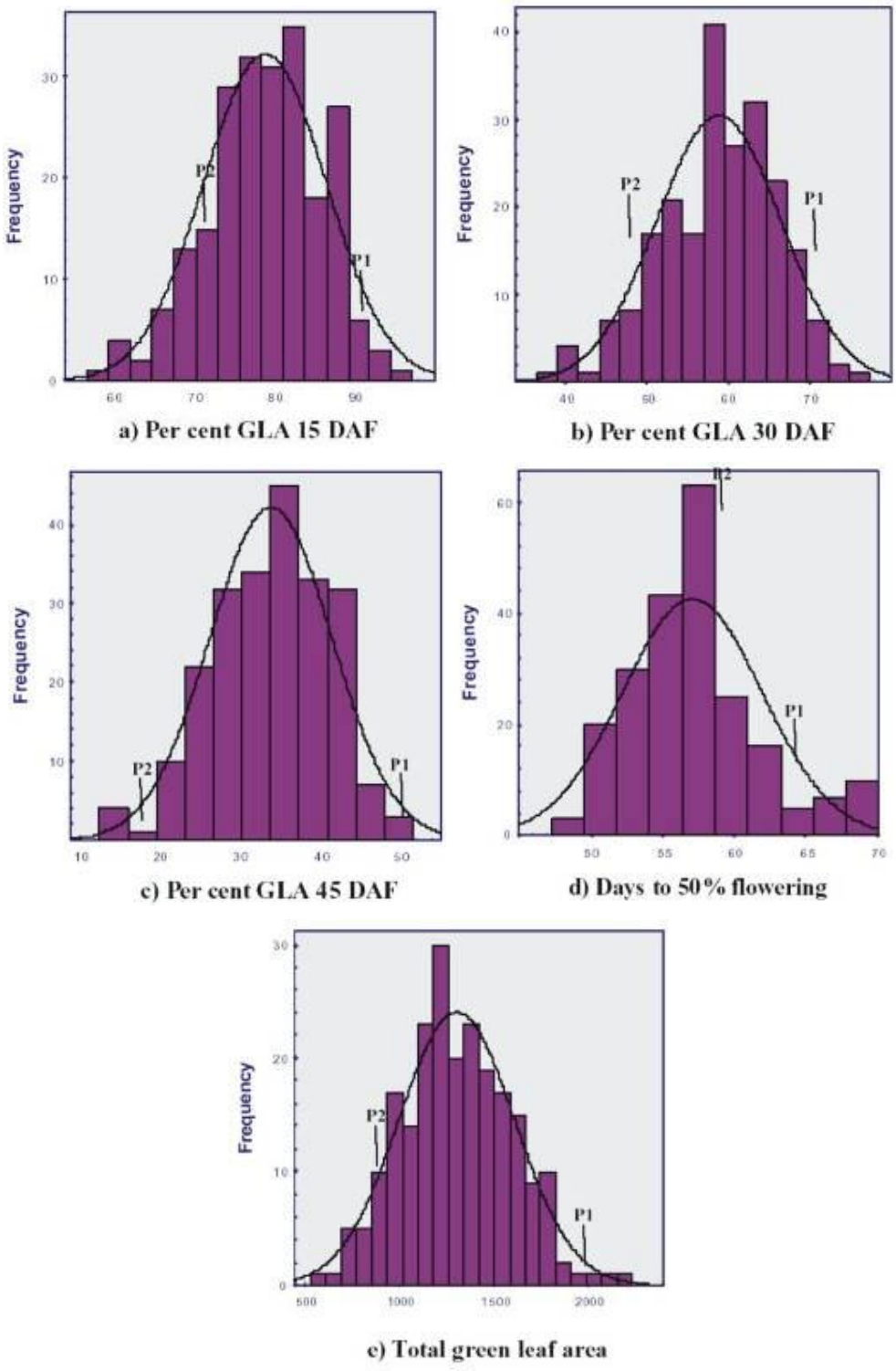
$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Table 11d: Genotypic correlation coefficients (pooled) for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2008 and 2009 at Bheemarayanagudi location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.457** | 0.253** | -0.293** | 0.269** | 0.024 | 0.218** | -0.094 | -0.005 | 0.117 | 0.213* | 0.306** | 0.073 |
| $X_2$ | | 1 | 0.480** | -0.025 | 0.238** | 0.005 | 0.251** | -0.062 | 0.026 | 0.032 | 0.121 | 0.068 | 0.062 |
| $X_3$ | | | 1 | -0.045 | 0.236* | 0.097 | 0.249* | -0.139* | 0.119 | -0.016 | 0.070 | 0.011 | 0.145* |
| $X_4$ | | | | 1 | -0.098 | 0.024 | -0.008 | 0.110 | -0.150 | -0.021 | -0.076 | -0.232* | -0.002 |
| $X_5$ | | | | | 1 | 0.233** | 0.542** | -0.211** | 0.590** | 0.332** | 0.292** | 0.032 | 0.074 |
| $X_6$ | | | | | | 1 | 0.096 | 0.069 | -0.061 | 0.119 | 0.076 | -0.175* | 0.019 |
| $X_7$ | | | | | | | 1 | -0.171* | 0.323** | 0.179* | 0.199* | -0.009 | 0.071 |
| $X_8$ | | | | | | | | 1 | -0.203** | 0.448** | -0.087 | 0.053 | -0.048 |
| $X_9$ | | | | | | | | | 1 | 0.144* | 0.155* | -0.040 | 0.101 |
| $X_{10}$ | | | | | | | | | | 1 | 0.152* | 0.078 | 0.017 |
| $X_{11}$ | | | | | | | | | | | 1 | -0.060 | 0.029 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.174* |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

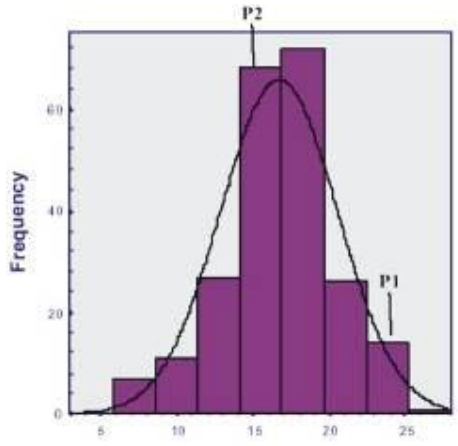
$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant



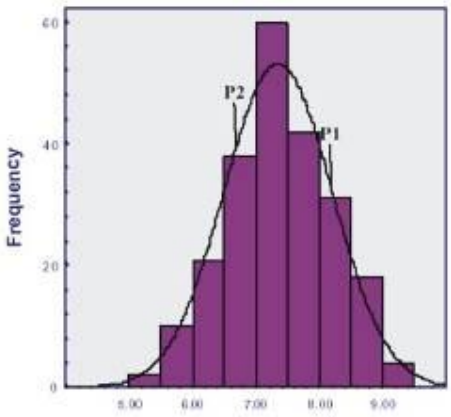

Legend : P1 – E36-1 P2 – IS9830

**Fig. 5: Frequency distribution (pooled) of stay-green and yield related traits in recombinant inbred lines derived from cross IS9830 × E36-1 evaluated at Bheemarayanagudi location**

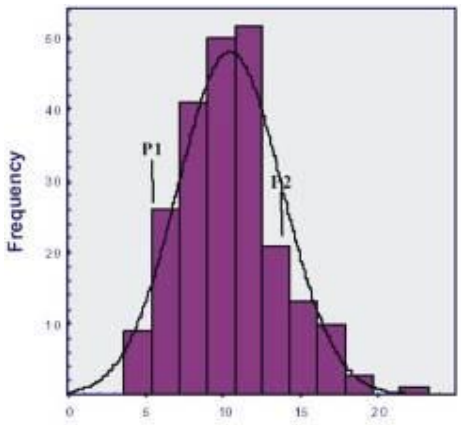
**Fig. 5 Contd….**



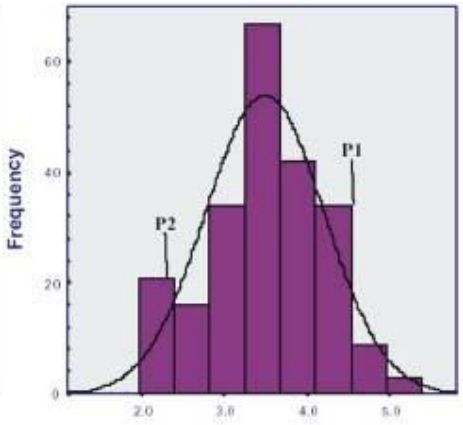

g) Earhead length




h) Number of leaves/plant

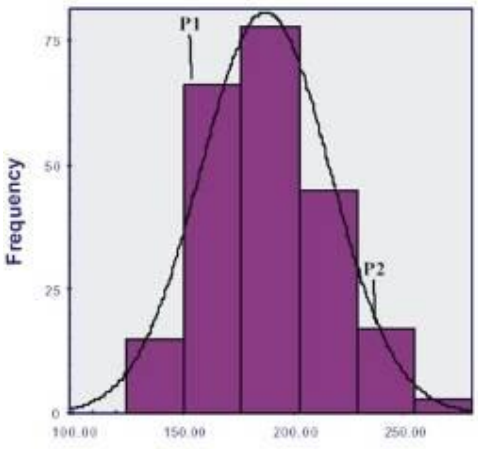



i) Panicle exertion

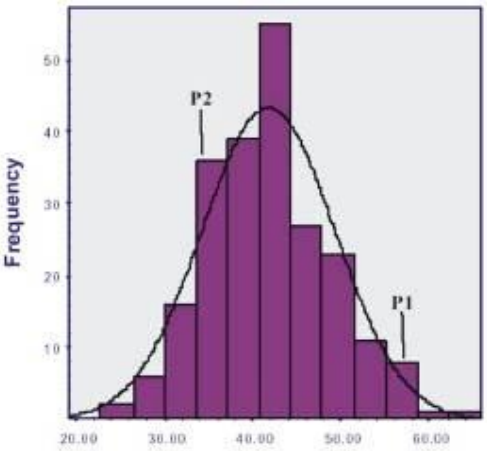



j) Stem girth

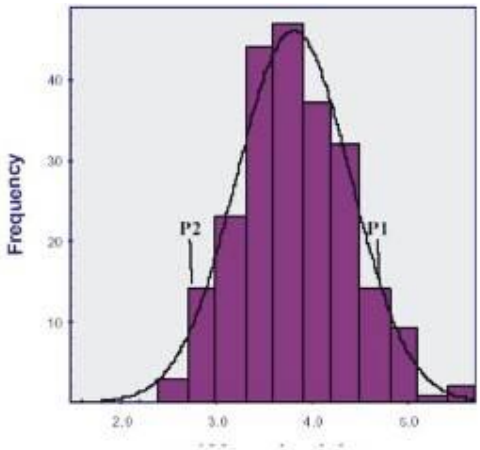
**Fig. 5 Contd….**



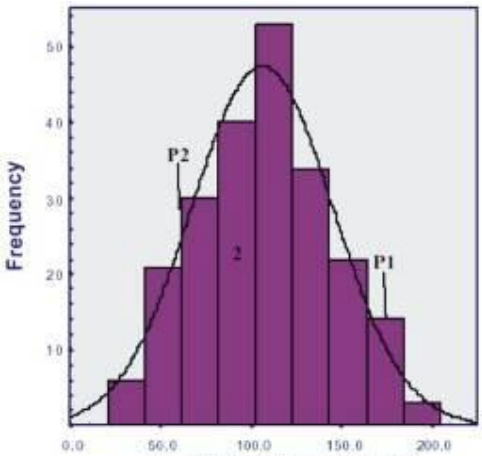

k) Plant height




l) Spicklets per head




m) 100 grain weight




n) Grain yield per plant

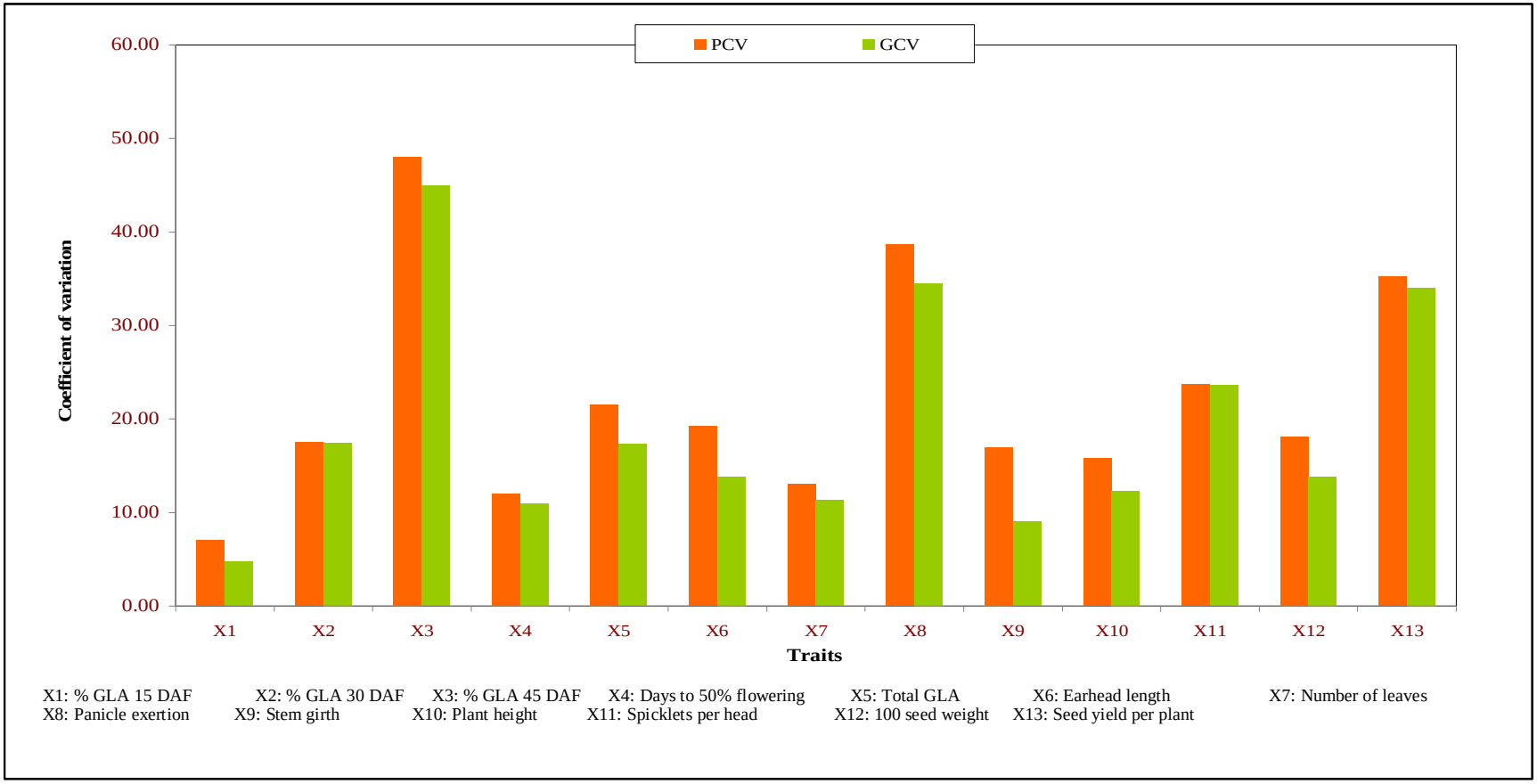



**Fig. 6: Pooled PCV and GCV for stay-green and yield related traits of recombinent inbred lines derived from cross IS9830 x E36-1 at Bheemarayanagudi location**

A positive correlation with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, earhead length, number of leaves, stem girth, plant height, spicklets per head, 100 grain weight and grain yield per plant was observed. And it was negatively correlated with days to 50 per cent flowering and panicle exertion.

Earhead length

There was a significant G x E interaction with significant differences among the RILs for length of head, which ranged from 12.81 to 34.00 cm with mean length of 17.70 cm. The per cent PCV and GCV of 19.22 and 13.87 were recorded respectively. Heritability estimate of 52.06 per cent and GA over mean of 20.62 per cent were recorded in respect of this trait.

Earhead length was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, number of leaves, panicle exertion, plant height, spicklets per head, grain yield per plant, but was negatively correlated with stem girth and 100 grain weight.

Number of leaves

RILs differed significantly in their values with significant G x E interaction for number of leaves with mean leaves of 7.27 and ranged from 5.17 to 13.17. The GA over mean of 20.35 per cent was recorded. The per cent PCV and GCV of 13.02 and 11.34 were recorded respectively with heritability estimates of 75.90 per cent.

Number of leaves was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, earhead length, total green leaf area, stem girth, plant height, spicklets per head and was negatively correlated with days to 50 per cent flowering, panicle exertion and 100 grain weight.

Panicle exertion

Significant difference among the RILs was recorded with significant G x E interaction for length of panicle exertion. The range of variation from 0.75 to 32.02 cm with mean length of 16.20 cm was recorded. The GCV and PCV of 34.54 and 38.64 per cent was recorded respectively with per cent heritability of 79.90. The GA over mean of 63.51 per cent was recorded.

Panicle exertion was positively correlated with days to 50 per cent flowering, plant height, 100 grain weight and was negatively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, number of leaves, stem girth, spicklets per head and grain yield per plant.

Stem girth

Significant difference among the RILs was recorded for girth of plant. The range from 2.91 to 6.78 cm with mean girth of 4.11 was recorded. The per cent GCV and PCV of 9.03 and 16.96 were recorded respectively with heritability estimate of 28.37 per cent and GA over mean of 9.97 per cent was recorded.

Stem girth has positive correlation with per cent GLA 30 DAF, per cent 45 DAF, total green leaf area, number of leaves, plant height, spicklets per head and grain yield per plant and negative correlation was found with per cent GLA 15 DAF , earhead length, panicle exertion and 100 grain weight.

Plant height

Significant variation among the RILs with significant G x E interaction was recorded for plant height. The mean height of 184.93 cm range from 118.33 to 278.58 cm was recorded. The per cent GCV and PCV of 12.31 and 15.81 was recorded; respectively with per cent heritability of 60.64 and 19.76 per cent of GA over mean was recorded.

Plant height was positively correlated with per cent GLA 15 DAF, per cent 30 DAF, total green leaf area, earhead length, number of leaves, panicle exertion, stem girth, spicklets per head, 100 grain weight, grain yield per plant, but negative correlation found with per cent GLA 45 DAF, and days to 50 per cent flowering.

Spicklets per head

RILs differed significantly in their values with significant G x E interaction for number of spicklets per head. The mean value of 36.26 with range from 18.50 to 62.58 spicklets per head was recorded. The per cent PCV and GCV of 23.72 and 23.61, respectively with per cent heritability of 99.10 was recorded and 48.51 per cent of GA over mean was recorded.

Spicklets per head was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, earhead length, number of leaves, stem girth, plant height, grain yield per plant and negatively correlated with days to 50 per cent flowering, panicle exertion and 100 grain weight.

100 grain weight

There was a significant G x E interaction with significant difference among the RILs for weight of 100 seeds at Bheemarayanagudi. The range from 1.32 to 4.47 g with mean 100 grain weight of 3.63 g was recorded in respect of this trait. The per cent PCV and GCV of 18.13 and 13.84 respectively was recorded with per cent heritability of 58.33. GA over mean of 21.76 per cent was recorded.

Hundred seed weight was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, panicle exertion, plant height, grain yield per plant, but negatively correlated with days to 50 per cent flowering, earhead length, number of leaves, stem girth, spicklets per head.

Grain yield per plant

Significant difference among the RILs was recorded along with significant G x E interaction forgrain yield per plant. The mean yield of 94.63 g ranged from 23.38 to 165.89 g was recorded; the per cent PCV and GCV values were 35.24 and 34.07 respectively. Very high heritability estimates of 93.47 per cent and GA over mean of 22.42 per cent were recorded.

Grain yield per plant was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, earhead length, number of leaves, stem girth, plant height, spicklets per head and 100 grain weight, but was found negative correlation with days to 50 per cent flowering and panicle exertion.

4.1.1.4 Pooled analysis of component traits of post-flowering drought tolerance and yield related traits in N13 × E36-1 derived RILs at Bheemarayanagudi location

Pooled analysis of variance was carried out and the estimates of variability parameters of post-flowering drought tolerance and yield related traits are presented in Table 12a to 12d. The frequency distribution patterns for all the traits are presented in Figure 7 and graphical presentation of PCV and GCV are shown in Figure 8. Analysis of variance and variability parameters were computed for Bheemarayanagudi location and presented in Appendices VIIa-VIIh.

Per cent GLA 15 DAF

Both mean performance and G x E interaction significantly differed among the RILs for per cent GLA 15 DAF at Bheemarayanagudi. The range from 46.30 to 96.41 per cent with mean of 79.90 was recorded. The per cent PCV and GCV of 8.99 and 6.31 per cent respectively with per cent heritability estimate of 36.07 and GA over mean of 8.02 per cent was recorded.

This trait was significantly and positively correlated with per cent GLA 30 DAF, per cent 45 DAF, CID, total green leaf area, number of leaves, spicklets per head, grain yield per plant but recorded negative correlation with days to 50 per cent flowering, panicle exertion, plant height and earhead length.

Per cent GLA of 30 DAF

RILs differed significantly among themselves for mean performance and G x E interaction. The mean value of 58.13 per cent and variability range of 17.73 to 88.81 per cent was recorded.

The per cent GCV and PCV of 16.25 and 16.18 respectively was recorded for this trait. The heritability estimate of 99.16 and GA over mean of 36.19 per cent were recorded at Bheemarayanagudi.

It was positively and significantly correlated with per cent GLA 15 DAF, per cent 45 DAF, CID, total green leaf area, number of leaves, spicklets per head, grain yield per plant but negatively correlated with days to 50 per cent flowering, panicle exertion, plant height and earhead length.

Per cent GLA 45 DAF

There was a significant difference among RILs for GLA at 45 days after flowering. Similarly G x E interaction was significant at Bheemarayanagudi. Mean value of 31.74 per cent and range of 18.31 to 80.30 per cent was recorded. The per cent PCV and GCV of 41.69 and 37.65 with heritability of 81.54 per cent was recorded. The GA over mean of 67.45 per cent was recorded.

Trait was positively and significantly correlated with per cent GLA 15 DAF, per cent 30 DAF, CID, total green leaf area, number of leaves, spicklets per head, grain yield per plant but negative correlated with days to 50 per cent flowering, panicle exertion, plant height and earhead length.

Days to 50 per cent flowering

RILs differed significantly with significant G x E interaction for days to 50 per cent flowering with range from 58.25 to 75.00 days with mean of 63.77 days was recorded. The GCV and PCV per cent of 10.13 and 10.74 was recorded respectively for this trait. The heritability estimate of 88.85 per cent and GA over mean of 16.35 per cent was recorded.

Days to 50 per cent flowering negatively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, total green leaf area, grain yield per plant but was found to be positively correlated with earhead length, number of leaves, spicklets per head, plant height and stem girth.

Total green leaf area

Analysis of variance showed significant G x E interaction and significant difference among the RILs for total green leaf area with mean area of 1400.41 $cm^2$ and range from 462.39 to 2090.82 $cm^2$. The per cent PCV and GCV of 17.12 and 9.88 respectively with per cent heritability of 33.32 and GA over mean of 3.81 per cent were recorded.

Total green leaf area was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, number of leaves, stem girth, spicklets per head, grain yield per plant but negatively correlated with days to 50 per cent flowering, earhead length, panicle exertion and plant height.

Earhead length

There was significant difference between the RILs with significant G x E interaction for length of head. GA over mean of 17.93 per cent and range from 10.13 to 34.36 cm with mean length of 18.01 cm was noted for this trait. The GCV and PCV of 12.81 and 19.25 per cent respectively with per cent heritability of 44.26 was recorded.

Earhead length was negatively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, stem girth, number of leaves but correlated positively with days to 50 per cent flowering, stem girth, plant height, spicklets per head and grain yield per plant.

Number of leaves

RILs differed significantly in their values with significant G x E interaction for number of leaves with mean value of 7.80 leaves and range of 5.00 to 10.50. The per cent PCV and GCV of 10.24 and 5.36 were recorded respectively with per cent heritability of 27.43. A very low genetic advance over mean of 7.56 per cent was recorded for this trait.

Number of leaves had positive correlation with plant height, total green leaf area, per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, grain yield per plant and negatively correlated with panicle exertion, stem girth and earhead length.

**Table 12a: Pooled analysis of variance of stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2008 and 2009 at Bheemarayanagudi location**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
| 1. | Replication | 3 | 13241.17 | 1970.37 | 971.76 | 3815.03 | 4553744.60 | 207.19 | 27.08 | 128.21 | 30.94 | 31754.51 | 1275.03 | 16.77 | 68.82 | 0.40 |
| 2. | Environment (E) | 1 | 4.09** | 36357.65** | 6305.17** | 1461.38** | 4192463.86** | 650.83** | 299.12** | 1235.48** | 119.71** | 102655.49** | 0.24** | 66.85** | 718.18** | 0.01* |
| 3. | Genotypes (G) | 225 | 752.43** | 1963.50** | 2500.50** | 285.11** | 285227.76** | 102.98** | 4.88** | 199.61** | 2.01** | 5856.40** | 695.46** | 1.94** | 954.08** | 0.13* |
| 4. | E x G | 225 | 8.64** | 1.09** | 1.67** | 0.11** | 205025.19** | 5.98** | 1.47** | 26.52** | 0.53** | 2331.45** | 0.59** | 0.05** | 5.27** | 0.01* |
| 5. | Error | 1353 | 130.58 | 1.39 | 32.16 | 1.92 | 66401.27 | 12.09 | 0.82 | 6.82 | 0.71 | 2026.06 | 21.50 | 0.34 | 9.80 | 0.13 |
| 6. | SEm± | | 2.98 | 0.43 | 2.89 | 1.05 | 99.10 | 1.30 | 0.35 | 1.57 | 0.30 | 17.58 | 1.15 | 0.18 | 1.00 | 0.26 |
| 7. | CD (1%) | | 10.84 | 1.56 | 10.53 | 3.83 | 361.03 | 4.73 | 1.26 | 5.72 | 1.10 | 64.05 | 4.19 | 0.65 | 3.64 | 0.96 |
| 8. | CD (5%) | | 8.25 | 1.19 | 8.02 | 2.91 | 274.70 | 3.60 | 0.96 | 4.35 | 0.84 | 48.73 | 3.19 | 0.49 | 2.77 | 1.73 |
| 9. | CV (%) | | 14.30 | 2.03 | 17.87 | 2.17 | 18.40 | 19.30 | 11.60 | 21.19 | 20.45 | 23.75 | 12.60 | 13.89 | 7.54 | 8.85 |

** - Significance at 1% probability level

$X_1$: % GLA 15 DAF
$X_2$: % GLA 30 DAF
$X_3$: % GLA 45 DAF
$X_4$: Days to 50 % flowering
$X_5$: Total GLA
$X_6$: Earhead length
$X_7$: Number of leaves
$X_8$: Panicle exertion
$X_9$: Stem girth
10: Plant height
$X_{11}$: Spicklets per head
$X_{12}$: 100 grain weight
$X_{13}$: Grain yield per plant
$X_{14}$: Carbon isotope discrimination

**Table 12b: Magnitude of genetic variability parameters of stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 × E36-1 during 2008 and 2009 at Bheemarayanagudi location**

| Sl. No. | Traits | Range | | Grand mean | PCV (%) | GCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Minimum | Maximum | | | | | |
| 1. | % GLA 15 DAF | 46.30 | 96.41 | 79.90 | 8.99 | 6.31 | 36.07 | 8.02 |
| 2. | % GLA 30 DAF | 17.73 | 88.81 | 58.13 | 16.25 | 16.18 | 99.16 | 36.19 |
| 3. | % GLA 45 DAF | 18.31 | 80.30 | 31.74 | 41.69 | 37.65 | 81.54 | 67.45 |
| 4. | Days to 50 % flowering | 58.25 | 75.00 | 63.77 | 10.74 | 10.13 | 88.85 | 16.35 |
| 5. | Total green leaf area ($cm^2$) | 462.39 | 2090.82 | 1400.41 | 17.12 | 9.88 | 33.32 | 3.81 |
| 6. | Earhead length (cm) | 10.13 | 34.36 | 18.01 | 19.25 | 12.81 | 44.26 | 17.93 |
| 7. | Number of leaves | 5.00 | 10.50 | 7.80 | 10.24 | 5.36 | 27.43 | 7.56 |
| 8. | Panicle exertion (cm) | 0.51 | 24.33 | 12.32 | 32.88 | 24.61 | 56.03 | 41.72 |
| 9. | Stem girth (cm) | 2.32 | 6.45 | 4.12 | 16.66 | 5.24 | 9.91 | 4.12 |
| 10. | Plant height (cm) | 123.67 | 267.42 | 189.53 | 20.17 | 10.06 | 20.17 | 8.15 |
| 11. | Spicklets per head | 12.50 | 58.40 | 36.80 | 18.80 | 17.72 | 88.82 | 36.41 |
| 12. | 100 grain weight (g) | 2.70 | 5.15 | 4.21 | 11.34 | 7.40 | 42.64 | 11.16 |
| 13. | Grain yield per plant (g/plant) | 22.41 | 164.73 | 93.57 | 25.39 | 24.81 | 95.53 | 20.78 |
| 14. | Carbon isotope discrimination | 3.19 | 5.14 | 3.92 | 8.73 | 3.32 | 1.02 | 20.66 |

**Table 12c: Phenotypic correlation coefficients (pooled) for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2008 and 2009 at Bheemarayanagudi location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.336** | 0.279** | 0.024 | -0.093 | 0.019 | -0.016 | 0.071 | -0.022 | 0.014 | 0.047 | 0.001 | 0.068 | 0.039 |
| $X_2$ | | 1 | 0.455** | 0.012 | -0.122 | 0.028 | -0.06 | 0.041 | -0.016 | 0.049 | 0.046 | 0.102 | 0.099 | 0.068 |
| $X_3$ | | | 1 | 0.055 | -0.025 | 0.048 | -0.011 | 0.054 | -0.001 | 0.040 | -0.022 | -0.007 | 0.014 | 0.067 |
| $X_4$ | | | | 1 | -0.054 | 0.064 | 0.032 | 0.041 | 0.046 | -0.021 | -0.016 | 0.027 | 0.016 | 0.014 |
| $X_5$ | | | | | 1 | -0.046 | 0.021 | 0.055 | 0.044 | 0.051 | 0.007 | 0.057 | -0.120 | -0.005 |
| $X_6$ | | | | | | 1 | -0.067 | 0.150* | -0.011 | 0.158* | -0.126* | 0.150* | 0.029 | 0.094 |
| $X_7$ | | | | | | | 1 | -0.009 | -0.148* | 0.007 | 0.042 | 0.005 | -0.013 | 0.031 |
| $X_8$ | | | | | | | | 1 | -0.076 | -0.110 | 0.025 | 0.033 | 0.083 | 0.117 |
| $X_9$ | | | | | | | | | 1 | -0.078 | 0.140* | -0.113 | -0.032 | -0.092 |
| $X_{10}$ | | | | | | | | | | 1 | -0.003 | 0.012 | 0.045 | 0.148* |
| $X_{11}$ | | | | | | | | | | | 1 | 0.038 | -0.041 | -0.057 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.016 | 0.203* |
| $X_{13}$ | | | | | | | | | | | | | 1 | 0.036 |
| $X_{14}$ | | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

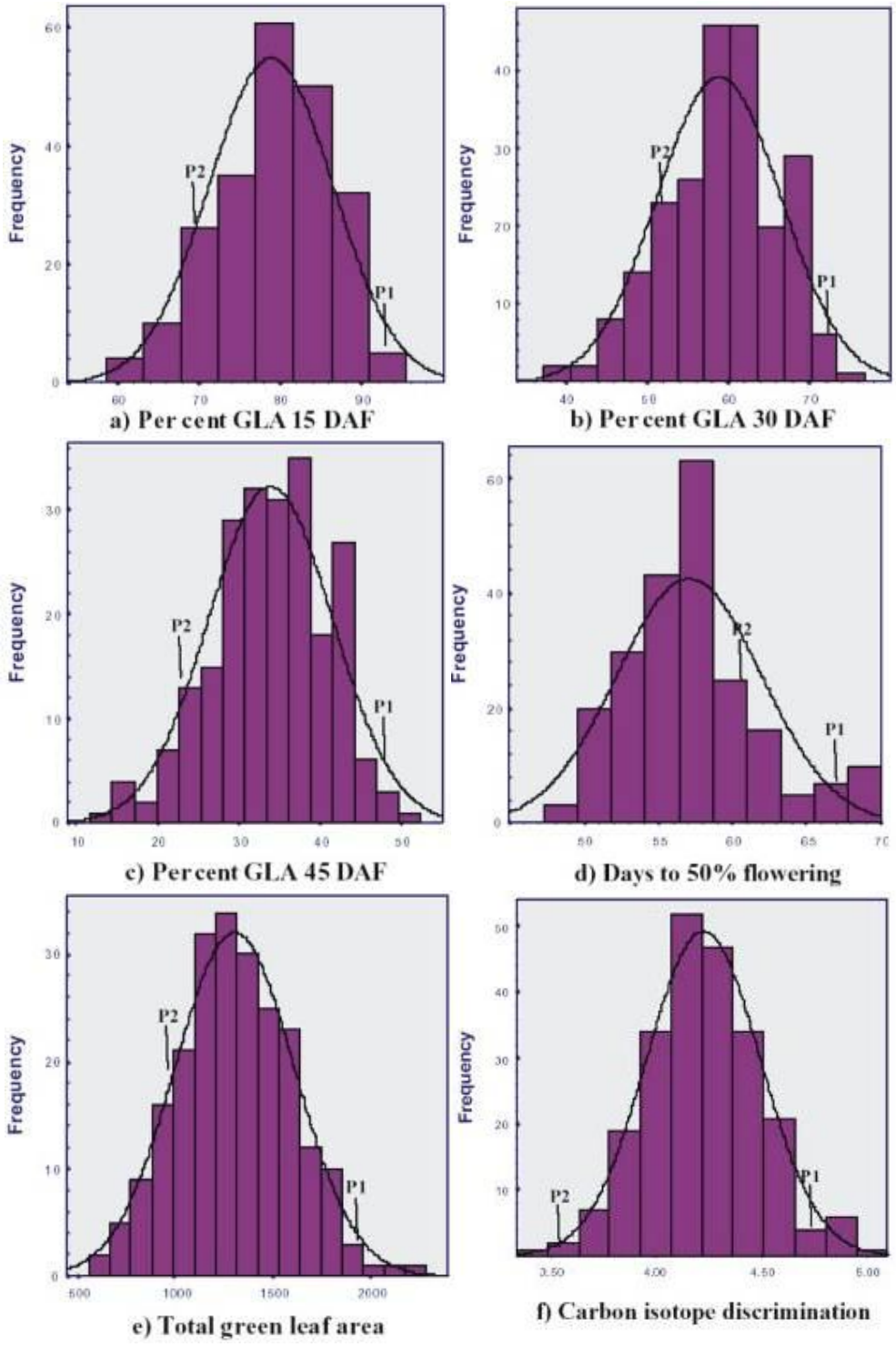
$X_1$: % GLA 15 DAF
$X_2$: % GLA 30 DAF
$X_3$: % GLA 45 DAF
$X_4$: Carbon isotope discrimination
$X_5$: Days to 50 % flowering
$X_6$: Total GLA
$X_7$: Earhead length
$X_8$: Number of leaves
$X_9$: Panicle exertion
$X_{10}$: Stem girth
$X_{11}$: Plant height
$X_{12}$: Spicklets per head
$X_{13}$: 100 grain weight
$X_{14}$: Grain yield per plant

**Table 12d: Genotypic correlation coefficients (pooled) for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2008 and 2009 at Bheemarayanagudi location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.564** | 0.474** | 0.142* | -0.171* | 0.068 | -0.014 | 0.253* | -0.048 | 0.17 | 0.045 | 0.013 | 0.187** | 0.056 |
| $X_2$ | | 1 | 0.508** | 0.063 | -0.128 | 0.045 | -0.09 | 0.085 | -0.024 | 0.107 | 0.092 | 0.110 | 0.132 | 0.07 |
| $X_3$ | | | 1 | 0.327** | -0.027 | 0.105 | -0.009 | 0.186* | -0.027 | 0.083 | -0.057 | -0.013 | 0.012 | 0.081 |
| $X_4$ | | | | 1 | -0.33** | 0.448** | 0.259* | 0.025 | 0.495** | -0.094 | -0.089 | 0.191* | 0.121 | -0.046 |
| $X_5$ | | | | | 1 | -0.077 | 0.008 | 0.027 | 0.066 | 0.102 | 0.013 | 0.068 | -0.21** | -0.007 |
| $X_6$ | | | | | | 1 | -0.058 | 0.308** | -0.036 | 0.644** | -0.251** | 0.243** | 0.033 | 0.154* |
| $X_7$ | | | | | | | 1 | -0.007 | -0.362** | 0.007 | 0.229** | -0.021 | -0.029 | 0.056 |
| $X_8$ | | | | | | | | 1 | -0.138* | -0.685** | 0.055 | 0.111 | 0.179* | 0.255** |
| $X_9$ | | | | | | | | | 1 | -0.323** | 0.378** | -0.183* | -0.031 | -0.112 |
| $X_{10}$ | | | | | | | | | | 1 | -0.319** | 0.131 | 0.105 | 0.59** |
| $X_{11}$ | | | | | | | | | | | 1 | 0.081 | -0.021 | -0.109 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.058 | 0.221** |
| $X_{13}$ | | | | | | | | | | | | | 1 | 0.045 |
| $X_{14}$ | | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

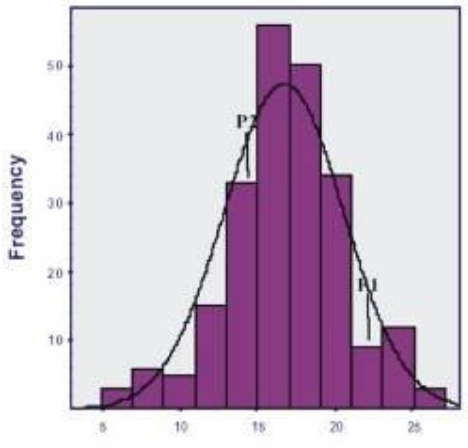
$X_1$: % GLA 15 DAF
$X_2$: % GLA 30 DAF
$X_3$: % GLA 45 DAF
$X_4$: Carbon isotope discrimination
$X_5$: Days to 50 % flowering
$X_6$: Total GLA
$X_7$: Earhead length
$X_8$: Number of leaves
$X_9$: Panicle exertion
$X_{10}$: Stem girth
$X_{11}$: Plant height
$X_{12}$: Spicklets per head
$X_{13}$: 100 grain weight
$X_{14}$: Grain yield per plant



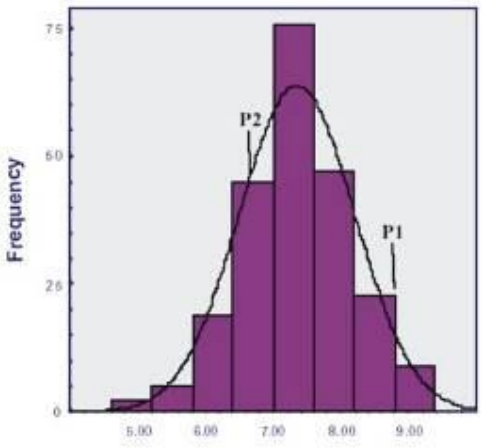

Legend : P1 – E36-1 P2-N13

**Fig. 7: Frequency distribution (pooled) of stay-green and yield related traits in recombinant inbred lines derived from cross N13 × E36-1 evaluated at Bheemarayanagudi location**

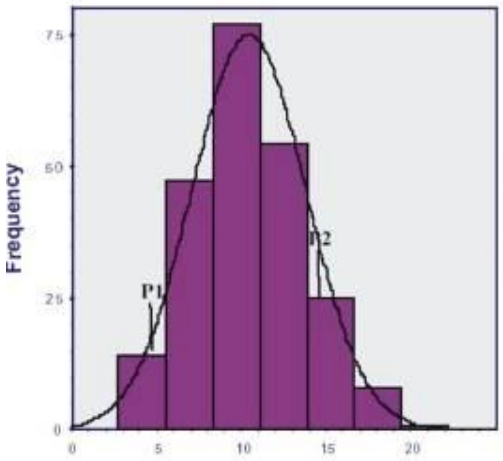
**Fig. 7 Contd….**



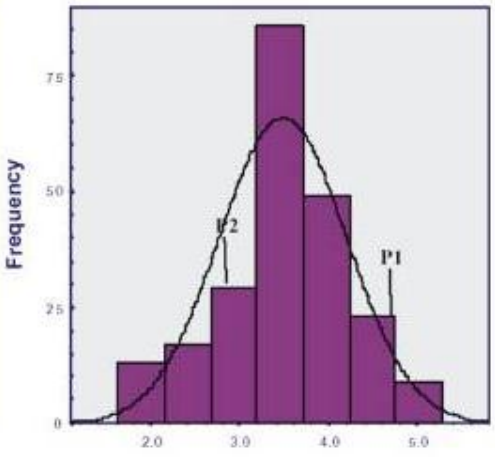

g) Earhead length




h) Number of leaves

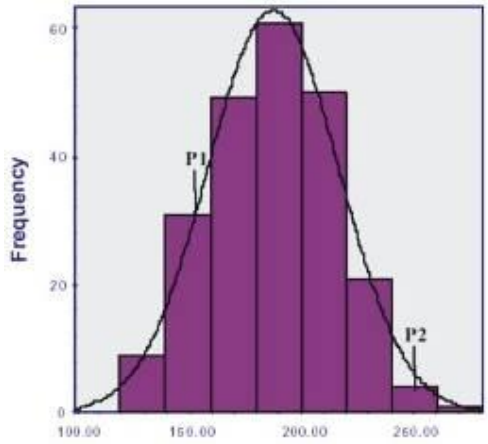



i) Panicle exertion

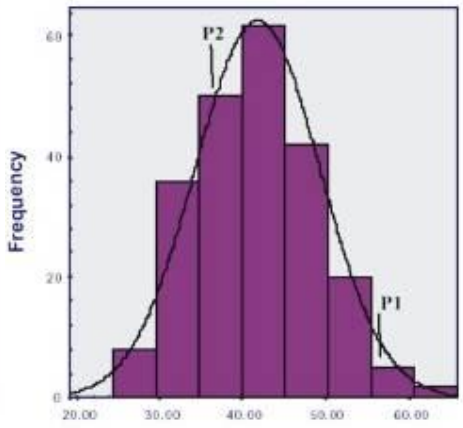



j) Stem girth

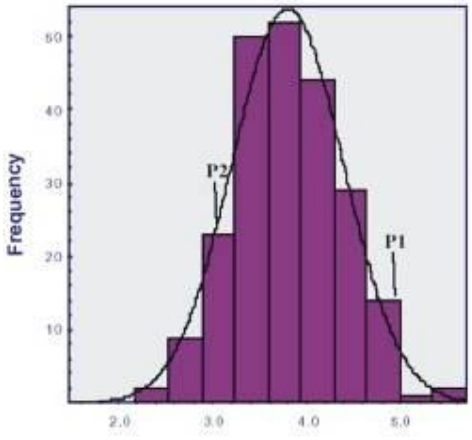
**Fig. 7 Contd…**



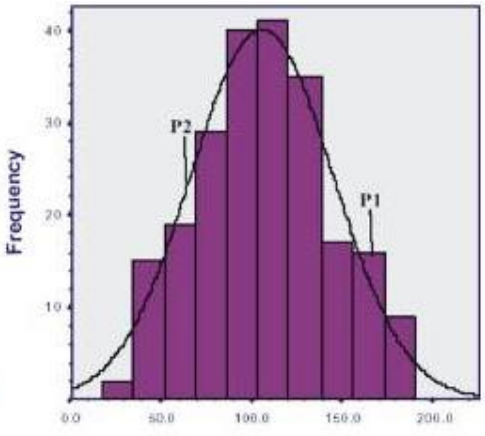

k) Plant height




l) Spicklets per head




m) 100 grain weight




n) Grain yield per plant

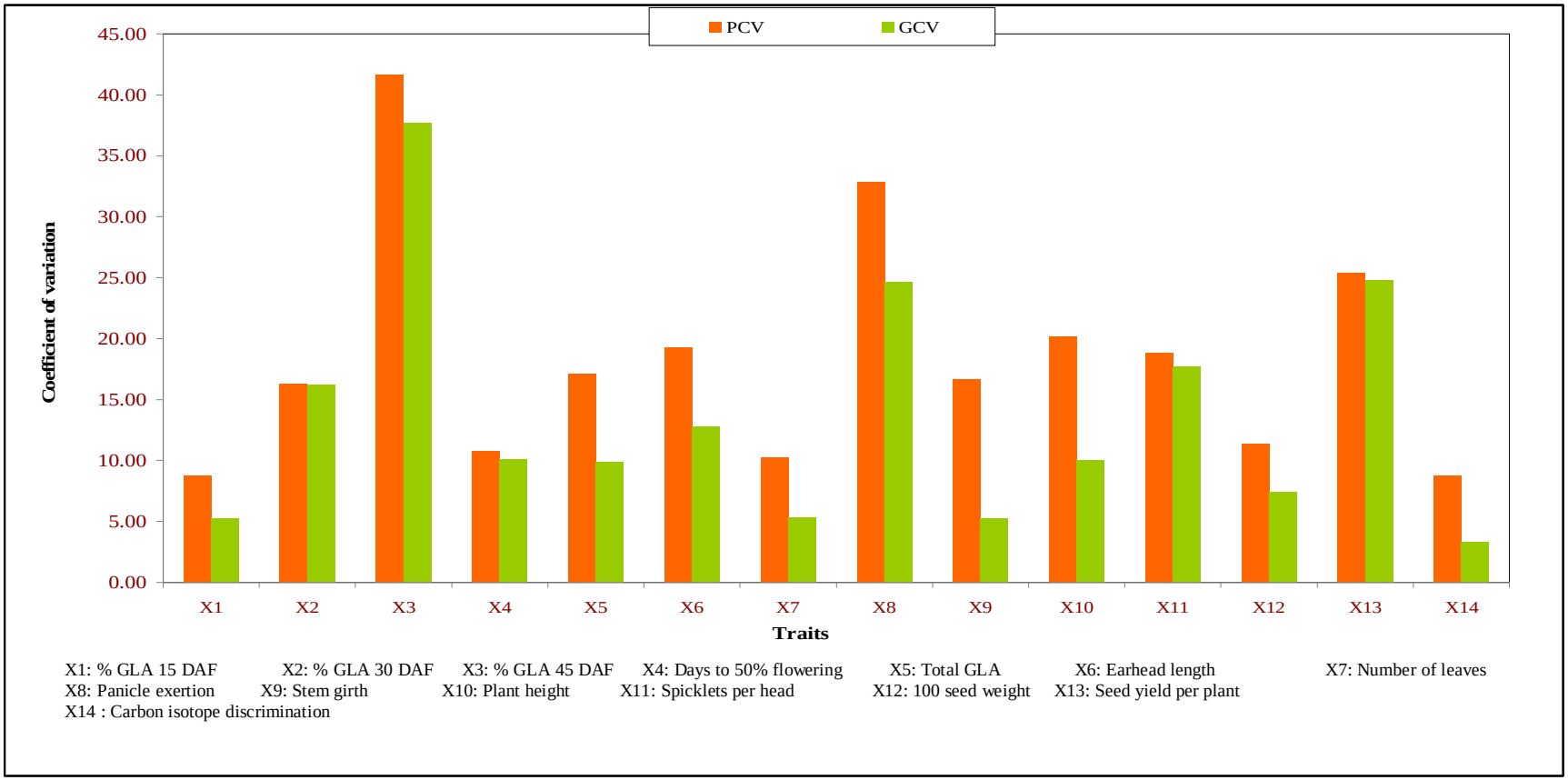



**Fig. 8: Pooled PCV and GCV for stay-green and yield related traits of recombinent inbred lines derived from cross N13 x E36-1 at Bheemarayanagudi location**

Panicle exertion

A significant G x E interaction and difference among RILs were observed for panicle exertion length with mean of 12.32 cm and range of 0.51 to 24.33 cm. The GCV and PCV per cent of 24.61 and 32.88 were recorded respectively with per cent heritability of 56.03 and 41.72 per cent of GA over mean.

Panicle exertion had positive correlation with plant height, days to 50 per cent flowering but correlated negatively with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, total green leaf area, grain yield per plant, stem girth and earhead length.

Stem girth

RILs differed significantly with significant G x E interaction for stem girth with mean girth of 4.12 and range from 2.32 to 6.45. The per cent PCV and GCV of 16.66 and 5.24 were recorded respectively. The heritability estimates of 9.91 per cent and GA over mean of 4.12 per cent were recorded for stem girth.

Stem girth had positive correlation with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, total green leaf area, grain yield per plant but negatively correlated with plant height, spicklets per head and number of leaves.

Plant height

Significant G x E interaction and significant difference among the RILs were recorded for height of the plant. The mean height of 189.53 cm and range from 123.67 to 267.42 cm were recorded. The per cent PCV and GCV values were of 20.17 and 10.06 respectively with heritability per cent of 20.17 for this trait. The GA over mean of 8.15 per cent was recorded.

Plant height correlated positively with per cent GLA 15 DAF, per cent 30 DAF, number of leaves, total green leaf area but negatively with stem girth, 100 grain weight and grain yield per plant.

Spicklets per head

High G x E interaction along with significant difference among the RILs were recorded for number of spicklets per head, the range of 12.50 to 58.40 spicklets with mean spicklets number of 36.80 was recorded. The per cent PCV and GCV of 18.80 and 17.72 respectively with heritability estimate of 88.82 per cent, the per cent GA over mean of 36.41 was recorded.

Spicklets per head has positive correlation with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, total green leaf area, panicle exertion, grain yield per plant but negatively correlated with stem girth, plant height and panicle exertion.

100 grain weight

RILs differed significantly for 100 grain weight with significant G x E interaction. The trait ranged from 2.70 to 5.15 g with mean weight of 4.21 g. The PCV and GCV of 11.34 and 7.40 per cent with per cent heritability of 42.64 were noted for this trait.

100 grain weight has positive correlation with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, stem girth, spicklets per head, grain yield per plant but correlated negatively with panicle exertion, days to 50 per cent flowering and plant height.

Grain yield per plant

Significant difference among the RILs was recorded forgrain yield per plant and G x E interaction with mean weight of 93.57 g. The range for this trait was from 22.41 to 164.73 g per plant. The per cent GCV and PCV of 24.81 and 25.39 were recorded respectively with heritability estimate of 95.53 and GA over per cent mean of 20.78 was recorded.

Grain yield per plant was positively correlated with per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, CID, spicklets per head, 100 grain weight, but correlated negatively with days to 50 per cent flowering, panicle exertion and plant height.

Carbon isotope discrimination

RILs differed significantly for CID with value ranged from 3.19 to 5.14 with mean CID value of 3.92. The per cent PCV and GCV of 8.73 and 3.32 were recorded respectively. Heritability estimate of 1.02 and GA over per cent mean of 20.66 per cent.

CID positively correlated with the trait recorded for per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, spicklets per head, 100 grain weight, grain yield per plant and contrary negatively correlated with days to 50 per cent flowering, earhead length, panicle exertion, stem girth.

## 4.2 Mapping of DNA markers and construction of genetic linkage maps

### 4.2.1 Parental polymorphism for nuclear and genic SSRs markers

In the present study, a set of 530 genic SSRs with annotated functions developed in house from genomic resources of sorghum, about 270 nuclear SSRs selected from published genetic linkage maps of sorghum and a set of 130 single nucleotide polymorphism markers representing a larger set of SNP markers recently developed at IABT, were used to screen the parents of both the RIPs, IS9830 x E36-1 and N13 x E36-1 to identify the polymorphic ones. PCR amplification of individual nuclear and genic SSR markers was done and fractioned first on 2.5 per cent agarose (Plate 3a) followed by 6 per cent PAGE gels (Plate 3b). On PAGE gels alleles were visualized through silver staining procedure. The markers found monomorphic on agarose were taken through the PAGE gels to check if any of they could exhibit polymorphism between parents.

In RIP1 out of 530 genic SSRs 40 markers and out of 270 nuclear SSR 71 markers were shown polymorphism between parents (Table 13a & 13b) and were genotyped across RIL and the pattern of segregation of marker on agarose and PAGE is presented in Plate 4a and 4b respectively. In RIP2, out of 530 genic SSR 68 markers and out of 270, 70 markers showed polymorphism between parents. The polymorphic markers were genotyped across the RILs of mapping population and the pattern of segregation of marker on agarose and PAGE is presented in Plate 5a and 5b respectively. And gels were scored into excel format suitable for QTL mapping.

### 4.2.2 Segregation of nuclear and genic SSR markers

During genotyping, banding pattern obtained across RILs was scored against respective parents and noted. Marker segregation pattern of all the polymorphic SSR markers in RIP1 and RIP2 was checked for their fitness to expected 1:1 ratio. The calculated $\chi^2$ values for all the markers genotyped in RIP1 and RIP2 are presented in Table 14 and 15 respectively. The calculated $\chi^2$ values were computed for each marker locus.

### 4.2.3 Construction of genetic linkage maps

Mapping populations used for construction of linkage maps are recombinant inbred line populations derived from IS9830 x E36-1 (RIP1) and N13 x E36-1 (RIP2) cross. In both the populations, 46 anchor marker data was included along with genotyped markers. The scored genotypic data of 134 markers in RIP1 and 160 markers in RIP2 were used for assigning the linkage positions and construction of linkage map using MAPMAKER/EXP (3.0) software (Londer *et al.*, 1987). Linkage distance in terms of centimorghan (cM) values was calculated using Kosambi mapping function. A total of 134 markers in RIP1 and 160 markers in RIP2 were associated among themselves with varied distances on ten linkage groups; the details are given in Table 16 and 17.

In RIP1, all 134 markers mapped on to the ten linkage group *viz.,* LG-A, LG-B, LG-C, LG-D, LG-E, LG-F, LG-G, LG-H, LG-I and LG-J the genetic map had a total length of 1661.1 cM distributed over ten linkage groups (Fig. 9), with number of markers assigned to linkage group were 15, 23, 16, 17, 15, 6, 16, 7, 8 and 11. The number of markers mapped per linkage group ranged from 6 in LG-F to 23 in LG-B with average distance covered of 12.39 cM. The highest and lowest distances covered were between 77.6 cM on LG-D flanked by Xsnp83 – Xiabt353 and 1.7 cM on LG-A flanked by Xtxp248 – Xtxp340 markers respectively.


**Plate 3a: Parental survey for polymorphic SSR markers on 2.5% agarose stained with Ethidium bromide**

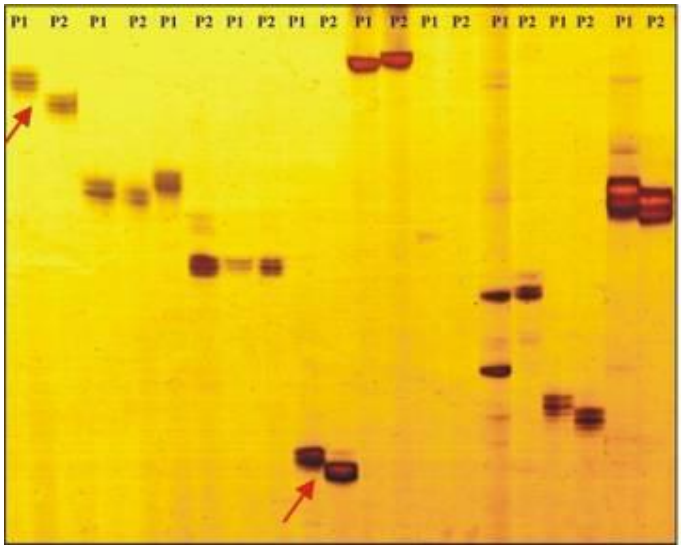



**Plate 3b: Parental survey for polymorphic SSR markers on 6% PAGE with silver staining**

**Table 13a: Summary statistics of all markers used to develop a genetic linkage map of RIP1 derived from the cross IS9830 × E36-1**

| Primer type | Total | Primers shown polymorphism | % polymorphism | Primers genotyped |
|---|---|---|---|---|
| Genic SSR | 530 | 40 | 7.55 | 40 |
| Nuclear SSR | 270 | 71 | 26.30 | 26 |
| Total | 800 | 111 | 33.85 | 66 |

**Table 13b: Summary statistics of all markers used to develop a genetic linkage map of RIP2 derived from the cross N13 × E36-1**

| Primer type | Total | Primers shown polymorphism | % polymorphism | Primers genotyped |
|---|---|---|---|---|
| Genic SSR | 530 | 68 | 12.83 | 68 |
| Nuclear SSR | 270 | 70 | 25.93 | 25 |
| Total | 800 | 138 | 38.76 | 93 |

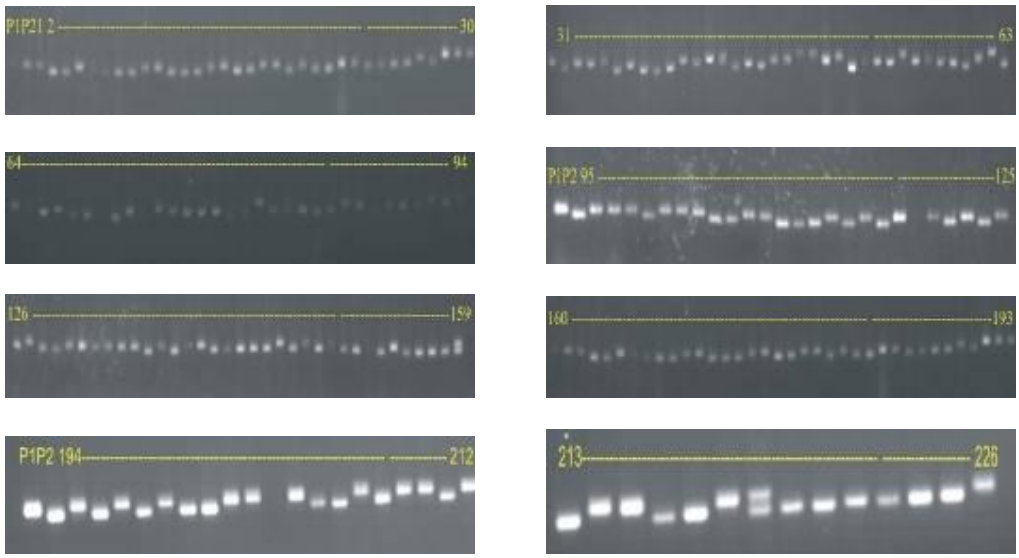



**Plate 4a: Segregation pattern of Xtxp73 marker across RIP1 on 2.5% agarose gel with Ethidium bromide staining (P1 : E36-1; P2 : IS9830; 1-226 : RILs derived from cross IS9830 x E36-1)**

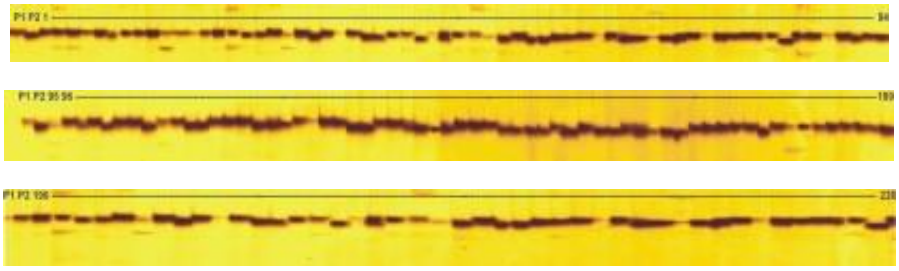


**Plate 4b: Segregation pattern of Xiabt224 marker across RIP1 on 6 % PAGE gel with silver staining (P1 : E36-1; P2 : IS9830; 1-226 : RILs derived from cross IS9830 x E36-1)**

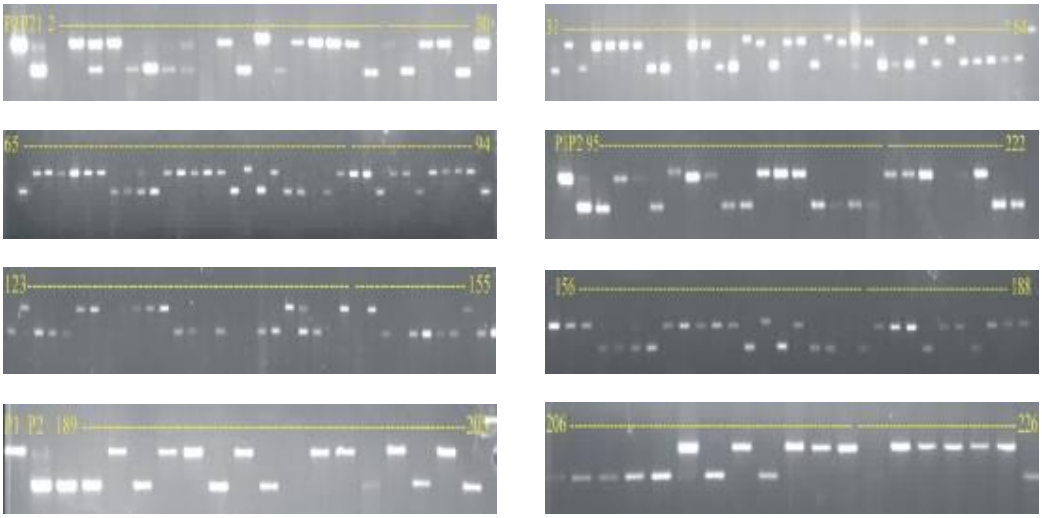


**Plate 5a: Segregation pattern of Xtxp50 marker across RIP2 on 2.5% agarose gel with Ethidium bromide staining (P1 : E36-1; P2 : N13; 1-226 : RILs derived from cross N13 x E36-1)**

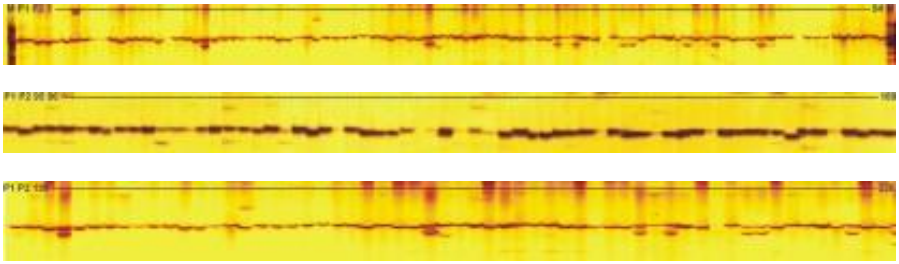


**Plate 5b: Segregation pattern of Xiabt450 marker across RIP2 on 6 % PAGE gel with silver staining (P1 : E36-1; P2 : N13; 1-226 : RILs derived from cross N13 x E36-1)**

**Table 14: Test of significance for segregation of polymorphic genic, nuclear SSR and SNP markers in RILs derived from the cross IS9830 x E36-1**

| Sl. No. | Marker | P1 allele | P2 allele | $\chi^2$ | Sl. No. | Marker | P1 allele | P2 allele | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Xtxp250 | 105 | 121 | 1.133** | 46 | Xiabt426 | 101 | 125 | 2.549** |
| 2 | Xtxp307 | 116 | 110 | 0.159** | 47 | Xiabt377 | 102 | 124 | 2.142** |
| 3 | Xtxp34 | 119 | 107 | 0.637** | 48 | Xiabt454 | 111 | 115 | 0.071** |
| 4 | Xtxp285 | 114 | 112 | 0.018** | 49 | Xiabt343 | 109 | 104 | 0.858** |
| 5 | Xtxp297 | 122 | 104 | 1.434** | 50 | Xiabt430 | 112 | 108 | 0.230** |
| 6 | Xtxp207 | 110 | 116 | 0.159** | 51 | Xiabt51 | 104 | 112 | 0.726** |
| 7 | Xtxp14 | 116 | 110 | 0.159** | 52 | Xiabt195 | 99 | 116 | 1.814** |
| 8 | Xtxp160 | 112 | 114 | 0.018** | 53 | Xiabt184 | 104 | 112 | 0.726** |
| 9 | Xtxp22 | 104 | 121 | 1.283** | 54 | Xiabt176 | 114 | 112 | 0.018** |
| 10 | Xtxp35 | 104 | 116 | 0.796** | 55 | Xiabt113 | 110 | 116 | 0.159** |
| 11 | Xtxp270 | 110 | 115 | 0.115** | 56 | Xiabt147 | 108 | 110 | 0.301** |
| 12 | Xtxp73 | 123 | 103 | 1.770** | 57 | Xiabt342 | 102 | 110 | 1.150** |
| 13 | Xtxp205 | 110 | 116 | 0.159** | 58 | Xiabt399 | 104 | 122 | 1.434** |
| 14 | Xtxp196 | 107 | 119 | 0.637** | 59 | Xiabt438 | 113 | 113 | 0.000** |
| 15 | Xtxp298 | 105 | 120 | 1.000** | 60 | Xiabt95 | 114 | 103 | 0.894** |
| 16 | Xtxp196 | 111 | 114 | 0.044** | 61 | Xiabt187 | 110 | 116 | 0.159** |
| 17 | Xtxp298 | 110 | 116 | 0.159** | 62 | Xiabt312 | 103 | 105 | 1.451** |
| 18 | Xtxp58 | 107 | 112 | 0.327** | 63 | Xiabt302 | 100 | 125 | 2.770** |
| 19 | Xtxp6 | 110 | 102 | 1.150** | 64 | Xiabt111 | 118 | 106 | 0.655** |
| 20 | Xtxp201 | 100 | 120 | 1.929** | 65 | Xiabt364 | 115 | 103 | 0.920** |
| 21 | Xtxp100 | 112 | 108 | 0.230** | 66 | Xiabt146 | 111 | 115 | 0.071** |
| 22 | Xtxp8 | 112 | 114 | 0.018** | 67 | Xsnp48 | 112 | 108 | 0.230** |
| 23 | Xtxp50 | 110 | 116 | 0.159** | 68 | Xsnp104 | 107 | 117 | 0.460** |
| 24 | Xtxp10 | 115 | 111 | 0.071** | 69 | Xsnp135 | 103 | 122 | 1.602** |
| 25 | Xtxp47 | 113 | 113 | 0.000** | 70 | Xsnp57 | 107 | 117 | 0.460** |
| 26 | Xtxp65 | 103 | 105 | 1.451** | 71 | Xsnp133 | 124 | 101 | 2.345** |
| 27 | Xiabt303 | 108 | 115 | 0.257** | 72 | Xsnp19 | 105 | 117 | 0.708** |
| 28 | Xiabt32 | 111 | 115 | 0.071** | 73 | Xsnp130 | 120 | 105 | 1.000** |
| 29 | Xiabt445 | 112 | 113 | 0.009** | 74 | Xsnp41 | 117 | 104 | 0.858** |
| 30 | Xiabt224 | 109 | 117 | 0.283** | 75 | Xsnp127 | 107 | 117 | 0.460** |
| 31 | Xiabt323 | 112 | 114 | 0.018** | 76 | Xsnp53 | 104 | 116 | 0.796** |
| 32 | Xiabt371 | 118 | 105 | 0.788** | 77 | Xsnp36 | 110 | 115 | 0.115** |
| 33 | Xiabt422 | 104 | 116 | 0.796** | 78 | Xsnp56 | 108 | 107 | 0.540** |
| 34 | Xiabt509 | 110 | 120 | 0.513** | 79 | Xsnp83 | 109 | 117 | 0.283** |
| 35 | Xiabt60 | 104 | 121 | 1.283** | 80 | Xsnp49 | 121 | 105 | 1.133** |
| 36 | Xiabt397 | 121 | 105 | 1.133** | 81 | Xsnp33 | 115 | 111 | 0.071** |
| 37 | Xiabt356 | 115 | 111 | 0.071** | 82 | Xsnp31 | 105 | 113 | 0.566** |
| 38 | Xiabt94 | 110 | 116 | 0.159** | 83 | Xsnp91 | 109 | 117 | 0.283** |
| 39 | Xiabt332 | 105 | 121 | 1.133** | 84 | Xsnp117 | 101 | 125 | 2.549** |
| 40 | Xiabt370 | 105 | 113 | 0.566** | 85 | Xsnp67 | 119 | 107 | 0.637** |
| 41 | Xiabt352 | 109 | 117 | 0.283** | 86 | Xsnp96 | 123 | 103 | 1.770** |
| 42 | Xiabt227 | 103 | 123 | 1.770** | 87 | Xsnp78 | 100 | 120 | 1.929** |
| 43 | Xiabt54 | 102 | 119 | 1.389** | 88 | Xsnp54 | 107 | 119 | 0.637** |
| 44 | Xiabt358 | 105 | 121 | 1.133** | 89 | Xsnp28 | 105 | 120 | 1.000** |
| 45 | Xiabt439 | 109 | 117 | 0.283** | | | | | |

** - Significant at 1% level of probability

**Table 15: Test of significance for segregation of polymorphic genic, nuclear SSR and SNP markers in RILs derived from the cross N13 x E36-1**

| Sl. No. | Primer No | P1 allele | P2 allele | $\chi^2$ | Sl. No. | Primer No | P1 allele | P2 allele | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Xtxp250 | 117 | 109 | 0.283** | 41 | Xiabt388 | 101 | 125 | 2.549** |
| 2 | Xtxp183 | 109 | 104 | 0.858** | 42 | Xiabt232 | 102 | 124 | 2.142** |
| 3 | Xtxp34 | 109 | 129 | 2.407** | 43 | Xiabt444 | 111 | 130 | 2.593** |
| 4 | Xtxp285 | 114 | 112 | 0.018** | 44 | Xiabt82 | 107 | 119 | 0.637** |
| 5 | Xtxp1 | 104 | 116 | 0.796** | 45 | Xiabt241 | 111 | 115 | 0.071** |
| 6 | Xtxp211 | 111 | 115 | 0.071** | 46 | Xiabt477 | 120 | 97 | 2.699** |
| 7 | XtxpK1f2 | 119 | 107 | 0.637** | 47 | Xiabt177 | 104 | 112 | 0.726** |
| 8 | Xtxp73 | 105 | 120 | 1.000** | 48 | Xiabt321 | 109 | 117 | 0.283** |
| 9 | Xtxp31 | 112 | 108 | 0.230** | 49 | Xiabt455 | 112 | 114 | 0.018** |
| 10 | Xtxp104 | 110 | 102 | 1.150** | 50 | Xiabt31 | 110 | 116 | 0.159** |
| 11 | Xtxp303 | 100 | 120 | 1.929** | 51 | Xiabt450 | 110 | 116 | 0.159** |
| 12 | Xtxp71 | 115 | 111 | 0.071** | 52 | Xiabt370 | 112 | 108 | 0.230** |
| 13 | Xtxp210 | 116 | 110 | 0.159** | 53 | Xiabt51 | 116 | 110 | 0.159** |
| 14 | Xtxp200 | 104 | 112 | 0.726** | 54 | Xiabt207 | 114 | 103 | 0.894** |
| 15 | Xtxp213 | 118 | 108 | 0.442** | 55 | Xiabt234 | 110 | 116 | 0.159** |
| 16 | Xtxp296 | 114 | 112 | 0.018** | 56 | Xiabt353 | 103 | 105 | 1.451** |
| 17 | Xtxp205 | 102 | 124 | 2.142** | 57 | Xiabt227 | 110 | 116 | 0.159** |
| 18 | Xtxp231 | 106 | 120 | 0.867** | 58 | Xiabt79 | 106 | 120 | 0.867** |
| 19 | Xtxp132 | 110 | 116 | 0.159** | 59 | Xiabt353 | 103 | 105 | 1.451** |
| 20 | Xtxp159 | 104 | 122 | 1.434** | 60 | Xiabt147 | 111 | 115 | 0.071** |
| 21 | Xtxp45 | 114 | 96 | 2.566** | 61 | Xiabt364 | 107 | 117 | 0.460** |
| 22 | Xtxp298 | 112 | 114 | 0.018** | 62 | Xiabt466 | 103 | 122 | 1.602** |
| 23 | Xtxp282 | 107 | 119 | 0.637** | 63 | Xiabt134 | 115 | 111 | 0.071** |
| 24 | Xtxp324 | 104 | 122 | 1.434** | 64 | Xiabt187 | 117 | 104 | 0.858** |
| 25 | Xtxp15 | 124 | 102 | 2.142** | 65 | Xiabt377 | 105 | 121 | 1.133** |
| 26 | Xiabt71 | 110 | 116 | 0.159** | 66 | Xiabt215 | 110 | 116 | 0.159** |
| 27 | Xiabt445 | 99 | 116 | 1.814** | 67 | Xiabt18 | 113 | 113 | 0.000** |
| 28 | Xiabt358 | 114 | 112 | 0.018** | 68 | Xiabt511 | 102 | 124 | 2.142** |
| 29 | Xiabt243 | 118 | 108 | 0.442** | 69 | Xiabt32 | 111 | 115 | 0.071** |
| 30 | Xiabt263 | 109 | 117 | 0.283** | 70 | Xiabt430 | 110 | 116 | 0.159** |
| 31 | Xiabt451 | 112 | 114 | 0.018** | 71 | Xiabt78 | 110 | 116 | 0.159** |
| 32 | Xiabt350 | 104 | 110 | 0.796** | 72 | Xiabt521 | 113 | 113 | 0.000** |
| 33 | Xiabt206 | 106 | 120 | 0.867** | 73 | Xiabt437 | 111 | 115 | 0.071** |
| 34 | Xiabt435 | 116 | 110 | 0.159** | 74 | Xiabt341 | 113 | 113 | 0.000** |
| 35 | Xiabt424 | 111 | 115 | 0.071** | 75 | Xiabt178 | 124 | 102 | 2.142** |
| 36 | Xiabt54 | 114 | 112 | 0.018** | 76 | Xiabt302 | 101 | 125 | 2.549** |
| 37 | Xiabt72 | 105 | 121 | 1.133** | 77 | Xiabt488 | 116 | 110 | 0.159** |
| 38 | Xiabt509 | 110 | 115 | 0.115** | 78 | Xiabt294 | 110 | 115 | 0.115** |
| 39 | Xiabt80 | 107 | 119 | 0.637** | 79 | Xiabt267 | 108 | 107 | 0.540** |
| 40 | Xiabt73 | 105 | 121 | 1.133** | 80 | Xiabt380 | 112 | 114 | 0.018** |

*Contd……*

| Sl. No. | Primer No | P1 allele | P2 allele | $\chi^2$ | Sl. No. | Primer No | P1 allele | P2 allele | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|---|
| 81 | Xiabt310 | 104 | 116 | 0.796** | 99 | Xsnp41 | 103 | 123 | 1.770** |
| 82 | Xiabt298 | 112 | 114 | 0.018** | 100 | Xsnp127 | 119 | 106 | 0.752** |
| 83 | Xiabt70 | 122 | 104 | 1.434** | 101 | Xsnp58 | 118 | 108 | 0.442** |
| 84 | Xiabt86 | 110 | 116 | 0.159** | 102 | Xsnp53 | 109 | 117 | 0.283** |
| 85 | Xiabt143 | 108 | 118 | 0.442** | 103 | Xsnp36 | 110 | 116 | 0.159** |
| 86 | Xiabt29 | 109 | 117 | 0.283** | 104 | Xsnp56 | 115 | 111 | 0.071** |
| 87 | Xiabt14 | 114 | 112 | 0.018** | 105 | Xsnp83 | 112 | 114 | 0.018** |
| 88 | Xiabt148 | 120 | 106 | 0.867** | 106 | Xsnp108 | 110 | 116 | 0.159** |
| 89 | Xiabt301 | 122 | 104 | 1.434** | 107 | Xsnp33 | 103 | 105 | 1.451** |
| 90 | Xiabt338 | 108 | 118 | 0.442** | 108 | Xsnp31 | 111 | 115 | 0.071** |
| 91 | Xiabt58 | 108 | 118 | 0.442** | 109 | Xsnp91 | 115 | 111 | 0.071** |
| 92 | Xiabt16 | 110 | 116 | 0.159** | 110 | Xsnp117 | 118 | 108 | 0.442** |
| 93 | Xiabt146 | 105 | 113 | 0.566** | 111 | Xsnp67 | 107 | 119 | 0.637** |
| 94 | Xsnp48 | 109 | 117 | 0.283** | 112 | Xsnp96 | 124 | 102 | 2.142** |
| 95 | Xsnp104 | 105 | 121 | 1.133** | 113 | Xsnp78 | 110 | 116 | 0.159** |
| 96 | Xsnp135 | 122 | 104 | 1.434** | 114 | Xsnp54 | 101 | 125 | 2.549** |
| 97 | Xsnp28 | 114 | 112 | 0.018** | 115 | Xsnp57 | 109 | 117 | 0.283** |
| 98 | Xsnp19 | 121 | 105 | 1.133** | | | | | |

P value = <0.01 >=3.81
** - Significant at 1% level of probability

**Table 16: Distance (cM) between pair of markers mapped to different linkage groups of IS9830 × E36-1 derived recombinant inbred lines**

| Sl. No. | LG | Marker interval | Distance (cM) | Sl. No. | LG | Marker interval | Distance (cM) |
|---|---|---|---|---|---|---|---|
| 1 | A | Xtxp248-Xtxp340 | 1.7 | 1 | C | Xtxp228-Xtxp14 | 22.8 |
| 2 | | Xtxp340-Xtxp61 | 6.3 | 2 | | Xtxp14-Xiumc063 | 6.8 |
| 3 | | Xtxp61-Xtxp37 | 16.4 | 3 | | Xiumc063-Xsnp57 | 5.5 |
| 4 | | Xtxp37-Xtxp88 | 11.4 | 4 | | Xsnp57-Xsnp133 | 16.2 |
| 5 | | Xtxp88-Xiabt303 | 12.9 | 5 | | Xsnp133-Xtxp33 | 43.6 |
| 6 | | Xiabt303-Xsnp48 | 10.7 | 6 | | Xtxp33-Xtxp38 | 21.3 |
| 7 | | Xsnp48-Xtxp250 | 8.3 | 7 | | Xtxp38-Xsnp19 | 13.3 |
| 8 | | Xtxp250-Xsnp104 | 9.4 | 8 | | Xsnp19-Xsnp130 | 7.9 |
| 9 | | Xsnp104-Xtxp40 | 8.8 | 9 | | Xsnp130-Xiabt370 | 14.1 |
| 10 | | Xtxp40-Xtxp307 | 7.4 | 10 | | Xiabt370-Xtxp71 | 9.6 |
| 11 | | Xtxp307-Xtxp34 | 8.3 | 11 | | Xtxp71-Xsnp41 | 8.3 |
| 12 | | Xtxp34-Xtxp285 | 4.6 | 12 | | Xsnp41-Xtxp160 | 9.6 |
| 13 | | Xtxp285-Xiabt32 | 5.2 | 13 | | Xtxp160-Xtxp22 | 6.8 |
| 14 | | Xiabt32-Xtxp208 | 9.4 | 14 | | Xtxp22-Xsnp127 | 9.3 |
| 1 | B | Xtxp297-Xtxp197 | 22.1 | 15 | | Xsnp127-Xiabt352 | 12.5 |
| 2 | | Xtxp197-Xtxp207 | 7.8 | 1 | D | Xtxp41-Xsnp53 | 15.8 |
| 3 | | Xtxp207-Xtxp331 | 19.9 | 2 | | Xsnp53-Xtxp327 | 13.6 |
| 4 | | Xtxp331-Xiabt445 | 9.2 | 3 | | Xtxp327-Xisp229 | 3.8 |
| 5 | | Xiabt445-Xtxp201 | 29.5 | 4 | | Xisp229-Xsnp36 | 11.5 |
| 6 | | Xtxp201-Xiumc136 | 2.6 | 5 | | Xsnp36-Xtxp27 | 9.5 |
| 7 | | Xiumc136-Xsnp28 | 19.6 | 6 | | Xtxp27-Xiabt227 | 11.1 |
| 8 | | Xsnp28-Xtxp100 | 21.4 | 7 | | Xiabt227-Xsnp56 | 12.8 |
| 9 | | Xtxp100-Xtxp8 | 7.4 | 8 | | Xsnp56-Xiabt54 | 16.4 |
| 10 | | Xtxp8-Xiabt224 | 7.5 | 9 | | Xiabt54-Xsnp83 | 42.2 |
| 11 | | Xiabt224-Xiabt323 | 12.8 | 10 | | Xsnp83-Xiabt353 | 77.6 |
| 12 | | Xiabt323-Xsnp135 | 28.4 | 11 | | Xiabt353-Xtxp35 | 21.5 |
| 13 | | Xsnp135-Xiabt371 | 22.6 | 12 | | Xtxp35-Xtxp270 | 13.5 |
| 14 | | Xiabt371-Xiabt422 | 24.9 | 13 | | Xtxp270-Xiabt358 | 10.3 |
| 15 | | Xiabt422-Xiabt509 | 12.4 | 14 | | Xiabt358-Xiabt439 | 12.2 |
| 16 | | Xiabt509-Xiabt60 | 15.4 | 15 | | Xiabt439-Xsnp49 | 12.5 |
| 17 | | Xiabt60-Xiabt397 | 15 | 16 | | Xsnp49-Xiabt426 | 13.2 |
| 18 | | Xiabt397-Xiabt356 | 47.6 | | | | |
| 19 | | Xiabt356-Xiabt94 | 8.3 | | | | |
| 20 | | Xiabt94-Xtxp50 | 16 | | | | |
| 21 | | Xtxp50-Xiabt332 | 5.2 | | | | |
| 22 | | Xiabt332-Xtxp96 | 5.2 | | | | |

*Contd…….*

| Sl. No. | LG | Marker interval | Distance (cM) | Sl. No. | LG | Marker interval | Distance (cM) |
|---|---|---|---|---|---|---|---|
| 1 | E | Xisp344-Xisu074 | 3.1 | 1 | H | Xtxp273-Xtxp47 | 4.7 |
| 2 | | Xisu074-Xisp362 | 6 | 2 | | Xtxp47-Xtxp18 | 3.6 |
| 3 | | Xisp362-Xiumc085 | 2.6 | 3 | | Xtxp18-Xtxp105 | 13.4 |
| 4 | | Xiumc085-Xtxp372 | 15.6 | 4 | | Xtxp105-Xiabt399 | 18.3 |
| 5 | | Xtxp372-Xtxp159 | 15.3 | 5 | | Xiabt399-Xtxp298 | 13.1 |
| 6 | | Xtxp159-Xiabt377 | 22.9 | 6 | | Xtxp298-Xtxp58 | 10.7 |
| 7 | | Xiabt377-Xsnp33 | 15.4 | 1 | I | Xiumc044-Xtxp176 | 2.7 |
| 8 | | Xsnp33-Xiabt454 | 12.2 | 2 | | Xtxp176-Xtxp57 | 2.7 |
| 9 | | Xiabt454-Xtxp73 | 15.7 | 3 | | Xtxp57-Xsnp78 | 5.7 |
| 10 | | Xtxp73-Xtxp295 | 7 | 4 | | Xsnp78-Xtxp145 | 13.6 |
| 11 | | Xtxp295-Xtxp168 | 3.2 | 5 | | Xtxp145-Xtxp6 | 11.8 |
| 12 | | Xtxp168-Xtxp231 | 5.4 | 6 | | Xtxp6-Xiabt438 | 9.8 |
| 13 | | Xtxp231-Xtxp205 | 7.1 | 7 | | Xiabt438-Xiabt95 | 10.1 |
| 14 | | Xtxp205-Xiabt343 | 9 | 1 | J | Xtxp225-Xtxp23 | 12.2 |
| 1 | F | Xtxp289-Xtxp10 | 37.3 | 2 | | Xtxp23-Xsnp54 | 6.2 |
| 2 | | Xtxp10-Xsnp31 | 9.3 | 3 | | Xsnp54-Xtxp65 | 17.6 |
| 3 | | Xsnp31-Xtxp67 | 5.8 | 4 | | Xtxp65-Xiabt187 | 22.3 |
| 4 | | Xtxp67-Xiabt430 | 8.8 | 5 | | Xiabt187-Xiabt312 | 10.8 |
| 5 | | Xiabt430-Xtxp284 | 10.2 | 6 | | Xiabt312-Xiabt302 | 10.8 |
| 1 | G | Xtxp217-Xtxp141 | 14.2 | 7 | | Xiabt302-Xiabt111 | 15.6 |
| 2 | | Xtxp141-Xsnp91 | 15.3 | 8 | | Xiabt111-Xtxp324 | 19.5 |
| 3 | | Xsnp91-Xiabt175 | 18 | 9 | | Xtxp324-Xiabt364 | 17.5 |
| 4 | | Xiabt175-Xiabt51 | 16.9 | 10 | | Xiabt364-Xiabt146 | 13.8 |
| 5 | | Xiabt51-Xiabt195 | 19.9 | | | | |
| 6 | | Xiabt195-Xtxp196 | 20.6 | | | | |
| 7 | | Xtxp196-Xsnp117 | 18.1 | | | | |
| 8 | | Xsnp117-Xiabt184 | 10.6 | | | | |
| 9 | | Xiabt184-Xiumc113 | 5.5 | | | | |
| 10 | | Xiumc113-Xiabt176 | 7 | | | | |
| 11 | | Xiabt176-Xsnp67 | 9.1 | | | | |
| 12 | | Xsnp67-Xiabt113 | 10 | | | | |
| 13 | | Xiabt113-Xiabt147 | 13.3 | | | | |
| 14 | | Xiabt147-Xsnp96 | 4.8 | | | | |
| 15 | | Xsnp96-Xiabt342 | 12.8 | | | | |

**Table 17: Distance (cM) between pair of markers mapped to different linkage groups of N13 × E36-1 derived recombinant inbred lines**

| Sl. No. | LG | Marker interval | Distance (cM) | Sl. No. | LG | Marker interval | Distance (cM) |
|---|---|---|---|---|---|---|---|
| 1 | A | Xtxp248-Xtxp340 | 2.5 | 23 | B | Xtxp104-Xiabt477 | 41.6 |
| 2 | | Xtxp340-Xtxp61 | 8.4 | 24 | | Xiabt477-Xtxp96 | 20.6 |
| 3 | | Xtxp61-Xtxp37 | 16.1 | 25 | | Xtxp96-Xiabt177 | 14 |
| 4 | | Xtxp37-Xtxp88 | 12.3 | 26 | | Xiabt177-Xtxp303 | 11.3 |
| 5 | | Xtxp88-Xtxp250 | 16 | 27 | | Xtxp303-Xiabt321 | 6.8 |
| 6 | | Xtxp250-Xiabt71 | 9.1 | 28 | | Xiabt321-Xiabt455 | 8.9 |
| 7 | | Xiabt71-Xsnp48 | 9.6 | 29 | | Xiabt455-Xiabt31 | 6 |
| 8 | | Xsnp48-Xtxp183 | 6.8 | 1 | C | Xtxp228-Xiabt450 | 22.2 |
| 9 | | Xtxp183-Xsnp104 | 8.8 | 2 | | Xiabt450-Xiumc063 | 7.4 |
| 10 | | Xsnp104-Xiabt445 | 8.2 | 3 | | Xiumc063-Xsnp57 | 5.9 |
| 11 | | Xiabt445-Xtxp34 | 8.3 | 4 | | Xsnp57-Xtxp33 | 22.7 |
| 12 | | Xtxp34-Xiabt358 | 4.6 | 5 | | Xtxp33-Xtxp38 | 20.1 |
| 13 | | Xiabt358-Xtxp285 | 5.4 | 6 | | Xtxp38-Xsnp19 | 13.7 |
| 14 | | Xtxp285-Xiabt243 | 4.9 | 7 | | Xsnp19-Xiabt370 | 15.9 |
| 15 | | Xiabt243-Xiabt263 | 7.8 | 8 | | Xiabt370-Xtxp71 | 8.9 |
| 16 | | Xiabt263-Xtxp208 | 7 | 9 | | Xtxp71-Xsnp41 | 8.3 |
| 17 | | Xtxp208-Xiabt451 | 6.3 | 10 | | Xsnp41-Xtxp210 | 9.9 |
| 18 | | Xiabt451-Xiabt350 | 18.6 | 11 | | Xtxp210-Xsnp127 | 8.9 |
| 19 | | Xiabt350-Xiabt206 | 27.7 | 12 | | Xsnp127-Xiabt51 | 9.3 |
| 20 | | Xiabt206-Xiabt435 | 36.7 | 13 | | Xiabt51-Xiabt207 | 8.9 |
| 21 | | Xiabt435-Xiabt424 | 25.3 | 14 | | Xiabt207-Xsnp58 | 17 |
| 22 | | Xiabt424-Xiabt54 | 22.5 | 15 | | Xsnp58-Xtxp200 | 18.5 |
| 23 | | Xiabt54-Xiabt72 | 27.2 | 16 | | Xtxp200-Xiabt234 | 13.7 |
| 1 | B | Xtxp197-Xsnp135 | 14.7 | 1 | D | Xtxp41-Xsnp53 | 14.7 |
| 2 | | Xsnp135-Xtxp50 | 32.6 | 2 | | Xsnp53-Xtxp327 | 11.1 |
| 3 | | Xtxp50-Xtxp201 | 3 | 3 | | Xtxp327-Xisp229 | 4.3 |
| 4 | | Xtxp201-Xiumc136 | 3.1 | 4 | | Xisp229-Xsnp36 | 11.5 |
| 5 | | Xiumc136-Xsnp28 | 19.1 | 5 | | Xsnp36-Xtxp27 | 8.8 |
| 6 | | Xsnp28-Xtxp100 | 20.8 | 6 | | Xtxp27-Xiabt353 | 11.1 |
| 7 | | Xtxp100-Xtxp8 | 7.4 | 7 | | Xiabt353-Xiabt227 | 12.8 |
| 8 | | Xtxp8-Xtxp1 | 7.5 | 8 | | Xiabt227-Xsnp56 | 16.4 |
| 9 | | Xtxp1-Xiabt509 | 30.8 | 9 | | Xsnp56-Xiabt79 | 42 |
| 10 | | Xiabt509-Xtxp201 | 19.7 | 10 | | Xiabt79-Xiabt353 | 37.6 |
| 11 | | Xtxp201-Xtxp211 | 22.2 | 11 | | Xiabt353-Xiabt147 | 12 |
| 12 | | Xtxp211-Xiabt80 | 20.1 | 12 | | Xiabt147-Xsnp83 | 12.9 |
| 13 | | Xiabt80-Xiabt73 | 9.2 | 13 | | Xsnp83-Xiabt364 | 10.8 |
| 14 | | Xiabt73-XtxpK1f2 | 28.9 | 14 | | Xiabt364-Xtxp213 | 12.6 |
| 15 | | XtxpK1f2-Xiabt388 | 14.8 | 15 | | Xtxp213-Xiabt466 | 12.9 |
| 16 | | Xiabt388-Xiabt232 | 24.9 | 16 | | Xiabt466-Xiabt134 | 13.6 |
| 17 | | Xiabt232-Xiabt444 | 9.6 | | | | |
| 18 | | Xiabt444-Xiabt82 | 15 | | | | |
| 19 | | Xiabt82-Xtxp73 | 47.9 | | | | |
| 20 | | Xtxp73-Xtxp31 | 8.3 | | | | |
| 21 | | Xtxp31-Xiabt241 | 20.2 | | | | |
| 22 | | Xiabt241-Xtxp104 | 5.3 | | | | |

*Contd……*

| Sl. No. | LG | Marker interval | Distance (cM) | Sl. No. | LG | Marker interval | Distance (cM) |
|---|---|---|---|---|---|---|---|
| 1 | E | Xisp344-Xisu074 | 2.3 | 1 | I | Xiumc044-Xtxp176 | 2.5 |
| 2 | | Xisu074-Xiabt187 | 5.6 | 2 | | Xtxp176-Xtxp57 | 3 |
| 3 | | Xiabt187-Xsnp108 | 11.3 | 3 | | Xtxp57-Xtxp145 | 18 |
| 4 | | Xsnp108-Xtxp295 | 4.9 | 4 | | Xtxp145-Xtxp6 | 11.1 |
| 5 | | Xtxp295-Xtxp168 | 2.7 | 5 | | Xtxp6-Xiabt70 | 10 |
| 6 | | Xtxp168-Xisp362 | 6.9 | 6 | | Xiabt70-Xiabt86 | 9.9 |
| 7 | | Xisp362-Xumc085 | 2 | 7 | | Xiabt86-Xiabt143 | 4.9 |
| 8 | | Xumc085-Xtxp372 | 16.5 | 8 | | Xiabt143-Xsnp78 | 4.6 |
| 9 | | Xtxp372-Xiabt377 | 24.3 | 9 | | Xsnp78-Xiabt29 | 9.6 |
| 10 | | Xiabt377-Xsnp33 | 15.4 | 10 | | Xiabt29-Xiabt14 | 11.1 |
| 11 | | Xsnp33-Xiabt215 | 12.2 | 11 | | Xiabt14-Xiabt148 | 17.1 |
| 12 | | Xiabt215-Xtxp296 | 15.6 | 12 | | Xiabt148-Xiabt301 | 27.9 |
| 13 | | Xtxp296-Xtxp205 | 13.6 | 13 | | Xiabt301-Xiabt338 | 24.2 |
| 14 | | Xtxp205-Xiabt18 | 7.1 | 14 | | Xiabt338-Xtxp225 | 2.8 |
| 15 | | Xiabt18-Xtxp231 | 8.7 | 1 | J | Xtxp23-Xsnp54 | 6.3 |
| 16 | | Xtxp231-Xiabt511 | 12.4 | 2 | | Xsnp54-Xtxp65 | 18.7 |
| 17 | | Xiabt511-Xiabt32 | 5.5 | 3 | | Xtxp65-Xtxp324 | 25.1 |
| 1 | F | Xtxp289-Xtxp10 | 35.6 | 4 | | Xtxp324-Xiabt58 | 12.3 |
| 2 | | Xtxp10-Xsnp31 | 8.4 | 5 | | Xiabt58-Xtxp15 | 10.8 |
| 3 | | Xsnp31-Xtxp67 | 5.7 | 6 | | Xtxp15-Xiabt16 | 10.8 |
| 4 | | Xtxp67-Xiabt430 | 8.2 | 7 | | Xiabt16-Xiabt146 | 11.3 |
| 5 | | Xiabt430-Xiabt78 | 9.6 | | | | |
| 6 | | Xiabt78-Xtxp132 | 8.2 | | | | |
| 7 | | Xtxp132-Xiabt521 | 6.7 | | | | |
| 1 | G | Xiumc113-Xsnp91 | 4.5 | | | | |
| 2 | | Xsnp91-Xtxp141 | 15 | | | | |
| 3 | | Xtxp141-Xiabt437 | 12 | | | | |
| 4 | | Xiabt437-Xiabt341 | 12 | | | | |
| 5 | | Xiabt341-Xiabt178 | 16.9 | | | | |
| 6 | | Xiabt178-Xiabt302 | 19.9 | | | | |
| 7 | | Xiabt302-Xsnp117 | 13.6 | | | | |
| 8 | | Xsnp117-Xiabt488 | 17.9 | | | | |
| 9 | | Xiabt488-Xiabt294 | 16.7 | | | | |
| 10 | | Xiabt294-Xiabt267 | 14.1 | | | | |
| 11 | | Xiabt267-Xtxp159 | 15 | | | | |
| 12 | | Xtxp159-Xsnp67 | 9.4 | | | | |
| 13 | | Xsnp67-Xsnp96 | 12.3 | | | | |
| 14 | | Xsnp96-Xtxp45 | 17.4 | | | | |
| 1 | H | Xtxp273-Xtxp47 | 4.6 | | | | |
| 2 | | Xtxp47-Xtxp18 | 3.6 | | | | |
| 3 | | Xtxp18-Xtxp298 | 10.2 | | | | |
| 4 | | Xtxp298-Xiabt380 | 13.1 | | | | |
| 5 | | Xiabt380-Xiabt310 | 11.9 | | | | |
| 6 | | Xiabt310-Xtxp282 | 5.4 | | | | |
| 7 | | Xtxp282-Xiabt298 | 5.8 | | | | |

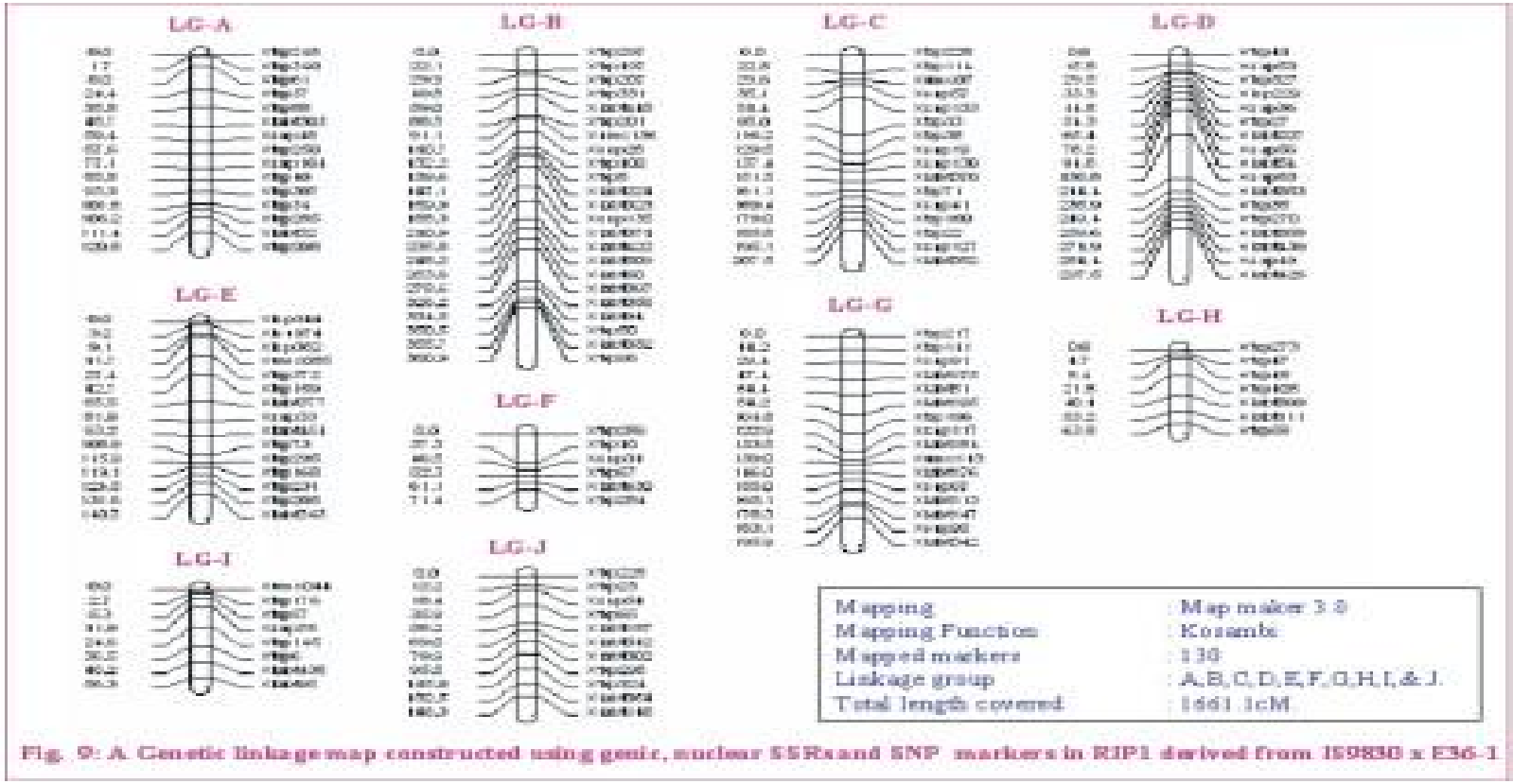



Fig. 9: A Genetic linkage map constructed using genic, nuclear SSRs and SNP markers in RIP1 derived from IS9830 x E36-1

**Fig. 9: A Genetic linkage map constructed using genic, nuclear SSRs and SNP markers in RIP1 derived from IS9830 x E36-1**

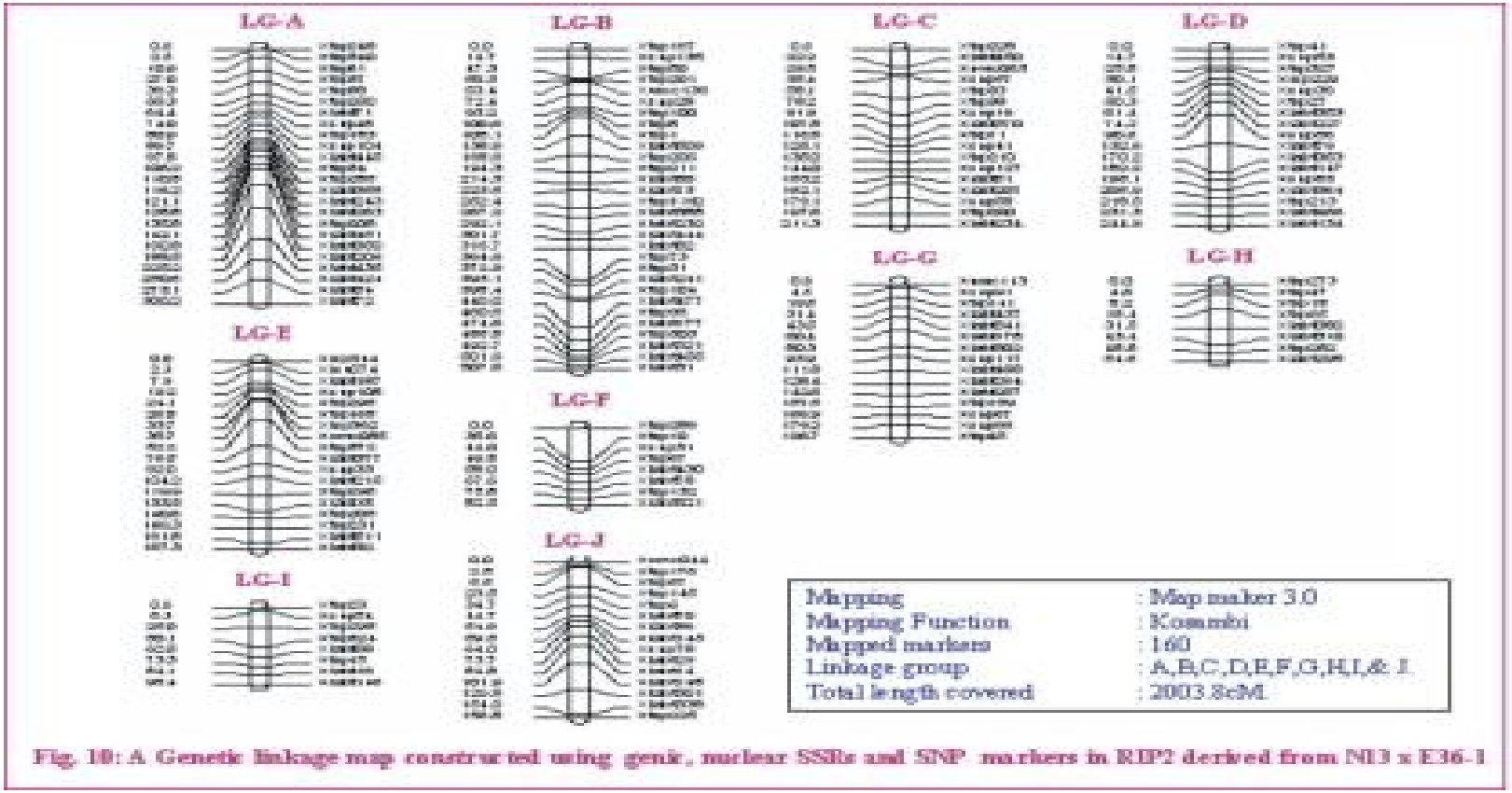



Fig. 10: A Genetic linkage map constructed using genic, nuclear SSRs and SNP markers in RIP2 derived from N13 x E36-1

**Fig. 10: A Genetic linkage map constructed using genic, nuclear SSRs and SNP markers in RIP2 derived from N13 x E36-1**

In RIP2, a total of 160 markers used for linkage map construction with help of MAPMAKER/EXP (3.0b) software. And all markers mapped on to ten linkage group genetic map had a total length of 2003.8 cM distributed over ten linkage groups (Fig. 10) with number of markers assigned to different linkage group were 24, 30, 17, 17, 18, 8, 15, 8, 15 and 8. The markers mapped per linkage group varied from 8 in LG-F, LG-H, LG-J to 30 in LG-B, with average distance covered was 12.52 cM. The highest distance observed was 47.9 cM which is between Xiabt82 – Xtxp73 on LG-B, the lowest distance was 2 cM LG-E flanked between Xisp362-Xumc085 markers.

### 4.2.4 QTL analysis and mapping

Linkage maps thus constructed using 226 RILs each in RIP1 and RIP2 were used for QTL analysis and mapping on to linkage maps. Both IS9830 and N13, the female parents were senescent types, highly susceptible to drought and non-stay green types. E36-1 was male parent, stay-green type, non-senescent and highly drought tolerant. Resulting RILs varied in their reaction to target traits and followed normal distribution with a few transgressive segregants.

QTL Cartographer (2.5b) was used to analyze data by Composite Interval Mapping (CIM) with LOD score of 2.0 as threshold value for QTL significance. The threshold values, with permutation times 300 and significance at 0.05 per cent. The additive effect and per cent phenotypic variation explained by each and individual QTL were computed.

QTL analysis was carried out for individual season data; 2007, 2008 and 2009 season data sets separately for Dharwad and simultaneously at Bheemarayanagudi, 2008 and 2009 season data was available. QTLs were computed and compared for their consistency and robustness. QTLs for 13 traits in RIP1 and 14 in RIP2 were computed separately and compared for Dharwad and Bheemarayanagudi locations. The QTLs were checked for their consistency across seasons within a location and finally between locations and mapping populations.

Finally, common and stable QTLs which found consistent over different season and both locations were noted down for both populations. The QTLs common for both populations were noted to account as stable QTLs.

### 4.2.5 Genetic features of QTLs for post-flowering drought tolerance and yield related traits

QTLs associated with 13 different traits during different season, in both locations were analysed and computed separately for each RIP.

a) Features of QTLs in IS9830 × E36-1 derived RIP at Dharwad location

QTLs associated with 13 different traits at Dharwad location in RIP1 are presented in Table 18a, 18b, 18c, 18d. QTLs were homed on to respective linkage groups (Fig. 11).

Per cent GLA 15 DAF

Four QTLs conditioned by the marker pairs Xtxp34-Xtxp285, Xtxp38-Xsnp19, Xiabt227-Xsnp56 and Xtxp248-Xtxp340 were detected for per cent GLA 15 DAF in RIP1 at Dharwad. Two of them were mapped on to LG-A (Xtxp34- Xtxp285 and Xtxp248- Xtxp300) and others were on LG-C and LG-D. Three QTLs were consistent for all the season except Xtxp248-Xtxp340. Three QTLs common were Xtxp34-Xtxp285, Xtxp38-Xsnp19 and Xiabt227-Xsnp56 with marker intervals of 4.6, 13.3 and 12.9 respectively and phenotypic variance of 11.04, 7.92 and 8.83 respectively. These QTLs recorded additive effect for all contributing allele from E36-1 with LOD score of 2.24, 2.51 and 3.13, respectively.

Per cent GLA 30 DAF

Three QTLs bracketed by the pairs of markers *viz.,* Xtxp114-Xumc7, Xtxp205-Xtxp231 and Xtxp196-Xsnp117 were detected for per cent GLA 30 DAF, of which two QTLs flanked by Xtxp114-Xumc7 and Xtxp205-Xtxp231 were found on LG-C and LG-E, respectively were common across season at Dharwad location. They showed positive additive effect contributing allele from E36-1 parent with 2.39 – 3.32 and 3.12– 3.14 LOD score accounting for per cent phenotypic variation of 8.27 – 10.24 and 8.21 – 10.48, respectively.

**Table 18a: Features of the QTLs computed by composite interval mapping for stay-green and yield related traits in the RILs derived from the cross IS9830 × E36-1 at Dharwad location evaluated during 2007**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_i b^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 103.9 | 4.6 | 2.24 | 2.18 | 11.04 |
| | C | Xtxp38-Xsnp19 | 123.03 | 13.3 | 2.51 | 3.02 | 7.92 |
| | D | Xiabt227-Xsnp56 | 72.02 | 12.9 | 3.13 | 3.34 | 8.83 |
| | A | Xtxp248-Xtxp340 | 0.85 | 1.7 | 2.65 | 3.24 | 4.35 |
| % GLA 30 DAF | C | Xtxp114-Xiumc7 | 26.3 | 6.8 | 2.48 | 4.13 | 9.35 |
| | E | Xtxp205- Xtxp231 | 128.27 | 7.1 | 3.14 | 2.87 | 10.48 |
| | G | Xtxp196-Xsnp117 | 113.85 | 18.8 | 2.83 | 4.56 | 9.82 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 105.32 | 19.71 | 3.35 | 3.84 | 18.94 |
| | E | Xtxp295-Xtxp168 | 117.72 | 3.42 | 4.6 | 4.39 | 10.46 |
| Days to 50 % flowering | E | Xtxp159-Xiabt377 | 54.28 | 22.9 | 2.9 | -2.12 | 13.67 |
| | B | Xiumc136-Xsnp28 | 99.9 | 20.24 | 2.85 | -1.22 | 7.83 |
| Total green leaf area | B | Xtxp331-Xiabt445 | 54.4 | 9.2 | 2.85 | 1.88 | 10.8 |
| | C | Xiabt370-Xtxp71 | 157.34 | 10.21 | 3.54 | 1.45 | 6.34 |
| | A | Xtxp340-Xtxp61 | 4.85 | 7.12 | 2.71 | 1.92 | 10.32 |
| | D | Xiabt353-Xtxp35 | 225.15 | 21.5 | 3.19 | 9.74 | 8.88 |
| Earhead length | A | Xtxp40-Xtxp307 | 89.63 | 7.4 | 2.27 | 0.61 | 22.59 |
| | D | Xisp229-Xsnp36 | 40.73 | 11.71 | 2.33 | 2.54 | 12.65 |
| Number of leaves | G | Xiabt113-Xiabt147 | 171.73 | 13.52 | 3.55 | 0.28 | 13.32 |
| | I | Xtxp145-Xtxp6 | 31.23 | 11.8 | 2.42 | 4.27 | 11.45 |
| Panicle exertion | C | Xtxp71-Xtxp160 | 169.92 | 18.23 | 3.59 | -2.13 | 14.54 |
| | I | Xtxp176-Xtxp57 | 4 | 2.7 | 3.23 | -2.03 | 12.54 |
| | H | Xtxp47-Xtxp18 | 6.84 | 3.6 | 3.13 | 1.89 | 9.91 |
| Stem girth | G | Xiabt176-Xiabt113 | 155.26 | 29.32 | 3.72 | 2.45 | 13.27 |
| | F | Xtxp67-Xiabt430 | 56.7 | 8.8 | 2.43 | 5.38 | 10.45 |
| | B | Xtxp201-Xiumc136 | 90.13 | 2.88 | 2.88 | 2.53 | 10.24 |
| | B | Xiabt422-Xiabt60 | 248.9 | 28.1 | 2.99 | 2.98 | 12.03 |
| Plant height | A | Xtxp88-Xiabt303 | 42.32 | 12.9 | 2.01 | 2.78 | 11.98 |
| | A | Xtxp61-Xtxp37 | 16.9 | 16.98 | 2.58 | -3.09 | 13.11 |
| | G | Xiabt113-Xiabt147 | 171.7 | 13.97 | 3.89 | 2.86 | 17.22 |
| Spicklets per head | B | Xtxp297-Xtxp197 | 11.28 | 22.5 | 2.64 | 2.79 | 14.9 |
| | H | Xtxp47-Xtxp18 | 6.98 | 3.6 | 3.29 | 5.2 | 9.12 |
| 100 grain weight | A | Xtxp32-Xtxp208 | 116.72 | 9.4 | 2.42 | 0.19 | 12.05 |
| | D | Xtxp327-Xisp229 | 31.56 | 4.6 | 2.28 | 4.42 | 7.52 |
| | E | Xtxp231-Xtxp205 | 129.34 | 7.1 | 2.96 | 2.18 | 12.66 |
| Grain yield per plant | J | Xiabt364-Xiabt146 | 140.54 | 14.38 | 3.29 | 1.19 | 14.23 |
| | I | Xtxp145-Xtxp6 | 30.5 | 12.73 | 2.43 | 1.92 | 7.72 |
| | D | Xsnp36-Xtxp27 | 49.55 | 9.92 | 2.77 | 1.43 | 8.92 |
| | G | Xtxp141-Xsnp91 | 21.9 | 16.24 | 2.69 | 4.74 | 14.67 |

GLA – Total green leaf area DAF – Days after flowering

**Table 18b: Features of the QTLs computed by composite interval mapping for stay-green and yield related traits in the RILs derived from the cross IS9830 × E36-1 at Dharwad location evaluated during 2008**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_i b^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 104.11 | 4.56 | 2.65 | 2.35 | 9.05 |
| | C | Xtxp38-Xsnp19 | 123.07 | 13.52 | 2.73 | 3.89 | 8.38 |
| | D | Xiabt227-Xsnp56 | 72 | 12.8 | 2.7 | 4.39 | 9.39 |
| | A | Xtxp248-Xtxp340 | 0.85 | 1.7 | 2.84 | 3.25 | 4.75 |
| % GLA 30 DAF | C | Xtxp114-Xiumc7 | 26.42 | 7.02 | 3.32 | 4.11 | 10.24 |
| | E | Xtxp205- Xtxp231 | 128.05 | 7.2 | 3.12 | 1.91 | 9.98 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 105.47 | 19.5 | 2.85 | 1.82 | 19.49 |
| | E | Xtxp295-Xtxp168 | 117.5 | 3.2 | 4.8 | 5.72 | 10.12 |
| Days to 50 % flowering | E | Xtxp159-Xiabt377 | 54.32 | 22.45 | 2.58 | -,2.3 | 12.84 |
| | E | Xisp344-Xisp074 | 1.6 | 3.1 | 2.22 | -2.34 | 9.34 |
| | B | Xiumc136-Xsnp28 | 103.24 | 19.6 | 2.32 | -1.28 | 6.34 |
| Total green leaf area | B | Xtxp331-Xiabt445 | 54.63 | 9.25 | 3.07 | 1.76 | 11.22 |
| | C | Xiabt370-Xtxp71 | 156.3 | 9.6 | 3.12 | 1.24 | 8.36 |
| | A | Xtxp340-Xtxp61 | 5.84 | 6.3 | 2.94 | 2.86 | 11.12 |
| Earhead length | A | Xtxp40-Xtxp307 | 89.55 | 7.62 | 2.19 | 0.39 | 22.98 |
| | D | Xisp229-Xsnp36 | 39.05 | 12.84 | 2.58 | 3.24 | 11.83 |
| Number of leaves | G | Xiabt113-Xiabt147 | 171.71 | 13.3 | 3.48 | 0.59 | 11.83 |
| | I | Xtxp145-Xtxp6 | 31.7 | 11.98 | 3.12 | 4.25 | 10.18 |
| Panicle exertion | C | Xtxp71-Xtxp160 | 169.5 | 18.76 | 3.61 | -2.76 | 13.97 |
| | H | Xtxp47-Xtxp18 | 6.55 | 3.92 | 2.82 | 0.23 | 10.34 |
| Stem girth | G | Xiabt176-Xiabt113 | 155.27 | 29.1 | 3.94 | 3.12 | 13.49 |
| | B | Xtxp201-Xiumc136 | 89.93 | 3.12 | 2.74 | 2.44 | 9.32 |
| | B | Xiabt422-Xiabt60 | 249.3 | 28.6 | 2.56 | 3.25 | 11.99 |
| Plant height | A | Xtxp88-Xiabt303 | 42.47 | 13.12 | 2.54 | 1.9 | 12.69 |
| | A | Xtxp61-Xtxp37 | 16.2 | 17.1 | 3.09 | -2.52 | 11.52 |
| | G | Xiabt113-Xiabt147 | 180.27 | 13.3 | 3.56 | 3.29 | 17.05 |
| Spicklets per head | B | Xtxp297-Xtxp197 | 11.32 | 22.2 | 2.86 | 2.68 | 13.68 |
| | H | Xtxp47-Xtxp18 | 7.14 | 4.2 | 4.14 | 4.56 | 8.42 |
| | J | Xsnp54-Xtxp65 | 27.15 | 17.6 | 2.94 | 4.24 | 12.33 |
| 100 grain weight | A | Xtxp32-Xtxp208 | 116.1 | 9.62 | 2.38 | 0.41 | 11.83 |
| | D | Xtxp327-Xisp229 | 31.9 | 4.2 | 3.13 | 5.14 | 10.31 |
| | B | Xtxp100-Xtxp8 | 135.9 | 7.4 | 2.54 | 3.87 | 9.47 |
| | E | Xtxp231-Xtxp205 | 129.12 | 8.2 | 3.2 | 1.29 | 10.54 |
| Grain yield per plant | J | Xiabt364-Xiabt146 | 139.4 | 14.76 | 3.51 | 1.47 | 14.45 |
| | H | Xtxp273-Xtxp47 | 2.35 | 4.7 | 3.42 | 3.37 | 14.53 |
| | I | Xtxp145-Xtxp6 | 30.23 | 12.44 | 2.36 | 1.89 | 8.43 |
| | D | Xsnp36-Xtxp27 | 50.11 | 10.11 | 2.65 | 2.98 | 10.45 |
| | G | Xtxp141-Xsnp91 | 22.04 | 15.4 | 2.02 | 3.49 | 12.88 |

GLA – Total green leaf area DAF – Days after flowering

**Table 18c: Features of the QTLs computed by composite interval mapping for stay-green and yield related traits in the RILs derived from the cross IS9830 × E36-1 at Dharwad location evaluated during 2009**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_ib^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 104.12 | 4.82 | 2.85 | 2.57 | 10.45 |
| | C | Xtxp38-Xsnp19 | 122.85 | 13.49 | 2.67 | 3.67 | 7.56 |
| | D | Xiabt227-Xsnp56 | 71.8 | 13.02 | 2.98 | 4.39 | 7.49 |
| % GLA 30 DAF | C | Xtxp114-Xiumc7 | 26.2 | 6.8 | 2.39 | 4 | 8.27 |
| | E | Xtxp205- Xtxp231 | 128.12 | 7.32 | 3.13 | 2.67 | 8.21 |
| | G | Xtxp196-Xsnp117 | 113.85 | 18.8 | 3.24 | 3.59 | 5.32 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 105.25 | 19.72 | 2.73 | 4.28 | 18.38 |
| | E | Xtxp295-Xtxp168 | 117.68 | 3.3 | 4.7 | 1.29 | 9.1 |
| Days to 50 % flowering | E | Xtxp159-Xiabt377 | 54.1 | 23.12 | 2.41 | -1.3 | 14.06 |
| | B | Xiumc136-Xsnp28 | 101.32 | 19.8 | 2.48 | -0.94 | 5.97 |
| Total green leaf area | B | Xtxp331-Xiabt445 | 54.52 | 9.2 | 2.95 | 1.73 | 11.48 |
| | C | Xiabt370-Xtxp71 | 158.41 | 10.37 | 3.39 | 0.32 | 7.11 |
| | A | Xtxp340-Xtxp61 | 5.55 | 6.84 | 2.63 | 1.67 | 12.43 |
| Earhead length | A | Xtxp40-Xtxp307 | 89.77 | 7.5 | 2.34 | 0.54 | 22.47 |
| | H | Xtxp105-Xiabt399 | 30.95 | 18.3 | 3.54 | 2.32 | 15.73 |
| | D | Xisp229-Xsnp36 | 39.74 | 11.5 | 2.21 | 2.71 | 11.72 |
| Number of leaves | G | Xiabt113-Xiabt147 | 171.71 | 13.45 | 3.19 | 0.46 | 12.38 |
| | I | Xtxp145-Xtxp6 | 30.5 | 12.3 | 2.84 | 4.21 | 12.41 |
| | B | Xiabt94-Xtxp50 | 342.5 | 16 | 2.43 | 3.54 | 9.45 |
| Panicle exertion | C | Xtxp71-Xtxp160 | 169.7 | 17.9 | 2.39 | -2.36 | 13.22 |
| | H | Xtxp47-Xtxp18 | 7.02 | 4.3 | 2.35 | 2.43 | 9.35 |
| Stem girth | G | Xiabt176-Xiabt113 | 155.22 | 29.22 | 3.85 | 3.24 | 13.33 |
| | B | Xtxp201-Xiumc136 | 89.8 | 2.6 | 2.91 | 1.29 | 8.53 |
| | B | Xiabt422-Xiabt60 | 250.23 | 27.8 | 2.37 | 2.43 | 11.56 |
| Plant height | A | Xtxp88-Xiabt303 | 42.25 | 13 | 2.39 | 2.53 | 10.47 |
| | A | Xtxp61-Xtxp37 | 16.39 | 16.4 | 2.98 | -3.42 | 12.45 |
| | F | Xtxp289-Xtxp10 | 18.65 | 37.3 | 3.98 | 2.46 | 10.38 |
| | G | Xiabt113-Xiabt147 | 173.78 | 14.28 | 3.02 | 2.98 | 17.28 |
| Spicklets per head | B | Xtxp297-Xtxp197 | 11.05 | 22.1 | 2.74 | 2.9 | 14.02 |
| | H | Xtxp47-Xtxp18 | 6.55 | 3.8 | 3.12 | 4.41 | 9.51 |
| 100 grain weight | A | Xtxp32-Xtxp208 | 116.52 | 9.5 | 2.2 | 0.37 | 11.94 |
| | D | Xtxp327-Xisp229 | 31.4 | 3.8 | 3.49 | 2.24 | 9.45 |
| | E | Xtxp231-Xtxp205 | 128.05 | 7.7 | 2.18 | 1.95 | 9.38 |
| Grain yield per plant | J | Xiabt364-Xiabt146 | 139.8 | 13.8 | 3.48 | 1.68 | 13.23 |
| | I | Xtxp145-Xtxp6 | 30.8 | 11.8 | 2.22 | 2.42 | 9.36 |
| | D | Xsnp36-Xtxp27 | 49.89 | 9.5 | 2.93 | 2.55 | 9.76 |
| | G | Xtxp141-Xsnp91 | 21.8 | 15.89 | 3.13 | 3.2 | 13.02 |

GLA – Total green leaf area DAF – Days after flowering

**Table 18d: Features of the QTLs that were commonly detected across three seasons for stay-green and yield related traits in RILs derived from the cross IS9830 × E36-1 at Dharwad location evaluated during 2007, 2008 and 2009**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_i b^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 103.9-104.12 | 4.6-4.82 | 2.24-2.85 | 2.18-2.57 | 9.05-11.04 |
| | C | Xtxp38-Xsnp19 | 122.85-123.07 | 13.3-13.52 | 2.51-2.73 | 3.02-3.89 | 7.56-8.38 |
| | D | Xiabt227-Xsnp56 | 71.8-72.02 | 12.8-13.02 | 2.70-3.13 | 3.34-4.39 | 7.49-9.39 |
| % GLA 30 DAF | C | Xtxp114-Xiumc7 | 26.2-26.42 | 6.8-7.02 | 2.39-3.32 | 4.0-4.13 | 8.27-10.24 |
| | E | Xtxp205- Xtxp231 | 128.05-128.27 | 7.1-7.32 | 3.12-3.14 | 1.91-2.67 | 8.21-10.48 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 105.25-105.47 | 19.5-19.72 | 2.73-3.35 | 1.82-4.28 | 18.34-19.49 |
| | E | Xtxp295-Xtxp168 | 117.5-117.72 | 3.2-3.42 | 4.6-4.8 | 1.29-5.72 | 9-10.46 |
| Days to 50 % flowering | E | Xtxp159-Xiabt377 | 54.1-54.32 | 22.9-23.12 | 2.41-2.90 | -1.34 | 12.84-14.06 |
| | B | Xiumc136-Xsnp28 | 99.9-103.24 | 19.6-20.24 | 2.32-2.85 | -1.28-1.22 | 5.97-7.83 |
| Total green leaf area | B | Xtxp331-Xiabt445 | 54.4-54.63 | 9.2-9.25 | 2.85-3.07 | 1.73-1.88 | 10.8-11.48 |
| | C | Xiabt370-Xtxp71 | 156.3-158.41 | 9.6-10.37 | 3.12-3.54 | 0.32-1.45 | 6.34-8.36 |
| | A | Xtxp340-Xtxp61 | 4.85-5.84 | 6.3-7.12 | 2.63-2.94 | 1.67-2.86 | 10.32-12.43 |
| Earhead length | A | Xtxp40-Xtxp307 | 89.55-89.77 | 7.4-7.62 | 2.19-2.34 | 0.39-0.61 | 22.47-22.98 |
| | D | Xisp229-Xsnp36 | 39.05-40.73 | 11.5-12.84 | 2.21-2.58 | 2.54-3.24 | 11.83-12.65 |
| Number of leaves | G | Xiabt113-Xiabt147 | 171.71-171.73 | 13.3-13.52 | 3.19-3.55 | 0.28-0.59 | 11.83-13.32 |
| | I | Xtxp145-Xtxp6 | 30.5-31.7 | 11.8-12.3 | 2.42-3.12 | 4.21-4.27 | 10.18-12.41 |
| Panicle exertion | C | Xtxp71-Xtxp160 | 169.7-169.92 | 17.9-18.76 | 2.39-3.61 | -4.12 | 13.22-14.54 |
| | H | Xtxp47-Xtxp18 | 6.55-7.02 | 3.6-4.30 | 2.35-3.13 | 0.23-2.43 | 9.35-10.34 |
| Stem girth | G | Xiabt176-Xiabt113 | 155.22-155.27 | 29.1-29.32 | 3.72-3.94 | 0.28-0.55 | 13.27-13.49 |
| | B | Xtxp201-Xiumc136 | 89.8-90.13 | 2.6-3.12 | 2.74-2.91 | 1.29-2.53 | 8.53-10.24 |
| | B | Xiabt422-Xiabt60 | 248.9-250.23 | 27.8-28.6 | 2.37-2.99 | 0.37-0.54 | 11.56-12.03 |
| Plant height | A | Xtxp88-Xiabt303 | 42.25-42.47 | 12.9-13.12 | 2.01-2.54 | 1.9-2.78 | 10.47-12.69 |
| | A | Xtxp61-Xtxp37 | 16.2-16.9 | 16.4-17.1 | 2.58-3.09 | -2.52-3.42 | 11.52-13.11 |
| | G | Xiabt113-Xiabt147 | 171.7-180.27 | 13.3-14.28 | 3.02-3.89 | 2.86-3.29 | 17.05-17.28 |
| Spicklets per head | B | Xtxp297-Xtxp197 | 11.05-11.32 | 22.1-22.5 | 2.64-2.86 | 2.68-2.9 | 13.68-14.90 |
| | H | Xtxp47-Xtxp18 | 6.55-7.14 | 3.6-4.2 | 3.21-4.14 | 4.41-5.2 | 8.42-9.51 |
| 100 grain weight | A | Xtxp32-Xtxp208 | 116.1-116.72 | 9.4-9.62 | 2.2-2.42 | 0.19-0.41 | 11.83-12.05 |
| | D | Xtxp327-Xisp229 | 31.4-31.9 | 3.8-4.6 | 2.28-3.49 | 2.24-5.14 | 7.52-10.31 |
| | E | Xtxp231-Xtxp205 | 128.05-129.34 | 7.1-8.2 | 2.18-3.2 | 1.29-2.18 | 9.38-12-66 |
| Grain yield per plant | J | Xiabt364-Xiabt146 | 139.4-140.54 | 13.8-14.38 | 3.29-3.51 | 1.19-1.68 | 13.23-14.45 |
| | I | Xtxp145-Xtxp6 | 30.5-30.8 | 11.8-12.73 | 2.22-2.43 | 1.89-2.42 | 7.72-9.36 |
| | D | Xsnp36-Xtxp27 | 49.55-50.11 | 9.5-10.11 | 2.65-2.93 | 1.43-2.98 | 8.92-10.45 |
| | G | Xtxp141-Xsnp91 | 21.8-22.04 | 15.4-16.24 | 2.02-3.13 | 3.2-4.74 | 13.02-14.67 |

GLA – Total green leaf area DAF – Days after flowering

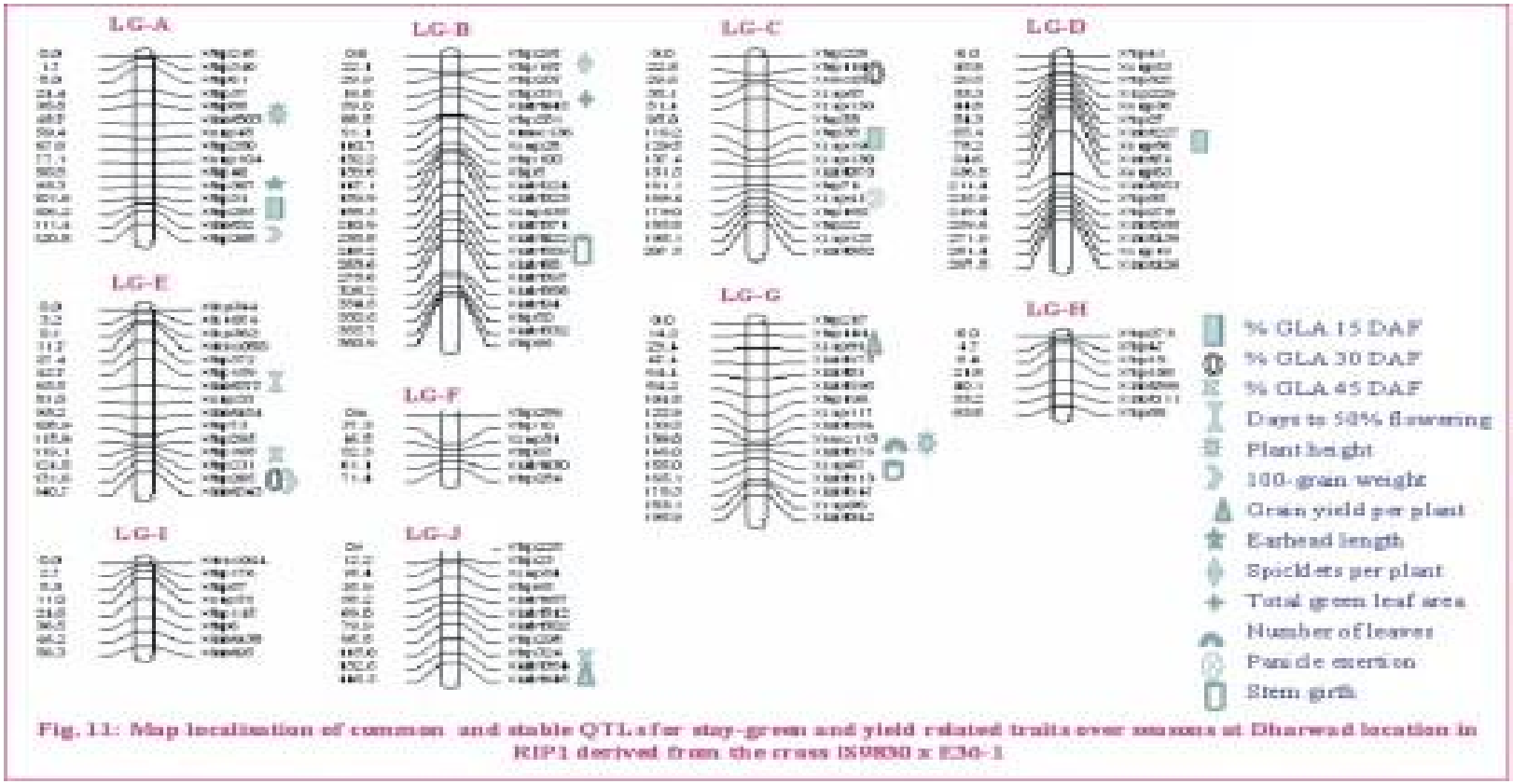



Fig. 11: Map localisation of common and stable QTLs for stay-green and yield related traits over seasons at Dharwad location in RIP1 derived from the cross IS9830 x E36-1

**Fig. 11: Map localisation of common and stable QTLs for stay-green and yield related traits over seasons at Dharwad location in RIP1 derived from the cross IS9830 x E36-1**

Per cent GLA 45 DAF

Two QTLs were detected for per cent GLA 45 DAF and were common for all three seasons. These QTLs were spanned by Xtxp298-Xtxp324 on LG-J and Xtxp295- Xtxp168 on LG-E with per cent phenotypic variation range of 18.34 – 19.49 and 9.10 – 10.46 and LOD score of 2.73 – 3.35 and 4.6 – 4.8, respectively. These QTLs recorded positive additive effect contributing allele from E36-1 parent for this trait.

Days to 50 per cent flowering

Two QTLs were detected for this trait one on each of LG-E (Xtxp159 – Xiabt377) and on LG-B (Xumc136-Xsnp28) with phenotypic variation of 12.84 – 14.06 and 5.97 – 7.83 and LOD score of 2.41 – 2.90 and 2.32 – 2.85 respectively. They recorded negative effect with favorable allele contributing from IS9830 parent.

Total green leaf area

Total of 4 QTLs were detected on LG-B (Xtxp331-Xiabt445), LG-C (Xiabt370-Xtxp71), LG-A (Xtxp340- Xtxp61) and LG-D (Xiabt353- Xtxp35), of which first three were common across season in Dharwad location accounting for a phenotypic variation per cent of 10.8 – 11.48, 6.34 – 8.36 and 10.32 – 12.43 respectively with additive effect contributing allele from E36-1. The LOD score of 2.85 – 3.07, 3.12 – 3.54 and 2.63 – 2.94 respectively were recorded for this trait.

Earhead length

Three QTLs were detected for earhead length in Dharwad location on LG-A (Xtxp40-Xtxp307), LG-H (Xtxp105-Xiabt399) and LG-D (Xisp229-Xsnp36). Among them two QTL, one from LG-A and another from LG-D were found to be common across season with additive effect accounting for a per cent phenotypic variation range 22.43 – 22.98 and 11.83 – 12.65. The LOD score of 2.19 – 2.34 and 2.21 – 2.58 respectively were recorded for these QTLs.

Number of leaves

Three QTLs conferring and conditioning number of leaves per plant were detected on LG-G (Xiabt113-Xiabt147), LG-I (Xtxp145- Xtxp6) and on LG-B (Xiabt94- Xtxp50). The QTLs found on LG-G and LG-I were common across season with LOD score of 3.19 – 3.55 and 2.42 – 3.12. They accounted for phenotypic variation ranged from 11.83 – 13.32 and 10.18 – 12.41, respectively.

Panicle exertion

A set of three QTLs were detected of which two QTLs, one each on LG-C (Xtxp71-Xtxp160) and on LG-H (Xtxp47-Xtxp18) were consistent across seasons at Dharwad location. QTL found on LG-C showed negative effect contributing allele from IS9830 while another one showed positive additive effect. The LOD score of 2.39 - 3.61 and 2.35 – 3.13 and per cent phenotypic variation of 13.22 – 14.54 and 9.35 – 10.34 for QTLs found on LG-C and LG-H respectively was recorded for this trait.

Stem girth

Four QTLs conferring to stem girth were identified on LG-G (Xiabt176-Xiabt113), LG-F (Xtxp67-Xiabt430), LG-B (Xtxp201-Xumc136) and again on LG-B (Xiabt422-Xiabt60). The LOD score of 3.72 – 3.94, 2.74 – 2.91 and 2.37 – 2.99 were recorded with per cent phenotypic variation of 13.27 – 13.49, 8.53 – 10.24 and 11.56 – 12.03 respectively. Of which, three QTL mapped on LG-G and LG-B were common across season with positive additive effect.

Plant height

A total of four QTLs detected for this trait; two QTLs mapped on LG-A (Xtxp88-Xiabt303, Xtxp61-Xtxp37) and one LG-G (Xiabt113-Xiabt147) were common across season with LOD score of 2.01 – 2.54, 2.58 – 3.09 and 3.02 – 3.89 respectively. The phenotypic variation of 10.47 – 12.69, 11.52 – 13.11 and 17.05 – 17.28 respectively were recorded for these QTLs of plant height.

Spicklets per head

Three QTLs were detected for number of spicklets per head in RIP1 at Dharwad. Two QTLs flanked by Xtxp297- Xtxp197 on LG-B and Xtxp47- Xtxp18 on LG-H were found common across season, with per cent phenotypic variation of 13.68 – 14.90 and 8.42 – 9.51 and LOD score of 2.64–2.86 and 4.41-5.2 respectively. E36-1 had allele to contribute for this trait.

100 grain weight

A total of four QTLs were mapped for 100 grain weight, of which three QTLs found on LG-A (Xtxp32- Xtxp208), LG-D (Xtxp327-Xisp229) and on LG-E (Xtxp231- Xtxp205) were common across season with LOD of 2.20 – 2.42, 2.28 – 3.49 and 2.18 – 3.20 respectively. These QTLs accounted for per cent phenotypic variation of 11.83 – 12.05, 7.52 – 10.31 and 9.38 – 12.66, respectively with positive additive effect from E36-1.

Grain yield per plant

Five QTLs with higher LOD scores over set threshold were detected for this trait among them four QTLs found on LG-J (Xiabt364-Xiabt146), LG-I (Xtxp145- Xtxp6), LG-D (*x*Xsnp36- Xtxp27) and on LG-G (Xtxp141-Xsnp91) were consistent over seasons. They accounted for per cent phenotypic variation of 13.23 – 14.45, 7.72 – 9.36, 8.92 – 10.45 and 13.02 – 14.67 with LOD score of 3.29 – 3.51, 2.22 – 2.43, 2.65 – 2.93 and 2.02 – 3.13 respectively.

b) Features of QTLs in IS9830 × E36-1 derived RIP at Bheemarayanagudi location

QTLs associated with 13 different traits at Bheemarayanagudi location in RIP1 are presented in Table 19a, 19b, 19c. QTLs were homed on to respective linkage groups (Fig. 12).

Per cent GLA 15 DAF

Four QTLs bracketed by the marker pairs Xtxp34-Xtxp285 on LG-A, Xtxp38-Xsnp19 on LG-C, Xiabt227-Xsnp56 on LG-D and Xtxp141-Xsnp91 on LG-G were detected for per cent GLA 15 DAF. Among them first three QTLs were found common across seasons in Bheemarayanagudi with LOD score of 2.15 – 2.89, 2.14 – 2.59 and 2.69 – 3.26 respectively, expressing positive additive effect conferring allele from E36-1 parent with per cent phenotypic variation of 7.15 – 10.23, 8.14 – 9.32 and 6.49 – 9.39 respectively.

Per cent GLA 30 DAF

Totally three QTLs were detected for this trait on different LGs. The QTLs found on LG-C (Xtxp114-Xumc7) and LG-E (Xtxp205- Xtxp231) were found common across two seasons expressing positive additive effect conferring allele from E36-1 with LOD score of 2.33 – 3.13 and 3.00 – 3.34 and per cent phenotypic variation of 8.27 – 9.08 and 8.21 – 10.48, respectively.

Per cent GLA 45 DAF

Two QTLs flanked by Xtxp298-Xtxp324 on LG-J and Xtxp295-Xtxp168 on LG-E were detected for per cent GLA 45 DAF trait and were found common across season with LOD score of 2.34 – 3.12 and 4.10 – 4.80 expressing per cent phenotypic variation of 10.34 – 10.49 and 9.00 – 10.46 with positive additive effect conferring allele from E36-1 parent.

Days to 50 per cent flowering

Three QTLs flanked by Xtxp159-Xiabt377 and Xisp074-Xisp362 on LG-E and Xumc136-Xsnp28 on LG-B were detected for this trait and were found common across season with LOD score of 2.51 – 2.90, 2.23 – 3.14 and 2.11 – 2.43 with negative effect conferring allele from IS9830 expressing per cent phenotypic effects of 10.84 – 11.06, 4.86 – 8.34 and 5.28 – 6.34 respectively.

**Table 19a: Features of the QTLs computed by composite interval mapping for stay-green and yield related traits in the RILs derived from the cross IS9830 × E36-1 at Bheemarayanagudi location evaluated during 2008**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_ib^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 103.8 | 4.82 | 2.15 | 3.32 | 10.23 |
| | C | Xtxp38-Xsnp19 | 122.35 | 13.3 | 2.56 | 4.99 | 9.32 |
| | D | Xiabt227-Xsnp56 | 71.23 | 13.32 | 3.26 | 9.22 | 9.39 |
| | G | Xtxp141-Xsnp91 | 21.8 | 15.3 | 2.65 | 2.34 | 5.76 |
| % GLA 30 DAF | C | Xtxp114-Xiumc7 | 26.47 | 6.34 | 3.13 | 3 | 8.27 |
| | E | Xtxp205- Xtxp231 | 128.09 | 7.22 | 3 | 1.29 | 10.48 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 105.21 | 19.5 | 2.34 | 1.99 | 10.49 |
| | E | Xtxp295-Xtxp168 | 117.3 | 3.2 | 4.8 | 5.72 | 10.46 |
| Days to 50 % flowering | E | Xtxp159-Xiabt377 | 54.22 | 22.9 | 2.9 | -2.4 | 11.06 |
| | B | Xiumc136-Xsnp28 | 103.24 | 19.53 | 2.43 | -0.23 | 6.34 |
| | E | Xisu074-Xisp362 | 7.05 | 6.23 | 2.23 | -1.23 | 8.34 |
| Total green leaf area | B | Xtxp331-Xiabt445 | 54.3 | 9.65 | 3.07 | 1.73 | 10.8 |
| | C | Xiabt370-Xtxp71 | 158.41 | 10.34 | 2.56 | 1.87 | 8.35 |
| | A | Xtxp340-Xtxp61 | 4.84 | 6.92 | 2.59 | 2.12 | 11.39 |
| Earhead length | A | Xtxp40-Xtxp307 | 89.38 | 7.4 | 2.23 | 0.61 | 21.48 |
| | D | Xisp229-Xsnp36 | 40.43 | 11.47 | 2.94 | 1.56 | 10.35 |
| Number of leaves | G | Xiabt113-Xiabt147 | 171.45 | 13.47 | 3.78 | 0.28 | 11.98 |
| | I | Xtxp145-Xtxp6 | 31.7 | 12.3 | 3.32 | 4.27 | 10.41 |
| | C | Xsnp127-Xiabt352 | 201.3 | 12.5 | 2.98 | 0.42 | 10.76 |
| | F | Xtxp67-Xiabt430 | 58.69 | 9.23 | 3.99 | -0.5 | 13.57 |
| Panicle exertion | C | Xtxp71-Xtxp160 | 169.7 | 17.9 | 2.04 | -3.45 | 14.54 |
| | B | Xsnp135-Xiabt371 | 199.6 | 22.6 | 2.25 | 3.48 | 9.54 |
| | H | Xtxp47-Xtxp18 | 7.12 | 4.01 | 2.12 | 2.86 | 10.67 |
| | A | Xtxp37-Xiabt303 | 72.2 | 24.2 | 3.28 | 2.83 | 10.73 |
| Stem girth | G | Xiabt176-Xiabt113 | 155.32 | 29.1 | 3.13 | 0.55 | 14.86 |
| | B | Xtxp201-Xiumc136 | 90.21 | 3.18 | 3.42 | 3.24 | 11.47 |
| Plant height | A | Xtxp88-Xiabt303 | 42.25 | 13.12 | 2.56 | 2.78 | 11.04 |
| | A | Xtxp61-Xtxp37 | 16.92 | 17.23 | 2.6 | -3.42 | 10.34 |
| | I | Xtxp57-Xsnp78 | 8.15 | 5.7 | 3.13 | 1.04 | 8.45 |
| Spicklets per head | B | Xtxp297-Xtxp197 | 11.04 | 22.3 | 2.86 | 3.92 | 12.9 |
| | H | Xtxp47-Xtxp18 | 6.55 | 4.29 | 4.25 | 5.2 | 9.51 |
| | H | Xtxp273-Xtxp47 | 2.35 | 4.7 | 2.67 | 2.22 | 10.23 |
| 100 grain weight | A | Xtxp32-Xtxp208 | 116.72 | 9.62 | 2.42 | 2.19 | 12.99 |
| | D | Xtxp327-Xisp229 | 31.4 | 3.38 | 3.58 | 5.14 | 7.52 |
| Total green leaf area | D | Xsnp53-Xtxp327 | 22.65 | 13.6 | 3.43 | 3.25 | 9.44 |
| Grain yield per plant | J | Xiabt364-Xiabt146 | 140.54 | 13.18 | 3.51 | 1.19 | 10.45 |
| | I | Xtxp145-Xtxp6 | 31.12 | 12.31 | 2.45 | 3.26 | 9.34 |
| | D | Xsnp36-Xtxp27 | 50.12 | 10.05 | 2.64 | 2.01 | 9.93 |
| | B | Xiabt94-Xiabt332 | 342.63 | 21.2 | 2.29 | 3.27 | 11.74 |

GLA – Total green leaf area DAF – Days after flowering

**Table 19b: Features of the QTLs computed by composite interval mapping for stay-green and yield related traits in the RILs derived from the cross IS9830 × E36-1 at Bheemarayanagudi location evaluated during 2009**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_ib^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 104.11 | 4.7 | 2.89 | 3.57 | 7.15 |
| | C | Xtxp38-Xsnp19 | 123.07 | 13.42 | 2.14 | 4.04. | 8.14 |
| | D | Xiabt227-Xsnp56 | 72.52 | 13.27 | 2.69 | 8.23 | 6.49 |
| | G | Xtxp141-Xsnp91 | 21.8 | 15.3 | 2.45 | 2.58 | 6.93 |
| % GLA 30 DAF | C | Xtxp114-Xiumc7 | 26.21 | 7.12 | 2.33 | 4.13 | 9.08 |
| | E | Xtxp205- Xtxp231 | 128.35 | 7.1 | 3.34 | 2.68 | 8.21 |
| | D | Xiabt439-Xsnp49 | 278.15 | 12.5 | 3.1 | 1.49 | 4.65 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 105.45 | 19.72 | 3.12 | 4.08 | 10.34 |
| | E | Xtxp295-Xtxp168 | 117.82 | 3.32 | 4.1 | 2.29 | 9.1 |
| Days to 50 % flowering | E | Xtxp159-Xiabt377 | 54.76 | 23.12 | 2.51 | -1.34 | 10.84 |
| | B | Xiumc136-Xsnp28 | 100.3 | 20.1 | 2.11 | -0.93 | 5.28 |
| | E | Xisu074-Xisp362 | 6.23 | 6.94 | 3.14 | -1.89 | 4.86 |
| Total green leaf area | B | Xtxp331-Xiabt445 | 54.8 | 9.2 | 2.15 | 1.88 | 11.48 |
| | C | Xiabt370-Xtxp71 | 156.3 | 9.42 | 2.94 | 1.23 | 9.93 |
| | A | Xtxp340-Xtxp61 | 5.82 | 6.12 | 3.12 | 1.56 | 10.35 |
| Earhead length | A | Xtxp40-Xtxp307 | 89.79 | 7.72 | 2.37 | 0.39 | 21.12 |
| | D | Xisp229-Xsnp36 | 39.01 | 11.79 | 2.67 | 1.98 | 12.45 |
| Number of leaves | G | Xiabt113-Xiabt147 | 171.78 | 13.52 | 3.1 | 0.59 | 11.56 |
| | I | Xtxp145-Xtxp6 | 30.5 | 11.8 | 2.12 | 4.21 | 9.23 |
| | F | Xtxp67-Xiabt430 | 56.45 | 8.8 | 3.74 | -0.35 | 12.48 |
| Panicle exertion | C | Xtxp71-Xtxp160 | 169.92 | 18.98 | 3.67 | -2.89 | 13.19 |
| | H | Xtxp47-Xtxp18 | 6.23 | 3.44 | 2.43 | 2.14 | 9.04 |
| | A | Xtxp37-Xiabt303 | 73.56 | 25.76 | 2.68 | 1.94 | 9.48 |
| Stem girth | G | Xiabt176-Xiabt113 | 155.97 | 29.32 | 3.25 | 0.28 | 14.06 |
| | F | Xtxp289-Xtxp10 | 18.65 | 37.3 | 2.12 | 3.27 | 4.25 |
| | B | Xtxp201-Xiumc136 | 89.4 | 2.64 | 2.97 | 2.96 | 10.36 |
| Plant height | A | Xtxp88-Xiabt303 | 42.47 | 12.9 | 2.11 | 1.9 | 9.47 |
| | A | Xtxp61-Xtxp37 | 16.32 | 16.54 | 3.21 | -2.52 | 13.01 |
| Spicklets per head | B | Xtxp297-Xtxp197 | 11.32 | 22.1 | 2.13 | 3.68 | 12.34 |
| | H | Xtxp47-Xtxp18 | 7.34 | 3.58 | 3.21 | 5.41 | 8.42 |
| 100 grain weight | A | Xtxp32-Xtxp208 | 116.01 | 9.4 | 2.2 | 2.41 | 12.83 |
| | D | Xtxp327-Xisp229 | 31.82 | 4.61 | 2.12 | 3.24 | 10.31 |
| | J | Xtxp23-Xsnp54 | 15.3 | 6.2 | 2.67 | 2.22 | 7.47 |
| Grain yield per plant | J | Xiabt364-Xiabt146 | 139.4 | 14.38 | 3.29 | 1.68 | 10.23 |
| | G | Xtxp196-Xsnp117 | 113.85 | 18.1 | 2.85 | 4.25 | 10.56 |
| | I | Xtxp145-Xtxp6 | 30.5 | 11.73 | 2.97 | 3.76 | 8.93 |
| | D | Xsnp36-Xtxp27 | 49.55 | 9.64 | 2.03 | 1.66 | 8.36 |
| | B | Xiabt94-Xiabt332 | 344.89 | 23.8 | 3.64 | 2.48 | 9.73 |

GLA – Total green leaf area DAF – Days after flowering

**Table 19c: Features of the QTLs that were commonly detected across two seasons for stay-green and yield related traits in RILs derived from the cross IS9830 × E36-1 at Bheemarayanagudi location evaluated during 2008 and 2009**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_i b^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 103.8-104.11 | 4.7-4.82 | 2.15-2.89 | 3.32-3.57 | 7.15-10.23 |
| | C | Xtxp38-Xsnp19 | 122.35-123.07 | 13.3-13.42 | 2.14-2.59 | 4.02-4.99 | 8.14-9.32 |
| | D | Xiabt227-Xsnp56 | 71.23-72.52 | 12.8-13.32 | 2.69-3.26 | 8.23-9.22 | 6.49-9.39 |
| % GLA 30 DAF | C | Xtxp114-Xiumc7 | 26.21-26.47 | 6.34-7.12 | 2.33-3.13 | 3.0-4.13 | 8.27-9.08 |
| | E | Xtxp205- Xtxp231 | 128.09-128.35 | 7.1-7.22 | 3.00-3.34 | 1.29-2.68 | 8.21-10.48 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 105.21-105.45 | 19.5-19.72 | 2.34-3.12 | 1.99-4.08 | 10.34-10.49 |
| | E | Xtxp295-Xtxp168 | 117.3-117.82 | 3.2-3.32 | 4.10-4.8 | 2.29-5.72 | 9.10.46 |
| Days to 50 % flowering | E | Xtxp159-Xiabt377 | 54.22-54.76 | 22.9-23.12 | 2.51-2.90 | -1.38 | 10.84-11.06 |
| | B | Xiumc136-Xsnp28 | 100.3-103.24 | 19.53-20.10 | 2.11-2.43 | -0.23-0.93 | 5.28-6.34 |
| | E | Xisu074-Xisp362 | 6.23-7.05 | 6.23-6.94 | 2.23-3.14 | -0.66 | 4.86-8.34 |
| Total green leaf area | B | Xtxp331-Xiabt445 | 54.30-54.83 | 9.2-9.65 | 2.15-3.07 | 1.73-1.88 | 10.8-11.48 |
| | C | Xiabt370-Xtxp71 | 156.3-158.41 | 9.42-10.34 | 2.56-2.94 | 1.23-1.87 | 8.35-9.93 |
| | A | Xtxp340-Xtxp61 | 4.84-5.82 | 6.12-6.92 | 2.59-3.12 | 1.56-2.12 | 10.35-11.39 |
| Earhead length | A | Xtxp40-Xtxp307 | 89.38-89.79 | 7.4-7.72 | 2.23-2.37 | 0.39-0.61 | 21.12-21.48 |
| | D | Xisp229-Xsnp36 | 39.01-40.43 | 11.47-11.79 | 2.67-2.94 | 1.56-1.98 | 10.35-12.45 |
| Number of leaves | G | Xiabt113-Xiabt147 | 171.45-171.78 | 13.3-13.52 | 3.10-3.78 | 0.28-0.59 | 11.56-11.98 |
| | I | Xtxp145-Xtxp6 | 30.5-31.7 | 11.8-12.3 | 2.12-3.32 | 4.21-4.27 | 9.23-10.41 |
| | F | Xtxp67-Xiabt430 | 56.45-58.69 | 8.8-9.23 | 3.74-3.99 | -0.35 | 12.48-13.57 |
| Panicle exertion | C | Xtxp71-Xtxp160 | 169.7-169.92 | 17.9-18.98 | 2.04-3.67 | -4.12 | 13.19-14.54 |
| | H | Xtxp47-Xtxp18 | 6.23-7.12 | 3.44-4.01 | 2.12-2.43 | 2.14-2.86 | 9.04-10.67 |
| | A | Xtxp37-Xiabt303 | 72.2-73.56 | 24.2-25.76 | 2.68-3.28 | -3.15 | 9.48-10.73 |
| Stem girth | G | Xiabt176-Xiabt113 | 155.32-155.97 | 29.1-29.32 | 3.13-3.25 | 0.28-0.55 | 14.09-14.86 |
| | B | Xtxp201-Xiumc136 | 89.4-90.21 | 2.64-3.18 | 2.97-3.42 | 2.96-3.24 | 10.36-11.47 |
| Plant height | A | Xtxp88-Xiabt303 | 42.25-42.47 | 12.9-13.12 | 2.11-2.56 | 1.9-2.78 | 9.47-11.04 |
| | A | Xtxp61-Xtxp37 | 16.32-16.92 | 16.54-17.23 | 2.60-3.21 | -2.52-3.42 | 10.34-13.01 |
| Spicklets per head | B | Xtxp297-Xtxp197 | 11.04-11.32 | 22.1-22.5 | 2.13-2.86 | 3.68-3.92 | 12.34-12.90 |
| | H | Xtxp47-Xtxp18 | 6.55-7.34 | 3.58-4.29 | 3.21-4.25 | 5.41-5.2 | 8.42-9.51 |
| 100 grain weight | A | Xtxp32-Xtxp208 | 116.01-116.72 | 9.4-9.62 | 2.2-2.42 | 2.19-2.41 | 12.83-12.99 |
| | D | Xtxp327-Xisp229 | 31.4-31.82 | 3.38-4.61 | 2.12-3.58 | 3.24-5.14 | 7.52-10.31 |
| Grain yield per plant | J | Xiabt364-Xiabt146 | 139.4-140.54 | 13.18-14.38 | 3.29-3.51 | 1.19-1.68 | 10.23-10.45 |
| | I | Xtxp145-Xtxp6 | 30.5-31.12 | 11.73-12.31 | 2.45-2.97 | 3.26-3.76 | 8.93-9.34 |
| | D | Xsnp36-Xtxp27 | 49.55-50.21 | 9.64-10.05 | 2.03-2.64 | 1.66-2.01 | 8.36-9.93 |
| | B | Xiabt94-Xiabt332 | 342.63-344.89 | 21.2-23.80 | 2.29-3.64 | 2.48-3.27 | 9.73-11.74 |

GLA – Total green leaf area DAF – Days after flowering

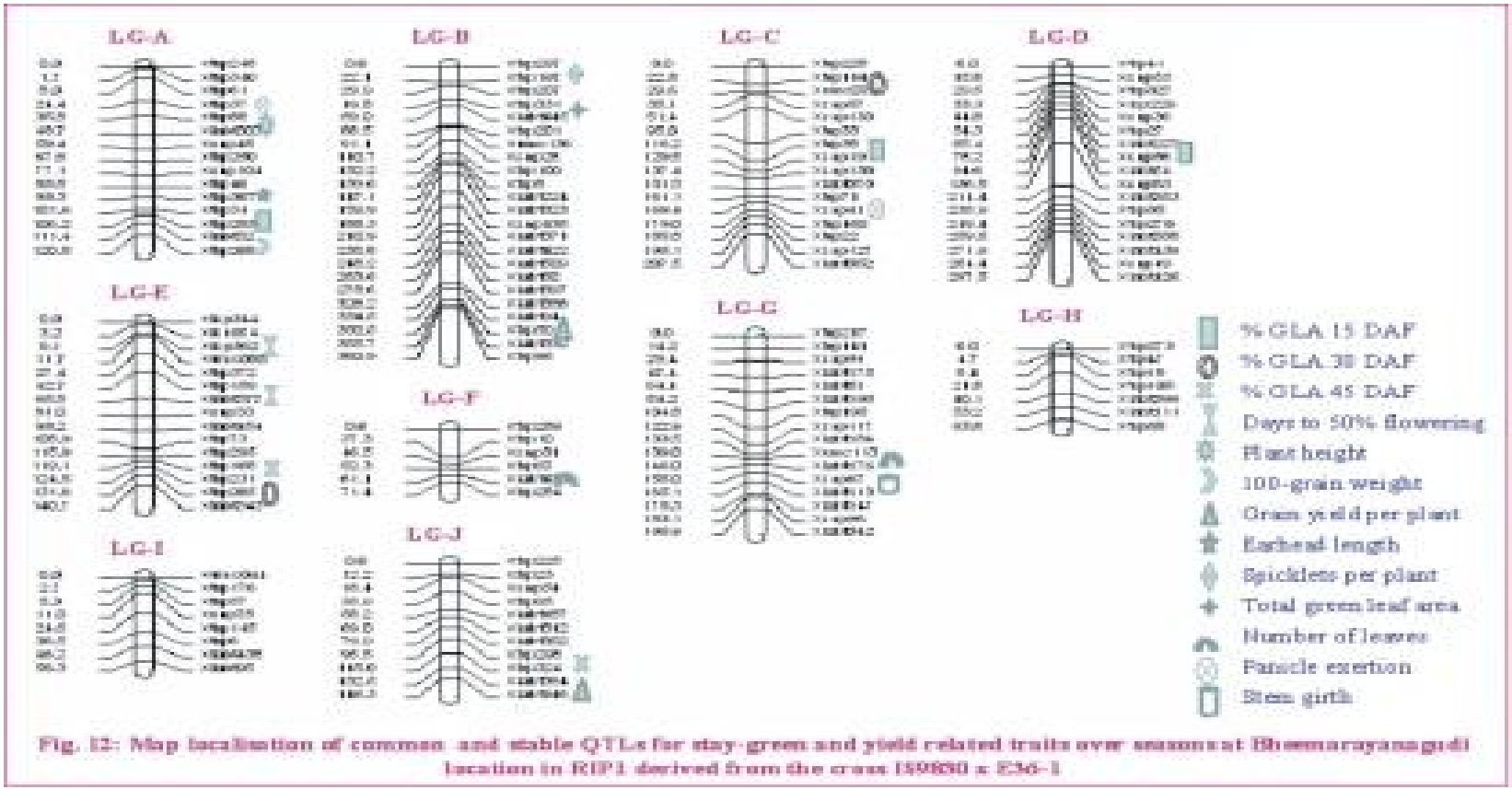



Fig. 12: Map localisation of common and stable QTLs for stay-green and yield related traits over seasons at Bheemarayanagudi location in RIP1 derived from the cross IS9830 x E36-1

**Fig. 12: Map localisation of common and stable QTLs for stay-green and yield related traits over seasons at Bheemarayanagudi location in RIP1 derived from the cross IS9830 x E36-1**

Total green leaf area

A set of four QTLs were detected for total green leaf area, among which three QTLs were common across season and were flanked by Xtxp331-Xiabt445 on LG-B, Xiabt370-Xtxp71 on LG-C and Xtxp340-Xtxp61 on LG-A with per cent phenotypic variation of 10.8 – 11.48, 8.35 – 9.93 and 10.35 – 11.39 and LOD score of 2.15 – 3.07, 2.56 – 2.94 and 2.59 – 3.12 respectively. They had positive additive effect conferring allele from E36-1 parent.

Earhead length

Two QTLs spanned by Xtxp40- Xtxp307 (LG-A) and Xisp229-Xsnp36 (LG-D) were detected for length of head were found common across season with LOD score of 2.23 – 2.37 and 2.67 – 2.94 with per cent phenotypic variation of 21.12 – 21.48 and 10.35 – 12.45 respectively.

Number of leaves

For number of leaves, four QTLs were detected, among them three QTLs bracketed by Xiabt113-Xiabt147 (on LG-G), Xtxp145- Xtxp6 (LG-I) and Xtxp67-Xiabt430 (LG-F) were found common across season with per cent phenotypic variation of 11.56 – 11.98, 9.23 – 10.41 and 12.48 – 13.57. These QTLs recorded LOD score of 3.10 – 3.78, 2.12 – 3.32 and 3.74 – 3.99 respectively and had positive additive effect conferring allele from E36-1 by first two QTLs and last one had negative effect conferring allele from IS9830.

Panicle exertion

As many as four QTLs were detected for length of panicle exertion, three QTLs from LG-C (Xtxp71- Xtxp160), LG-H (Xtxp47- Xtxp18) and LG-G (Xtxp37-Xiabt303) were found common across seasons with LOD score of 2.04 – 3.67, 2.12 – 2.43 and 2.68 – 3.28 with phenotypic variation of 13.19 – 14.54, 9.04 – 10.67 and 9.48 – 10.73, respectively. The QTLs from LG-C and LG-G showed negative effect conferring allele from IS9830 and QTL from LG-C showed positive additive effect conferring allele from E36-1.

Stem girth

Three QTLs spanned by Xiabt176-Xiabt113 on LG-G, Xtxp289- Xtxp10 on LG-F and Xtxp201-Xumc136 on LG-B were detected for stem girth. QTLs found on LG-G and LG-B were found common across season with positive additive effect conferring allele from E36-1 parent, with LOD score of 3.13 – 3.25 and 2.97 – 3.42 and expressing phenotypic variation of 14.09 – 14.86 and 10.36 – 11.47 respectively.

Plant height

Plant height recorded three QTLs. Among them two QTLs mapped on LG-A were found common across season; they were flanked by Xtxp88-Xiabt303 and Xtxp61- Xtxp37 with LOD score of 2.11 – 2.56 and 2.60 – 3.21 and phenotypic variation of 9.47 – 11.04 and 10.34 – 13.01 respectively.

Spicklets per head

QTLs spanned by the markers pairs Xtxp297- Xtxp197 on LG-B and Xtxp47- Xtxp18, Xtxp273- Xtxp47 on LG-H were detected for spicklets per head. The QTL from LG-B (Xtxp297- Xtxp197) and LG-H (Xtxp47- Xtxp18) were found common across season with positive additive effect conferring allele from E36-1 parent and accounting for12.34 – 12.90 and 8.42 – 9.51 per cent phenotypic variance, with LOD score of 2.13 – 2.86 and 3.21 – 4.25 respectively.

100 grain weight

For 100 grain weight, three QTLs were detected and among them two QTLs flanking Xtxp32-Xtxp208 on LG-A and Xtxp327-Xisp229 on LG-D were found common across season with LOD score of 2.20 – 2.42 and 2.12 – 3.58 and phenotypic variation of 12.83 – 12.99 and 7.52 – 10.31 per cent respectively. The positive additive effect conferring allele from E36-1 parent was recorded for this trait.

Grain yield per plant

Out of five QTLs detected for grain yield per plant, four QTLs were found common across season flanked by Xiabt364-Xiabt146 (LG-J), Xtxp145- Xtxp6 (LG-I), Xsnp36- Xtxp27 (LG-D) and Xiabt94-Xiabt332 (LG-B) with LOD score of 3.29 - 3.51, 2.45 – 2.97, 2.03 – 2.64 and 2.29 - 3.64. The per cent phenotypic variation of 10.23 – 10.45, 8.93 – 9.34, 8.36 – 9.93 and 9.73 – 11.74 respectively with positive additive effect conferring allele from E36-1 parent was recorded.

c) Features of QTLs in IS9830 × E36-1 derived RIP at Dharwad and Bheemarayanagudi over seasons

Common QTLs associated with 13 different traits at Dharwad and Bheemarayanagudi locations in RIP1 are presented in Table 20. QTLs were homed on to respective linkage groups (Fig. 13).

Per cent GLA 15 DAF

Three QTLs conferring to per cent GLA 15 DAF were found common across season and locations. The QTLs were flanked by Xtxp34-Xtxp285 (LG-A), Xtxp38-Xsnp19 (LG-C) and Xiabt227-Xsnp56 (LG-D) with LOD value of 2.24 – 2.85, 2.51 – 2.73 and 2.70 – 3.13, per cent phenotypic variation of 9.05 - 11.04, 7.56 - 8.38 and 7.49 – 9.39, respectively. All QTLs showed positive additive affect conferring favorable allele from E36-1.

Per cent GLA 30 DAF

Two QTLs were detected common across season and locations for per cent GLA 30 DAF, bracketed by Xtxp114-Xumc7 on LG-C and Xtxp205-Xtxp231 on LG-E with LOD value of 2.39 – 3.32 and 3.12 – 3.14, phenotypic variation of 8.27 – 10.24 and 8.21 – 10.48 per cent respectively. QTLs were of positive additive effect type from E36-1 parent in this cross.

Per cent GLA 45 DAF

For per cent GLA 45 DAF, two QTLs on LG-J (Xtxp298-Xtxp324) and on LG-E (Xtxp295-Xtxp168) were mapped and were common over season and across locations with LOD value of 2.73 – 3.35 and 4.60 – 4.80, per cent phenotypic variation of 10.34 – 19.49 and 9.00 – 10.46 respectively with positive additive effect conferring favorable allele from E36-1.

Days to 50 per cent flowering

For days to 50 per cent flowering, two QTLs were found common across seasons and locations mapped on LG-E (Xtxp159-Xiabt377) and on LG-B (Xumc136-Xsnp28) with LOD value of 2.41 – 2.90 and 2.11 – 2.85, per cent phenotypic variation of 12.84 – 14.06 and 5.28 - 8.83, respectively. QTLs recorded negative effect conferring favorable allele from IS9830.

Total green leaf area

Three QTLs were found common across seasons and locations for total green leaf area. The QTLs were bracketed by Xtxp331-Xiabt445 (LG-B), Xiabt370-Xtxp71 (LG-C) and on Xtxp340-Xtxp61 (LG-A) with LOD of 2.85 – 3.07, 2.56 - 3.54 and 2.59 – 3.12, phenotypic variation of 10.81 – 11.48, 6.34 – 9.93 and 10.32 – 12.43 per cent respectively.

Earhead length

Two QTLs flanked by Xtxp40-Xtxp307 (LG-A) and Xisp229-Xsnp36 (LG-D) were found common across seasons and locations with LOD score of 2.19 – 2.34 and 2.21 – 2.94, per cent phenotypic variation of 22.47 – 22.98 and 10.35 – 12.65 respectively.

Number of leaves

Two QTLs detected common across seasons and across locations for number of leaves. The QTLs flanked by Xiabt113-Xiabt147 (LG-G) and Xtxp145-Xtxp6 (LG-I) with LOD score of 3.19 – 3.55 and 2.42 – 3.12, phenotypic variation of 11.83 – 13.32 and 10.18 – 12.41 per cent with positive additive effect were stable across seasons.

**Table 20: Features of the QTLs that were commonly detected across three years and two locations for stay-green and yield related traits in RILs derived from the cross IS9830 × E36-1**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_i b^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 103.9-104.12 | 4.6-4.82 | 2.24-2.85 | 2.18-2.57 | 9.05-11.04 |
| | C | Xtxp38-Xsnp19 | 122.85-123.07 | 13.3-13.52 | 2.51-2.73 | 3.02-3.89 | 7.56-8.38 |
| | D | Xiabt227-Xsnp56 | 71.8-72.02 | 12.8-13.02 | 2.70-3.13 | 8.34-9.39 | 7.49-9.39 |
| % GLA 30 DAF | C | Xtxp114-Xiumc7 | 26.2-26.42 | 6.8-7.02 | 2.39-3.32 | 4.0-413 | 8.27-10.24 |
| | E | Xtxp205- Xtxp231 | 128.05-128.27 | 7.1-7.32 | 3.12-3.14 | 1.91-2.67 | 8.21-10.48 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 105.25-105.47 | 19.5-19.72 | 2.73-3.35 | 1.82-4.28 | 18.34-19.49 |
| | E | Xtxp295-Xtxp168 | 117.5-117.72 | 3.2-3.42 | 4.6-4.8 | 1.29-5.72 | 9-10.46 |
| Days to 50 % flowering | E | Xtxp159-Xiabt377 | 54.1-54.32 | 22.9-23.12 | 2.41-2.90 | -.0.38.0.96 | 12.84-14.06 |
| | B | Xiumc136-Xsnp28 | 99.9-103.24 | 19.53-20.24 | 2.11-2.85 | -1.28-0.23 | 5.28-8.83 |
| Total green leaf area | B | Xtxp331-Xiabt445 | 54.4-54.63 | 9.2-9.252 | 2.85-3.07 | 1.73-1.88 | 10.8-11.48 |
| | C | Xiabt370-Xtxp71 | 156.3-158.41 | 9.42-10.37 | 2.56-3.54 | 0.32-1.87 | 6.34-9.93 |
| | A | Xtxp340-Xtxp61 | 4.84-5.84 | 6.12-7.12 | 2.59-3.12 | 1.56-2.86 | 10.32-12.43 |
| Earhead length | A | Xtxp40-Xtxp307 | 89.55-89.77 | 7.4-7.62 | 2.19-2.34 | 0.39-0.61 | 22.47-22.98 |
| | D | Xisp229-Xsnp36 | 39.01-40.73 | 11.47-12.84 | 2.21-2.94 | 1.56-3.24 | 10.35-12.65 |
| Number of leaves | G | Xiabt113-Xiabt147 | 171.71-71.73 | 13.3-13.52 | 3.19-3.55 | 0.28-0.59 | 11.83-13.32 |
| | I | Xtxp145-Xtxp6 | 30.5-31.7 | 11.8-12.3 | 2.42-3.12 | 4.21-4.27 | 10.18-12.41 |
| Panicle exertion | C | Xtxp71-Xtxp160 | 169.7-169.92 | 17.9-18.76 | 2.39-3.61 | -4.12 | 13.22-14.54 |
| | H | Xtxp47-Xtxp18 | 6.23-7.12 | 3.44-4.30 | 2.12-3.13 | 0.23-2.86 | 9.04-10.76 |
| Stem girth | G | Xiabt176-Xiabt113 | 155.22-155.27 | 29.1-29.32 | 3.72-3.94 | 0.28-0.55 | 13.27-13.49 |
| | B | Xtxp201-Xiumc136 | 89.4-90.21 | 2.6-3.18 | 2.74-3.42 | 1.29-3.24 | 8.53-11.47 |
| Plant height | A | Xtxp88-Xiabt303 | 42.25-42.47 | 12.9-13.12 | 2.01-2.54 | 1.9-2.78 | 10.47-12.69 |
| | A | Xtxp61-Xtxp37 | 16.2-16.9 | 16.4-17.1 | 2.58-3.09 | -2.52-3.42 | 11.52-13.11 |
| Spicklets per head | B | Xtxp297-Xtxp197 | 11.05-11.32 | 22.1-22.5 | 2.64-2.86 | 2.68-2.9 | 13.68-14.90 |
| | H | Xtxp47-Xtxp18 | 6.55-7.14 | 3.6-4.2 | 3.21-4.14 | 4.41-5.2 | 8.42-9.51 |
| 100 grain weight | A | Xtxp32-Xtxp208 | 116.1-116.72 | 9.4-9.62 | 2.2-2.42 | 0.19-0.41 | 11.83-12.05 |
| | D | Xtxp327-Xisp229 | 31.4-31.9 | 3.8-4.6 | 2.28-3.49 | 2.24-5.14 | 7.52-10.31 |
| Grain yield per plant | J | Xiabt364-Xiabt146 | 139.4-140.54 | 13.8-14.38 | 3.29-3.51 | 1.19-1.68 | 13.23-14.45 |
| | I | Xtxp145-Xtxp6 | 30.5-31.12 | 11.73-12.73 | 2.22-2.97 | 1.89-3.76 | 7.72-9.34 |
| | D | Xsnp36-Xtxp27 | 49.55-50.21 | 9.5-10.11 | 2.03-2.93 | 1.43-2.98 | 8.36-10.45 |

GLA – Total green leaf area DAF – Days after flowering

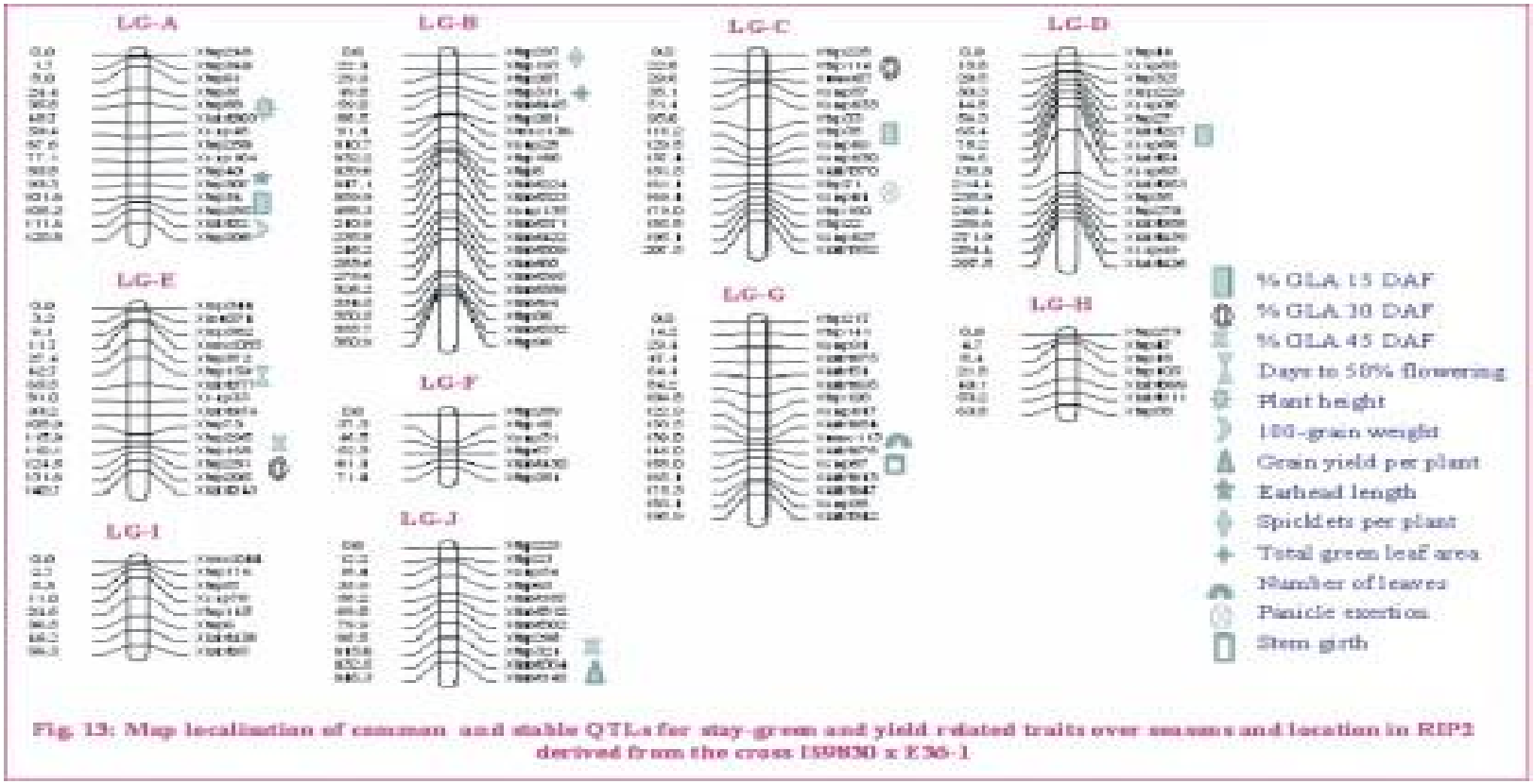



Fig. 13: Map localisation of common and stable QTLs for stay-green and yield related traits over seasons and location in RIP2 derived from the cross IS9830 x E36-1

**Fig. 13: Map localisation of common and stable QTLs for stay-green and yield related traits over seasons and location in RIP2 derived from the cross IS9830 x E36-1**

Panicle exertion

QTLs mapped on LG-C (Xtxp71-Xtxp160) and LG-H (Xtxp47-Xtxp18) were found common across locations and seasons with LOD of 2.39 – 3.61 and 2.12 – 3.13, per cent phenotypic variation of 13.22 – 14.54 and 9.04 – 10.76 respectively. QTL found on LG-C showed negative effect allele from IS9830 while QTL on LG-H showed positive additive effect allele from E36-1.

Stem girth

Two QTLs bracketed by Xiabt176-Xiabt113 (LG-G) and Xtxp201-Xumc136 (LG-B) were found common across seasons and locations for this trait with per cent phenotypic variation of 13.27 – 13.49 and 8.53 – 11.47 and LOD value of 3.72 – 3.94 and 2.74 – 3.42, respectively.

Plant height

For plant height, two QTLs on LG-G flanked by Xtxp88-Xiabt303 and Xtxp61-Xtxp37 were detected common across seasons and locations with LOD value of 2.01 – 2.54 and 2.58 – 3.09, per cent phenotypic variation of 10.47 – 12.69 and 11.52 - 13.11 respectively.

Spicklets per head

Two QTLs found common across seasons and location for this trait, flanked by Xtxp297-Xtxp197 (LG-B) and Xtxp47-Xtxp18 (LG-H) with LOD of 2.64 – 2.86 and 3.21 – 4.14, phenotypic variation of 13.68 – 14.90 and 8.42 – 9.51 per cent respectively with positive additive effect conferring favorable allele from E36-1. There were no genic SSR or SNP markers conditioning QTLs for this trait.

100 grain weight

QTLs bracketed by Xtxp32-Xisp229 (LG-A) and Xtxp327-Xisp229 (LG-D) were found common across seasons and locations with LOD of 2.20 – 2.42 and 2.28 – 3.49, phenotypic variation of 11.83 – 12.05, 7.52 – 10.32 per cent respectively.

Grain yield per plant

Totally three QTLs found common across season and across locations and were flanked by Xiabt364-Xiabt146 (LG-J), Xtxp145-Xtxp6 (LG-I) and Xsnp36-Xtxp27 (LG-D), with per cent phenotypic variation of 13.23 – 14.45, 7.72 – 9.34 and 8.36 – 10.45, LOD value of 3.29 – 3.51, 2.22 – 2.97 and 2.03 – 2.93 respectively. All these QTLs showed positive additive effect conferring favorable allele from E36-1 parent.

d. Features of QTLs in N13 × E36-1 derived RIP at Dharwad location

QTLs associated with 14 different traits at Dharwad location in RIP2 presented in Table 21a, 21b, 21c, 21d. QTLs were homed on to respective linkage groups (Fig. 14).

Per cent GLA 15 DAF

A total of four QTLs detected for per cent GLA 15 DAF, three QTL bracketed by Xtxp34- Xtxp285 (LG-A), Xtxp38-Xsnp19 (LG-C) and Xiabt227-Xsnp56 (LG-D) were found common over seasons with LOD score of 3.15 – 3.97, 3.08 – 3.68 and 2.63 – 2.68 showing per cent phenotypic variation of 10.05 – 12.46, 6.39 – 8.47 and 7.56 – 8.49 respectively with positive additive effect conferring favorable allele from E36-1 parent.

Per cent GLA 30 DAF

Three QTLs were detected for this trait at Dharwad location of which two QTLs from LG-E (Xtxp205- Xtxp231) and LG-B (Xtxp8-Xtxp1) were found common across season with per cent phenotypic variation of 9.00 – 10.37 and 8.00 – 9.94 and exhibiting LOD score of 2.89 – 2.29 and 2.32 – 2.79, respectively. The QTLs had positive additive effect with favorable allele from E36-1 parent.

**Table 21a: Features of the QTLs computed by composite interval mapping for stay-green and yield related traits in the RILs derived from the cross N13 × E36-1 at Dharwad location evaluated during 2007**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_ib^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 108.25 | 4.98 | 3.15 | 2.18 | 12.46 |
| | C | Xtxp38-Xsnp19 | 85.05 | 13.82 | 3.52 | 2.31 | 8.47 |
| | D | Xiabt227-Xsnp56 | 82.1 | 16.4 | 2.68 | 3.83 | 7.56 |
| % GLA 30 DAF | E | Xtxp205-Xtxp231 | 144.82 | 8.7 | 2.89 | 6.32 | 9 |
| | B | Xtxp8-Xtxp1 | 103.96 | 7.63 | 2.32 | 8.34 | 8 |
| | E | Xisp344-Xisp74 | 1.15 | 2.3 | 3.16 | 1.26 | 4.55 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 37.81 | 25.63 | 2.56 | 2.49 | 11.57 |
| | G | Xiabt341-Xiabt178 | 52.11 | 17.19 | 3.26 | 2.28 | 16.98 |
| | A | Xtxp248-Xtxp340 | 1.25 | 2.5 | 2.34 | 3.25 | 5.25 |
| Carbon isotope discrimination | G | Xtxp141-Xiabt437 | 25.45 | 12.39 | 2.53 | -2.34 | 3.16 |
| | I | Xtxp6-Xiabt70 | 39.93 | 10.48 | 3.1 | 0.76 | 7.23 |
| Days to 50 % flowering | E | Xiabt511-Xtxp231 | 156.96 | 12.57 | 2.85 | -1.02 | 14.55 |
| | B | Xiumc136-Xsnp28 | 62.31 | 19.23 | 2.45 | -0.84 | 6.23 |
| | A | Xiabt424-Xiabt54 | 262.16 | 23.48 | 2.89 | 1.73 | 27.43 |
| Total green leaf area | B | Xiabt80-Xtxp73 | 218.9 | 9.31 | 2.69 | 1.24 | 13.33 |
| | C | Xiabt370-Xtxp71 | 112.7 | 9.04 | 2.97 | 2.53 | 7.11 |
| | A | Xtxp340-Xtxp61 | 6.23 | 8.42 | 2.95 | 3.6 | 10.65 |
| Earhead length | D | Xsnp56-Xiabt79 | 111.6 | 42.96 | 2.22 | -1.23 | 17.59 |
| | D | Xisp229-Xsnp36 | 35.81 | 11.49 | 2.53 | 3.67 | 11.62 |
| Number of leaves | I | Xtxp6-Xiabt438 | 41.31 | 9.91 | 2.71 | 1.45 | 10.45 |
| | I | Xtxp145-Xtxp6 | 30.2 | 12.4 | 3.32 | 2.15 | 9.08 |
| | B | Xtxp96-Xiabt177 | 467.6 | 14 | 2.87 | 2.74 | 12.84 |
| Panicle exertion | F | Xtxp67-Xiabt430 | 54.09 | 8.42 | 2.69 | 0.74 | 19.65 |
| | H | Xtxp47-Xtxp18 | 6.69 | 3.59 | 2.96 | 2.12 | 8.55 |
| | D | Xiabt364-Xiabt466 | 219.45 | 25.5 | 3.12 | 0.95 | 12.12 |
| Stem girth | G | Xiabt178-Xiabt302 | 70.35 | 19.9 | 2.54 | 1.05 | 9.5 |
| | B | Xtxp201-Xiumc136 | 51.73 | 3.23 | 2.92 | 0.93 | 9.93 |
| | E | Xtxp205-Xiabt511 | 150.47 | 21.1 | 2.95 | 2.42 | 22.43 |
| Plant height | B | Xiumc136-Xsnp28 | 63.12 | 19.23 | 3.42 | 3.12 | 14.28 |
| | A | Xtxp61-Xtxp37 | 18.95 | 17.44 | 3.12 | 1.45 | 9.34 |
| Spicklets per head | A | Xtxp208- Xiabt263 | 142.3 | 7.12 | 2.02 | 2.58 | 9.83 |
| | H | Xtxp47-Xtxp18 | 6.93 | 3.6 | 2.44 | 1.74 | 9.51 |
| 100 grain weight | D | Xtxp27-Xiabt353 | 56.07 | 12 | 4.35 | 0.63 | 19.48 |
| | D | Xtxp327-Xisp229 | 28.13 | 4.3 | 2.46 | 2.34 | 12.09 |
| | I | Xiabt301-Xiabt338 | 141.9 | 24.2 | 2.77 | 1.03 | 10.54 |
| | D | Xiabt353-Xiabt147 | 177.23 | 12 | 4.13 | 4.44 | 20.14 |
| Grain yield per plant | E | Xtxp372-Xiabt377 | 64.24 | 29.3 | 3.19 | 2.49 | 21.53 |
| | I | Xtxp145-Xtxp6 | 30.01 | 11.36 | 3.12 | 3.67 | 7.45 |
| | D | Xsnp36-Xtxp27 | 45.88 | 8.82 | 2.88 | 2.88 | 9.52 |

GLA – Total green leaf area DAF – Days after flowering

**Table 21b: Features of the QTLs computed by composite interval mapping for stay-green and yield related traits in the RILs derived from the cross N13 × E36-1 at Dharwad location evaluated during 2008**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_ib^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 108.87 | 4.77 | 3.97 | 2.65 | 10.05 |
| | C | Xtxp38-Xsnp19 | 85.27 | 13.92 | 3.08 | 2.38 | 7.84 |
| | D | Xiabt227-Xsnp56 | 82.4 | 16.55 | 2.63 | 3.02 | 8.12 |
| | G | Xiabt267-Xtxp159 | 150.05 | 15 | 2.28 | 2.11 | 4.98 |
| % GLA 30 DAF | E | Xtxp205-Xtxp231 | 144.95 | 9.01 | 2.55 | 5.38 | 9.93 |
| | B | Xtxp8-Xtxp1 | 103.35 | 7.5 | 2.79 | 6.45 | 9.94 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 37.55 | 25 | 2.34 | 3.29 | 11.34 |
| | G | Xiabt341-Xiabt178 | 51.95 | 16.9 | 2.23 | 1.35 | 16 |
| | A | Xtxp248-Xtxp340 | 1.25 | 2.5 | 2.19 | 1.37 | 5.36 |
| Carbon isotope discrimination | G | Xtxp141-Xiabt437 | 25.97 | 12.14 | 2.99 | -1.67 | 3.74 |
| | I | Xtxp6-Xiabt70 | 39.7 | 10.22 | 2.69 | 0.93 | 7.19 |
| Days to 50 % flowering | E | Xiabt511-Xtxp231 | 155.95 | 12.62 | 2.97 | -1.78 | 14.87 |
| | B | Xiumc136-Xsnp28 | 61.9 | 19.84 | 2.25 | -0.32 | 7.19 |
| | A | Xiabt424-Xiabt54 | 261.85 | 23.32 | 2.73 | 1.82 | 27.12 |
| Total green leaf area | B | Xiabt80-Xtxp73 | 219.11 | 9.42 | 2.53 | 1.38 | 13.38 |
| | C | Xiabt370-Xtxp71 | 112.9 | 8.87 | 3.2 | 2.84 | 7.05 |
| | A | Xtxp340-Xtxp61 | 6.18 | 8.38 | 2.53 | 2.64 | 10.37 |
| Earhead length | D | Xsnp56-Xiabt79 | 111.7 | 42 | 2.1 | -0.92 | 17.41 |
| | D | Xisp229-Xsnp36 | 36.12 | 11.46 | 3.61 | 4.27 | 12.46 |
| Number of leaves | I | Xtxp6-Xiabt438 | 41.2 | 9.8 | 2.54 | 1.33 | 10.6 |
| | I | Xtxp145-Xtxp6 | 29.1 | 11.1 | 3.01 | 3.14 | 8.53 |
| Panicle exertion | F | Xtxp67-Xiabt430 | 53.9 | 8.39 | 2.56 | 0.63 | 19.52 |
| | H | Xtxp47-Xtxp18 | 6.74 | 4.12 | 2.54 | 1.49 | 9.37 |
| | D | Xiabt364-Xiabt466 | 219.12 | 26.23 | 3.54 | 1.26 | 12.34 |
| Stem girth | G | Xiabt178-Xiabt302 | 70.49 | 20.34 | 2.07 | 0.98 | 9.28 |
| | J | Xiabt16-Xiabt146 | 89.75 | 11.3 | 2.74 | 2.81 | 6.33 |
| | B | Xtxp201-Xiumc136 | 52.12 | 3.18 | 2.66 | 1.78 | 9.81 |
| | E | Xtxp205-Xiabt511 | 151.34 | 21.96 | 2.11 | 1.43 | 22.84 |
| Plant height | B | Xiumc136-Xsnp28 | 63.06 | 19.1 | 3.3 | 3.34 | 14.5 |
| | A | Xtxp61-Xtxp37 | 19.14 | 16.1 | 2.24 | 1.96 | 8.45 |
| Spicklets per head | A | Xtxp208- Xiabt263 | 142.37 | 7.22 | 2.19 | 2.49 | 9.72 |
| | H | Xtxp47-Xtxp18 | 6.4 | 4.2 | 3.01 | 1.87 | 10.43 |
| | F | Xtxp289-Xtxp10 | 17.8 | 35.6 | 2.89 | 0.83 | 4.63 |
| 100 grain weight | D | Xtxp27-Xiabt353 | 55.92 | 12.17 | 4.26 | 0.86 | 19.53 |
| | D | Xtxp327-Xisp229 | 27.35 | 5.1 | 2.58 | 1.09 | 11.53 |
| | I | Xiabt301-Xiabt338 | 141.9 | 24.2 | 2.34 | 1.93 | 5.34 |
| | D | Xiabt353-Xiabt147 | 176.83 | 12.24 | 3.87 | 4.23 | 19.83 |
| Grain yield per plant | E | Xtxp372-Xiabt377 | 64.57 | 29.58 | 3.28 | 2.71 | 21.6 |
| | I | Xtxp145-Xtxp6 | 29.77 | 11.29 | 2.84 | 3.89 | 7.04 |
| | D | Xsnp36-Xtxp27 | 45.93 | 8.78 | 3.23 | 3.12 | 9.76 |

GLA – Total green leaf area DAF – Days after flowering

**Table 21c: Features of the QTLs computed by composite interval mapping for stay-green and yield related traits in the RILs derived from the cross N13 × E36-1 at Dharwad location evaluated during 2009**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_i b^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 108.58 | 4.6 | 3.82 | 3.25 | 11.76 |
| | C | Xtxp38-Xsnp19 | 85.18 | 13.7 | 3.68 | 2.26 | 6.39 |
| | D | Xiabt227-Xsnp56 | 82.88 | 16.79 | 2.65 | 3.46 | 8.49 |
| % GLA 30 DAF | E | Xtxp205-Xtxp231 | 145.73 | 8.91 | 2.29 | 8.32 | 10.37 |
| | B | Xtxp8-Xtxp1 | 103.84 | 7.79 | 2.54 | 4.67 | 9.23 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 37.68 | 25.42 | 2.97 | 3.11 | 11.93 |
| | G | Xiabt341-Xiabt178 | 52.63 | 17.23 | 2.21 | 2.36 | 16.27 |
| Days to 50 % flowering | E | Xiabt511-Xtxp231 | 156.32 | 12.4 | 3.39 | -1.52 | 14.29 |
| | B | Xiumc136-Xsnp28 | 62.1 | 20.12 | 2.13 | -0.12 | 6.52 |
| | A | Xiabt424-Xiabt54 | 262.38 | 22.5 | 2.54 | 2.04 | 26.38 |
| Total green leaf area | B | Xiabt80-Xtxp73 | 219.39 | 9.2 | 2.47 | 1.49 | 13.27 |
| | C | Xiabt370-Xtxp71 | 112.3 | 9.21 | 2.93 | 1.35 | 6.38 |
| | A | Xtxp340-Xtxp61 | 6.13 | 8.4 | 2.74 | 2.22 | 9.34 |
| Earhead length | D | Xsnp56-Xiabt79 | 111.6 | 42.54 | 2.32 | -0.42 | 17.37 |
| | D | Xisp229-Xsnp36 | 35.52 | 11.53 | 2.04 | 2.43 | 11.03 |
| Number of leaves | I | Xtxp6-Xiabt438 | 41.35 | 10.02 | 2.49 | 1.23 | 10.38 |
| | I | Xtxp145-Xtxp6 | 29.65 | 12.93 | 3.45 | 3.09 | 9.14 |
| Panicle exertion | F | Xtxp67-Xiabt430 | 54.12 | 8.2 | 2.47 | 0.85 | 19.43 |
| | I | Xiumc44-Xtxp176 | 1.25 | 2.5 | 2.34 | 1.63 | 10.34 |
| | H | Xtxp47-Xtxp18 | 6.15 | 4.09 | 2.46 | 1.82 | 8.79 |
| | D | Xiabt364-Xiabt466 | 218.5 | 25.83 | 3.33 | 1.43 | 11.63 |
| Stem girth | G | Xiabt178-Xiabt302 | 70.57 | 20.69 | 2.85 | 0.83 | 9.44 |
| | B | Xtxp201-Xiumc136 | 51.63 | 3.04 | 3.01 | 0.35 | 9.56 |
| | E | Xtxp205-Xiabt511 | 150.92 | 21.54 | 2.74 | 2.58 | 23.15 |
| Plant height | B | Xiumc136-Xsnp28 | 62.9 | 19.32 | 3.52 | 3.48 | 14.47 |
| | A | Xtxp61-Xtxp37 | 19.11 | 17.37 | 2.89 | 1.57 | 10.58 |
| | H | Xtxp273-Xtxp47 | 2.3 | 4.6 | 2.22 | 0.94 | 9.35 |
| Spicklets per head | A | Xtxp208- Xiabt263 | 142.48 | 7 | 2.28 | 2.71 | 9.54 |
| | H | Xtxp47-Xtxp18 | 7.1 | 3.92 | 2.87 | 2.51 | 10.22 |
| 100 grain weight | D | Xtxp27-Xiabt353 | 55.85 | 12.22 | 4.13 | 0.76 | 19.7 |
| | D | Xtxp327-Xisp229 | 27.89 | 4.79 | 2.13 | 2.41 | 12.14 |
| | D | Xiabt353-Xiabt147 | 176.2 | 12.35 | 3.42 | 3.48 | 19.48 |
| Grain yield per plant | E | Xtxp372-Xiabt377 | 64.35 | 29.42 | 3.06 | 2.57 | 21.38 |
| | C | Xtxp200-Xiabt234 | 2.4.45 | 13.7 | 2.84 | 1.02 | 9.54 |
| | I | Xtxp145-Xtxp6 | 29.03 | 11.23 | 2.78 | 2.58 | 6.34 |
| | D | Xsnp36-Xtxp27 | 45.84 | 8.8 | 2.79 | 2.79 | 8.34 |

GLA – Total green leaf area DAF – Days after flowering

**Table 21d: Features of the QTLs that were commonly detected across three seasons for stay-green and yield related traits in RILs derived from the cross N13 × E36-1 at Dharwad location evaluated during 2007, 2008 and 2009**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_i b^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 108.25-108.87 | 4.6-4.98 | 3.15-3.97 | 2.18-3.25 | 10.05-12.46 |
| | C | Xtxp38-Xsnp19 | 85.05-85.27 | 13.7-13.92 | 3.08-3.68 | 2.26-2.38 | 6.39-8.47 |
| | D | Xiabt227-Xsnp56 | 82.4-82.88 | 16.4-16.79 | 2.63-2.68 | 3.02-3.83 | 7.56-8.49 |
| % GLA 30 DAF | E | Xtxp205-Xtxp231 | 144.95-145.73 | 8.7-9.01 | 2.89-2.29 | 5.38-8.32 | 9.0-10.37 |
| | B | Xtxp8-Xtxp1 | 103.35-103.96 | 7.5-7.79 | 2.32-2.79 | 4.67-8.34 | 8.0-9.94 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 37.55-37.81 | 25-25.63 | 2.34-2.97 | 2.49-3.29 | 11.34-11.93 |
| | G | Xiabt341-Xiabt178 | 51.95-52.11 | 16.9-17.23 | 2.21-3.26 | 1.35-2.36 | 16-16.98 |
| Carbon isotope discrimination | G | Xtxp141-Xiabt437 | 25.45-25.97 | 12-12.39 | 2.4-2.99 | -0.903- 1-121 | 2.4-3.74 |
| | I | Xtxp6-Xiabt70 | 39.7-41.02 | 10-10.48 | 2.69-3.1 | 0.76-0.93 | 6.8-7.23 |
| Days to 50 % flowering | E | Xiabt511-Xtxp231 | 155.95-156.96 | 12.4-12.62 | 2.85-3.39 | -1.64- -0.71 | 14.29-14.87 |
| | B | Xiumc136-Xsnp28 | 61.9-62.31 | 19.23-20.12 | 2.13-2.45 | -0.32-0.84 | 6.23-7.19 |
| | A | Xiabt424-Xiabt54 | 261.85-262.38 | 22.5-23.48 | 2.54-2.89 | 1.73-2.04 | 26.38-27.43 |
| Total green leaf area | B | Xiabt80-Xtxp73 | 218.9-219.39 | 9.2-9.42 | 2.47-2.69 | 1.49-1.24 | 13.27-13.38 |
| | C | Xiabt370-Xtxp71 | 112.3-112.9 | 8.87-9.21 | 2.93-3.02 | 1.35-2.84 | 6.38-7.11 |
| | A | Xtxp340-Xtxp61 | 6.13-6.23 | 8.38-8.42 | 2.53-2.95 | 2.64-3.60 | 9.34-10.65 |
| Earhead length | D | Xsnp56-Xiabt79 | 111.6-111.70 | 42-42.96 | 2.1-2.32 | -0.22- -1.01 | 17.37-17.59 |
| | D | Xisp229-Xsnp36 | 35.52-36.12 | 11.46-11.53 | 2.04-3.61 | 2.43-4.27 | 11.03-12.46 |
| Number of leaves | I | Xtxp6-Xiabt438 | 41.35-41.20 | 9.8-10.02 | 2.49-2.71 | 1.23-1.45 | 10.38-10.60 |
| | I | Xtxp145-Xtxp6 | 29.1-30.2 | 11.1-12.4 | 3.01-3.45 | 2.15-3.14 | 8.53-9.14 |
| Panicle exertion | F | Xtxp67-Xiabt430 | 53.9-54.12 | 8.2-8.42 | 2.47-2.69 | 0.63-0.85 | 19.43-19.65 |
| | H | Xtxp47-Xtxp18 | 6.15-6.69 | 3.59-4.12 | 2.46-2.96 | 1.49-2.12 | 8.55-9.37 |
| | D | Xiabt364-Xiabt466 | 218.5-219.45 | 25.5-26.23 | 3.12-3.54 | 0.95-1.43 | 11.63-12.34 |
| Stem girth | G | Xiabt178-Xiabt302 | 70.35-70.57 | 19.9-20.69 | 2.07-2.85 | 0.83-1.05 | 9.28-9.5 |
| | B | Xtxp201-Xiumc136 | 51.63-52.12 | 3.04-3.23 | 2.66-3.01 | 0.35-1.78 | 9.56-9.93 |
| | E | Xtxp205-Xiabt511 | 150.47-151.34 | 21.1-21.96 | 2.11-2.95 | 1.43-2.58 | 22.43-23.15 |
| Plant height | B | Xiumc136-Xsnp28 | 62.9-63.12 | 19.1-19.32 | 3.3-3.52 | 3.12-3.48 | 14.28-14.5 |
| | A | Xtxp61-Xtxp37 | 18.95-19.14 | 16.1-17.44 | 2.24-3.12 | 1.45-1.96 | 8.45-10.58 |
| Spicklets per head | A | Xtxp208- Xiabt263 | 142.3-142.48 | 7-7.22 | 2.02-2.28 | 2.49-2.71 | 9.54-9.83 |
| | H | Xtxp47-Xtxp18 | 6.4-7.1 | 3.6-4.2 | 2.44-3.01 | 1.74-2.51 | 9.51-10.43 |
| 100 grain weight | D | Xtxp27-Xiabt353 | 55.85-56.07 | 12-12.22 | 4.13-4.35 | 0.63-0.86 | 19.48-19.7 |
| | D | Xtxp327-Xisp229 | 27.35-28.13 | 4.3-5.1 | 2.13-2.58 | 1.09-2.41 | 11.53-12.14 |
| | D | Xiabt353-Xiabt147 | 176.2-177.23 | 12-12.35 | 3.42-4.13 | 3.48-4.44 | 19.48-20.14 |
| Grain yield per plant | E | Xtxp372-Xiabt377 | 64.35-64.57 | 29.3-29.58 | 3.06-3.28 | 2.49-2.71 | 21.38-21.6 |
| | I | Xtxp145-Xtxp6 | 29.03-30.01 | 11.23-11.36 | 2.78-3.12 | 2.58-3.67 | 6.34-7.45 |
| | D | Xsnp36-Xtxp27 | 45.84-45.93 | 8.78-8.82 | 2.79-3.23 | 2.88-3.12 | 8.34-9.76 |

GLA – Total green leaf area DAF – Days after flowering

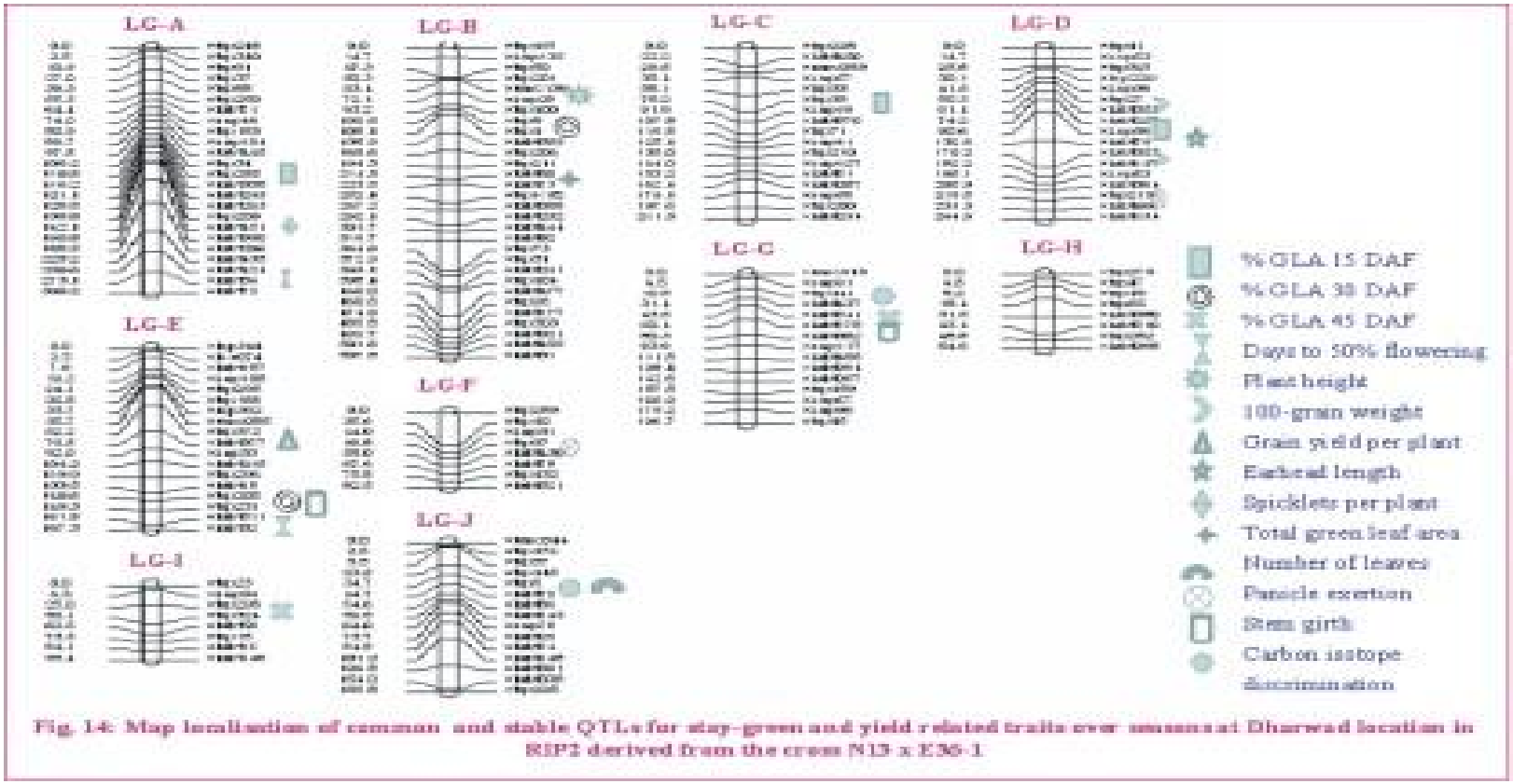



Fig. 14: Map localisation of common and stable QTLs for stay-green and yield related traits over seasons at Dharwad location in RIP2 derived from the cross N13 x E36-1

**Fig. 14: Map localisation of common and stable QTLs for stay-green and yield related traits over seasons at Dharwad location in RIP2 derived from the cross N13 x E36-1**

Per cent GLA 45 DAF

Three QTLs were detected for per cent GLA 45 DAF, among which two QTLs bracketed by Xtxp298- Xtxp324 (LG-J) and Xiabt341-Xiabt178 (LG-G) were detected commonly across seasons, QTLs showed positive additive effect with favorable allele from E36-1 parent. The QTLs from LG-G and LG-J showed LOD score of 2.34 – 2.97 and 2.21 – 3.26 with per cent phenotypic variation of 11.34 – 11.93 and 16.00 – 16.98, respectively.

Carbon isotope discrimination

QTLs bracketed by Xtxp141-Xiabt437 (on LG-G) and Xtxp6-Xiabt70 (LG-I) were detected for this trait and were found to be common across seasons with LOD score of 2.40 – 2.99 and 2.69 – 3.10 per cent phenotypic variation of 2.40 – 3.74 and 6.80 – 7.23, respectively. The QTLs mapped on LG-G showed negative effect with favorable allele from N13 parent and the QTL mapped on LG-I had positive additive effect with favorable allele from E36-1 parent.

Days to 50 per cent flowering

Totally three QTLs spanned by Xiabt511- Xtxp231 (LG-E) Xumc136-Xsnp28 (LG-B) and Xiabt424-Xiabt54 (LG-A) were detected for days to 50 per cent flowering and same were common across seasons. The QTLs were present on LG-E, LG-B and LG-A showed per cent phenotypic variation of 14.29 – 14.87, 6.23 – 7.19 and 26.38 – 27.43 and LOD score of 2.85 – 3.39, 2.13 – 2.45 and 2.54 – 2.89 respectively. First two QTLs showed negative effect conferring favorable allele from N13 parent but latter QTL showed positive additive effect with favorable allele from E36-1 parent.

Total green leaf area

A total of three QTLs were detected for total green leaf area which were common across seasons as well as QTLs were spanned by Xiabt80-Xtxp73 (LG-B), Xtxp370-Xtxp71 (LG-C) and Xtxp340-Xtxp61 (LG-A) with LOD score of 2.47 – 2.69, 2.93 – 3.02 and 2.53 – 2.95 and expressing per cent phenotypic variation from 13.27 – 13.80, 6.37 – 7.11 and 9.34 – 10.65 respectively with positive additive effect conferring stay green allele from E36-1.

Earhead length

Two QTLs mapped on LG-D spanned by Xsnp56-Xiabt79 and Xisp229-Xsnp36 were detected and are found common across season with LOD score of 2.10 – 2.32 and 2.04 – 3.61 per cent phenotypic variation of 17.37 – 17.59 and 11.03 – 12.46 respectively. QTL Xsnp56-Xiabt79 has negative effect conferring allele from N13 parent and another QTL Xisp229-Xsnp36 showed positive additive effect conferring favorable allele from E36-1 parent.

Number of leaves per plant

Two QTLs were detected for number of leaves and are found common across seasons and were mapped on LG-I spanned by Xtxp6-Xiabt438 and Xtxp145-Xtxp6 with LOD score of 2.49 – 2.71 and 3.01 – 3.45 per cent phenotypic variation of 10.38 – 10.60 and 8.53 – 9.14, respectively showing positive additive effect conferring favorable allele from E36-1 parent.

Panicle exertion

Totally four QTLs were detected for panicle exertion, among which three QTLs bracketed by Xtxp67-Xiabt430 (LG-F), Xtxp47-Xtxp18 (LG-H) and Xiabt364-Xiabt466 (LG-D) with LOD score of 2.47 – 2.69, 2.46 – 2.96 and 3.12 – 3.54 exhibiting per cent phenotypic variation of 19.43 – 19.65, 8.55 – 9.37 and 11.63 – 12.34 respectively with positive additive effect conferring favorable allele from E36-1.

Stem girth

For stem girth, three QTLs out of four QTLs detected were found common across seasons and are flanked by Xiabt178-Xiabt302 (LG-G), Xtxp201-Xumc136 (LG-B) and Xtxp205-Xiabt511 (LG-E) with LOD score of 2.07 – 2.85, 2.66 – 3.01 and 2.11 – 2.95 showing per cent phenotypic variation of 9.28 – 9.50, 9.56 – 9.93 and 22.43 – 23.15, respectively with positive additive effect conferring favorable allele from E36-1 parent.

Plant height

Three QTLs were detected for plant height out of these, two QTLs on LG-B (Xumc136-Xsnp28) and LG-A (Xtxp61-Xtxp37) were found common across season with LOD score of 3.30 – 3.52 and 2.24 – 3.12 per cent, phenotypic variation of 14.28 – 14.50 and 8.45 – 10.58, respectively showed positive additive effect conferring favorable allele from E36-1 parent.

Spicklets per plant

Two QTLs out of three QTLs detected were found common across seasons, which are spanned by Xtxp208-Xiabt263 (LG-A) and Xtxp47-Xtxp18 (LG-H) with 2.07 – 2.28 and 2.44 – 3.01 expressing per cent phenotypic variation of 9.54 – 9.83 and 9.51 – 10.43 respectively with positive additive effect conferring favorable allele from E36-1 parent.

100 grain weight

Totally four QTLs were detected for 100 grain weight, three QTLs on LG-D were found common across season which were spanned by Xtxp27-Xiabt353, Xtxp327-Xisp229 and Xiabt353-Xiabt147 with LOD score of 4.13 – 4.35, 2.13 – 2.58 and 3.42 – 4.13 per cent phenotypic variation of 19.48 – 19.70, 11.53 – 12.14 and 19.48 – 20.14, respectively. QTLs expressed positive additive effect conferring favorable allele from E36-1.

Grain yield per plant

Three QTLs out of detected four QTLs were found common across seasons with flanking markers Xtxp372-Xiabt377 (LG-E), Xtxp145-Xtxp6 (LG-I) and Xsnp36-Xtxp27 (LG-D) expressing per cent phenotypic variation of 21.38 – 21.60, 6.34 – 7.45 and 8.34 – 9.76 with LOD score of 3.06 – 3.28, 2.78 – 3.12 and 2.79 – 3.23 respectively. The QTLs have positive additive effect conferring favorable allele from E36-1 parent.

e) Features of QTLs in N13 × E36-1 derived RIP at Bheemarayanagudi location

The QTLs associated with 14 different traits in RIP2 at Bheemarayanagudi location are presented in Table 22a, 22b, 22c. QTLs were homed on to respective linkage groups (Fig. 15).

Per cent GLA 15 DAF

A set of three QTLs were detected for per cent GLA 15 DAF and are found to be common across seasons. The QTLs bracketed by Xtxp34-Xtxp285 (LG-A), Xtxp38-Xsnp19 (LG-C) and Xiabt227-Xsnp56 (LG-D) with LOD score of 2.03 – 2.97, 3.91 – 3.99 and 2.12 – 2.77 expressing per cent phenotypic variation of 9.56 – 11.23, 6.53 – 8.17 and 5.96 – 7.99, respectively. The QTLs showed positive additive effect conferring favorable allele from E36-1 parent.

Per cent GLA 30 DAF

Out of three QTLs detected two QTLs found to be common across seasons with positive additive effect. The two QTLs on LG-E (Xtxp205-Xtxp231) and LG-B (Xtxp8-Xtxp1) with LOD score of 2.01 – 2.19 and 2.91 – 2.80 per cent phenotypic variation of 8.0 – 9.37 and 8.0 – 9.94, respectively.

Per cent GLA 45 DAF

Three QTLs spanned by Xtxp298-Xtxp324 (LG-J), Xiabt341-Xiabt178 (LG-G) and Xtxp176-Xtxp57 (LG-I) were detected for per cent GLA 45 DAF. Among them, two QTLs from linkage groups LG-J and LG-G found common across seasons. QTL on LG-J (Xtxp298-Xtxp324) showed per cent phenotypic variation of 8.34 - 11.93 with 2.00 – 2.17 LOD score and QTL on LG-G spanned by Xiabt341-Xiabt178 exhibited per cent phenotypic variation of 10.23 – 11.98 with LOD of 2.11 – 3.25. The QTLs showed positive additive effect with conferring favorable allele from E36-1 parent.

**Table 22a: Features of the QTLs computed by composite interval mapping for stay-green and yield related traits in the RILs derived from the cross N13 × E36-1 at Bheemarayanagudi location evaluated during 2008**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_i b^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 108.11 | 4.88 | 2.97 | 3.12 | 11.23 |
| | C | Xtxp38-Xsnp19 | 85.17 | 13.82 | 3.99 | 2.32 | 6.53 |
| | D | Xiabt227-Xsnp56 | 82.78 | 16.4 | 2.12 | 3.33 | 5.96 |
| % GLA 30 DAF | E | Xtxp205-Xtxp231 | 145.85 | 9.21 | 2.01 | 5.39 | 9.37 |
| | B | Xtxp8-Xtxp1 | 103.23 | 7.23 | 2.8 | 4.84 | 9.94 |
| | A | Xtxp340-Xtxp61 | 6.7 | 8.4 | 2.19 | 1.76 | 5.24 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 37.48 | 25.58 | 2 | 2.19 | 8.34 |
| | G | Xiabt341-Xiabt178 | 51.67 | 17.33 | 3.25 | 2.16 | 11.98 |
| Carbon isotope discrimination | G | Xtxp141-Xiabt437 | 25.97 | 12 | 2.23 | -0.1 | 2.4 |
| | I | Xtxp6-Xiabt70 | 39.67 | 10.45 | 2.7 | 0.76 | 7.23 |
| Days to 50 % flowering | E | Xiabt511-Xtxp231 | 156.96 | 12.63 | 3.19 | -2.39 | 14.45 |
| | B | Xiumc136-Xsnp28 | 63.2 | 19.12 | 3.12 | -0.94 | 6.94 |
| | B | Xiabt241-Xiabt477 | 406.47 | 5.65 | 2.92 | 1.3 | 18.66 |
| Total green leaf area | B | Xiabt80-Xtxp73 | 218.43 | 9.2 | 2.22 | 2.24 | 13.38 |
| | C | Xiabt370-Xtxp71 | 112.8 | 8.98 | 2.87 | 2.42 | 8.88 |
| | A | Xtxp340-Xtxp61 | 6.9 | 8.41 | 3.22 | 2.84 | 11.37 |
| | E | Xiabt187-Xsnp108 | 13.55 | 11.3 | 2.05 | 1.48 | 8.38 |
| Earhead length | D | Xsnp56-Xiabt79 | 111.56 | 42 | 2.23 | -0.22 | 13.55 |
| | D | Xisp229-Xsnp36 | 35.8 | 11.92 | 2.14 | 1.94 | 9.86 |
| Number of leaves | I | Xtxp6-Xiabt438 | 41.83 | 10.02 | 2.19 | 2.34 | 10.4 |
| | I | Xtxp145-Xtxp6 | 30.22 | 12.4 | 3.01 | 3.19 | 9.14 |
| Panicle exertion | F | Xtxp67-Xiabt430 | 54.12 | 8.54 | 2.69 | 1.73 | 16.15 |
| | C | Xiabt207-Xsnp58 | 170.6 | 17 | 2.57 | 2.48 | 14.74 |
| | H | Xtxp47-Xtxp18 | 6.4 | 3.82 | 3.78 | 2.75 | 10.67 |
| | D | Xsnp36-Xtxp27 | 46.13 | 8.62 | 3.01 | -0.019 | 12.89 |
| Stem girth | G | Xiabt178-Xiabt302 | 70.15 | 20.59 | 2.85 | 2.56 | 9.51 |
| | B | Xtxp201-Xiumc136 | 51.85 | 3.26 | 2.99 | 1.65 | 11.86 |
| Plant height | B | Xiumc136-Xsnp28 | 62.9 | 19.34 | 3.12 | 3.33 | 11.28 |
| | A | Xtxp61-Xtxp37 | 18.9 | 17.42 | 3.34 | 2.45 | 10.58 |
| | G | Xsnp96-Xtxp45 | 187.95 | 17.4 | 2.78 | 1.34 | 9.73 |
| | A | Xtxp208-Xiabt263 | 143.2 | 7.25 | 3.12 | -2.31 | 10.73 |
| Spicklets per head | A | Xtxp208- Xiabt263 | 142.5 | 7 | 2.02 | 2.49 | 9.83 |
| | H | Xtxp47-Xtxp18 | 6.2 | 3.3 | 3.01 | 2.51 | 9.51 |
| 100 grain weight | D | Xtxp27-Xiabt353 | 55.23 | 12.22 | 3.12 | 2.22 | 19.7 |
| | D | Xtxp327-Xisp229 | 28.54 | 4.3 | 2.58 | 3.09 | 12.24 |
| | F | Xtxp289-Xtxp10 | 17.8 | 35.6 | 2.22 | 1.24 | 11.37 |
| | A | Xiabt350-Xiabt206 | 175.54 | 28.13 | 3.84 | -1.25 | 15.63 |
| Grain yield per plant | E | Xtxp372-Xiabt377 | 64.35 | 29.54 | 3.12 | 2.49 | 20.26 |
| | H | Xtxp282-Xiabt298 | 51.7 | 5.8 | 3.2 | 2.42 | 10.67 |
| | I | Xtxp145-Xtxp6 | 29.7 | 11.19 | 3.04 | 2.32 | 10.66 |
| | D | Xsnp36-Xtxp27 | 46.04 | 8.88 | 2.84 | 2.96 | 10.87 |

GLA – Total green leaf area DAF – Days after flowering

**Table 22b: Features of the QTLs computed by composite interval mapping for stay-green and yield related traits in the RILs derived from the cross N13 × E36-1 at Bheemarayanagudi location evaluated during 2009**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_i b^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 108.17 | 4.4 | 2.03 | 2.1 | 9.56 |
| | C | Xtxp38-Xsnp19 | 85.03 | 13.17 | 3.91 | 2.88 | 8.17 |
| | D | Xiabt227-Xsnp56 | 82.5 | 16.79 | 2.77 | 3.14 | 7.99 |
| % GLA 30 DAF | E | Xtxp205-Xtxp231 | 144.73 | 7.5 | 2.19 | 8.44 | 8 |
| | B | Xtxp8-Xtxp1 | 103.43 | 7.79 | 2.91 | 4.55 | 8 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 37.96 | 25.02 | 2.17 | 3.2 | 11.93 |
| | G | Xiabt341-Xiabt178 | 52.42 | 16.9 | 2.11 | 1.4 | 10.23 |
| | I | Xtxp176-Xtxp57 | 4 | 3 | 2.93 | 1.46 | 3.87 |
| Days to 50 % flowering | E | Xiabt511-Xtxp231 | 155.95 | 12.3 | 2.15 | -1.94 | 12.3 |
| | B | Xiumc136-Xsnp28 | 62.8 | 19.33 | 2.45 | -1.23 | 6.24 |
| | B | Xiabt241-Xiabt477 | 409.38 | 5.3 | 2.47 | 2.43 | 19.03 |
| Total green leaf area | B | Xiabt80-Xtxp73 | 219.89 | 9.52 | 2.8 | 2.49 | 12.33 |
| | C | Xiabt370-Xtxp71 | 112.3 | 8.92 | 3.12 | 1.48 | 9.45 |
| | A | Xtxp340-Xtxp61 | 6.7 | 9.52 | 2.94 | 2.33 | 10.43 |
| | H | Xtxp273-Xtxp47 | 2.3 | 4.6 | 2.9 | 2.43 | 8.88 |
| Earhead length | D | Xsnp56-Xiabt79 | 111.7 | 42.76 | 2.32 | -0.16 | 10.17 |
| | I | Xiabt29-Xiabt14 | 79.25 | 11.1 | 3.1 | 1.59 | 9.74 |
| | D | Xisp229-Xsnp36 | 36.1 | 11.53 | 2.22 | 2.47 | 10.34 |
| Number of leaves | I | Xtxp6-Xiabt438 | 41.35 | 9.83 | 2.71 | 2.58 | 10.6 |
| | I | Xtxp145-Xtxp6 | 29.34 | 11.23 | 3.45 | 2.79 | 8.53 |
| | J | Xiabt16-Xiabt146 | 89.75 | 11.3 | 2.54 | 1.92 | 9.46 |
| Panicle exertion | F | Xtxp67-Xiabt430 | 53.19 | 8.2 | 2.17 | 2.75 | 15.24 |
| | H | Xtxp47-Xtxp18 | 6.49 | 3.61 | 3.16 | 3.12 | 10.38 |
| | D | Xsnp36-Xtxp27 | 45.9 | 8.3 | 3.22 | -0.28 | 11.04 |
| Stem girth | G | Xiabt178-Xiabt302 | 70.36 | 19.9 | 2.03 | 3.05 | 8.28 |
| | B | Xtxp201-Xiumc136 | 51.92 | 3.21 | 2.84 | 1.42 | 10.23 |
| Plant height | B | Xiumc136-Xsnp28 | 63.12 | 19.1 | 3.52 | 3.48 | 13.32 |
| | A | Xtxp61-Xtxp37 | 19.19 | 16.2 | 2.1 | 2.96 | 8.45 |
| | A | Xtxp208-Xiabt263 | 142.3 | 7 | 3.42 | -,2.13 | 11.45 |
| Spicklets per head | A | Xtxp208- Xiabt263 | 142.8 | 7.23 | 2.28 | 2.71 | 9.54 |
| | H | Xtxp47-Xtxp18 | 7.1 | 4.18 | 2.44 | 1.74 | 10.43 |
| | B | Xtxpk1f2-Xiabt388 | 259.8 | 14.8 | 2.22 | 0.87 | 10.53 |
| 100 grain weight | D | Xtxp27-Xiabt353 | 56.07 | 12.1 | 4.35 | 1.72 | 15.48 |
| | D | Xtxp327-Xisp229 | 27.35 | 5.23 | 2.13 | 4.41 | 11.47 |
| | A | Xiabt350-Xiabt206 | 174.65 | 27.7 | 4.02 | -1.82 | 17.82 |
| Grain yield per plant | E | Xtxp372-Xiabt377 | 64.57 | 29.3 | 3.87 | 2.83 | 11.42 |
| | I | Xtxp145-Xtxp6 | 29.1 | 12.01 | 2.47 | 2.17 | 9.23 |
| | D | Xsnp36-Xtxp27 | 45.9 | 8.82 | 2.45 | 2.54 | 9.36 |

GLA – Total green leaf area DAF – Days after flowering

**Table 22c: Features of the QTLs that were commonly detected across two seasons for stay-green and yield related traits in RILs derived from the cross N13 × E36-1 at Bheemarayanagudi location evaluated during 2008 and 2009**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_i b^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 108.11-108.17 | 4.4-4.88 | 2.03-2.97 | 2.10-3.12 | 9.56-11.23 |
| | C | Xtxp38-Xsnp19 | 85.03-85.17 | 13.17-13.82 | 3.91-3.99 | 2.32-2.88 | 6.53-8.17 |
| | D | Xiabt227-Xsnp56 | 82.5-82.78 | 16.4-16.79 | 2.12-2.77 | 3.14-3.33 | 5.96-7.99 |
| % GLA 30 DAF | E | Xtxp205-Xtxp231 | 144.73-145.85 | 7.5-9.21 | 2.01-2.19 | 5.39-8.44 | 8.0-9.37 |
| | B | Xtxp8-Xtxp1 | 103.23-103.43 | 7.23-7.79 | 2.91-2.80 | 4.55-4.84 | 8.0-9.94 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 37.48-37.96 | 25.02-25.58 | 2.00-2.17 | 2.19-3.20 | 8.34-11.93 |
| | G | Xiabt341-Xiabt178 | 51.67-52.42 | 16.9-17.33 | 2.11-3.25 | 1.40-2.16 | 10.23-11.98 |
| Carbon isotope discrimination | G | Xtxp141-Xiabt437 | 25.48-25.97 | 12-12.38 | 2.23-2.45 | -0.81-, 0.1 | 2.4-3.74 |
| | I | Xtxp6-Xiabt70 | 39.67-41.02 | 10-10.45 | 2.70-3.12 | 0.76-0.93 | 6.8-7.23 |
| Days to 50 % flowering | E | Xiabt511-Xtxp231 | 155.95-156.96 | 12.3-12.63 | 2.15-3.19 | -1.64- -1.94 | 12.30-14.45 |
| | B | Xiumc136-Xsnp28 | 62.8-63.2 | 19.12-19.33 | 2.45-3.12 | -0.94-1.23 | 6.24-6.94 |
| | B | Xiabt241-Xiabt477 | 406.47-409.38 | 5.3-5.65 | 2.47-2.92 | 1.3-2.43 | 18.66-19.03 |
| Total green leaf area | B | Xiabt80-Xtxp73 | 218.43-219.89 | 9.2-9.52 | 2.22-2.80 | 2.24-2.49 | 12.33-13.38 |
| | C | Xiabt370-Xtxp71 | 112.3-112.8 | 8.92-8.98 | 2.87-3.12 | 1.48-2.42 | 8.88-9.45 |
| | A | Xtxp340-Xtxp61 | 6.7-6.9 | 8.41-9.52 | 2.94-3.22 | 2.33-2.84 | 10.43-11.37 |
| Earhead length | D | Xsnp56-Xiabt79 | 111.56-111.70 | 42-42.76 | 2.23-2.32 | -0.22- -0.16 | 10.17-13.55 |
| | D | Xisp229-Xsnp36 | 35.8-36.1 | 11.53-11.92 | 2.14-2.22 | 1.47-1.94 | 9.86-10.34 |
| Number of leaves | I | Xtxp6-Xiabt438 | 41.35-41.83 | 9.83-10.02 | 2.19-2.71 | 2.34-2.58 | 10.40-10.60 |
| | I | Xtxp145-Xtxp6 | 29.34-30.22 | 11.23-12.4 | 3.01-3.45 | 2.79-3.19 | 8.53-9.14 |
| Panicle exertion | F | Xtxp67-Xiabt430 | 53.19-54.12 | 8.2-8.54 | 2.17-2.69 | 1.73-2.75 | 15.24-16.15 |
| | H | Xtxp47-Xtxp18 | 6.4-6.49 | 3.61-3.82 | 3.16-3.78 | 2.75-3.12 | 10.38-10.67 |
| | D | Xsnp36-Xtxp27 | 45.9-46.13 | 8.3-8.62 | 3.01-3.22 | -0.28-0.019 | 11.04-12.89 |
| Stem girth | G | Xiabt178-Xiabt302 | 70.15-70.36 | 19.9-20.59 | 2.03-2.85 | 2.56-3.05 | 8.28-9.51 |
| | B | Xtxp201-Xiumc136 | 51.85-51.92 | 3.21-3.26 | 2.84-2.99 | 1.42-1.65 | 10.23-11.86 |
| Plant height | B | Xiumc136-Xsnp28 | 62.9-63.12 | 19.1-19.34 | 3.12-3.52 | 3.33-3.48 | 11.28-13.32 |
| | A | Xtxp61-Xtxp37 | 18.90-19.19 | 16.2-17.42 | 2.10-3.34 | 2.45-2.96 | 8.45-10.58 |
| | A | Xtxp208-Xiabt263 | 142.3-143.2 | 7-7.25 | 3.12-3.42 | -2.06 | 10.73-11.45 |
| Spicklets per head | A | Xtxp208- Xiabt263 | 142.5-142.80 | 7-7.23 | 2.02-2.28 | 2.49-2.71 | 9.54-9.83 |
| | H | Xtxp47-Xtxp18 | 6.2-7.1 | 3.3-4.18 | 2.44-3.01 | 1.74-2.51 | 9.51-10.43 |
| 100 grain weight | D | Xtxp27-Xiabt353 | 55.23-56.07 | 12.10-12.22 | 3.12-4.35 | 1.72-2.22 | 15.48-19.7 |
| | D | Xtxp327-Xisp229 | 27.35-28.54 | 4.3-5.23 | 2.13-2.58 | 3.09-4.41 | 11.47-12.24 |
| | A | Xiabt350-Xiabt206 | 174.65-175.54 | 27.7-28.13 | 3.84-4.02 | -2.8 | 15.63-17.82 |
| Grain yield per plant | E | Xtxp372-Xiabt377 | 64.35-64.57 | 29.3-29.54 | 3.12-3.87 | 2.49-2.83 | 11.42-20.26 |
| | I | Xtxp145-Xtxp6 | 29.1-29.7 | 11.19-12.01 | 2.47-3.04 | 2.17-2.32 | 9.23-10.66 |
| | D | Xsnp36-Xtxp27 | 45.90-46.04 | 8.82-8.88 | 2.45-2.84 | 2.54-2.96 | 9.36-10.87 |

GLA – Total green leaf area DAF – Days after flowering

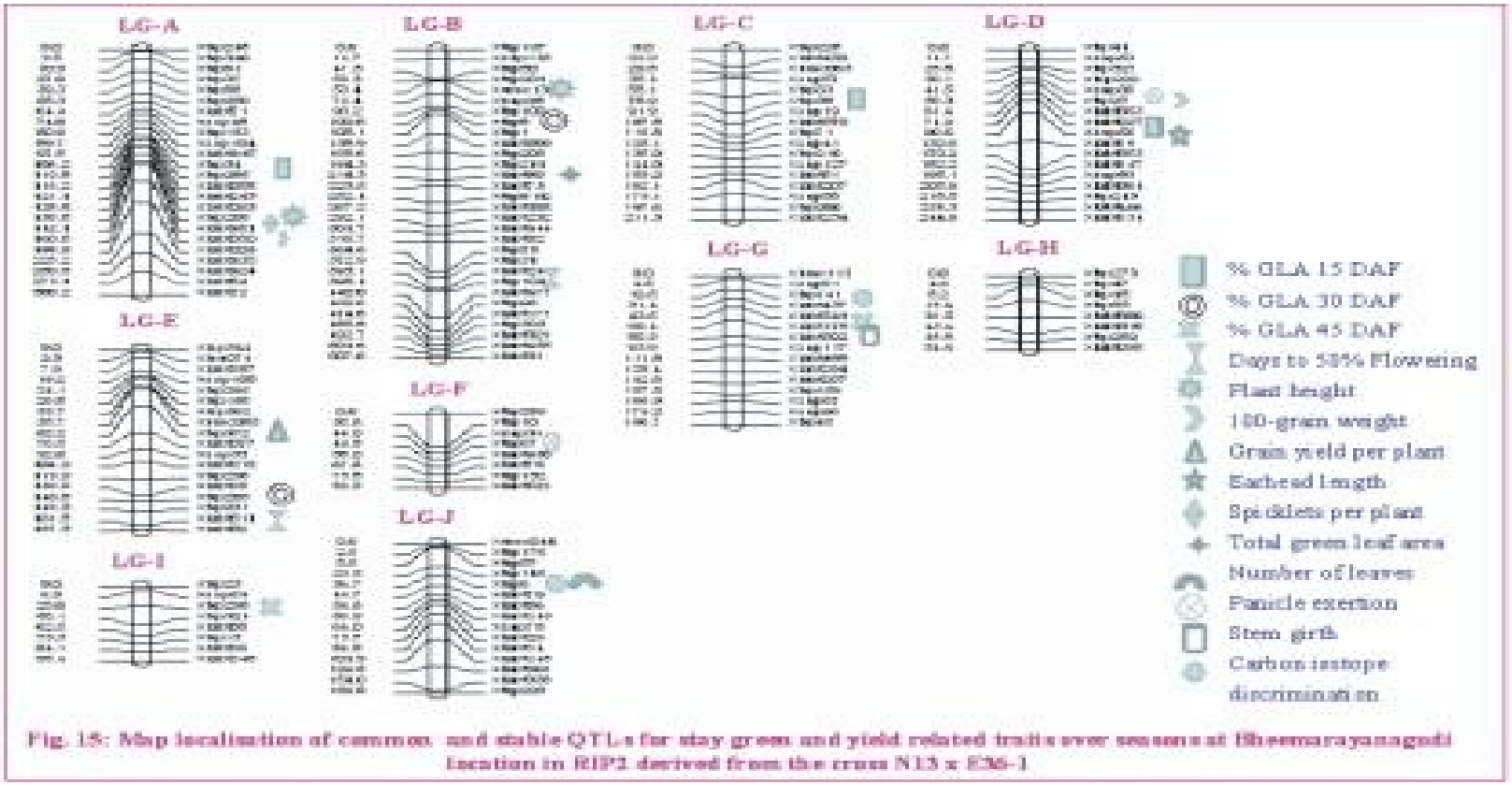



Fig. 15: Map localisation of common and stable QTLs for stay green and yield related traits over seasons at Bheemarayanagudi location in RIP2 derived from the cross N13 x E36-1

**Fig. 15: Map localisation of common and stable QTLs for stay green and yield related traits over seasons at Bheemarayanagudi location in RIP2 derived from the cross N13 x E36-1**

Carbon isotope discrimination

Two QTLs bracketed by Xtxp141-Xiabt437 (LG-G) and Xtxp6-Xiabt70 (LG-I) were detected for this trait. QTL on LG-G (Xtxp141-Xiabt437) had negative effect conferring favorable allele from Xsnp13 parent with LOD of 2.23 – 2.45 and per cent phenotypic variation of 2.40 – 3.74. The QTL mapped on LG-I (Xtxp6-Xiabt70) had positive additive effect conferring favorable allele from E36-1 parent with LOD of 2.70 – 3.12 and per cent phenotypic variation of 6.80 – 7.23.

Days to 50 per cent flowering

Totally three QTLs were detected for days to 50 per cent flowering and were common across seasons. Two QTLs mapped on LG-B bracketed by Xumc136-Xsnp28 and Xiabt241-Xiabt477 had LOD score of 2.45 – 3.12 and 2.47 – 2.92 with phenotypic variation of 6.24 – 6.94 and 18.66 – 19.03, respectively with negative and positive additive effect, respectively. The QTL on LG-E (Xiabt511-Xtxp231) had negative effect conferring favorable allele from N13 with LOD score of 2.15 – 3.19 and phenotypic variation of 12.30 – 14.45 per cent.

Total green leaf area

Totally five QTLs were detected for total green leaf area. Among them, three QTLs flanked by Xiabt80-Xtxp73 (LG-B), Xiabt370-Xtxp71 (LG-C) and Xtxp340-Xtxp61 (LG-A) were found common across seasons with LOD score of 2.22 – 2.80, 2.87 – 3.12 and 2.94 – 3.22 and per cent phenotypic variation of 12.33 – 13.38, 8.88 – 9.45 and 10.43 – 11.37, respectively. All QTLs showed positive additive effect conferring favorable allele from E36-1 parent.

Earhead length

Out of three QTLs detected, two QTLs found common across seasons. The QTLs were detected on LG-D spanned by Xsnp56-Xiabt79 and Xisp229-Xsnp36 markers with LOD score of 2.23 – 2.32 and 2.14 – 2.22 expressing per cent phenotypic variation of 10.17 – 13.55 and 9.86 – 10.34, respectively.

Number of leaves

Two QTLs out of detected three QTLs were found to be common across seasons. Two QTLs for number of leaves mapped on LG-I flanked by Xtxp6-Xiabt438 and Xtxp145-Xtxp6 with LOD score of 2.19 – 2.71 and 3.01 – 3.45, per cent phenotypic variation of 10.40 – 10.60 and 8.53 – 9.14, respectively with positive additive effect were common across seasons.

Panicle exertion

Totally four QTLs were detected for this trait and among which, three QTLs found common across seasons. The QTLs flanked by Xtxp67-Xiabt430 (LG-F), Xtxp47-Xtxp18 (LG-H) and Xsnp36-Xtxp27 (LG-D) with LOD score of 2.17 – 2.69, 3.16 – 3.78 and 3.01 – 3.22 per cent phenotypic variation of 15.24 – 16.15, 10.38 – 10.67 and 11.04 - 12.89, respectively. First two QTLs showed positive additive effect and QTL on LG-D showed negative effect.

Stem girth

Two QTLs spanned by Xiabt178-Xiabt302 (LG-G) and Xtxp201-Xumc136 (LG-B) were found detected across seasons with LOD score of 2.03 – 2.85 and 2.84 – 2.99 per cent phenotypic variation of 8.28 – 9.51 and 10.23 – 11.86 respectively. QTLs showed positive additive effect conferring favorable allele from E36-1 parent.

Plant height

Totally four QTLs were detected for plant height trait. Among them, three QTLs were found common across seasons. Two QTLs were found on LG-A spanned by Xtxp61-Xtxp37 and Xtxp208-Xiabt263 markers with LOD score of 2.10 – 3.34, 3.12 – 3.42, per cent phenotypic variation of 8.45 – 10.58 and 10.73 – 11.45, respectively. The QTL on LG-B flanked by Xumc136-Xsnp28 with LOD score of 3.12 – 3.52, per cent phenotypic variation of 11.28 – 13.32. All QTLs showed positive additive effect.

Spicklets per head

Totally three QTLs were detected for spicklets per head. Two QTLs found common across seasons. The QTL flanking markers of Xtxp208-Xiabt263 (LG-A), Xtxp47-Xtxp18 (LG-H). With LOD score of 2.02 – 2.28 and 2.44 – 3.04, per cent phenotypic variation of 9.54 – 9.83 and 9.51 – 10.43, respectively. The QTLs showed positive additive effect conferring favorable allele from E36-1 parent.

100 grain weight

Three QTLs out of four QTLs detected were found common across seasons. The two QTLs on LG-D spanned by Xtxp27-Xiabt353 and Xtxp327-Xisp229 with LOD score of 3.12 – 4.35 and 2.13 – 2.58, per cent phenotypic variation of 15.48 – 19.70 and 11.47 – 12.24, respectively showed positive additive effect conferring favorable allele from E36-1. The third QTL on LG-A flanked by Xiabt350-Xiabt206 with LOD of 3.84 – 4.02 with phenotypic variation of 15.63 – 17.82 with negative effect found conferring favorable allele from N13 parent.

Grain yield per plant

Totally four QTLs were detected for this trait. Three QTLs were common across seasons and were found on LG-E (Xtxp372-Xiabt377), LG-I (Xtxp145-Xtxp6) and LG-D (Xsnp36-Xtxp27) with LOD score of 3.12 – 3.87, 2.47 – 3.04 and 2.45 – 2.84, per cent phenotypic variation of 11.42 – 20.26, 9.23 – 10.66 and 9.36 – 10.87 respectively. All QTLs showed positive additive effect conferring favorable allele from E36-1 parent.

f) Features of QTLs in N13 × E36-1 derived RIP at Dharwad and Bheemarayanagudi locations

Stable QTLs associated with 14 different traits at Dharwad and Bheemarayanagudi location in RIP2 are presented in Table 23. QTLs were homed on to respective linkage groups (Fig. 16).

Per cent GLA 15 DAF

Totally three QTLs found common across seasons and locations for per cent GLA 15 DAF. QTLs were flanked by Xtxp34-Xtxp285 (LG-A), Xtxp38-Xsnp19 (LG-C) and Xiabt227-Xsnp56 (LG-D) with LOD score of 3.15 – 3.97, 3.08 – 3.68 and 2.63 – 2.68, per cent phenotypic variation of 13.05 – 14.46, 6.39 – 8.47 and 7.56 – 8.49, respectively. All QTLs showed positive additive affect conferring favorable allele from E36-1.

Per cent GLA 30 DAF

Two QTLs bracketed by Xtxp205-Xtxp231 (LG-E) and Xtxp8-Xtxp1 (LG-B) were found common across season and locations for per cent GLA 30 DAF with per cent phenotypic variation of 16.0 – 17.37 and 8.00 – 9.94, LOD value of 2.89 – 2.29 and 2.32 – 2.79 respectively.

Per cent GLA 45 DAF

Two QTLs from LG-J (Xtxp298-Xtxp324) and LG-G (Xiabt341-Xiabt178) were found common across seasons and locations with per cent phenotypic variation of 17.34 – 17.93 and 16.00 – 16.99. The LOD score of 2.34 – 2.97 and 2.21 – 3.26, respectively. All QTLs showed positive additive effect conferring favorable allele from E36-1 parent.

Carbon isotope discrimination

Two QTLs were found common across seasons and across locations, flanked by Xtxp141-Xiabt437 (LG-G) and Xtxp6-Xiabt70 (LG-I) with LOD score of 2.40 – 2.99 and 2.69 – 3.10, per cent phenotypic variation of 2.40 – 3.74, 6.80 – 7.23, respectively. The QTL from LG-G showed negative effect conferring favorable allele from N13 while QTL from LG-I showed positive additive effect conferring favorable allele from E36-1 parent.

Days to 50 per cent flowering

Two QTLs were found common across seasons and across locations found on LG-E (Xiabt511-Xtxp231) and LG-B (Xumc136-Xsnp28) with LOD score of 2.85 – 3.39 and 2.13 – 3.12, per cent phenotypic variation of 14.29 – 14.87 and 6.23 – 7.19 with negative effect conferring favorable allele from N13 parent.

**Table 23: Features of the QTLs that were commonly detected across three years and two locations for stay-green and yield related traits in RILs derived from the cross N13 × E36-1**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_i b^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 108.25-108.87 | 4.6-4.98 | 3.15-3.97 | 2.18-3.25 | 13.05-14.46 |
| | C | Xtxp38-Xsnp19 | 85.05-85.27 | 13.7-13.92 | 3.08-3.68 | 2.26-2.38 | 6.39-8.47 |
| | D | Xiabt227-Xsnp56 | 82.4-82.88 | 16.4-16.79 | 2.63-2.68 | 3.02-3.83 | 7.56-8.49 |
| % GLA 30 DAF | E | Xtxp205-Xtxp231 | 144.95-145.73 | 8.7-9.01 | 2.89-2.29 | 5.38-8.32 | 16.0-17.37 |
| | B | Xtxp8-Xtxp1 | 103.35-103.96 | 7.5-7.79 | 2.32-2.79 | 4.67-8.34 | 8.0-9.94 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 37.55-37.81 | 25-25.63 | 2.34-2.97 | 2.49-3.29 | 17.34-17.93 |
| | G | Xiabt341-Xiabt178 | 51.95-52.11 | 16.9-17.23 | 2.21-3.26 | 1.35-2.36 | 16-16.98 |
| Carbon isotope discrimination | G | Xtxp141-Xiabt437 | 25.45-25.97 | 12-12.39 | 2.4-2.99 | -0.90- -0.12 | 2.4-3.74 |
| | I | Xtxp6-Xiabt70 | 39.7-41.02 | 10-10.48 | 2.69-3.1 | 0.76-0.93 | 6.8-7.23 |
| Days to 50 % flowering | E | Xiabt511-Xtxp231 | 155.95-156.96 | 12.4-12.62 | 2.85-3.39 | -1.64- -1.94 | 14.29-14.87 |
| | B | Xiumc136-Xsnp28 | 61.9-63.2 | 19.12-20.12 | 2.13-3.12 | -1.23--0.32 | 6.23-7.19 |
| Total green leaf area | B | Xiabt80-Xtxp73 | 218.9-219.39 | 9.2-9.42 | 2.47-2.69 | 1.49-1.24 | 13.27-13.38 |
| | C | Xiabt370-Xtxp71 | 112.3-112.9 | 8.87-9.21 | 2.87-3.12 | 1.35-2.84 | 6.38-9.45 |
| | A | Xtxp340-Xtxp61 | 6.13-6.9 | 8.38-9.52 | 2.53-3.22 | 2.33-3.60 | 9.34-11.37 |
| Earhead length | D | Xsnp56-Xiabt79 | 111.6-111.70 | 42-42.96 | 2.1-2.32 | -0.22- -0.16 | 17.37-17.59 |
| | D | Xisp229-Xsnp36 | 35.52-36.12 | 11.46-11.92 | 2.04-3.61 | 1.47-4.27 | 9.86-12.46 |
| Number of leaves | I | Xtxp6-Xiabt438 | 41.35-41.20 | 9.8-10.02 | 2.49-2.71 | 1.23-1.45 | 10.38-10.60 |
| | I | Xtxp145-Xtxp6 | 29.1-30.2 | 11.1-12.4 | 3.01-3.45 | 2.15-3.14 | 8.53-9.14 |
| Panicle exertion | F | Xtxp67-Xiabt430 | 53.9-54.12 | 8.2-8.42 | 2.47-2.69 | 0.63-0.85 | 19.43-19.65 |
| | H | Xtxp47-Xtxp18 | 6.15-6.69 | 3.59-4.12 | 2.46-3.78 | 1.49-3.12 | 8.55-10.67 |
| Stem girth | G | Xiabt178-Xiabt302 | 70.35-70.57 | 19.9-20.69 | 2.07-2.85 | 0.83-1.05 | 9.28-9.5 |
| | B | Xtxp201-Xiumc136 | 51.63-52.12 | 3.04-3.26 | 2.66-3.01 | 0.35-1.65 | 9.56-11.86 |
| Plant height | B | Xiumc136-Xsnp28 | 62.9-63.12 | 19.1-19.32 | 3.3-3.52 | 3.12-3.48 | 14.28-14.5 |
| | A | Xtxp61-Xtxp37 | 18.95-19.14 | 16.1-17.44 | 2.24-3.12 | 1.45-1.96 | 8.45-10.58 |
| Spicklets per head | A | Xtxp208- Xiabt263 | 142.3-142.48 | 7-7.22 | 2.02-2.28 | 2.49-2.71 | 9.54-9.83 |
| | H | Xtxp47-Xtxp18 | 6.4-7.1 | 3.6-4.2 | 2.44-3.01 | 1.74-2.51 | 9.51-10.43 |
| 100 grain weight | D | Xtxp27-Xiabt353 | 55.85-56.07 | 12-12.22 | 4.13-4.35 | 0.63-0.86 | 19.48-19.7 |
| | D | Xtxp327-Xisp229 | 27.35-28.13 | 4.3-5.1 | 2.13-2.58 | 1.09-2.41 | 11.53-12.14 |
| Grain yield per plant | E | Xtxp372-Xiabt377 | 64.35-64.57 | 29.3-29.58 | 3.06-3.28 | 2.49-2.71 | 21.38-21.6 |
| | I | Xtxp145-Xtxp6 | 29.03-30.01 | 11.19-12.01 | 2.47-3.12 | 2.17-3.67 | 6.34-10.66 |
| | D | Xsnp36-Xtxp27 | 45.84-46.04 | 8.78-8.88 | 2.45-3.23 | 2.54-3.12 | 8.34-10.87 |

GLA – Total green leaf area DAF – Days after flowering

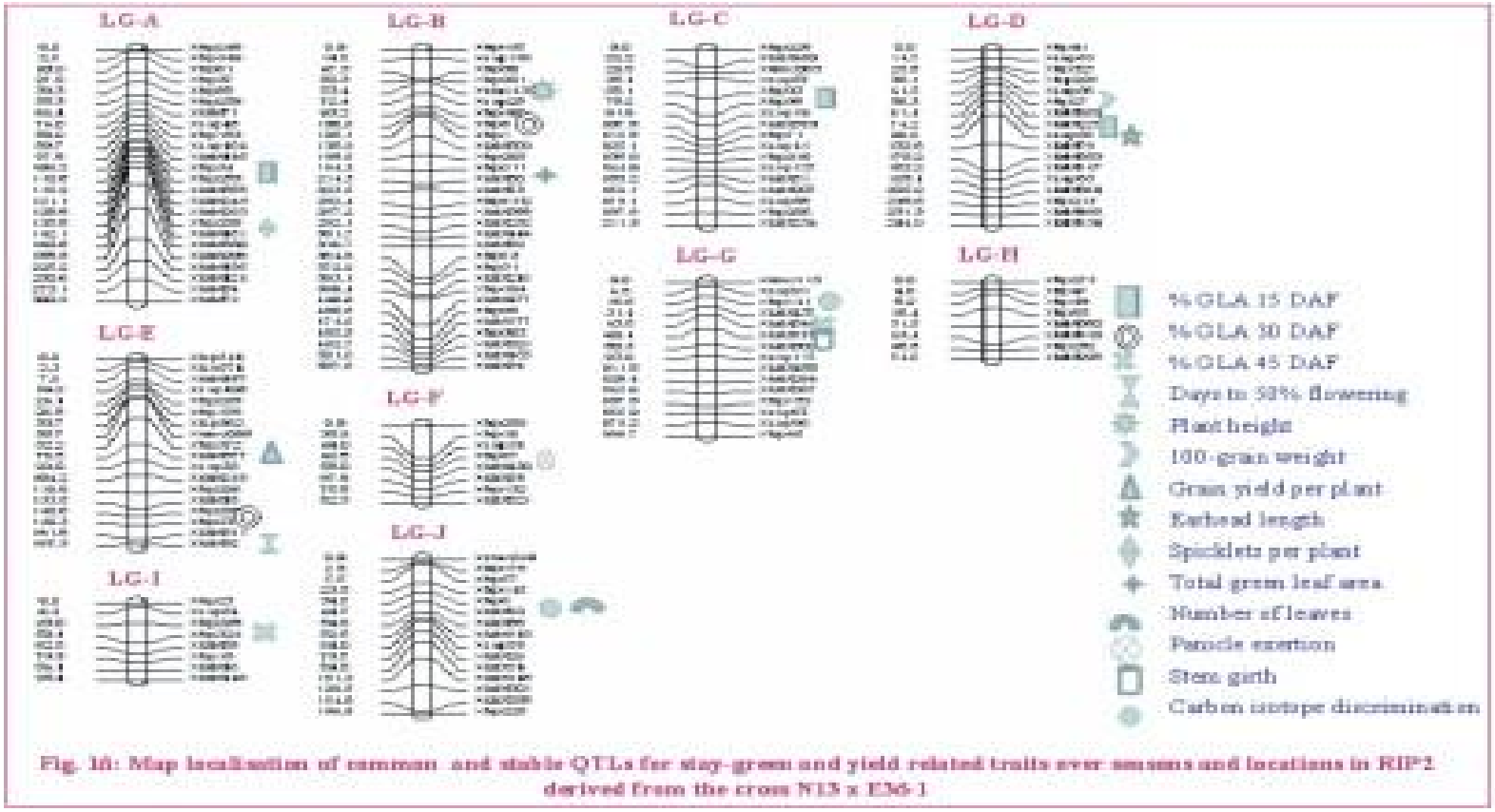



Fig. 16: Map localisation of common and stable QTLs for stay-green and yield related traits over seasons and locations in RIP2 derived from the cross N13 x E36-1

**Fig. 16: Map localisation of common and stable QTLs for stay-green and yield related traits over seasons and locations in RIP2 derived from the cross N13 x E36-1**

Total green leaf area

Totally three QTLs found common across seasons and locations flanked by Xiabt80-Xtxp73 (LG-B), Xiabt370-Xtxp71 (LG-C) and Xtxp340-Xtxp61 (LG-A), LOD score of 2.47 – 2.69, 2.87 – 3.12 and 2.53 – 3.22, phenotypic variation of 13.27 – 13.88, 6.38 – 9.45 and 9.34 – 11.37 per cent respectively. All QTLs showed positive additive affect.

Earhead length

Two QTLs from LG-D flanked by Xsnp56-Xiabt79 and Xisp229-Xsnp36 were found common across seasons and locations with LOD score of 2.10 – 2.32 and 2.04 – 3.61, per cent phenotypic variation of 17.37 – 17.59 and 9.86 – 12.46, respectively.

Number of leaves

QTLs from LG-I spanned by Xtxp6-Xiabt438 and Xtxp145-Xtxp6 on LG-I were found common across locations and across seasons. LOD score of 2.49 – 2.71 and 3.01 – 3.45, phenotypic variation of 10.38 – 10.60 and 8.53 – 9.14, respectively with positive additive effect.

Panicle exertion

Two QTLs found common for panicle exertion across locations and seasons. QTLs were found on LG-F (Xtxp67-o430) and LG-H (Xtxp47-Xtxp18) with LOD score of 2.47 – 2.69 and 2.46 – 3.78, per cent phenotypic variation of 19.43 – 19.65 and 8.55 – 10.67.

Stem girth

QTLs found on LG-G flanked by Xiabt178-Xiabt302 and LG-B flanked by Xtxp201-Xumc136 were found common across seasons and locations with LOD score of 2.07 – 2.85 and 2.66 – 3.01, per cent phenotypic variation of 9.28 – 9.50 and 9.56 – 11.86.

Plant height

Two QTLs found common across seasons and locations, flanked by Xumc136-Xsnp28 on LG-B and Xtxp61-Xtxp37 on LG-A with phenotypic variation of 14.28 – 14.50 and 8.45 – 10.58, LOD score of 3.30 – 3.52 and 2.24 – 3.12, respectively. The QTLs showed positive additive effect conferring favorable allele from N13 parent.

Spicklets per head

The QTLs bracketed by Xtxp208-Xiabt263 on LG-A and Xtxp47-Xtxp18 on LG-H found common across seasons and locations with LOD score of 2.02 – 2.28 and 2.44 – 3.01, phenotypic variation of 4.54 – 9.83 and 9.51 – 10.43, respectively with positive additive effect.

100 grain weight

Two QTLs found common across locations and seasons found on LG-D spanned by Xtxp27-Xiabt353 and Xtxp327-Xisp229 with per cent phenotypic variation of 19.48 – 19.70 and 11.53 – 12.14, LOD value of 4.13 – 4.35 and 2.13 – 2.58 with positive additive effect conferring favorable allele from E36-1 parent.

Grain yield per plant

Totally three QTLs found common across locations and seasons found on LG-E (Xtxp372-Xiabt377), LG-I (Xtxp145-Xtxp6) and LG-D (Xsnp36-Xtxp27) with LOD score of 3.06 – 3.28, 2.47 – 3.12 and 2.45 – 3.23, per cent phenotypic variation of 21.38 – 21.60, 6.34 – 10.66 and 8.34 – 10.87, respectively. The QTLs showed positive additive effect conferring favorable allele from E36-1 parent.

g) Features of stable QTLs in IS9830 × E36-1 and N13 × E36-1 evaluated at Dharwad and Bheemarayanagudi locations over seasons

Stable QTLs associated with 14 different traits at Dharwad and Bheemarayanagudi location in both RIP1 and RIP2 are presented in Table 24. These are the common QTLs mapped over seasons and across locations of phenotyping.

**Table 24: Features of the QTLs that were commonly detected across three years and two locations for stay-green and yield related traits in RILs derived from the cross IS9830 × E36-1 and N13 × E36-1**

| Traits | LG | Marker interval | Position (cM) | Length (cM) | LOD | $a_i b^2$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| % GLA 15 DAF | A | Xtxp34-Xtxp285 | 103.9-108.87 | 4.6-4.98 | 2.24-3.97 | 2.18-3.25 | 9.05-14.46 |
| | C | Xtxp38-Xsnp19 | 85.05-123.07 | 13.3-13.92 | 2.51-3.68 | 2.26-3.89 | 6.39-8.47 |
| | D | Xiabt227-Xsnp56 | 71.8-82.88 | 12.8-16.79 | 2.63-3.13 | 3.02-9.39 | 7.49-9.39 |
| % GLA 30 DAF | E | Xtxp205- Xtxp231 | 128.05-145.73 | 7.1-9..01 | 2.89-3.14 | 1.91-8.32 | 8.21-17.37 |
| % GLA 45 DAF | J | Xtxp298-Xtxp324 | 37.81-105.47 | 19.5-25.63 | 2.34-3.35 | 1.82-4.28 | 17.34-19.49 |
| Days to 50 % flowering | B | Xiumc136-Xsnp28 | 61.9-103.24 | 19.12-20.24 | 2.11-3.12 | -1.28-0.23 | 5.28-7.19 |
| Total green leaf area | C | Xiabt370-Xtxp71 | 112.3-158.41 | 8.87-10.37 | 2.56-3.54 | 0.32-2.84 | 6.34-9.93 |
| | A | Xtxp340-Xtxp61 | 4.84-6.9 | 6.12-9.52 | 2.53-3.22 | 1.56-3.6 | 9.34-12.43 |
| Earhead length | D | Xisp229-Xsnp36 | 35.52-40.73 | 11.46-12.84 | 2.04-3.61 | 1.47-4.27 | 9.86-12.65 |
| Number of leaves | I | Xtxp145-Xtxp6 | 29.1-31.7 | 11.1-12.4 | 2.42-3.45 | 2.15-4.27 | 8.53-12.41 |
| Panicle exertion | H | Xtxp47-Xtxp18 | 6.15-7.12 | 3.44-4.30 | 2.12-3.78 | 0.23-3.12 | 8.55-10.76 |
| Stem girth | B | Xtxp201-Xiumc136 | 51.63-90.21 | 2.6-3.26 | 2.66-3.42 | 0.35-3.24 | 8.53-11.86 |
| Plant height | A | Xtxp61-Xtxp37 | 16.2-19.14 | 16.1-17.44 | 2.24-3.12 | ,-3.42-1.96 | 8.54-13.11 |
| Spicklets per head | H | Xtxp47-Xtxp18 | 6.4-7.14 | 3.6-4.2 | 2.44-4.14 | 1.74-5.2 | 8.42-10.43 |
| 100 grain weight | D | Xtxp327-Xisp229 | 27.35-31.9 | 3.8-5.1 | 2.13-3.49 | 1.09-5.14 | 7.52-12.14 |
| Grain yield per plant | I | Xtxp145-Xtxp6 | 29.03-31.12 | 11.19-12.73 | 2.22-3.12 | 1.89-3.76 | 6.34-10.66 |
| | D | Xsnp36-Xtxp27 | 45.84-50.21 | 8.78-10.11 | 2.03-3.23 | 1.43-3.12 | 8.34-10.87 |

GLA – Total green leaf area DAF – Days after flowering

Per cent GLA 15 DAF

Totally three QTLs on linkage groups A (Xtxp34-Xtxp285), C (Xtxp38-Xsnp19) and D (Xiabt227-Xsnp56) were found stable across seasons, across locations and across populations for GLA 15 DAF. The QTL found on LG-A, LG-C and LG-D had their LOD value of 2.24 – 3.97, 2.51 – 3.68 and 2.63 – 3.13 with positive additive effect conferring favorable allele from E36-1 parent expressing per cent phenotypic variation of 9.05 – 14.46, 6.39 – 8.47 and 7.49 – 9.39 respectively.

Per cent GLA 30 DAF

Only one QTL for per cent GLA 30 DAF was found common across season, across location and populations. The QTL found on LG-E flanked by Xtxp205-Xtxp231 at 128.05 – 145.73 position with length of 7.10 – 9.01 cM recorded LOD value of 2.89 – 3.14 expressing positive additive effect with phenotypic variation of 8.21 – 17.37 per cent.

Per cent GLA 45 DAF

One QTL for per cent GLA 45 DAF was found common across seasons, across locations and across populations with length of 19.5 – 25.63, LOD value of 2.34 – 3.35, per cent phenotypic variation of 17.34 – 19.49 expressing positive additive effect conferring favorable allele from E36-1 parent.

Days to 50 per cent flowering

A QTL mapped on LG-B flanked by Xumc136-Xsnp28 markers found common across season, across location and across populations with length ranged from 19.12 – 20.24, LOD value of 2.11 – 3.12 expressing negative effect conferring favorable allele from N13/IS9830 parent, with per cent phenotypic variation of 5.28 – 7.19.

Total green leaf area

Two QTLs were found common across season, across location and across populations for total green leaf area. They were mapped on LG-C (Xiabt370-Xtxp71) and LG-A (Xtxp340-Xtxp61) with LOD value of 2.56 – 3.54 and 2.53 – 3.22 and per cent phenotypic variation of 6.34 – 9.93 and 9.34 – 12.43 respectively. These QTLs showed positive additive effect conferring favorable allele from E36-12 parent.

Earhead length

The QTL on LG-D spanned by Xisp229-Xsnp36, length varying from 11.465 – 12.84 cM with LOD value of 2.04 – 3.61 and per cent phenotypic variation of 9.86 – 12.65 expressing positive additive effect conferring favorable allele from E36-1 was found common across season, across location and across populations.

Number of leaves

For this trait, QTL flanked by Xtxp145-Xtxp6 marker on LG-I was found common across season, across location and population with length varying from 11.10 – 12.40 cM with LOD value of 2.42 – 3.45, per cent phenotypic variation of 8.53 – 12.41 expressing positive additive effect conferring favorable allele from E36-1 parent.

Panicle exertion

One QTL found common across season, across location and population for panicle exertion length. The QTL bracketed by Xtxp47-Xtxp18 marker on LG-H, length varying from 3.44 – 4.30 cM, LOD value of 2.12 – 3.78, per cent phenotypic variation of 8.55 – 10.76 expressing positive additive effect conferring favorable allele from E36-1 parent.

Stem girth

A QTL for stem girth on LG-B flanked by Xtxp201-numc136 found common across season, across location and across population. The length of QTL varying from 2.60 – 3.26, LOD value of 2.66 – 3.42 with per cent phenotypic variation of 8.53 – 11.86 expressing positive additive effect conferring favorable allele from E36-1.

Plant height

For plant height, QTL found common across seasons, across locations and across populations flanked by Xtxp61-Xtxp37 on LG-A with LOD value of 2.24 - 3.12, per cent phenotypic variation of 8.54 – 13.11, length varying from 16.10 – 17.44 expressing negative effect conferring favorable allele from N13/IS9830 parent.

Spicklet per head

One QTL found common across season, across location and population was on LG-H (Xtxp47-Xtxp18), length varying from 3.6 – 4.2, LOD value of 2.44 – 4.14, phenotypic variation of 8.42 – 10.43 per cent with positive additive effect conferring favorable allele from E36-1 parent.

100 grain weight

A QTL on LG-D (Xtxp327-Xisp229) was found common across season, across location and across population with length varying from 3.80 – 5.10, LOD value of 2.13 – 3.49, per cent phenotypic variation of 7.52 – 12.14 with positive additive effect.

Grain yield per plant

Two QTLs were found common across season, across location and population forgrain yield per plant. Two QTLs found on LG-I (Xtxp145-Xtxp6) and LG-D (Xsnp36-Xtxp27) with length 11.19 – 12.73 and 8.78 – 10.11, LOD value of 2.22 – 3.12 and 2.03 – 3.23, phenotypic variation of 6.34 – 10.66 and 8.34 – 10.87 per cent respectively. QTLs showed positive additive effect conferring favorable allele from E36-1 parent.

Carbon isotope discrimination

This trait was mapped only in RIP2. Two QTLs were found common across season and locations. The QTLs on LG-G flanked by Xtxp141-Xiabt437, length varying from 12.40 – 12.62, LOD value of 2.40 – 2.99 and per cent phenotypic variation of 2.40 – 3.74 with negative effect conferring favorable allele from N13 parent. Another QTL found on LG-I flanked by Xtxp6-Xiabt70, length of 10.00 – 10.48, LOD value of 2.69 – 3.10, per cent phenotypic variation of 6.80 – 7.23 with positive additive effect conferring favorable allele from E36-1 parent.

## 4.3 Introgression and field evaluation of stable charcoal rot resistant QTLs in two recurrent backgrounds

Three stable QTLs, one each for lodging per cent (Xtxp176 – Xiabt312, (*cr1*)) on LG-I, number of internodes crossed by fungus (Xtxp297 – Xiabt 73, (*cr2*)) on LG-B and length of infection (Xiabt 275 – Xiabt241, (*cr3*)) on LG-B were handled in this study. These three QTLs accounted for a collective of 43.52 per cent of phenotypic variation for charcoal rot resistance. For all the three QTLs the favorable allele was found to be contributed by E36-1 parent. At first level the target markers conditioning the QTLs were screened among the recurrent parents, M35-1 and SPV86 and the donor parent (E36-1) to achieve the foreground selection. Selecting backcross progeny with the target QTLs and tightly-linked flanking markers in order to minimize linkage drag, was done which is referred to as ‘recombinant selection’ or limited background selection was done. Further, the Marker Assisted Backcrossing (MAB) involved selecting the backcross progeny (that have already been selected for the target trait) with ‘background’ markers to accelerate the recovery of the recurrent parent genome (background selection). Two recurrent parents, M35-1 and SPV 86, both highly susceptible for charcoal rot disease while being agronomically superior were backcrossed with donor parent E36-1 and subsequently advanced to $BC_3F_2$ generations separately and their performance was evaluated in field condition under sick plot. The $BC_2F_1$ was the stage at which material was included into this study and taken ahead to its logical end.

### 4.3.1 Foreground selection in M35-1 derived progenies

The target QTLs from donor parent were confirmed in recurrent parent background by selecting for heterozygous state using the flanking marker at all the three targeted QTL region till $BC_3F_1$. Heterozygote identification in $BC_3F_1$ progenies in M35-1 background with Xiabt*275* marker presented in Plate 6.

**Table 25: Score sheet indicating the genotypic status for flanking markers for three charcoal resistance QTLs (*cr1*, *cr2* and *cr3*) among $BC_3F_1$ progenies derived from M35-1 × E36-1 crosses (foreground selection and limited background selection )**

| Progeny No. | *cr1* | | *cr1* | | *cr2* | | *cr2* | | *cr3* | | *cr3* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | |
| | Xtxp176 | Xiabt312 | Xtxp96 | Xtxp274 | Xtxp297 | Xiabt73 | Xtxp48 | Xiabt82 | Xiabt275 | Xiabt241 | Xiabt60 | Xiabt87 |
| 1 | H | - | H | B | H | B | H | H | B | A | 0 | B |
| 2 | B | H | B | A | - | - | A | H | H | A | H | H |
| 3 | B | H | - | H | B | B | H | B | B | B | 0 | H |
| 4 | 0 | H | H | 0 | - | B | - | H | B | 0 | H | - |
| 5 | 0 | H | A | H | B | B | 0 | B | H | H | B | H |
| 6 | A | - | - | - | B | B | B | B | H | A | B | - |
| 7 | H | 0 | B | B | H | - | B | B | H | H | B | B |
| 8 | 0 | H | - | H | 0 | H | - | 0 | B | H | B | A |
| 9 | B | H | H | 0 | H | H | - | 0 | B | B | H | B |
| 10 | 0 | B | B | - | H | H | 0 | - | H | B | B | H |
| 11 | H | H | H | A | 0 | B | H | H | H | H | 0 | B |
| 12 | A | 0 | - | H | - | - | B | 0 | H | B | - | B |
| 13 | H | B | B | 0 | B | B | H | H | 0 | B | A | A |
| 14 | B | - | - | 0 | A | B | H | B | B | B | H | B |
| 15 | B | H | H | - | B | - | A | - | H | H | B | H |
| 16 | H | H | A | A | B | H | H | H | B | 0 | H | H |
| 17 | H | H | B | B | H | B | - | A | A | 0 | - | B |
| 18 | B | 0 | H | B | H | H | 0 | B | H | B | - | H |
| 19 | B | - | B | B | A | B | 0 | H | - | - | H | H |
| 20 | B | B | H | B | B | - | H | B | B | B | B | B |
| 21 | H | 0 | B | B | 0 | H | H | A | - | B | - | H |
| 22 | B | B | B | B | H | H | H | B | B | B | H | - |
| 23 | A | B | - | B | H | H | B | H | B | B | B | A |

*Contd…*

| Progeny No. | *cr1* | | *cr1* | | *cr2* | | *cr2* | | *cr3* | | *cr3* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | |
| | **Xtxp176** | **Xiabt312** | **Xtxp96** | **Xtxp274** | **Xtxp297** | **Xiabt73** | **Xtxp48** | **Xiabt82** | **Xiabt275** | **Xiabt241** | **Xiabt60** | **Xiabt87** |
| 24 | B | - | B | B | B | B | A | B | - | B | H | B |
| 25 | B | H | - | - | B | - | 0 | B | 0 | H | B | H |
| 26 | H | B | H | - | B | B | A | H | H | H | 0 | B |
| 27 | H | H | H | A | B | H | B | 0 | H | H | - | B |
| 28 | - | - | H | H | B | 0 | H | H | 0 | B | H | H |
| 29 | B | B | B | A | B | H | B | B | - | - | B | B |
| 30 | A | B | 0 | 0 | B | H | B | - | B | B | B | A |
| 31 | B | - | H | H | H | B | H | B | A | B | H | - |
| 32 | B | H | H | B | B | B | B | A | B | - | H | H |
| 33 | H | B | B | B | - | H | - | H | B | H | B | B |
| 34 | H | H | B | - | A | H | H | 0 | H | B | B | H |
| 35 | A | B | - | - | H | H | A | H | H | H | - | 0 |
| 36 | B | - | B | H | H | B | - | - | A | B | B | H |
| 37 | - | - | H | B | B | B | 0 | H | B | - | A | B |
| 38 | B | B | 0 | - | B | B | - | H | 0 | H | B | B |
| 39 | - | B | H | B | B | B | H | 0 | H | H | H | B |
| 40 | B | B | B | H | H | H | B | - | H | H | B | B |
| 41 | B | B | - | B | - | H | H | A | B | B | B | B |
| 42 | - | B | H | H | A | A | - | H | B | - | A | B |
| 43 | 0 | H | A | H | H | H | B | 0 | B | B | B | H |
| 44 | H | H | H | B | - | H | - | 0 | B | H | B | H |
| 45 | H | H | - | H | A | H | H | - | B | 0 | 0 | B |
| 46 | 0 | B | 0 | B | H | H | A | A | B | H | H | H |
| 47 | - | - | B | B | H | B | B | B | B | H | 0 | H |
| 48 | B | B | A | H | B | B | H | B | H | B | H | - |
| 49 | A | B | - | 0 | B | B | B | B | B | B | B | H |

*Contd…*

| Progeny No. | *cr1* | | *cr1* | | *cr2* | | *cr2* | | *cr3* | | *cr3* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | |
| | Xtxp176 | Xiabt312 | Xtxp96 | Xtxp274 | Xtxp297 | Xiabt73 | Xtxp48 | Xiabt82 | Xiabt275 | Xiabt241 | Xiabt60 | Xiabt87 |
| 50 | B | - | - | 0 | B | H | H | B | B | 0 | B | - |
| 51 | B | H | 0 | - | B | 0 | B | B | B | B | 0 | - |
| 52 | H | B | H | H | B | B | B | B | H | H | B | A |
| 53 | H | H | B | 0 | H | - | - | B | - | H | H | B |
| 54 | A | B | H | H | B | H | B | B | A | A | B | H |
| 55 | B | - | H | B | B | H | - | - | H | H | 0 | B |
| 56 | 0 | H | A | - | 0 | H | H | - | - | H | - | B |
| 57 | H | H | H | H | 0 | H | H | 0 | A | H | A | A |
| 58 | H | H | - | A | A | - | B | 0 | H | H | H | B |
| 59 | B | B | 0 | B | B | B | B | H | H | B | B | H |
| 60 | B | - | 0 | H | 0 | H | H | 0 | B | B | H | H |
| 61 | B | B | H | B | B | H | B | A | B | B | - | B |
| 62 | H | H | B | B | H | H | B | B | H | H | B | B |
| 63 | B | 0 | H | B | H | H | H | B | B | 0 | H | H |
| 64 | B | H | B | H | A | 0 | B | H | B | B | B | B |
| 65 | B | H | A | B | H | B | A | B | H | - | - | H |
| 66 | H | B | 0 | B | B | - | A | H | B | H | H | - |
| 67 | B | B | A | H | B | H | H | B | B | H | B | A |
| 68 | B | 0 | B | 0 | H | H | - | H | 0 | H | H | B |
| 69 | B | B | H | H | H | H | 0 | B | 0 | H | B | H |
| 70 | H | H | B | B | B | 0 | B | B | A | - | 0 | B |
| 71 | - | H | B | - | B | - | A | H | B | B | - | B |
| 72 | A | A | H | B | B | B | - | 0 | 0 | H | A | B |
| 73 | H | H | B | H | H | 0 | - | 0 | B | H | B | H |
| 74 | - | H | 0 | B | 0 | B | 0 | - | 0 | B | B | H |
| 75 | A | H | - | B | 0 | B | H | H | H | H | 0 | B |

*Contd…*

| Progeny No. | *cr1* | | *cr1* | | *cr2* | | *cr2* | | *cr3* | | *cr3* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | |
| | **Xtxp176** | **Xiabt312** | **Xtxp96** | **Xtxp274** | **Xtxp297** | **Xiabt73** | **Xtxp48** | **Xiabt82** | **Xiabt275** | **Xiabt241** | **Xiabt60** | **Xiabt87** |
| 76 | H | H | A | B | H | B | B | 0 | A | 0 | H | H |
| 77 | H | B | B | H | B | A | H | H | H | B | 0 | H |
| 78 | B | B | B | H | H | A | H | B | B | - | H | - |
| 79 | B | B | 0 | B | B | B | A | - | B | H | B | H |
| 80 | B | H | H | H | B | 0 | H | H | H | H | B | - |
| 81 | B | 0 | 0 | H | H | H | - | A | H | H | 0 | - |
| 82 | B | B | H | - | H | A | 0 | B | B | 0 | B | A |
| 83 | H | - | B | H | B | H | 0 | H | B | - | H | B |
| 84 | B | H | B | - | B | H | H | B | B | B | B | H |
| 85 | B | H | 0 | - | B | B | H | A | H | 0 | 0 | B |
| 86 | 0 | H | B | A | H | B | H | B | 0 | B | - | B |
| 87 | 0 | H | 0 | B | H | H | B | H | 0 | B | A | A |
| 88 | A | - | 0 | H | H | B | A | B | H | B | H | B |
| 89 | B | B | H | B | 0 | B | 0 | B | B | A | B | H |
| 90 | 0 | H | B | H | B | B | - | B | H | A | H | H |
| 91 | B | H | B | H | H | H | B | H | B | B | - | B |
| 92 | 0 | B | 0 | B | B | 0 | H | B | B | 0 | - | H |
| 93 | H | H | H | H | A | 0 | 0 | B | H | H | H | H |
| 94 | A | 0 | 0 | H | B | H | A | H | H | A | B | B |
| 95 | H | B | H | - | 0 | A | B | 0 | B | H | - | H |
| 96 | B | - | B | H | B | H | B | B | B | H | H | - |
| 97 | B | H | B | - | 0 | H | B | H | B | B | B | A |
| 98 | H | H | 0 | - | H | B | - | H | H | B | H | B |
| 99 | H | H | B | A | B | B | - | H | H | H | B | H |
| 100 | B | 0 | H | B | H | H | H | B | H | B | 0 | B |
| 101 | B | - | B | H | B | B | B | A | 0 | B | - | B |

*Contd…*

| Progeny No. | *cr1* | | *cr1* | | *cr2* | | *cr2* | | *cr3* | | *cr3* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | |
| | **Xtxp176** | **Xiabt312** | **Xtxp96** | **Xtxp274** | **Xtxp297** | **Xiabt73** | **Xtxp48** | **Xiabt82** | **Xiabt275** | **Xiabt241** | **Xiabt60** | **Xiabt87** |
| 102 | B | B | 0 | B | H | B | - | H | B | B | - | - |
| 103 | H | H | B | B | H | H | B | B | H | H | B | B |
| 104 | 0 | B | A | A | - | - | A | H | B | 0 | H | B |
| 105 | 0 | B | H | B | 0 | H | - | - | A | 0 | 0 | - |
| 106 | H | B | B | H | 0 | H | 0 | H | B | H | H | B |
| 107 | B | A | H | H | A | - | - | H | 0 | A | B | H |
| 108 | H | A | - | B | B | B | H | 0 | B | H | - | B |
| 109 | B | B | - | H | 0 | H | B | - | 0 | H | H | H |
| 110 | B | 0 | H | H | B | H | H | A | H | B | A | H |
| 111 | H | H | B | B | 0 | B | - | H | B | B | H | B |
| 112 | H | A | - | H | H | H | B | 0 | H | H | - | H |
| 113 | B | H | H | - | A | 0 | - | 0 | B | B | 0 | B |
| 114 | B | H | B | A | H | B | H | - | H | B | B | B |
| 115 | B | B | H | B | B | - | A | A | H | H | A | H |
| 116 | H | B | B | H | B | H | B | B | - | - | - | 0 |
| 117 | H | H | 0 | B | H | H | H | B | B | A | - | 0 |
| 118 | H | B | - | B | H | H | B | B | H | A | 0 | - |
| 119 | 0 | B | H | H | B | 0 | H | B | B | B | H | H |
| 120 | B | B | A | H | B | - | B | B | B | 0 | B | 0 |
| 121 | H | H | H | B | B | B | B | B | H | H | H | H |
| 122 | B | 0 | - | H | H | 0 | - | B | H | A | H | B |
| 123 | A | 0 | 0 | B | B | H | B | B | B | H | A | - |

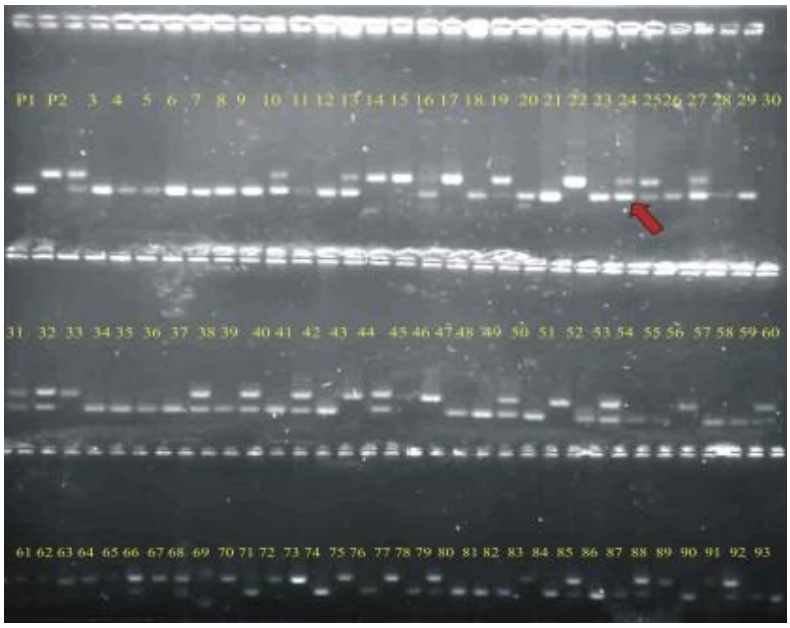



**Plate 6: Heterozygous plants identified in $BC_3F_1$ progenies in M35-1 background with Xiabt275 marker (P1 : E36-1; P2 : M35-1)**

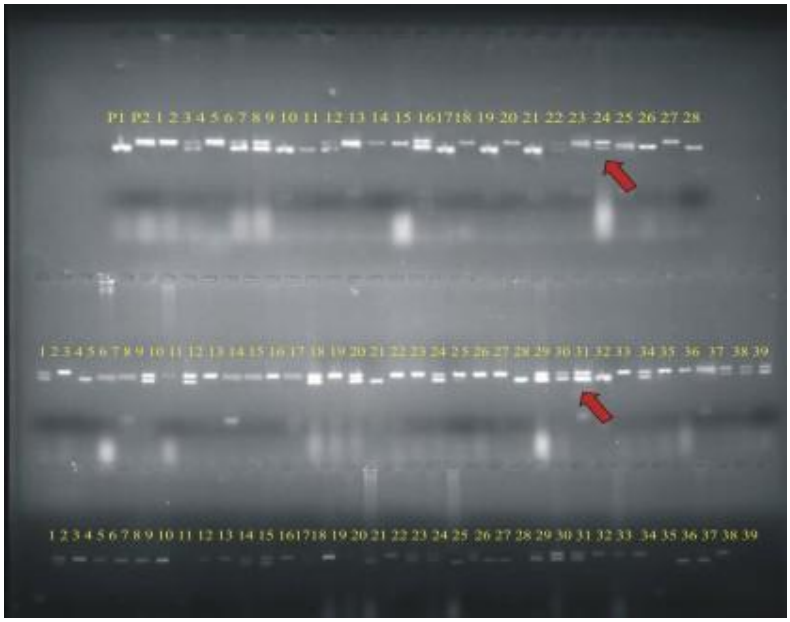



**Plate 7: Heterozygous plants identified in $BC_3F_1$ progenies in SPV86 background with Xtxp297 marker (P1 : E36-1; P2 : SPV-86)**

A total of 123 $BC_3F_1$ progenies were screened with six polymorphic foreground markers (Xtxp176, Xiabt312, Xtxp297, Xiabt73, Xiabt75, Xiabt241) flanking *cr1, cr2* and *cr3* QTLs on LG-B and LG-I. Scored marker data is presented in Table 25. The alleles in the gels were scored as A, B, H, O and "—" based on their pattern compared with those of the parents. "A" was defined as the presence of allele from the donor parent (E36-1), "B" as the presence of allele from recurrent parent M35-1, "H" as the heterozygous (presence of both recurrent and donor parent alleles), "O" as an allele from neither from donor parent nor from the recurrent parent allele and "—" was a missing sample. In order to overcome the linkage drag it was ensured that a strong selection with marker outside the donor QTL region was carried on chromosome and the progenies that showed banding pattern as that of recipient type (homozygote). Two flanking markers outside each targeted QTL region were used for identifying the backcross progenies having the recurrent parent allele at both the markers called as double homozygote at that specific locus. At $BC_3F_1$ generation only the progenies with marker status "H" and "B" for limited background for all the target marker pairs were scored and selected (Table 25). Two progenies were found to be heterozygous ("H") and double homozygous at limited background for all three targeted QTL region. Out of which one progeny (#62) was selfed to get $BC_3F_2$ and another progeny (#103) was selected for backcrossing with recurrent parent M35-1 and seeds were advanced to next round of backcross generation ($BC_4F_1$).

### 4.3.2 Foreground selection in SPV 86 derived progenies

The target QTLs from donor parent were confirmed in recurrent parent background by selecting for heterozygous state using the flanking marker at all the three targeted QTL region till $BC_3F_1$. Heterozygote identification in $BC_3F_1$ progenies in SPV86 background with Xtxp297 marker presented in Plate 7.

A total of 115 progeny were screened with six polymorphic foreground markers (Xtxp176, Xtxp312, Xtxp297, Xiabt73, Xiabt75, Xiabt241) flanking the three QTL regions (*cr1*, *cr2*, and c*r3*) on two linkage group (B and I). Scored marker data is presented in Table 26. The alleles on the gels were scored as presented in the previous section. Here the recurrent parent was SPV86. Thereafter, a second level of selection was performed to avoid the other genome region from donor parent from QTL carrying linkage group B and LG-I, *i.e.* limited background selection was exercised so as to take care of linkage drag around target QTL position. Two flanking markers outside each targeted QTL region were used for identifying the backcross progenies having the recurrent parent allele at both the markers termed as double homozygote at that particular locus. A total of two progeny were found to be heterozygous ("H") for all the three targeted QTL region and out of which one progeny (#14) was selected for emasculation and backcrossing with recurrent parent SPV 86, seed obtained from this cross were advanced to next generation $BC_4F_1$. Another progeny (#82) was allowed to self to get $BC_3F_2$ generation.

### 4.3.3 Screening of $BC_3F_2$ homozygous progenies for charcoal rot resistance in sick plot

The $BC_3F_2$ progenies were screened for their marker status (Table 27 and Table 28) and finally progenies which showed homozygosity for target alleles were selected. Selected plants were checked for their recurrent parent whole genome recovery and selfed to get $BC_3F_3$ seeds, which were further evaluated in field in replication under sick plot.

### 4.3.4 Screening of $BC_3F_2$ homozygous progenies carrying various QTL combinations for charcoal rot resistance

The $BC_3F_3$ progenies were screened for their zygosity status using pairs of markers at all three QTL positions. The $BC_3F_3$ progenies carrying *cr1+cr2+cr3, cr1+cr2, cr2+cr3, cr1+cr3, cr1, cr2, cr3*, Null *cr* were found to be homozygous without any exception. From each block a set of 10 plants chosen randomly were checked for this purpose.

**Table 26: Score sheet indicating the genotypic status for flanking markers for three charcoal resistance QTLs (*cr1*, *cr2* and *cr3*) among $BC_3F_1$ progenies derived from SPV86 × E36-1 crosses (foreground selection and limited background selection)**

| Progeny No. | *cr1* | | *cr1* | | *cr2* | | *cr2* | | *cr3* | | *cr3* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | |
| | Xtxp176 | Xiabt312 | Xtxp96 | Xtxp274 | Xtxp297 | Xiabt73 | Xtxp48 | Xiabt82 | Xiabt275 | Xiabt241 | Xiabt60 | Xiabt87 |
| 1 | 0 | - | - | - | B | A | B | - | B | H | 0 | B |
| 2 | H | H | H | - | H | B | B | H | A | B | B | B |
| 3 | B | 0 | H | 0 | B | 0 | H | - | 0 | B | - | H |
| 4 | H | H | B | 0 | H | B | 0 | 0 | A | H | B | A |
| 5 | H | B | B | H | H | B | B | B | B | 0 | H | B |
| 6 | A | - | H | 0 | - | H | B | B | H | H | B | 0 |
| 7 | H | H | B | A | 0 | B | B | 0 | B | B | B | 0 |
| 8 | - | A | B | B | 0 | B | B | B | B | - | - | 0 |
| 9 | 0 | B | H | B | - | - | B | B | B | H | H | - |
| 10 | 0 | H | B | H | A | B | B | 0 | H | - | A | A |
| 11 | H | B | A | B | H | H | H | H | 0 | 0 | B | B |
| 12 | H | A | H | B | B | 0 | 0 | H | B | B | H | B |
| 13 | H | B | H | 0 | 0 | B | H | H | B | B | B | B |
| 14 | H | H | B | B | H | H | B | B | H | H | B | B |
| 15 | A | B | A | B | - | H | B | - | B | B | B | B |
| 16 | 0 | B | H | - | B | A | H | H | B | B | B | B |
| 17 | A | H | 0 | H | H | B | B | H | B | 0 | - | B |
| 18 | B | 0 | - | H | B | 0 | A | H | H | H | B | B |
| 19 | H | H | B | A | B | 0 | H | H | 0 | H | - | - |
| 20 | B | B | H | B | - | 0 | B | B | H | H | H | - |

*Contd…*

| Progeny No. | *cr1* | | *cr1* | | *cr2* | | *cr2* | | *cr3* | | *cr3* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | |
| | **Xtxp176** | **Xiabt312** | **Xtxp96** | **Xtxp274** | **Xtxp297** | **Xiabt73** | **Xtxp48** | **Xiabt82** | **Xiabt275** | **Xiabt241** | **Xiabt60** | **Xiabt87** |
| 21 | B | - | B | 0 | H | - | B | B | B | B | H | 0 |
| 22 | B | H | H | B | A | A | B | 0 | B | - | B | 0 |
| 23 | H | - | H | B | B | B | B | B | H | H | B | H |
| 24 | 0 | 0 | - | H | H | B | B | B | B | H | H | 0 |
| 25 | B | B | 0 | B | B | B | H | - | A | H | B | A |
| 26 | B | B | 0 | B | H | B | 0 | H | H | H | B | B |
| 27 | B | 0 | - | - | B | B | H | H | H | B | H | B |
| 28 | B | B | A | B | B | B | - | H | A | - | B | H |
| 29 | B | B | H | H | - | B | H | H | H | H | A | B |
| 30 | B | 0 | B | 0 | B | B | A | B | - | A | H | B |
| 31 | H | H | 0 | B | - | - | B | H | 0 | B | H | 0 |
| 32 | 0 | H | B | B | H | - | H | B | 0 | H | B | H |
| 33 | H | H | - | H | H | 0 | B | - | H | B | A | B |
| 34 | B | B | B | A | B | 0 | B | - | H | A | H | - |
| 35 | B | - | H | B | B | H | A | H | H | B | 0 | H |
| 36 | H | H | B | 0 | H | 0 | B | B | B | H | H | H |
| 37 | B | H | B | 0 | B | A | H | H | A | B | - | H |
| 38 | A | H | - | 0 | B | B | 0 | H | 0 | B | H | H |
| 39 | H | H | H | - | H | B | H | H | A | H | A | B |
| 40 | B | B | A | A | B | H | H | - | B | 0 | B | H |
| 41 | B | B | B | B | A | B | H | 0 | H | H | H | B |
| 42 | B | 0 | H | B | H | H | B | 0 | B | B | B | - |
| 43 | B | B | B | B | H | - | B | H | B | - | B | - |
| 44 | B | B | H | B | H | 0 | B | - | B | H | A | H |

*Contd…*

| Progeny No. | *cr1* | | *cr1* | | *cr2* | | *cr2* | | *cr3* | | *cr3* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | |
| | **Xtxp176** | **Xiabt312** | **Xtxp96** | **Xtxp274** | **Xtxp297** | **Xiabt73** | **Xtxp48** | **Xiabt82** | **Xiabt275** | **Xiabt241** | **Xiabt60** | **Xiabt87** |
| 45 | H | B | B | B | B | 0 | 0 | - | H | - | B | B |
| 46 | B | - | B | B | B | H | B | A | 0 | 0 | H | H |
| 47 | B | - | - | B | B | - | H | B | B | B | 0 | H |
| 48 | A | H | B | B | 0 | - | B | H | B | B | H | H |
| 49 | B | B | - | - | B | A | 0 | B | B | 0 | H | - |
| 50 | H | H | H | - | H | B | - | B | B | B | H | 0 |
| 51 | 0 | H | H | 0 | B | H | A | A | B | B | B | 0 |
| 52 | H | H | B | 0 | 0 | B | H | B | B | 0 | B | H |
| 53 | H | - | B | H | - | B | B | H | H | H | B | - |
| 54 | H | 0 | H | 0 | A | A | H | H | 0 | H | 0 | - |
| 55 | B | 0 | B | A | H | B | - | B | H | H | B | A |
| 56 | B | H | B | B | B | H | - | H | B | B | H | B |
| 57 | B | - | H | B | H | H | H | H | B | - | B | H |
| 58 | 0 | - | B | H | - | B | B | B | H | H | 0 | B |
| 59 | B | A | A | B | - | H | - | H | B | H | - | B |
| 60 | H | B | H | B | H | H | H | - | A | H | A | A |
| 61 | B | H | H | 0 | B | B | B | A | H | H | H | B |
| 62 | 0 | B | B | H | - | H | H | B | B | B | B | H |
| 63 | - | B | A | B | H | - | B | H | B | B | H | H |
| 64 | A | A | H | - | B | A | 0 | B | B | 0 | - | B |
| 65 | H | B | 0 | H | H | B | - | B | B | B | - | H |
| 66 | B | H | H | H | B | H | B | H | B | B | H | H |
| 67 | H | H | - | H | 0 | B | A | B | A | A | B | B |
| 68 | - | B | H | H | - | B | 0 | B | H | B | - | H |

*Contd…*

| Progeny No. | *cr1* | | *cr1* | | *cr2* | | *cr2* | | *cr3* | | *cr3* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | |
| | **Xtxp176** | **Xiabt312** | **Xtxp96** | **Xtxp274** | **Xtxp297** | **Xiabt73** | **Xtxp48** | **Xiabt82** | **Xiabt275** | **Xiabt241** | **Xiabt60** | **Xiabt87** |
| 69 | - | H | A | B | H | H | A | H | B | H | H | - |
| 70 | A | B | B | H | B | B | B | 0 | H | H | B | A |
| 71 | H | - | H | B | B | - | H | H | - | B | H | B |
| 72 | 0 | H | B | - | H | H | B | B | - | H | B | H |
| 73 | H | H | B | - | B | H | B | - | H | H | 0 | B |
| 74 | - | H | A | H | A | H | B | H | B | B | - | B |
| 75 | H | H | B | B | H | H | H | - | - | H | H | B |
| 76 | A | B | H | H | H | B | 0 | 0 | H | - | B | - |
| 77 | B | H | 0 | H | A | - | B | B | B | A | B | - |
| 78 | H | B | H | H | H | H | B | B | H | B | A | H |
| 79 | B | - | H | - | - | A | B | 0 | B | H | B | B |
| 80 | B | - | H | 0 | 0 | B | B | B | 0 | H | H | H |
| 81 | A | H | B | 0 | 0 | H | B | B | H | H | 0 | H |
| 82 | H | H | B | B | H | H | B | B | H | H | B | B |
| 83 | H | H | B | - | H | A | H | H | H | H | H | - |
| 84 | 0 | H | 0 | - | H | B | 0 | H | A | B | H | 0 |
| 85 | H | H | B | A | B | H | H | H | B | H | B | 0 |
| 86 | H | - | H | B | A | B | B | B | H | B | B | H |
| 87 | H | 0 | B | H | 0 | B | B | - | B | - | B | - |
| 88 | B | 0 | 0 | B | A | H | H | H | B | - | 0 | - |
| 89 | B | H | - | B | B | 0 | B | H | A | H | B | A |
| 90 | B | - | A | A | H | H | A | H | B | B | H | B |
| 91 | 0 | - | H | B | B | B | H | H | H | H | B | H |
| 92 | B | A | B | H | B | - | H | H | 0 | H | 0 | B |

*Contd…*

| Progeny No. | *cr1* | | *cr1* | | *cr2* | | *cr2* | | *cr3* | | *cr3* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | | Flanking marker (foreground selection) | | Flanking marker (limited background selection) | |
| | Xtxp176 | Xiabt312 | Xtxp96 | Xtxp274 | Xtxp297 | Xiabt73 | Xtxp48 | Xiabt82 | Xiabt275 | Xiabt241 | Xiabt60 | Xiabt87 |
| 93 | H | B | H | H | B | H | - | H | H | H | - | B |
| 94 | B | H | - | B | H | - | H | H | H | - | A | A |
| 95 | 0 | B | - | H | 0 | 0 | A | B | H | 0 | H | B |
| 96 | - | B | H | H | B | B | B | H | B | 0 | B | H |
| 97 | A | A | B | B | B | B | H | B | B | H | H | H |
| 98 | H | B | - | H | B | 0 | B | - | B | - | - | B |
| 99 | A | B | H | - | B | B | B | - | 0 | - | - | H |
| 100 | B | H | B | A | B | B | A | H | B | A | A | B |
| 101 | H | B | H | B | B | 0 | B | B | H | B | B | H |
| 102 | B | - | B | H | H | H | H | H | B | H | H | B |
| 103 | B | - | 0 | B | H | B | 0 | H | 0 | B | B | - |
| 104 | A | H | - | B | B | - | H | H | - | B | B | - |
| 105 | B | B | B | H | B | - | H | - | A | A | A | H |
| 106 | H | H | 0 | B | A | H | H | 0 | H | B | B | B |
| 107 | 0 | H | - | B | B | B | B | 0 | A | B | H | H |
| 108 | H | H | A | A | H | H | B | H | B | H | 0 | H |
| 109 | H | - | H | B | 0 | H | B | - | H | B | H | H |
| 110 | H | 0 | B | H | H | H | 0 | - | B | - | H | - |
| 111 | B | 0 | H | H | H | - | B | A | B | - | H | 0 |
| 112 | B | H | - | B | H | 0 | H | B | A | H | B | 0 |
| 113 | B | - | - | H | B | 0 | B | H | B | B | B | H |
| 114 | 0 | - | H | H | B | H | 0 | B | H | H | B | - |
| 115 | B | A | B | B | B | - | - | B | 0 | H | 0 | - |

### 4.3.5 Screening of $BC_3F_3$ homozygous progenies carrying various QTL combinations for charcoal rot resistance

The $BC_3F_3$ progenies were screened for their zygosity status using pairs of markers at all three QTL positions. The $BC_3F_3$ propagules carrying *cr1+cr2+cr3, cr1+cr2, cr2+cr3, cr1+cr3, cr1, cr2, cr3*, Null *cr* were found to be homozygous without any exception in both the backgrounds and same presented in Plate 8 and Plate 9. From each block a set of 10 plants chosen randomly checked for this purpose. And the $BC_3F_3$ progenies subjected for their field performance along with parents in sick plot and tooth pick artificial inoculation, during *rabi* 2010 at Main Agricultural Research Station, Dharwad. At harvest time variation in charcoal rot disease length of infection and number of internodes crossed by the fungus in different combinations of introgressed lines were presented in Plate 10.

#### 4.3.5.1 Analysis of variance and variability parameters

The appropriate degrees of freedom (DF) and mean sum of squares (MSS) along with other genetic parameters *viz.,* range, mean, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability estimates and predicted genetic advance as per cent of mean were worked out for all the traits related to charcoal rot component. The analysis of variance revealed highly significant difference between the treatments (Table 29) and the estimates of variability parameters were presented in Table 30.

Per cent lodging

There was significant difference among treatments for lodging per cent due to fungus, with mean value of 17.96 per cent. The genotypic and phenotypic coefficients of variation for this trait were 81.99 and 83.87 respectively with heritability of 95.57 per cent and GA over per cent mean of 42.09 was recorded.

Number of internodes crossed by fungus

For number of internodes crossed by the fungus, the treatments differ significantly. Mean number of internodes crossed was of about 2.32. Genotypic and phenotypic coefficient of variation recorded was 36.81 and 39.98 per cent with per cent heritability 84.78 and GA over per cent mean of 72.19 per cent.

Length of infection

All the treatments were differing significantly with respect to length of infection by the fungus. Mean length of infection recorded was 22.36 cm. The genotypic and phenotypic coefficients of variation for this trait were 56.18 and 58.46 per cent respectively with 92.33 per cent heritability and GA over per cent mean of 30.44 was recorded.

Plant height

Significant difference between the treatments for plant height was recorded with mean height of 183.69 cm. The GCV and PCV values recorded were 8.02 and 11.96 per cent respectively. The heritability value of 44.97 per cent and GA over mean of 2.35 per cent was recorded for this trait.

Panicle exertion

There was significant difference for this trait among the treatments. The mean exertion length of 4.16 cm with genetic advance over per cent mean of 17.69 was recorded. The GCV and PCV for the trait were 34.58 and 77.03 per cent with heritability estimate of 17.69 per cent were recorded.

Earhead length

Earhead lengths per plant varied across treatments significantly with mean length of 19.52 cm. GCV and PCV for this trait were 11.03 and 17.10 per cent respectively. Heritability estimate of 41.61 and GA over per cent mean of 7.96 were recorded.

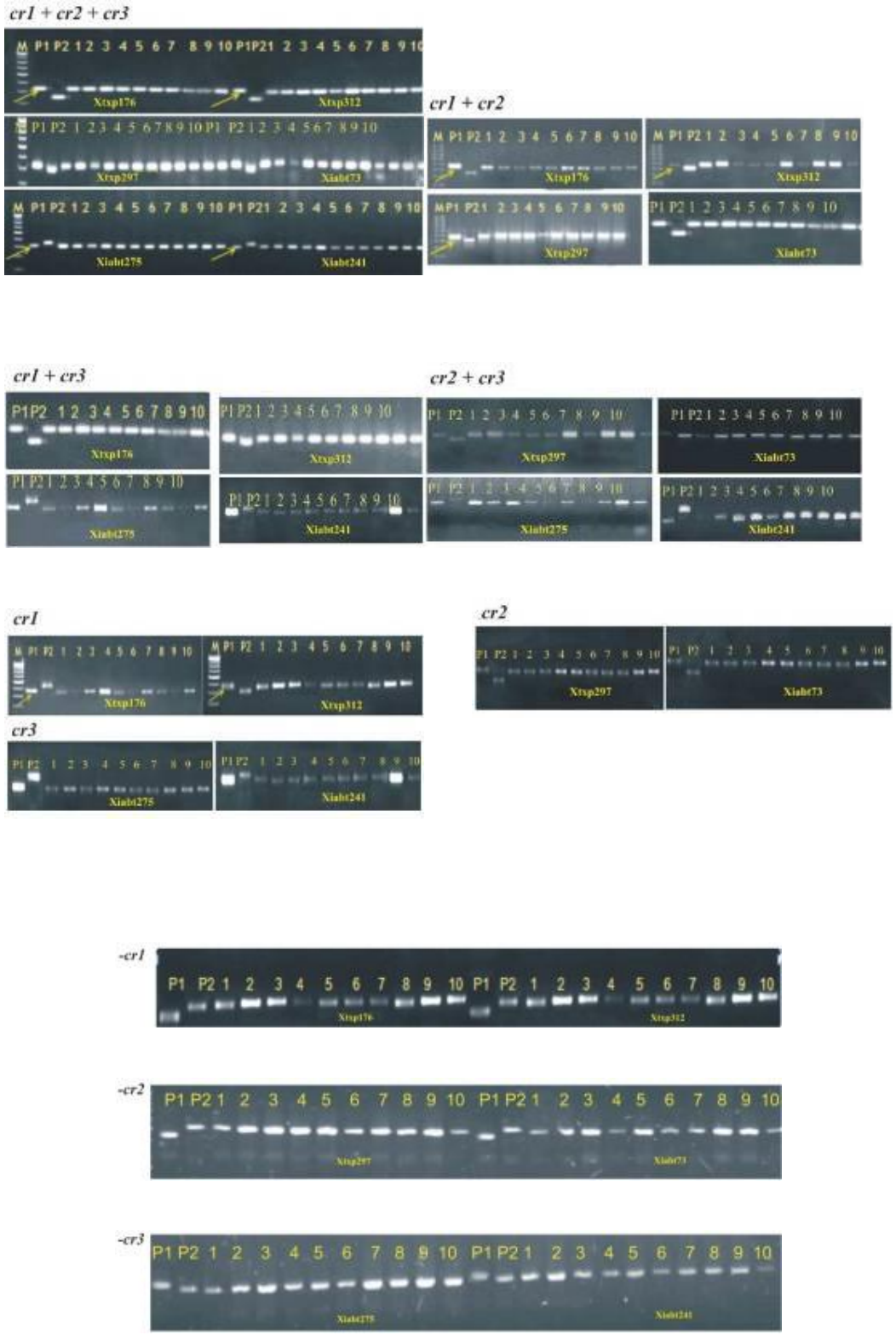



**Plate 8: Conformation of homozygosity of QTLs with different combination in $BC_3F_3$ progenies in M35-1 background (P1 : E36-1; P2 : M35-1)**

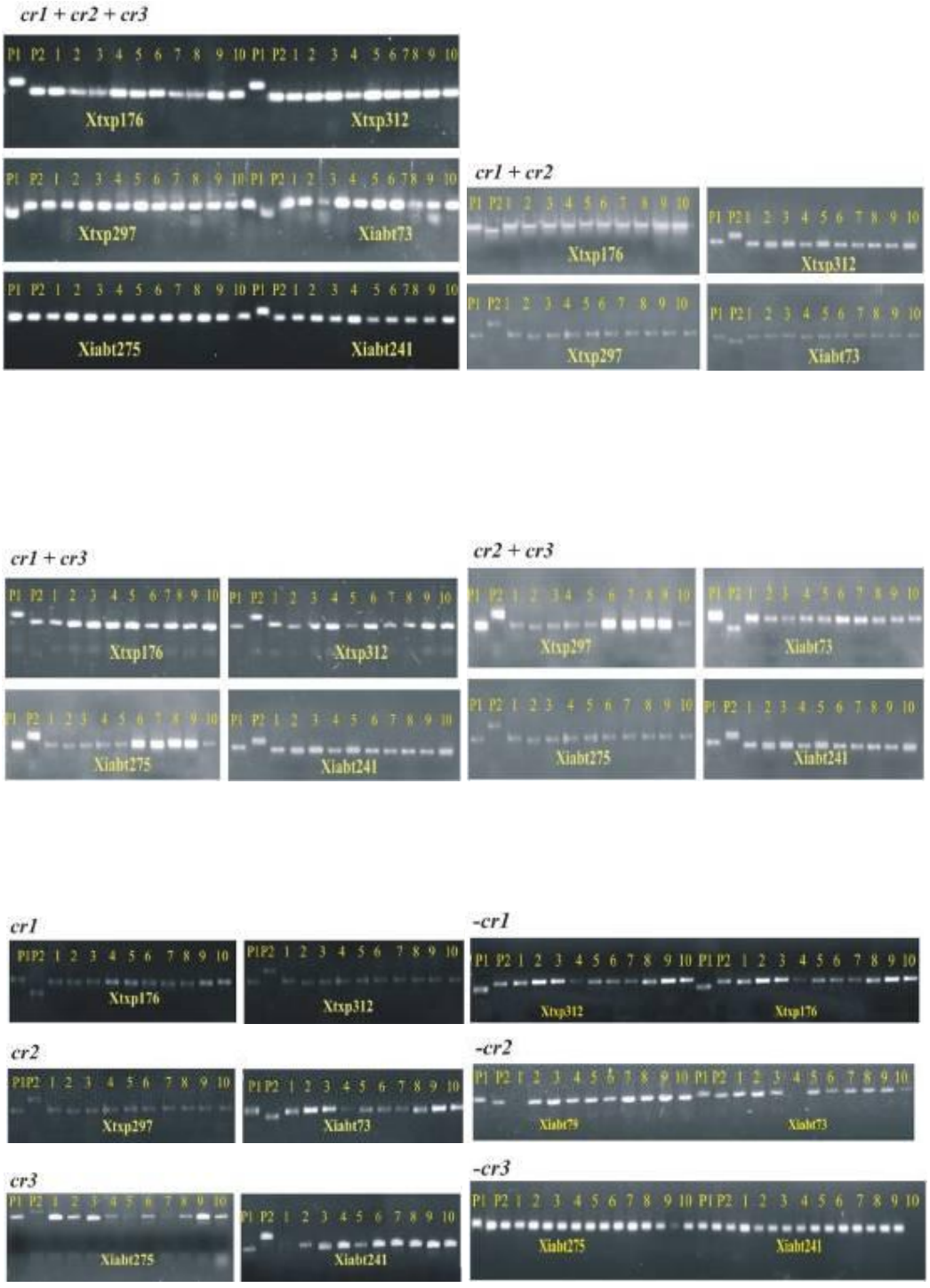



**Plate 9: Conformation of homozygosity of QTLs with different combination in $BC_3F_3$ progenies in SPV86 background (P1 : E36-1; P2 : SPV86)**


**Plate 10: Variation in charcoal rot disease manifestation in different combination of QTLs introgressed NILs**

Number of spicklets per head

Mean value for number of spicklets per plant was 63.23 with significant difference between treatments. For number of spicklets per plant GCV and PCV recorded were 11.02 and 15.98 per cent respectively with heritability of 47.51 per cent and GA over per cent mean of 4.87 were recorded.

Stem girth

There was variation between treatments for stem girth with mean girth of mean of 4.57 cm. The per cent GCV and PCV recorded were 3.51 and 15.60 respectively. Heritability estimates of 5.05 per cent and GA over per cent mean of 1.90 was recorded.

Number of leaves

Significant variation was observed between the treatments, with mean number of leaves of 7.45. The PCV and GCV recorded were 10.09 and 6.36 per cent, respectively. The heritability estimate of 39.75 per cent and genetic advance over per cent mean of 9.45 was recorded for this trait.

The components due to entries were highly significant for all the three attributes (Table 31). Mean lodging per cent was lower in M35-1 introgressed with all three QTLs (*cr1* + *cr2* + *cr3*) of about 0.9 per cent, but was 1.2 per cent in E36-1 parent, 41.46 per cent in M35-1 and 53.38 per cent in SPV86. Mean number of internodes crossed by the fungus was very low in M35-1 introgressed with all three QTLs of 1.26 and was 1.38 in M35-1 background with *cr1* + *cr3* combination and was 2.4 in M35-1, 1.0 in E36-1. Mean length of infection recorded was lower in M35-1 introgressed with all three QTLs (10.64 cm) and 11.18 cm in M35-1 background with *cr1* + *cr2* combination and 11.18 cm in same background with *cr1* + *cr3* combination of about 11.3 cm and it was 6.32 cm in E36-1 parent.

**Table 27 : Score sheet indicating the genotypic status for flanking markers for three charcoal resistance QTLs (*cr1*, *cr2* and *cr3*) among $BC_3F_2$ progenies derived from M35-1 × E36-1 crosses (foreground selection )**

| Progeny No. | *cr1* | | *cr2* | | *cr3* | |
|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | | | | |
| | Xtxp176 | Xiabt312 | Xtxp297 | Xiabt73 | Xiabt275 | Xiabt241 |
| 1 | A | B | B | A | A | H |
| 2 | H | H | A | A | H | H |
| 3 | B | A | A | H | B | H |
| 4 | B | H | B | 0 | H | B |
| 5 | A | H | H | - | A | 0 |
| 6 | B | 0 | B | H | H | B |
| 7 | A | B | A | B | H | A |
| 8 | B | 0 | B | A | A | H |
| 9 | H | 0 | A | 0 | H | B |
| 10 | H | A | H | A | B | A |
| 11 | B | A | 0 | A | H | B |
| 12 | A | 0 | A | B | A | H |
| 13 | A | A | H | H | H | H |
| 14 | H | H | A | A | A | A |
| 15 | A | H | B | 0 | H | 0 |
| 16 | B | H | A | H | 0 | H |
| 17 | A | B | H | A | B | 0 |
| 18 | H | H | A | A | H | H |
| 19 | B | H | H | 0 | A | H |
| 20 | A | H | B | H | B | A |
| 21 | B | A | A | 0 | A | H |
| 22 | A | H | 0 | B | H | B |
| 23 | B | 0 | A | B | H | - |
| 24 | H | H | H | H | A | A |
| 25 | H | - | B | A | B | 0 |
| 26 | B | H | B | H | H | B |
| 27 | A | B | H | B | 0 | B |
| 28 | B | A | H | B | H | B |
| 29 | A | 0 | A | H | H | - |
| 30 | H | A | B | A | B | A |
| 31 | A | A | H | H | A | A |
| 32 | B | B | B | B | B | B |
| 33 | 0 | A | H | - | A | B |
| 34 | A | B | B | H | 0 | A |
| 35 | A | A | H | H | H | H |

*Contd…*

| Progeny No. | *cr1* | | *cr2* | | *cr3* | |
|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | | | | |
| | **Xtxp176** | **Xiabt312** | **Xtxp297** | **Xiabt73** | **Xiabt275** | **Xiabt241** |
| 36 | B | 0 | H | 0 | B | A |
| 37 | A | H | 0 | H | A | H |
| 38 | H | A | B | 0 | H | A |
| 39 | H | 0 | H | A | A | 0 |
| 40 | B | H | H | 0 | A | B |
| 41 | A | 0 | B | A | B | A |
| 42 | 0 | B | 0 | B | A | H |
| 43 | H | H | A | A | H | H |
| 44 | A | B | A | B | H | A |
| 45 | B | A | H | B | B | H |
| 46 | A | A | H | H | H | H |
| 47 | B | H | H | 0 | H | B |
| 48 | H | H | A | A | A | A |
| 49 | H | B | H | B | B | A |
| 50 | H | B | A | 0 | 0 | A |
| 51 | A | H | B | H | H | B |
| 52 | B | A | H | B | A | H |
| 53 | H | - | H | B | B | H |
| 54 | H | H | H | H | A | A |
| 55 | A | A | A | A | A | A |
| 56 | B | H | H | A | B | A |
| 57 | A | A | H | H | A | A |
| 58 | H | 0 | H | B | A | H |
| 59 | 0 | H | B | 0 | B | H |
| 60 | B | 0 | H | - | A | H |
| 61 | H | H | A | A | H | H |
| 62 | H | A | A | B | B | 0 |
| 63 | H | 0 | H | B | A | B |
| 64 | B | A | H | B | B | 0 |
| 65 | 0 | B | A | B | H | 0 |
| 66 | A | B | A | H | H | A |
| 67 | H | B | A | 0 | B | A |
| 68 | H | 0 | 0 | H | A | 0 |
| 69 | H | B | H | 0 | A | H |
| 70 | A | 0 | B | A | B | H |
| 71 | B | H | A | 0 | A | B |
| 72 | H | B | A | 0 | B | 0 |
| 73 | H | H | A | A | A | A |
| 74 | H | B | H | A | H | - |
| 75 | H | A | A | H | A | B |

*Contd…*

| Progeny No. | *cr1* | | *cr2* | | *cr3* | |
|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | | | | |
| | **Xtxp176** | **Xiabt312** | **Xtxp297** | **Xiabt73** | **Xiabt275** | **Xiabt241** |
| 76 | H | H | H | H | A | A |
| 77 | H | B | B | H | H | B |
| 78 | B | 0 | H | B | H | B |
| 79 | H | H | H | H | A | A |
| 80 | H | - | A | 0 | A | B |
| 81 | A | B | H | B | A | H |
| 82 | A | A | H | H | H | H |
| 83 | H | B | H | A | A | 0 |
| 84 | H | B | A | H | 0 | H |
| 85 | A | B | H | B | H | 0 |
| 86 | A | H | B | A | B | A |
| 87 | A | 0 | H | B | A | 0 |
| 88 | 0 | H | A | H | A | 0 |
| 89 | H | 0 | H | 0 | H | A |
| 90 | H | H | A | A | H | H |
| 91 | B | A | 0 | H | A | H |
| 92 | A | A | A | A | H | H |
| 93 | A | 0 | B | 0 | A | H |
| 94 | A | 0 | A | H | B | H |
| 95 | H | A | B | A | H | B |
| 96 | H | H | H | H | A | A |
| 97 | 0 | A | A | H | A | 0 |
| 98 | A | B | H | B | H | B |
| 99 | H | B | H | - | A | 0 |
| 100 | A | H | B | 0 | 0 | H |
| 101 | B | H | H | B | H | 0 |
| 102 | H | H | H | H | A | A |
| 103 | H | B | 0 | B | B | A |
| 104 | A | 0 | H | B | A | 0 |
| 105 | H | B | H | - | A | 0 |
| 106 | H | A | B | A | H | A |
| 107 | A | H | A | B | 0 | A |
| 108 | H | B | 0 | A | A | B |
| 109 | B | A | B | A | H | B |
| 110 | H | B | A | H | A | H |
| 111 | A | A | A | A | H | H |
| 112 | A | H | H | A | B | H |
| 113 | H | 0 | A | 0 | H | B |
| 114 | 0 | H | A | B | A | 0 |
| 115 | H | H | A | A | H | H |

*Contd…*

| Progeny No. | *cr1* | | *cr2* | | *cr3* | |
|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | | | | |
| | **Xtxp176** | **Xiabt312** | **Xtxp297** | **Xiabt73** | **Xiabt275** | **Xiabt241** |
| 116 | B | 0 | B | A | H | B |
| 117 | A | A | A | A | A | A |
| 118 | A | H | A | H | H | A |
| 119 | B | A | H | A | A | H |
| 120 | A | H | B | H | H | B |
| 121 | H | B | H | B | B | A |
| 122 | A | A | A | A | A | A |
| 123 | H | - | B | A | H | B |
| 124 | H | A | 0 | A | A | H |
| 125 | 0 | B | H | B | H | 0 |
| 126 | B | A | A | H | 0 | H |
| 127 | H | H | A | A | H | H |
| 128 | A | A | H | H | A | A |
| 129 | H | B | B | H | B | 0 |
| 130 | A | B | B | A | H | B |
| 131 | H | 0 | A | H | B | A |
| 132 | H | A | 0 | H | A | H |
| 133 | A | A | H | H | H | H |
| 134 | B | H | B | 0 | B | 0 |
| 135 | H | B | H | A | H | - |
| 136 | A | H | H | 0 | B | H |
| 137 | 0 | A | B | A | A | B |
| 138 | B | 0 | 0 | B | B | A |
| 139 | H | B | A | B | A | 0 |
| 140 | B | H | H | B | H | A |
| 141 | A | A | A | A | H | H |
| 142 | B | B | B | B | B | B |
| 143 | 0 | A | H | 0 | 0 | A |
| 144 | H | H | A | A | H | H |
| 145 | H | 0 | H | B | A | B |
| 146 | A | B | A | 0 | B | 0 |
| 147 | H | - | B | H | A | H |
| 148 | H | A | H | B | H | A |
| 149 | H | B | H | B | H | 0 |
| 150 | B | 0 | H | A | B | H |
| 151 | A | 0 | H | B | A | 0 |
| 152 | A | A | H | H | A | A |
| 153 | H | A | B | 0 | 0 | B |
| 154 | 0 | B | 0 | H | A | B |
| 155 | H | B | B | 0 | B | A |

*Contd…*

| Progeny No. | *cr1* | | *cr2* | | *cr3* | |
|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | | | | |
| | **Xtxp176** | **Xiabt312** | **Xtxp297** | **Xiabt73** | **Xiabt275** | **Xiabt241** |
| 156 | H | B | 0 | A | B | H |
| 157 | B | A | H | 0 | H | B |
| 158 | H | A | A | B | H | B |
| 159 | H | H | A | A | H | H |
| 160 | A | H | A | H | A | H |
| 161 | 0 | A | A | 0 | B | A |
| 162 | B | A | 0 | H | H | - |
| 163 | H | B | H | 0 | B | H |
| 164 | B | H | B | A | H | 0 |
| 165 | H | H | H | H | A | A |
| 166 | A | B | A | 0 | 0 | H |
| 167 | H | A | A | 0 | B | 0 |
| 168 | A | A | H | H | A | A |
| 169 | 0 | H | H | A | H | A |
| 170 | B | H | 0 | A | H | 0 |
| 171 | A | B | A | B | B | A |
| 172 | H | 0 | H | B | 0 | B |
| 173 | B | 0 | A | H | A | B |
| 174 | H | B | B | H | H | B |
| 175 | 0 | B | H | B | H | 0 |
| 176 | B | B | B | B | B | B |
| 177 | H | B | A | 0 | H | B |
| 178 | H | H | A | A | A | A |
| 179 | H | - | H | B | A | 0 |
| 180 | B | A | H | A | B | H |
| 181 | A | B | A | H | H | B |
| 182 | 0 | A | H | B | H | B |
| 183 | B | A | B | A | H | A |
| 184 | A | H | H | B | H | B |
| 185 | H | A | A | H | B | 0 |
| 186 | A | 0 | H | 0 | H | - |
| 187 | A | B | 0 | H | A | B |
| 188 | A | A | H | H | H | H |
| 189 | B | A | B | 0 | H | B |
| 190 | A | H | - | - | H | B |
| 191 | H | A | B | 0 | A | B |
| 192 | A | A | A | A | H | H |
| 193 | B | H | H | B | A | H |
| 194 | H | B | 0 | B | A | 0 |
| 195 | B | A | H | B | 0 | H |

*Contd…*

| Progeny No. | *cr1* | | *cr2* | | *cr3* | |
|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | | | | |
| | **Xtxp176** | **Xiabt312** | **Xtxp297** | **Xiabt73** | **Xiabt275** | **Xiabt241** |
| 196 | 0 | A | H | - | H | 0 |
| 197 | H | H | H | H | A | A |
| 198 | H | B | B | A | B | A |
| 199 | A | H | A | B | A | 0 |
| 200 | B | H | 0 | A | A | 0 |
| 201 | B | A | B | A | H | A |
| 202 | H | H | A | A | A | A |
| 203 | H | H | A | A | A | A |
| 204 | A | H | A | H | B | 0 |
| 205 | H | H | A | A | H | H |
| 206 | A | B | H | A | H | B |
| 207 | 0 | H | A | 0 | 0 | B |
| 208 | H | A | A | B | H | B |
| 209 | B | 0 | B | A | H | - |
| 210 | A | A | H | H | H | H |
| 211 | B | H | A | H | B | A |
| 212 | A | B | H | A | A | B |
| 213 | 0 | A | B | H | 0 | A |
| 214 | H | H | A | A | A | A |
| 215 | A | H | H | B | B | A |
| 216 | H | H | A | A | H | H |
| 217 | B | A | B | A | A | H |
| 218 | H | A | 0 | A | H | A |
| 219 | B | H | H | B | A | 0 |
| 220 | A | B | A | H | A | B |
| 221 | H | B | B | H | B | A |
| 222 | A | A | H | H | H | H |
| 223 | A | H | B | A | A | H |
| 224 | H | 0 | A | H | H | A |
| 225 | H | A | A | H | B | H |
| 226 | A | H | A | 0 | H | B |
| 227 | B | A | 0 | H | B | A |
| 228 | A | B | H | 0 | 0 | A |
| 229 | B | A | B | A | H | B |
| 230 | A | B | A | 0 | A | H |
| 231 | A | H | A | 0 | B | H |
| 232 | 0 | H | H | A | B | A |
| 233 | A | A | A | A | H | H |
| 234 | H | - | 0 | A | A | H |
| 235 | B | A | A | B | A | 0 |
| 236 | H | H | H | H | A | A |
| 237 | A | H | H | B | H | B |
| 238 | B | H | A | H | H | A |
| 239 | A | A | H | H | A | A |
| 240 | 0 | B | B | H | A | H |
| 241 | B | A | H | B | H | B |

**Table 28: Score sheet indicating the genotypic status for flanking markers for three charcoal resistance QTLs (*cr1*, *cr2* and *cr3*) among $BC_3F_2$ progenies derived from SPV 86 × E 36-1 crosses (foreground selection)**

| Progeny No. | *cr1* | | *cr2* | | *cr3* | |
|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | | | | |
| | Xtxp176 | Xiabt312 | Xtxp297 | Xiabt73 | Xiabt275 | Xiabt241 |
| 1 | H | B | H | B | H | A |
| 2 | B | A | A | H | 0 | A |
| 3 | H | A | H | 0 | A | B |
| 4 | H | H | A | A | H | H |
| 5 | A | H | H | A | H | B |
| 6 | H | H | A | A | A | A |
| 7 | 0 | A | A | H | A | H |
| 8 | H | B | B | A | B | H |
| 9 | B | A | A | B | H | B |
| 10 | H | A | B | A | A | 0 |
| 11 | A | H | A | B | H | B |
| 12 | 0 | A | A | H | H | A |
| 13 | H | H | A | A | H | H |
| 14 | H | B | 0 | H | A | H |
| 15 | B | A | H | - | H | B |
| 16 | 0 | A | 0 | B | B | A |
| 17 | H | B | A | B | H | B |
| 18 | A | H | B | A | A | H |
| 19 | H | H | A | A | A | A |
| 20 | B | H | B | H | H | 0 |
| 21 | A | A | H | H | A | A |
| 22 | B | A | H | B | 0 | H |
| 23 | A | H | H | B | B | 0 |
| 24 | B | H | A | H | - | b |
| 25 | B | A | B | A | B | 0 |
| 26 | A | H | A | H | H | B |
| 27 | A | B | H | A | 0 | B |
| 28 | 0 | H | A | 0 | H | B |
| 29 | H | A | A | B | H | - |
| 30 | B | H | B | A | B | A |
| 31 | H | B | A | H | A | B |
| 32 | A | A | H | H | H | H |
| 33 | H | B | H | A | 0 | A |
| 34 | A | H | 0 | A | 0 | A |
| 35 | H | 0 | A | H | A | B |

*Contd……*

| Progeny No. | *cr1* | | *cr2* | | *cr3* | |
|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | | | | |
| | **Xtxp176** | **Xiabt312** | **Xtxp297** | **Xiabt73** | **Xiabt275** | **Xiabt241** |
| 36 | H | A | B | A | H | B |
| 37 | A | H | H | A | A | H |
| 38 | B | A | B | H | B | H |
| 39 | A | B | A | B | H | B |
| 40 | H | H | H | H | A | A |
| 41 | H | B | A | B | H | 0 |
| 42 | B | A | H | B | H | B |
| 43 | 0 | A | H | B | A | 0 |
| 44 | H | B | A | B | B | H |
| 45 | A | A | A | A | H | H |
| 46 | A | H | A | H | H | B |
| 47 | A | A | H | H | H | H |
| 48 | B | H | A | 0 | H | B |
| 49 | B | A | 0 | H | H | A |
| 50 | A | H | H | 0 | H | B |
| 51 | B | H | B | A | B | 0 |
| 52 | A | H | A | 0 | H | - |
| 53 | B | 0 | A | 0 | A | B |
| 54 | A | B | H | A | H | B |
| 55 | B | 0 | A | H | H | B |
| 56 | H | 0 | A | H | A | B |
| 57 | H | H | H | H | A | A |
| 58 | H | A | B | H | A | H |
| 59 | B | A | H | B | A | 0 |
| 60 | A | 0 | A | 0 | 0 | H |
| 61 | A | H | H | B | H | 0 |
| 62 | A | A | H | H | H | H |
| 63 | B | H | A | 0 | B | A |
| 64 | A | B | 0 | H | A | 0 |
| 65 | B | 0 | H | 0 | A | 0 |
| 66 | H | - | B | A | H | A |
| 67 | A | B | A | 0 | A | H |
| 68 | H | B | A | 0 | B | H |
| 69 | A | A | A | A | A | A |
| 70 | H | B | H | A | H | B |
| 71 | A | B | 0 | A | A | 0 |
| 72 | A | H | A | B | H | B |
| 73 | A | 0 | H | B | H | A |
| 74 | 0 | H | A | H | A | H |
| 75 | A | A | H | H | A | A |

*Contd……*

| Progeny No. | *cr1* | | *cr2* | | *cr3* | |
|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | | | | |
| | **Xtxp176** | **Xiabt312** | **Xtxp297** | **Xiabt73** | **Xiabt275** | **Xiabt241** |
| 76 | H | 0 | B | H | H | B |
| 77 | B | A | H | B | A | 0 |
| 78 | A | A | H | H | H | H |
| 79 | A | 0 | A | 0 | 0 | H |
| 80 | A | H | H | B | H | 0 |
| 81 | B | H | A | 0 | B | A |
| 82 | A | B | 0 | H | A | 0 |
| 83 | B | 0 | H | 0 | A | 0 |
| 84 | B | B | B | B | B | B |
| 85 | H | - | B | A | H | A |
| 86 | H | H | H | H | A | A |
| 87 | A | B | A | 0 | A | H |
| 88 | H | B | A | 0 | B | H |
| 89 | H | B | H | A | H | B |
| 90 | H | H | A | A | H | H |
| 91 | A | B | 0 | A | A | 0 |
| 92 | H | H | H | H | A | A |
| 93 | A | H | 0 | A | 0 | A |
| 94 | A | A | A | A | A | A |
| 95 | H | 0 | A | H | A | B |
| 96 | H | A | B | A | H | B |
| 97 | A | A | A | A | H | H |
| 98 | B | H | A | H | A | H |
| 99 | A | B | H | A | A | H |
| 100 | 0 | A | H | 0 | A | 0 |
| 101 | H | H | A | A | A | A |
| 102 | A | H | B | H | 0 | H |
| 103 | B | A | A | 0 | H | 0 |
| 104 | H | A | 0 | B | B | A |
| 105 | B | H | A | B | A | 0 |
| 106 | A | A | H | H | H | H |
| 107 | B | B | B | B | B | B |
| 108 | A | B | B | H | A | 0 |
| 109 | H | B | A | B | H | A |
| 110 | A | H | 0 | A | 0 | A |
| 111 | H | 0 | A | H | A | B |
| 112 | H | A | B | A | H | B |
| 113 | A | H | H | A | A | H |
| 114 | B | A | B | H | B | H |
| 115 | A | B | A | B | H | B |

*Contd……*

| Progeny No. | *cr1* | | *cr2* | | *cr3* | |
|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | | | | |
| | **Xtxp176** | **Xiabt312** | **Xtxp297** | **Xiabt73** | **Xiabt275** | **Xiabt241** |
| 116 | 0 | B | A | H | B | 0 |
| 117 | A | B | H | A | A | H |
| 118 | B | A | H | B | B | A |
| 119 | A | 0 | H | - | A | H |
| 120 | B | B | B | B | B | B |
| 121 | H | H | A | A | H | H |
| 122 | H | A | B | A | B | 0 |
| 123 | 0 | A | A | B | H | - |
| 124 | A | B | 0 | A | B | H |
| 125 | B | 0 | B | A | A | B |
| 126 | A | H | A | H | B | A |
| 127 | H | A | H | A | A | 0 |
| 128 | H | 0 | A | 0 | H | A |
| 129 | B | H | A | B | 0 | A |
| 130 | A | A | H | H | A | A |
| 131 | A | 0 | B | A | A | B |
| 132 | 0 | B | A | H | B | 0 |
| 133 | A | B | H | A | A | H |
| 134 | 0 | A | B | H | H | A |
| 135 | A | B | H | B | H | 0 |
| 136 | B | 0 | B | A | B | H |
| 137 | H | H | A | A | H | H |
| 138 | A | H | 0 | A | A | 0 |
| 139 | H | A | H | B | 0 | B |
| 140 | H | B | A | B | H | 0 |
| 141 | B | A | H | B | H | B |
| 142 | 0 | A | H | B | A | 0 |
| 143 | H | B | A | B | B | H |
| 144 | A | H | A | H | H | B |
| 145 | H | H | H | H | A | A |
| 146 | B | H | A | 0 | H | B |
| 147 | B | A | 0 | H | H | A |
| 148 | A | H | H | 0 | H | B |
| 149 | A | A | A | A | H | H |
| 150 | B | H | B | A | B | 0 |
| 151 | H | H | A | A | A | A |
| 152 | A | H | A | 0 | H | - |
| 153 | B | 0 | A | 0 | A | B |
| 154 | A | B | H | A | H | B |
| 155 | B | 0 | A | H | H | B |

*Contd……*

| Progeny No. | *cr1* | | *cr2* | | *cr3* | |
|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | | | | |
| | **Xtxp176** | **Xiabt312** | **Xtxp297** | **Xiabt73** | **Xiabt275** | **Xiabt241** |
| 156 | H | 0 | A | H | A | B |
| 157 | H | A | B | H | A | H |
| 158 | B | A | H | B | A | 0 |
| 159 | A | 0 | A | 0 | 0 | H |
| 160 | H | H | H | H | A | A |
| 161 | A | H | H | B | H | 0 |
| 162 | B | H | A | 0 | B | A |
| 163 | A | A | A | A | A | A |
| 164 | A | B | 0 | H | A | 0 |
| 165 | A | A | H | H | H | H |
| 166 | B | 0 | H | 0 | A | 0 |
| 167 | H | - | B | A | H | A |
| 168 | A | B | A | 0 | A | H |
| 169 | A | A | A | A | H | H |
| 170 | H | B | A | 0 | B | H |
| 171 | H | B | H | A | H | B |
| 172 | A | B | 0 | A | A | 0 |
| 173 | A | A | H | H | H | H |
| 174 | A | H | A | B | H | B |
| 175 | A | 0 | H | B | H | A |
| 176 | 0 | H | A | H | A | H |
| 177 | H | 0 | B | H | H | B |
| 178 | B | 0 | H | A | B | H |
| 179 | H | B | H | 0 | B | A |
| 180 | H | H | A | A | A | A |
| 181 | H | H | H | H | A | A |
| 182 | B | A | B | H | A | H |
| 183 | A | H | A | 0 | 0 | H |
| 184 | B | 0 | 0 | B | B | 0 |
| 185 | H | - | A | B | H | A |
| 186 | B | H | H | B | H | 0 |
| 187 | A | B | B | H | B | A |
| 188 | B | A | 0 | A | 0 | B |
| 189 | A | 0 | H | 0 | A | B |
| 190 | H | A | A | B | H | B |
| 191 | 0 | A | H | - | H | 0 |
| 192 | H | H | A | A | A | A |
| 193 | A | B | H | A | H | B |
| 194 | B | 0 | H | B | A | 0 |
| 195 | H | H | A | A | H | H |

*Contd……*

| Progeny No. | *cr1* | | *cr2* | | *cr3* | |
|---|---|---|---|---|---|---|
| | Flanking marker (foreground selection) | | | | | |
| | **Xtxp176** | **Xiabt312** | **Xtxp297** | **Xiabt73** | **Xiabt275** | **Xiabt241** |
| 196 | A | H | B | 0 | B | H |
| 197 | H | A | A | 0 | H | B |
| 198 | A | H | H | A | H | B |
| 199 | 0 | A | 0 | B | H | A |
| 200 | H | H | H | H | A | A |
| 201 | B | 0 | H | B | H | B |
| 202 | A | A | H | H | A | A |
| 203 | H | B | H | B | B | 0 |
| 204 | A | A | H | H | H | H |
| 205 | B | H | B | A | 0 | H |
| 206 | 0 | A | H | A | B | 0 |
| 207 | H | 0 | A | H | 0 | A |
| 208 | A | A | A | A | H | H |
| 209 | A | B | 0 | A | H | 0 |
| 210 | H | - | B | H | A | B |
| 211 | H | A | A | B | A | H |
| 212 | H | B | 0 | A | A | 0 |
| 213 | B | 0 | A | H | 0 | H |
| 214 | A | 0 | B | A | H | 0 |
| 215 | H | A | H | A | B | A |
| 216 | H | H | H | H | A | A |
| 217 | 0 | B | B | H | A | 0 |
| 218 | H | B | A | B | A | 0 |

**Table 29: Analysis of variance for charcoal rot component traits in $BC_3F_3$ progenies**

| Source | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean sum of squares | | | | | | | | |
| Treatments | 18 | 1093.84** | 3.77** | 801.89** | 1351.79** | 18.56* | 29.7** | 296.34** | 0.61 | 1.46** |
| Replications | 4 | 2.85 | 0.48 | 12.29 | 311.06 | 1.56 | 3.07 | 17.33 | 0.11 | 0.17 |
| Error | 72 | 10.06 | 0.13 | 13.1 | 265.83 | 8.2 | 6.51 | 53.6 | 0.48 | 0.34 |
| SEm+ | | 1.42 | 0.16 | 1.62 | 7.29 | 1.28 | 1.14 | 3.27 | 0.31 | 0.26 |
| CV (%) | | 17.66 | 15.6 | 16.19 | 8.88 | 28.84 | 13.07 | 11.58 | 15.2 | 7.83 |

** - Significant at 1% level of probability * - Significant at 5% level of probability

$X_1$ – Per cent lodging $X_2$ – No. of internodes crossed by the fungus $X_3$ – Length of infection
$X_4$ – Plant height $X_5$ – Panicle exertion $X_6$ – Earhead length
$X_7$ – No. of spicklets per plant $X_8$ – Stem girth $X_9$ – Number of leaves

**Table 30: Genetic variability parameters for charcoal rot component traits in $BC_3F_3$ progenies**

| Parameters | Mean | PCV | GCV | $h^2$ | GA over per cent mean |
|---|---|---|---|---|---|
| Per cent lodging | 17.96 | 83.87 | 81.99 | 95.57 | 42.09 |
| No. of internodes crossed by the fungus | 2.32 | 39.98 | 36.81 | 84.78 | 72.19 |
| Length of infection (cm) | 22.36 | 58.46 | 56.18 | 92.33 | 30.45 |
| Plant height (cm) | 183.69 | 11.96 | 8.02 | 44.97 | 2.35 |
| Panicle exertion (cm) | 4.16 | 77.03 | 34.58 | 20.15 | 17.70 |
| Earhead length (cm) | 19.52 | 17.10 | 11.03 | 41.61 | 7.96 |
| Spicklets per head | 63.23 | 15.98 | 11.02 | 47.52 | 4.88 |
| Stem girth (cm) | 4.57 | 15.60 | 3.51 | 5.05 | 1.91 |
| Number of leaves | 7.45 | 10.09 | 6.36 | 39.75 | 9.45 |

**Table 31: Mean performance of NILs introgressed with different QTLs and parents for attributes of charcoal rot**

| Treatments | Lodging per cent | Number of internodes crossed | Length of infection (cm) |
|---|---|---|---|
| $T_1$ - M35-1 (cr*1*+cr*2*+cr*3*) | 0.9 | 1.26 | 10.64 |
| $T_2$ - SPV86 (cr*1*+cr*2*+cr*3*) | 4.28 | 1.94 | 15.64 |
| $T_3$ - M35-1 (cr*1*+cr*2*) | 9.46 | 1.52 | 11.18 |
| $T_4$ - M35-1 (cr*2*+cr*3*) | 9.36 | 1.46 | 12.46 |
| $T_5$, M35-1 (cr*1*+cr*3*) | 6.82 | 1.38 | 11.3 |
| $T_6$ - SPV86 (cr*1*+cr*2*) | 15.28 | 2.78 | 23.3 |
| $T_7$ - SPV86 (cr*2*+cr*3*) | 14.76 | 3.04 | 25.12 |
| $T_8$ - SPV86 (cr*1*+cr*3*) | 11.54 | 2.78 | 24.22 |
| $T_9$ - M35-1 (cr*1*) | 12.94 | 1.78 | 15.14 |
| $T_{10}$ - M35-1 (cr*2*) | 11.24 | 1.78 | 14.18 |
| $T_{11}$ - M35-1 (cr*3*) | 13.3 | 1.7 | 11.52 |
| $T_{12}$ - SPV86 (cr*1*) | 21.26 | 3.28 | 22.36 |
| $T_{13}$ - SPV86 (cr*2*) | 15.5 | 3.04 | 23.74 |
| $T_{14}$ - SPV86 (cr*3*) | 20.2 | 3.06 | 26.38 |
| $T_{15}$- M35-1 (Null QTL) | 35.22 | 2.28 | 34.16 |
| $T_{16}$ - SPV86 (Null QTL) | 43.08 | 3.52 | 45.92 |
| $T_{17}$ - M35-1 | 41.46 | 2.4 | 40.2 |
| $T_{18}$ - SPV86 | 53.38 | 4.04 | 51.04 |
| $T_{19}$ - E36-1 | 1.2 | 1 | 6.32 |

# 5. DISCUSSION

Abiotic environmental factors are considered as the main source of yield reduction all over world (Ashrad *et al*., 2005). Drought stress is highly heterogenous in time, space, degree of stress, growth stage and time of stress exposure (Gupta and Sheoran, 1983) and is unpredictable. Drought is one of the most common environmental stresses that affects growth and development of plants through alterations in metabolism and gene expression. Breeding for drought tolerance is a major objective in arid and semiarid regions of the world due to inadequate precipitation, shortage of irrigation water and high water demand for crop evapotranspiration in such climates. Drought is the primary abiotic stress causing not only differences between the mean yield and potential yield but also causing variation from year to year, resulting in yield instability. Against this stress, plants adapt themselves by different mechanisms including change in morphological and developmental pattern as well as physiological and biochemical responses (Bohnert *et al*., 1995).

Worldwide, sorghum is the fifth most important grain crop (FAO, 2010). Sorghum is a C4 plant and exhibits tolerance to high temperatures and drought (Dogg *et al*., 1988). Improving sorghum's tolerance to various abiotic and biotic stresses would improve yield and yield stability in drought prone areas and thus increase production efficiency. Three growth stages (GS) have been identified in sorghum that are critical in understanding drought tolerance. GS1, seedling establishment (early vegetative stage), GS2, pre-flowering (panicle differentiation to flowering) and GS3, is post-flowering (grain fill to physiological maturity of grain). Two distinct types of drought stress responses have been identified and described in sorghum and are related to GS2 and GS3. Genotypes that are pre-flowering drought tolerance were necessarily did not show any terminal or post-flowering drought tolerance. The involvement of many genes in controlling complex traits each with very little individual effect is highly influenced by environment. Phenotypic selection for such traits will be generally difficult. Selection based on markers could theoretically facilitate the manipulation of such trait without affecting other important agronomic traits besides evaluating breeding progeny populations for the presence of gene/genes conditioning drought trait in sorghum.

Drought causes harmful physiological or metabolic changes in the plant. It reduces plant vigor, plants so affected are predisposed to attack by nonaggressive pathogens such as *Machrophamina phaseolina*. Drought tolerance in sorghum has been known to be positively associated with charcoal rot tolerance. This disease is influenced by drought stress in post flowering stages of the crop growth and causes furthermore yield losses including fodder quality. Drought stress is the primary factor that predisposes sorghum to charcoal rot. Both charcoal rot resistance and drought tolerance in sorghum are polygenic in nature and understanding of the complex nature of such traits by molecular markers, QTL mapping and introgressing them into elite sorghum cultivars through marker assisted selection leads to development of charcoal rot resistant cultivars.

In sorghum, the best characterized form of tolerance to stress during post-flowering stage of crop growth is the 'stay-green' trait. Stay-green is the ability of plant to withstand premature leaf and plant death, develop grain normally and resist charcoal rot and lodging when exposed to moisture stress during the late stages of grain development (Rosenow, 1984; Rosenow and Clark, 1981; Tenkouano *et al.,* 1993; Walulu *et al.,* 1994). Stay-green in sorghum has been associated with higher yield under water limited conditions. Such genotypes also contain increased levels of basal stem sugars (Duncan, 1984) and cytokinins (McBee, 1984) than senescent genotypes, which may reduce the rate of drought-induced senescence (Thomas and Smart, 1993).

Mapping and identification of genomic regions associated with stay-green and yield would serve as the basis to initiate marker assisted transfer of relevant genomic regions into elite genotypes. Development of a reasonably saturated genetic linkage map is essential with functional markers wherever possible and it would help in identification and understanding of genomic region responsible for a complex quantitative trait. This initial resource greatly form basis for introgression of stable QTLs in to elite sorghum cultivars by marker assisted selection. MAS has several evidence of increased efficiency of breeding efforts (Knapp, 1998; Bouchez *et al.,* 2002). With MAS it is possible for the breeder to conduct many rounds of selections in a year.

Gene introgression with marker technology can integrate into existing plant breeding programmes all over the world to allow researchers to access, transfer and combine genes at faster rate and with a precision not previously possible. In drought tolerance improvement, the identification and characterization of QTLs controlling the adaptive traits for drought tolerance are necessary to understand the control and expression of these complex polygenic traits. Drought traits like yield, yield components and morpho-physiological characters were used to identify QTLs controlling these traits. The QTL information then exploited as background information in transferring target QTLs to a traditional locally adopted variety.

MAS involves the selection of genotypes carrying a desirable gene or gene combination, *via* linked markers. Through MAS, more rapid transfer of traits from exotic donor parents to more elite locally-adapted crop cultivars is possible. Backcrossing is often the chosen method to introduce a new trait into a breeding programme, in particular when the trait of interest comes from a parent that has poor agronomic background. Backcrossing is the fastest way to recover the genome of the agronomically favourable recurrent parent. MAS has the potential to greatly reduce the time required for selecting desirable genotypes with traits of interest (Morris *et al.,* 2003; Joshi and Nayak, 2010).

In the present investigation the two RILs population derived from crosses IS9830 (non-stay green) x E36-1 (stay-green) and N13 (non-stay green) x E36-1 (stay- green) were characterized for the post-flowering drought tolerance and yield related traits. The potential polymorphism for both phenotypic traits and mapping of genic SSR, nuclear SSR, SNPs markers was done and later genotypic data for individual RILs was generated to construct a genetic linkage maps for these populations. A series of phenotypic evaluation of RIPs along with parents at Dharwad and Bheemarayanagudi over three and two years respectively was done. Then QTL mapping was done for post flowering drought, yield and yield related traits and finally stable QTLs listed out. Further, charcoal rot resistant stable QTLs were introgressed into elite locally adopted sorghum cultivars *i.e.*, M35-1 and SPV86 from E36-1 (exotic) and $BC_3F_3$ near isogenic lines were evaluated under field in sick plot by artificial inoculation to check for their field performance in terms of imposing charcoal rot resistance.

## 5.1 Genetic variability and character association studies for post-flowering drought and yield related traits

Drought tolerance in sorghum is polygenic in the nature of inheritance, involving a number of component traits with significant G x E interactions. Understanding the genetic nature of drought resistance, the phenotypic characterization of inbred lines and parents across locations is a prerequisite and such a data provides deeper understanding of the trait. The interaction of environment with genetic factors, whose individual effect leads to the expression of any quantitative trait is difficult to determine. To study genetic nature of any character sufficient variability is the prerequisite (Bello *et al*., 2007).

The phenotypic evaluation for post-flowering drought tolerance, yield and yield related traits was carried out in mapping populations derived from crosses IS9830 x E361 and N13 x E36-1 crosses. Field evaluations were carried out at MARS, Dharwad location for over three years (2007, 2008 and 2009 *rabi* seasons) and at RARS, Bheemarayanagudi for over two years (2008 and 2009 *rabi* seasons). Typically in *rabi* season, crop enters a reducing moisture situation where soil moisture keeps depleting leads to severe stress coincide post-flowering stage (GS2 and GS3). The progenies that carry stay-green trait survive and set reasonably good seeds compared to non stay-green types. Genetic expectations of means and variances of phenotypic data were obtained by pooled analysis within location over the years. In both RIPs, RILs displayed considerable differences in their mean performance with respect to all the 13 traits in RIP1 and 14 traits in RIP2 investigated. This variation is expected as the RILs were derived from contrasting parents for several traits including post-flowering drought resistance, yield and yield related traits.

Wide range of variation among the lines indicates the utility of RILs for further genetic investigation. Pooled analysis has shown that the phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits studied, pointing at their polygenic inheritance and high influence of environment.

In RIP1, high PCV and GCV were noticed for per cent GLA 30 DAF and per cent GLA 45 DAF. Similar results were obtained by Haussmann *et al.* (2002) and Gebre (2009). It was observed that the rate of leaf senescence from per cent GLA 15 DAF to per cent GLA 30 DAF was rapid compared to the rate of leaf senescence from per cent GLA 30 DAF to per cent GLA 45 DAF. Differences in patterns of senescence are common in many species that exhibit stay-green feature, even among genotypes that may retain same level of leaf area at maturity (Thomas and Howarth, 2000). This phenomenon was clearly shown by Borrell *et al.* (2000a) where hybrids involving line KS 19 had delayed onset and reduced rate of senescence, whereas those with B35 had only delayed onset of senescence, though GLA at maturity for both was similar.

The ability of sorghum plant to maintain green leaf area is positively associated with yield. High variability noticed for total green leaf area, extended greenness of leaves is expected to allow continued photosynthesis and better translocation from source to sink, which is likely to result in better grain fill and increased yield. In sorghum similar trend between stay-green and yield was noticed in many studies (Nimbalkar *et al.,* 1988; Khannure, 1993; Biradar *et al.,* 1996; Rao and Patil 1996; Reddy and Rao, 1978; Borrel *et al.,* 2000b; Haussmann *et al.,* 2002; Gebre, 2009).

PCV and GCV were also high for traits such as earhead length, panicle exertion and these observations were also in agreement with the studies of Swarup and Chaugale (1962); Khanure, (1993); Biradar, (1996), Mallinath *et al.,* (2004) and Arunkumar *et al.,* (2004). High PCV and GCV were also noticed for stem girth, spicklets per plant. Similar observations have been made in sorghum by Bereji, (1990); Khanure, (1993); Biradar, (1996); Punnuri, (2004); Reddy, (2006); Rajkumar *et al.* (2007); Mukri *et al.* (2007) and Kachapur and Salimath, (2009). For yield per plant high variability was noticed and these observations were also in agreement with the studies of Prabhakar, (2001); Mallinath *et al.* (2004); Arunkumar *et al.* (2004); Mukri *et al.* (2007); Rajkumar *et al.* (2007) and Kachapur and Salimath, (2009).

Moderate PCV and GCV noticed for traits such as 50 per cent flowering, number of leaves, plant height and 100 grain weight and these observations were in agreement with previous reports of Dhimer and Desai, (1978); Prabhakaran, (2001); Mallinath *et al.* (2004); Arunkumar *et al.* (2004); Mukri *et al.* (2007) and Chavan *et al.* (2010). Low PCV and GCV were reported for per cent GLA 15 DAF and were similar to the observations in the studies of Haussmann *et al.* (2002); Gebre, (2009) who also investigated the same populations as done in the present study.

High variability is necessary for improvement through selection. However, one of the parent, E36-1 has been used both in breeding and mapping efforts for stay-green, striga resistance (Haussmann *et al.,* 2002, 2004) and charcoal rot resistance (Reddy *et al*., 2008; Rajkumar *et al*., 2007; Patil, 2009) and drought tolerance (Yamane *et al*., 2009).

In RIP2, high variability as indicated by higher GCV and PCV values for per cent GLA 45 DAF, was similar to the observations made by Haussmann *et al.* (2002); Sundresha, (2006). High PCV and GCV also evident for this trait such as panicle exertion, yield per plant and several previous workers (Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Mukri *et al.,* 2007; Rajkumar *et al.,* 2007; Kachapur and Salimath, 2009) have also made similar observations for some of these traits in sorghum germplasm,

PCV and GCV were moderate for per cent GLA 30 DAF and these observations were also in agreement with the studies of Haussmann *et al.* (2002) and Sundresha, (2006). Traits such as days to 50 per cent flowering, total green leaf area, earhead length showed moderate PCV and GCV and several workers (Prabhakaran, 2001; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Mukri *et al.,* 2007; Rajkumar *et al.,* 2007; Kachapur and Salimath, 2009 Chavan *et al.,* 2010) have also made similar observations for some of these traits in sorghum germplasm and mapping populations. Moderate PCV and GCV were noticed for traits such as, stem girth, plant height, spicklets per head, 100 grain weight similar to the repors made by Dhimer and Desai, (1978); Mallinath *et al.* (2004); Arunkumar *et al.* (2004); Mukri *et al.* (2007); Chavan *et al.* (2010). Low per cent GCV and PCV were reported for traits such as per cent GLA 15 DAF and CID.

Presence of variability in the population is a prerequisite for selection to be effective, while heritability indicate how well this can be done. Heritability if on the higher side for any traits is indicative of the trait that those traits are controlled by additive genes and it can be assumed that they will remain stable under varied environmental conditions. In the present study, in RIP1, high heritability is evident for traits such as per cent GLA 15 DAF, per cent GLA 30 DAF and per cent GLA 45 DAF. Similar observations have been made in same RIP population in sorghum by, Haussmann *et al.* (2002) and Gebre, (2009). High heritability also noticed for the traits such as days to 50 per cent flowering, panicle exertion, plant height, spicklets per head and 100 grain weight. Similar observations have been made in sorghum by Khanure, (1993); Reddy *et al, (*1996); Prabhakar, (2001); Mallinath *et al.* (2004); Arunkumar *et al.* (2004); Bello *et al.* (2007); Mukri *et al.* (2007) and Chavan *et al.* (2010).

Total green leaf area showed high heritability and this observation was in agreement with the studies of Haussmann *et al.* (2002) and Gebre, (2009). High heritability was also evident for stem girth as previously reported in sorghum by Punnuri, (2004); Reddy, (2006); Rajkumar, *et al.* (2007) and Kachapur and Salimath, (2009). Moderate heritability was noticed for traits such as yield per plant, number of leaves, earhead length and these results are in accordance with earlier studies made by, Naphade, (1973); Dinakar, (1985); Rajkumar *et al.* (2007); Kachapur and Salimath, (2009) and Gebre, (2009).

In RIP2, high percentage of heritability was evident for per cent GLA 30 DAF and per cent GLA 45 DAF, which is in accordance with Haussman *et al.* (2002) and Sundresha, (2006) who also investigated the same stay-green doner sources. Traits such as spicklets per head and yield per plant have shown high heritability, and several workers (Khanure, 1993; Biradar, 1996; Mallinath *et al.,* 2004; Arunkumar *et al.,* 2004; Bello *et al.,* 2007; Mukri, *et al.,* 2007; Rajkumar *et al.,* 2007; Kachapur and Salimath, 2009; Mukri *et al.,* 2007 and Chavan *et al.,* 2010) have also made similar observations for these traits in sorghum germplasm.

Heritability was moderate for per cent GLA 15 DAF, total green leaf area (Sundresha, 2006) and for traits such as earhead length, number of leaves, stem girth, plant height and 100 grain weight, similar to the observations made by Vasudeva Rao, (1973); Janom, (1974); Eckebil *et al.* (1977); Mallinath *et al.* (2004); Rajkumar *et al.* (2007); Kachapur and Salimath, (2009). Low percentage of heritability was evident for CID.

Expected genetic advance over per cent mean is a parameter that considers both variability and heritability and provides a realistic estimate of gain achievable following selection. High heritability coupled with genetic advance indicates that additive gene effects are operating and selection for superior genotype is possible (Arunkumar *et al*., 2004). In the present study, high heritability coupled with high genetic advance over per cent mean was observed for stay-green parameters. In RIP1, high genetic advance over per cent mean for per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area (Haussmann *et al.,* 2002; Gebre, 2009) was noticed. High genetic advance over per cent mean was noticed for, days to 50 per cent flowering, earhead length, panicle exertion, plant height and similar observations have been made in sorghum by Biradar, (1996); Reddy *et al.* (1996) ; Nguyen *et al,* (1998); Mallinath *et al,* (2004); Arunkumar *et al.* (2004); Mukri *et al.* (2007) and Chavan *et al.* (2010).

Stem girth also shown high genetic advance over per cent mean which is also indicated in the studies of Punnuri, (2004); Reddy, (2006); Rajkumar, *et al.* (2007); Kachapur and Salimath, (2009). High genetic advance over per cent mean also evident for spicklets per head, 100 grain weight and yield these observations were also in agreement with the studies of Naphade, (1973); Shinde, (1979); Phul and Allah Rang, (1986); Nguyen *et al.,* (1990); Khanure, (1993); Biradar, (1996); Mukri *et al.* (2007) and Chavan *et al.* (2010).

Moderate genetic advance over per cent mean reported for number of leaves, yield similar observations were made by Mukri *et al.* (2007); Gebre, (2009). Low Genetic advance over per cent mean noticed for per cent GLA 15 DAF and similar observations have been made in same RIL population in sorghum by Gebre, (2009), Haussmann *et al.* (2002).

In RIP2, high genetic advance over per cent mean was evident for per cent GLA 30 DAF and per cent GLA 45 DAF as reported by earlier studies made by Haussmann *et al.,* (2002) and Sundresha, (2006). GA over per cent mean was high for panicle exertion and spicklets per head.

There is ample of evidence with the reports of Khanure (1993); Biradar (1996); Mukri *et al.* (2007) and Chavan *et al.* (2010) a similar observations for some of these traits in sorghum germplasm have been made. CID showed high genetic advance over per cent mean.

Moderate genetic advance over per cent mean exist for traits such as days to 50 per cent flowering, earhead length, 100 grain weight, and yield per plant, similar observations have been made in sorghum by Naphade, (1973); Shinde, (1979); Phul and Allah Rang, (1986); Rajkumar *et al.* (2007) ; Mukri *et al.* (2007); Kachapur and Salimath, (2009) and Chavan *et al.* (2010). Total green leaf area also showed moderate genetic advance over per cent mean and similar observations were also made by Haussmann *et al.,* (2002) and Sundresha, (2006). Low genetic advance over per cent mean was evident for traits such as GLA 15 DAF, number of leaves and plant height. These observations were also in agreement with the studies of Singh and Singh, (1973); Amirthadvarathinam *et al.* (1994); Mallinath *et al.* (2004); Arunkumar *et al.* (2004).

Expected genetic advance is a parameter that considers both variability and heritability and provides a realistic estimate of gain achievable following selection. Presence of variability in the population is a prerequisite for selection to be effective, while heritability indicate how well this can be done. In the present study, high heritability coupled with high genetic advance over per cent mean was observed for post flowering drought parameters *viz*., per cent GLA 15 DAF, per cent GLA 30 DAF and per cent GLA 45 DAF. Selection made through these characters would be effective as they are more likely to be controlled by additive gene effects. This kind of trend for the above traits is already known in sorghum (Haussmann *et al*., 2002; Sundresha, 2006; Gebre, 2009).

Drought is a complex trait and its expression depends on several other component characters. The phenotype of a plant is the result of interaction of a large number of factors. Therefore, it is important to know the extent and nature of interrelationship between grain yield and its contributory characters and also among themselves. Hence, the final yield is sum total of effects of several component factors. Correlation studies provide information on the nature and extent of association between any two pairs of metric characters. Grafius, (1959) opined that there may not be any gene for yield as such but operates only through its components. Correlation studies will surely help to identify the character to make selections for higher yield with a view to determine the extent and nature of relationship among the yield contributing characters. The correlation coefficient helps the breeder in determining the direction and number of characters to be considered in improving the grain yield. In the present investigation, both genotypic and phenotypic correlations were worked out for post flowering drought and yield related traits. The main purpose of correlation in crop plants is the detailed understanding of complex characters, such as yield per plant.

Among the post flowering drought parameters, in both RIPs, per cent GLA 15 DAF is positively correlated with per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, number of leaves, stem girth, plant height, spicklets per plant, 100 grain weight but has found negatively correlating with days to 50 per cent flowering, earhead length, panicle exertion. Similar findings were reported (Haussmann *et al.,* 2002; Sundresha, 2007; Gebre, 2009) in RIPs developed from same crosses and in RILs derived from R16 x B35 cross (Kassuhun *et al.,* 20090)

It was found that, per cent GLA 30 DAF had significant and positive association with number of leaves, stem thickness, total GLA, per cent GLA 45 DAF but had found negatively correlating with days to 50 per cent flowering, panicle exertion and earhead length. A highly significant and positive association was found between per cent GLA 45 DAF and number of leaves per plant; stem thickness, number of spicklets per head, total GLA. Similar findings were reported (Haussmann *et al.,* 2002; Sundresha, 2006; Gebre, 2009) in RIPs developed from same crosses and in RILs derived from R16 x B35 cross (Kassuhun *et al.,* 2009).

Per cent GLA 45 DAF was strongly associated with grain yield per plant. Similarly significant positive association of per cent GLA 30 DAF with yield at both genotypic and phenotypic level was made by Haussmann *et al.* (2002) in two sorghum RIPs. Significant negative association was observed between 50 per cent flowering and GLA30, similar to the observation of Kebede (2001) for this trait.

A negative correlation between days to 50 per cent flowering and post anthesis stay-green feature indicated by GLA15 and GLA45 under drought conditions clearly indicates the importance of GLA as a means to minimize the effects of post flowering moisture stress. The days to 50% flowering showed negative association with grain yield at phenotypic level indicating that high yielding and early maturing genotypes can be developed for *rabi* season.

Negative association was found between plant height and days to 50 per cent flowering at genotypic level. Stem thickness showed positive association with earhead length, number of leaves and leaf area. On the contrary, stem thickness had negative association with plant height at genotypic level. Similar observations were made by Esechia *et al.* (1977). Among the yield components, relationship of plant height was negative with days to 50 per cent flowering.

The earhead length was positively associated with grain yield. Its association with plant height was negative and agreed with the findings of Panchal *et al.* (1979). This means that the *rabi* genotypes with short earhead length prove to be better for high grain yield per plant. It had negative direct effect on grain yield. Similar to these results Patel *et al.* (1980) reported positive indirect influence on grain yield and Pokle *et al.* (1976), Kukadia *et al.* (1980) and Ivanar *et al.* (2001) reported positive direct effect on grain yield.

The 100 grain weight was positively associated with grain yield. This is in line with the observations of earlier workers Rao and Ranchi, (1964), Chavan and Singh (1975), Shrihari and Nagur (1980), Nimbalkar *et al.* (1988), Tourchi and Rizai, (1997) and Basheeruddin, (2003). These results revealed that 100 grain weight has important role in enhancing the grain yield. The positive significant influence on grain yield was exhibited by 100 grain weight at both the phenotypic and genotypic levels. Similar results were reported by Patel *et al.* (1979), Gomez *et al.* (1986). Berenji (1990), Patdukhe *et al.* (1994) and Gomez *et al.* (1986) and Potdukhe *et al.* (1992). Geremew and Gebeyehu (1993) reported positive indirect influence on grain yield.

Grain yield was highly significant and positively correlated with total green leaf area, earhead length, per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF and 100 grain weight and were contributed to grain yield positively and indirectly. Higher yields are due to a greater area of leaves which increases light interception and consequently increases photosynthate production and delayed leaf senescence. Higher photosynthate production and translocation to the grain results in higher yields. Similar observations made by Mahalaxmi and Bidinger (2000); Haussman *et al.* (2002); Sundresha (2006); Gebre (2009) and Kassahum *et al.* (2009).

In RIP2, carbon isotope discrimination was found to be positively correlated with total GLA, per cent GLA 15, per cent GLA 30, per cent GLA 45 DAF and spicklets per plant. A similar observation was made by Tulet *et al.* (2002) but they reported negative association with stem girth, panicle exertion, days to 50 per cent flowering, earhead length, grain yields and were affected by the stress in the latter flowering genotypes and as a result, there was less translocation from the leaves, which lead to difference among the genotypes in the escape from stress. Due to this differences in grain number/grain growth under grain filling drought stress resulting into negative correlation between yield and days to 50 per cent flowering.

Highly significant positive associations were observed between yield and stay-green at different stages in both RIPs. Among yield related traits, yield shown positive correlation with CID, number of leaves, stem girth, total grain leaf area, spicklets per head but had negative association with days to 50 per cent flowering, panicle exertion and plant height, which may be obvious when the plant possess more than expected height, the food allocation from source to sink of different part will be more. Less amount of processed food reaches to seed development and result in yield reduction.

## 5.2 Molecular mapping and construction of genetic linkage map

Molecular genetic markers represent one of the most powerful tools for the analysis of genomes and the association of heritable traits with underlying genetic variation. A variety of DNA markers such as nuclear and genic SSRs have been used as genetic markers for mapping. EST-SSR, unlike the genomic markers, shows the position of functional part of the genome in the relation to others.

Though these markers are expensive to develop, they are serving as useful additional markers in mapping and MAS to the marked regions of the genome. EST based SSRs can be developed directly from existing sequence resources and can often be transferred from one species to another, EST data bases are an attractive source of measures for the genetic analysis to understand the complex nature of the trait (Ellis and Burue, 2007; Chabane *et al.,* 2005). These will also provide opportunities to understand the genetic mechanisms underlying different stress responses (Jaques and Edawrds, 2009).

Rapid advances in molecular biology have helped in the development of saturated molecular maps in several crops. A good, permanent mapping population such as a set of RILs can be genotyped with respect to any available marker, their position genetically mapped and updated with more markers as they become available. Most of SSR markers available at present in sorghum have been mapped previously. Selection of some of SSR markers in the present study was based on the linkage map published by Bhattramakki *et al*., (2000), Taramino *et al.* (1997) and Haussmann *et al.* (2004) for sorghum. Further, through in-house efforts, several genic SSRs were identified in EST database of sorghum and some of them were also used in the present study.

### 5.2.1 Parental polymorphism

A total of 530 EST-SSR, 270 genome SSR and 130 SNP markers used to identify the potential polymorphism of which 40 EST-SSR (7.55 %) and 71 nuclear SSR (26.30 %) markers have shown polymorphism and are genotyped across RILs of RIP1. In RIP2, 68 EST-SSR (12.83 %) and 70 nuclear SSRs (25.93 %) markers showed polymorphism and are genotyped across the RILs. A set of 23 and 22 SNP polymorphic markers and their genotyped data from another study in laboratory was additionally adopted to facilitate linkage map construction and QTL mapping. The total polymorphic markers detected in RIP1 (33.85 %) were less as compared to RIP2 (38.76%). This is because i) the genetic distance between parents of RIP1 (IS9830 x E36-1) is less and ii) EST-SSR primers have been reported to be less polymorphic compared with genomic SSR in crop plants because of greater DNA sequence conservation in transcribed region (Cho *et al.,* 2000; Scott *et al.,* 2000; Eujayl *et al*., 2001; Rungis *et al*., 2004; Rugis *et al.,* 2004).

### 5.2.2 Construction of genetic linkage maps

Genetic linkage maps are fundamental for localization of genes responsible for biotic and abiotic stresses. Development of highly saturated linkage map is essential to accurately map both agronomically important major genes and QTL in any crop species and their efficient use in marker assisted breeding. In sorghum, several linkage maps have been developed (Subudhi and Nguyen, 2000). Hulbert *et al.* (1990) developed the first sorghum genome map using DNA probes that were previously mapped in the maize genome. Pereira *et al.* (1994) developed a sorghum linkage map with 10 complete linkage groups using maize and sorghum probes.

Tao *et al.* (1993) constructed sorghum genetic linkage map by utilizing a variety of probes, including sorghum, maize and sugarcane genomic DNA, maize and sugarcane cDNA, cereal anchor probes and eight SSR loci. One of the most complete sorghum genetic maps available today was published by Menz *et al.* (2002), who constructed a 1713 cM high-density map using 2454 AFLPs, 136 SSRs previously mapped in sorghum, and 203 cDNAs and genomic clones from rice, barley, oat, and maize. Nagaraj *et al.* (2005) mapped 60 SSR markers on sorghum map and also Mace *et al.* (2009) and Duan *et al.* (2009) mapped sorghum linkage map using 122 MSAP and 1190 DaRT, 839 SSR markers respectively. Patil (2009) constructed a genetic linkage map spanning 2905 cM, which is highest covered map of sorghum to date, wherein, a total of 141 polymorphic DNA markers (48 SSRs, 65 EST-SSRs, 28 RAPD) were genotyped in RILs derived from cross IS22380 x E36-1.

The present maps were constructed using genic, non-genic SSRs and SNP markers in the order as in the maps could by Haussman *et al.* (2002a) and Bhatramakki *et al*., 2000. Comparison of different sorghum genetic linkage maps developed in different crosses with QTL related to stay-green is presented in Table 32.

**Table 32: Relative comparison of various genetic linkage maps with their stay-green QTLs in sorghum**

| Sl. No. | Reference | Year | Population | LG1 | LG 2 | LG 3 | LG 4 | LG 5 | LG 6 | LG 7 | LG 8 | LG 9 | LG 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Haussman *et al.* | 2002 | IS9830 x E36-1 | A (*stg2*, *stg3*) | B | C(*stg1*,*stg2*) | D(*stg1*, *stg2*,*stg3*) | E (*stg1*) | F | G(*stg1*, *stg2*,*stg3*) | H(*stg2*, *stg3*) | I | J |
| | | | N13 x E36-1 | A(*stg1*, *stg2*,*stg3*) | B (*stg1*, *stg2*,*stg3*) | C | D | E(*stg1*, *stg2*,*stg3*) | F | G (*stg2*, *stg3*) | H | I | J |
| 2 | Harrys *et al.* | 2007 | BT642 x RT700 | A | B(*stg3*) | C(*stg1*,*stg2*) | D | E (*stg4*) | F | G | H | I | J |
| 3 | Xu *et al.* | 2000 | B35 x T700 | G | D (*stg3*) | A(*stg1*,*stg2*) | C | E | I | B | H | F | J(*stg4*) |
| 4 | Crasta *et al.* | 2000 | B35 x T430 | G | D(*stg3*) | A(*stg1*,*stg2*) | C | E | I | B | H | F | J(*stg4*) |
| 5 | Tao *et al.* | 1997 | QL93 x QL 41 | C | B | A | F | J | G+ | I+ | E | D | H |
| 6 | Tuinstra *et al.* | 1997 | B35 x T7078 | F | B(*stg3*) | G(*stg1*,st2) | N | D | K | E | | | H |
| 7 | Srinivas *et al.* | 2008 | 296B x IS18551 | A | B(*stg3*) | C(*stg1*,*stg2*) | D | E(*stg4*) | F | G | H | I | J |
| 8 | Menz *et al.* | 2002 | | A | B(*stg3*) | C(*stg1*,*stg2*) | D | E(*stg4*) | F | G | H | I | J |
| 9 | Bhattarmak *et al.* | 2002 | BT623 x IS3620C | A | B(*stg3*) | C(*stg1*,*stg2*) | D | E(*stg4*) | F | G | H | I | J |
| 10 | Sanchcz *et al.* | 2002 | B35 x T7000 | G | D(*stg3*) | A(*stg1*,*stg2*) | C | E | I | B | H | F | J(*stg4*) |
| 11 | Present study | 2011 | IS9830 x E36-1 | A | B | C(*stg1*) | D(*stg1*,*stg2*) | E(*stg2*,*stg3*) | F | G | H | I | J(*stg3*) |
| | | | N13 x E36-1 | A(*stg1*) | B(*stg2*) | C(*stg1*) | D(*stg1*) | E(*stg2*) | F | G(*stg3*) | H | I | J(*stg3*) |

*Stg1* – Per cent GLA 15 DAF *Stg2* – Per cent GLA 30 DAF *Stg3* – Per cent GLA 45 DAF

Present study revealed that, in both the RIPs, the markers on LG-A ( Xtxp248, Xtxp340, Xtxp61, Xtxp208), on LG-B (Xtxp50 and Xtxp96), on LG-C (Xumc07 and Xtxp33), on LG-D (Xtxp41, Xtxp327, Xtxp27), on LG-E (Xumc085, Xtxp 312, Xtxp295, Xtxp168), on LG-F (Xtxp10 and Xtxp67) on LG-G (Xtxp217, Xtxp141), on LG-H (Xtxp273, Xtxp47), on LG-I (Xtxp145 and Xtxp6) and on LG-J (Xtxp23 and Xtxp65) were located on corresponding linkage groups in the order as that of Haussman *et al.* (2002); Bhatramakki *et al.* (2000); Sundresha (2007) and Gebre (2009) with comparable deviations in distances.

On LG A, the distance between Xtxp340 to Xtxp37 was 22.7 cM wherein it was 53.9 cM in Haussman *et al*., 2002. And the distance between Xtxp50 and Xtxp96 was 10.4 cM in contrast to 33.4 cM in Haussman *et al.* (2002). On LG D, distance between Xtxp327 and Xtxp27 was 24.8 but it was 38.4 in Haussman *et al.* (2002). The markers Xtxp23 and Xtxp65 found on LG J were 23.7 cM apart in present study compared to 87.9 cM reported in Haussman *et al.* (2002). This clearly indicates better saturation of maps constructed in the study as distance between the markers was comparatively less.

In both the populations, 45 anchor marker data, kindly provided by Dr. C.T. Hash, ICRISAT were included along with genotyped markers. Some of EST-SSR markers represented in present study have been mapped previously in these RILs, 20 EST-SSR markers genotyped data is included from earlier study done by Gebre, (2009) in RIP1 and 26 EST-SSR marker data by Sundresha, (2006) in RIP2. The length of maps were 1558.1 cM in RIP I and 1602 cM in RIP 2 with larger gaps between the markers. In order to saturate the map we have used some more EST SSR, nuclear SSRs and SNP marker to genotype across these RIL populations. Finally, a total of 134 markers in RIP1 and 160 markers in RIP2 were mapped, constructed the genetic linkage map and QTL analysis was done. A linkage distance of 20 cM was considered as limit of resolution. The reason to select markers with linkage distance of about 20 cM as interval was that a QTL in such a segment might contain many loci affecting due trait loci necessarily in the same direction. Thus, more QTLs are likely to be detected when the alleles are in association than when they use in dispersion (Tanskley, 1993).

In RIP1, a total of 134 markers were used for linkage map construction with the help of MAPMAKER/EXP (3.0b) software. And all 134 markers mapped on to the ten linkage group *viz.,* A, B, C, D, E, F, G, H, I and J. The genetic map had a total length of 1661.1 cM distributed over ten linkage groups. The number of markers assigned to different linkage groups was 15, 23, 16, 17, 15, 6, 16, 7, 8 and 11. The highest distance observed was 47.9 cM which is between Xiabt82 – Xtxp73 on LG-B, the lowest distance was 2 cM on LG-E flanked between Xisp362-Xumc085 markers. The number of markers mapped per linkage group ranges from 6 in LG-F to 23 in LG-B with average distance covered was 12.39 cM.

In RIP2, a total of 160 markers were used for linkage map construction with help of MAPMAKER/EXP (3.0b) software. All the markers were mapped on to the ten linkage groups, genetic map had a total length of 2003.8 cM distributed over ten linkage groups. The number of markers assigned to different linkage group was 24, 30, 17, 17, 18, 8, 15, 8, 15 and 8. The highest and lowest distances observed were between 77.6 cM on LG-D is flanked by Xsnp83 – Xiabt353 and 1.7 cM on LG-A flanked by Xtxp248 – Xtxp340 markers respectively. Markers mapped per linkage group varied from 8 in LG-F, LG-H, LG-J to 30 in LG-B, with average distance covered was 12.52 cM.

Linkage maps are available in sorghum for striga resistance (Haussmann *et al.,* 2004), charcoal rot resistance (Rajkumar *et al*., 2007 and Patil, 2009), stay-green (Bhatramakhi *et al.,* 2000; Haussmann *et al.,* 2002 and Harris *et al.,* 2007). Using different mapping populations, a number of genomic regions associated with the stay-green component of post flowering drought tolerance were mapped (Tuinstra *et al.,* 1996, 1997a, 1998; Crasta *et al*., 1994; Tao *et al.* 2000; Xu *et al*., 2006; Subhudi *et al.,* 2000; Kebedu *et al.,* 2001; Sanchez *et al.,* 2002; Haussmann *et al*., 2002b and Harries *et al.,* 2007).

### 5.2.3 QTL analysis and mapping

Mapping QTLs for agronomcally important characters is to identify genomic regions with significant effects, positive or negative in the progeny of a given cross. To locate a QTL between flanking markers, analysis of co-segregation of marker and phenotypic information of traits is important and essentially measured.

If the proportion of recombinants with higher or/and lower phenotypic values is less, tight linkage between flanking markers and QTL is indicated in one approach.

It is possible to gather desired QTLs into a genotype by marker assisted selection. QTL mapping is likely to provide information for better and efficient selection strategies for any trait or traits in question, be it a complex trait. QTL analysis is a molecular tool available to dissect complex quantitative characters, governed by many genes with small individual effects, into recognizable genetic units or blows. Xu *et al.* (2000) reported that the extreme phenotypes of quantitative traits comes from the association of favorable or unfavourable QTL alleles, while a preponderance of intermediate phenotype usually indicate allelic dispersion. The genetic stocks with dispersed QTL alleles usually show similar phenotype, making it difficult to identify genetic differences only by phenotypic evaluation. The knowledge of QTL allele status in breeding population and the genome marker associated with them can greatly increase the efficiency of selection by picking individuals by their markers status. MAS is a powerful tool applicable to any simple or complex character such as resistance to disease, abiotic stresses, yield *etc*.

Linkage map thus constructed in the present study for the 226 RILs each of 2 RIL populations of crosses IS9830 x E36-1 and N13 x E36-1 were used for QTL mapping. QTL analysis and mapping was carried out for a set of 13 and 14 traits respectively in RIP1 and RIP2 which include complex traits of post-flowering drought and water use efficiency. The software QTL cartographer (2.5 b) was used for QTL analysis by composite interval mapping (CIM) procedure implemented with a LOD score of 2.0 as threshold value for QTL significance. The additive effect and the estimates of the phenotypic variation were computed.

A common feature of QTL analysis is that QTL effects depend on environment. The QTLs detected in one environment but not in other environments are considered as location specific QTLs. Identification of QTLs that occurs consistently in most of the environments would be important for practical breeding applications. Hence, stability of the QTLs is an important consideration for MAS. In the present study, the QTLs commonly detected at Dharwad for the three years (2007, 2008, 2009) and Bheemarayanagudi for two years (2008 and 2009) were considered as stable. Further co-localization of QTLs between RIP1 and RIP2 were also included.

In RIP1, three QTLs were found common over season and across locations for per cent GLA 15 DAF. These QTLs were flanked by Xtxp34-Xtxp285 (LG-A), Xtxp38-Xsnp19 (LG-C) and Xiabt227-Xsnp56 (LG-D) with LOD value of 2.24 – 2.85, 2.51 – 2.73 and 2.70 – 3.13, per cent phenotypic variation of 9.05 - 11.04, 7.56 - 8.38 and 7.49 – 9.39, respectively. In RIP2, three QTLs found common over seasons and across locations for per cent GLA 15 DAF, spanned by Xtxp34-Xtxp285 (LG-A), Xtxp38-Xsnp19 (LG-C) and Xiabt227-Xsnp56 (LG-D). The QTLs with higher phenotypic varience for per cent GLA 15 DAF conferring allele came from E36-1. Similar results were reported by Haussman *et al.* (2002) and Gebre (2009) in RIL derived from the cross with change in marker type and linkage map. Several other workers (Crasta *et al*., 1999; Xu *et al*., 2000; Subudhi *et al*., 2000; Harris *et al.,* 2007) in populations developed from different crosses also have reported QTL for stay-green trait at per cent GLA 50 DAF.

In RIP1, for per cent GLA 30 DAF two QTLs were detected as common over season and across locations which were bracketed by Xtxp114-Xumc7 on LG-C and Xtxp205-Xtxp231 on LG-E. For per cent GLA 45 DAF, two QTLs on LG-J (Xtxp298-Xtxp324) and on LG-E (Xtxp295-Xtxp168) were detected and were common over season and across locations. An increased per cent GLA conferring allele was contributed by the parent E36-1. On the other hand, in RIP2, QTLs bracketed by Xtxp205-Xtxp231 (LG-E) and Xtxp8-Xtxp1 (LG-B) were found common over season and across locations for per cent GLA 30 DAF and QTLs from LG-J (Xtxp298-Xtxp324), LG-G (Xiabt341-Xiabt178) were found common over seasons and across locations for per cent GLA 45 DAF, the QTLs shown positive effect meaning increased per cent GLA conferring allele from E36-1. Similar results were reported by Haussman *et al.* (2002); Sundresha (2006) and Gebre (2009) in RIL derived from same cross based on field evaluation at Dharwad location. Several other workers reported similar results in populations developed from different crosses of sorghum and evaluated for agronomically important and drought related traits (Crasta *et al*., 1999; Xu *et al*., 2000; Subudhi *et al*., 2000; Harris *et al.,* 2007).

Two QTLs were found common over seasons and across locations for days to 50 per cent flowering, found on LG-E and on LG-B with per cent phenotypic variation of 12.84 – 14.06 and 5.28 - 8.83 respectively. QTLs showed negative effect conferring favorable allele from IS9830 parent. In RIP2, two QTLs were found common across seasons and across locations and were located on LG-E (Xiabt511-Xtxp231) and LG-B (Xumc136-Xsnp28) with LOD score of 2.85 – 3.39 and 2.13 – 3.12, per cent phenotypic variation of 14.29 – 14.87 and 6.23 – 7.19. The QTLs showed negative effect conferring favorable allele from N13 parent. Similar studies made by Haussman *et al*. (2002) ; Sundresha, (2006) and Gebre (2009) in populations derived from same crosses also reported concurrent observations as that of present studies.

Three QTLs were found common over seasons and across locations for total green leaf area in RIP1. QTLs were bracketed by Xtxp331-Xiabt445 (LG-B), Xiabt370-Xtxp71 (LG-C) and on Xtxp340-Xtxp61 (LG-A) with positive additive effect *i.e.,* increased total green leaf area conferring allele came from E36-1 parent. In RIP2, QTLs flanked by Xiabt80-Xtxp73 (LG-B), Xiabt370-Xtxp71 (LG-C) and Xtxp340-Xtxp61 (LG-A) with LOD score of 2.47 – 2.69, 2.87 – 3.12 and 2.53 – 3.22, phenotypic variation of 13.27 – 13.88, 6.38 – 9.45 and 9.34 – 11.37 per cent respectively were noticed. For total green leaf area, which measures photosynthetic capacity at flowering stage, four QTLs were detected. Among these, QTLs on LG-A, LG-C, LG-D and SBI-05, corresponds to the major stay-green QTL identified from stay-green donor B35 *viz., StgG*, *Stg2*, and *Stg4*, respectively (Crasta *et al.* 1999; Xu *et al.* 2000, Subudhi *et al.* 2000).

A new additive QTL, *QGlaa-sbi04*, on SBI-04 was identified by Srinivas *et al*., 2009. Out of three QTLs identified for green leaf area, only one QTL on SBI-01 was common with the QTL identified for green leaf area at flowering, indicating the involvement of different genes responsible for the trait at these stages. Similarly, the two QTLs were mapped for Green Leaf Area at Maturity (GLAM) in the study at similar positions as the stay-green QTL *Stg2* by Xu *et al.* (2000); and the stay-green QTL *StgI.1* and *QLsn.txs-F* reported by Crasta *et al.* (1999) and Feltus *et al.* (2006) respectively. These results demonstrate that the stay-green QTLs *StgG*, *Stg2*, *Stg4*, *StgI.1* and *QLsn.txs-F* are common stay-green trait QTLs in sorghum. The QTL results for the stay-green components (GLAA and GLAM) suggests that though they are inherited independently, there exist some common genetic mechanism regulating these traits (such as at the *Stg2* QTL).

An independent inheritance of the stay-green trait components GLAA and GLAM had also been suggested by Van Oosterom *et al.* (1996) and Haussmann *et al.* (2002). The results also suggest that probability of presence of different alleles of the genes regulating the trait at the identified QTL regions in the 296B since they explained a minor portion of phenotypic variance. This is in contrast to the QTL alleles identified using high stay-green sources such as B35 which explained a major phenotypic variance (Crasta *et al.* 1999; Xu *et al.* 2000; Subudhi *et al.* 2000). Transferring these QTL alleles from the high stay-green donor to high yielding genetic backgrounds through MAS may improve the level of trait expression thereby increasing their agronomic performance under terminal drought stress conditions.

For earhead length, in RIP1 two QTLs flanked by Xtxp40-Xtxp307 (LG-A) and Xisp229-Xsnp36 (LG-D) were found common over seasons and across locations with per cent phenotypic variation of 22.47 – 22.98 and 10.35 – 12.65 respectively. In RIP2, QTLs from LG-D flanked by Xsnp56-Xiabt79 and Xisp229-Xsnp36 were found common across seasons and locations with positive additive effect conferring allele came from E36-1. A significant QTL, *QPle-sbi06-2*, for earhead length explaining 50.4% of phenotypic variation was mapped on SBI-06 from IS18551 (tall parent with short panicle). At the corresponding location on the linkage group LG-B, a QTL for earhead length was also identified (Rami *et al.,* 1998) which explained only 10–15% of phenotypic variation. Further, the QTLs on LG-B and LG-G for the trait correspond to the QTLs on LG-F and LG-A (Rami *et al.,* 1998), respectively.

Similarly, the QTL on SBI-07 also shared similar position with the QTL on LG-E by Hart *et al.* (2001). The *QPlesbi07* QTLs was mapped for earhead length on LG-G. Similar results were also observed for QTL between panicle length and plant height (Hart *et al.,* 2001) and Brown *et al.* (2006). For earhead length, QTLs detected in LG-A and LG-B in the study (Maradiaga, 2003) in sorghum is in accordance with present study.

In RIP1, two QTLs were common over seasons and across locations for number of leaves. The QTLs flanked by Xiabt113-Xiabt147 (LG-G) and Xtxp145-Xtxp6 (LG-I) with LOD score of 3.19 – 3.55 and 2.42 – 3.12, phenotypic variation of 11.83 – 13.32 and 10.18 – 12.41 per cent with positive additive effect. Number of leaves produced per plant is an important component of forage yield. Srinivas *et al*., (2009) identified four QTLs located on three linkage groups including two on LG-D, one each on LG-C and LG-G for total number of leaves. And in RIP2, QTLs from LG-I spanned by Xtxp6-Xiabt438 and Xtxp145-Xtxp6 on LG-I were found common across locations and across seasons for number of leaves.

QTLs from LG-C (Xtxp71-Xtxp160) and LG-H (Xtxp47-Xtxp18) were found common across locations and over seasons for panicle exertion in RIP1, QTL found on LG C showed negative effect allele from IS9830 while QTL from LG H showed positive additive effect allele from E36-1. In RIP2, QTLs were found on LG-F (Xtxp67-o430) and LG-H (Xtxp47-Xtxp18) with LOD score of 2.47 – 2.69 and 2.46 – 3.78, per cent phenotypic variation of 19.43 – 19.65 and 8.55 – 10.67. For panicle exertion, Klein *et al.* (2001) reported six genomic regions responsible for panicle exertion in a population derived from RT × 430 and Sureño. Sanchez-Gomez (2002) also has identified two QTL responsible for panicle exertion in sorghum, but such QTLs are not located in the same genomic regions responsible for variation in this RIL population. For panicle exertion, QTL detected in LG-B and LG-G in the study made (Maradiaga, 2003) in sorghum.

In RIP1, two QTLs bracketed by Xiabt176-Xiabt113 (LG-G) and Xtxp201-Xumc136 (LG-B) were found common over seasons and locations for stem girth with per cent phenotypic variation of 13.27 – 13.49 and 8.53 – 11.47 and LOD value of 3.72 – 3.94 and 2.74 – 3.42, respectively. In RIP2, QTLs found on LG-G flanked by Xiabt178-Xiabt302 and LG-B flanked by Xtxp201-Xumc136 were found common across seasons and locations for stem girth. QTLs showed positive additive effect, indicating that increased stem girth trait came from E36-1 parent.

For plant height, in RIP1, two QTLs on LG-G flanked by Xtxp88-Xiabt303 and Xtxp61-Xtxp37 were detected common across seasons and locations with LOD value of 2.01 – 2.54 and 2.58 – 3.09, per cent phenotypic variation of 10.47 – 12.69 and 11.52 - 13.11 respectively. In RIP2, by Xumc136-Xsnp28 on LG-B and Xtxp61-Xtxp37 on LG-A , with negative effect in both populations, indicating that increased plant height is due to N13 or IS9830 parent. In sorghum, four genes regulating plant height (*Dw1-Dw4*) have been identified (Quinby and Karper 1954). Pereira and Lee (1995) mapped four QTLs for plant height and based on their co-localization, pleiotrophy with other QTLs for morphology and yield components, they related their QTLs with dwarfing (*Dw)* genes. They inferred *Dw2*, *Dw3*, and *Dw4* genes with QTLs on their linkage groups LG-H, LG-A and LG-E, respectively.

A major QTL, *QPhe-sbi07-2*, explaining 24.2% of phenotypic variation identified by Srinivas *et al*., (2009) on SBI-07 corresponds to the genomic region where QTL conditioning *Dw3* gene was mapped on the linkage group by Brown *et al.* (2006). The QTL corresponding to the *Dw3* gene was also reported for plant height on LGs, LG-A (Rami *et al.,* 1998) and on LG-E by Klein *et al.* (2001) and Hart *et al.* (2001), respectively. The QTL, *QPhe-sbi06-1*, explaining 21.6% of phenotypic variance identified on LG-E in the Srinivas *et al*., (2009) studied population corresponds to the position of QTLs reported for plant height on LG-F (Brown *et al.,* 2006), and LG-I (Hart *et al.,* 2001). This QTL corresponds to the *Dw2* gene conditioning plant height QTL on LG-D (Lin *et al.,* 1995).

Plant height QTL on LG-F and LG-G observed in this study also supported the results of Hart *et al.* (2001) and Feltus *et al.* (2006) suggesting that these regions play an important role in the control of plant height variation in sorghum. For plant height QTL detected in LG-A and LG-B in the study conducted by Maradiaga, 2003 in sorghum are consistent with those reported (Pereira and Lee, 1995; Tuinstra *et al.,* 1996; Rami *et al.,* 1998 and Hart *et al.,* 2001) on LG-A for plant height.

Two QTLs in RIP1 found common over seasons and across locations for spicklets per head, flanked by Xtxp297-Xtxp197 (LG-B) and Xtxp47-Xtxp18 (LG-H) and QTLs bracketed by Xtxp208-Xiabt263 on LG-A and Xtxp47-Xtxp18 on LG-H found common over seasons and locations in RIP2 with positive additive effect conferring favorable allele from E36-1.

In RIP1, QTLs bracketed by Xtxp32-Xisp229 (LG-A) and Xtxp327-Xisp229 (LG-D) were found common over seasons and across locations for 100 grain weight and three QTLs for grain yield per plant and were flanked by Xiabt364-Xiabt146 (LG-J), Xtxp145-Xtxp6 (LG-I) and Xsnp36-y27 (LG-D) and in RIP2, QTLs found on LG-E (Xtxp312-Xiabt377), LG-I (Xtxp145-Xtxp6) and LG-D (Xsnp36-Xtxp27). Positive additive effect conferring alleles for increased 100 grain weight and yield per plant came from E36-1 parent. Srinivas *et al*., (2009) identified four QTLs for yield, two QTLs on LG-F, one each on LG-B and LG-G. Rami *et al.* (1998) reported a major grain yield QTL in LG-A. Tuinstra *et al.* (1996) reported one pre-flowering drought and full irrigation yield QTL in LG-G an LG-C respectively.

QTL network (2.0) was run to study interactions among the QTLs, but it was found that there were no interaction among the QTLs detected in present study.

## 5.3 Marker assisted introgression and field evaluation of introgressed recurrent progenies

Modern plant breeding may be different as systematic crop improvement by genetic change of specific and targeted regions of the genome. The use of DNA based markers for the genetic analysis and manipulation of important agronomic traits has become an increasingly useful tool in plant breeding. One of the greatest potential of molecular markers is to accurate the rate of gain from selection of desirable genotypes and in the manipulation of quantitative trait loci (QTL) that condition complex economic traits. MAS have the potential to greatly improve efficiency of selection of quantitative traits. MAS is applied when an economically important portion of variation for an agronomically important trait that is difficult to assess is tightly linked to Mendelian traits that can be easily scored. Through MAS, more rapid transfer of traits from exotic donor parents to more elite locally-adopted crop cultivars is possible. Use of molecular markers can thus offer the potential at achieving breeding goals more precisely than through direct phenotypic selection.

Improving drought tolerance has been considered as plant breeding targets of utmost importance to minimize yield losses resulting from moisture stress in sorghum. Delayed leaf senescence (stay-green) in sorghum has been associated with improved grain yields, particularly in environments in which available water during grain filling is not adequate (Borell *et al.,* 1999, 2000a, 2000b). This drastically reduces the effectiveness of conventional empirical selection for improved drought tolerance. MAS has the potential to greatly improve the efficiency of selection traits. Since, the components of charcoal rot resistance mostly quantitatively measured, it is important to analyze QTL from the point of view of breeding.

In an earlier study of our laboratory, the stable QTL for charcoal rot resistance were mapped from IS22380 x E-36-1 derived RILs. Marker assisted introgression of the favourable alleles for three QTLs from E36-1 parents into the genetic background of recurrent parent, M35-1 and SPV 86 was initiated. Both SPV 86 and M35-1 are highly susceptible and widely cultivated cultivars known for their agronomical eliteness and good grain quality.

Three QTLs of compound traits of charcoal rot resistance with major effect, confirmed stably across multiple environments were chosen for MAS. Targeted QTLs were one each for lodging per cent (*cr*1), number of internodes crossed by the fungus (*cr*2) and length of infection (*cr*3). These three QTLs collectively accounted for 43 per cent of phenotypic variation, where selection n marker assisted backcross breeding progeny of minimized linkage drag was done to minimize. Next, among these selections of progeny with background markers this would help accelerating the recovery of the recurrent parent genome (Hospital and Charcosset, 1997). Screening was ensured using SSR and EST-SSR markers flanking the target QTL regions, wherein the alleles for donor parent in both crosses were retained in heterozygous state, similar approach followed by Punna Ramu (2005) and Kassahun *et al.* (2009).

During initial backcrossing stages ($BC_1F_1$ and $BC_2F_1$) foreground and limited background selection of the marker for all the three QTL region was done at high frequency such that they can be selected in following backcrossing generation and the same could be fixed through one selfing generation. A similar study done in maize by Bouchez *et al.* (2002) for earliness and grain yield was of similar type and thus justified. QTL transferring for stay-green trait from donor B35 to recurrent parent R16 by Punna Ramu (2005) was also target a similar attempt in breeding.

### 5.3.1 Foreground and limited background selection in $BC_3F_1$

Present study started with screening $BC_3F_1$ plants. A total of 123 M35-1 derived and 115 SPV 86 derived $BC_3F_1$ progenies were screened for the six foreground polymorphic markers and simultaneously second level of selection was performed to avoid the other genome regions from donor parent from QTL carrying linkage group B and I, *i.e.* limited background selection to take care of linkage drag around target QTL position. Progenies which showed heterozygous for all the polymorphic markers flanking the three QTL region and double homozygote for markers flanking outside each targeted QTL region were identified as backcross progenies having the recurrent parent allele at both flanking markers. Such progenies were selected and selfed to get $BC_3F_2$ seeds in M35-1 and SPV86 backgrounds.

$BC_3F_2$ progenies of both crosses were screened for foreground and whole genome background selection and were selfed to get $BC_3F_3$ seeds. Whole genome background selection was also done to check for percentage of recurrent parent genome recovered in backcrossed progenies. Progenies showing highest percentage of recurrent parent genome recovery were selected for further field evaluation in charcoal rot sick plot. Similar studies were carried out by Hash *et al*., 2003; Lecomte *et al.,* 2004, where they have introgressed ruminant nutritional quality and fruit quality traits through MAS in sorghum.

A set of 34 RTX7000 NILs were developed by crossing BTX642 with the senescent genotype RTX7000 followed by subsequent introgression of one or more of the BTX642 stay-green QTL regions into the RTX7000 background by Harris *et al.,* 2007. NILs containing *Stg1, 2, 3*, and 4 were identified and found to have enhanced stay-green related phenotypes relative to RTX7000, Selection was continued until the $BC_4$ or $BC_6$ generation where the lines were selfed to create $BC_4F_{2-4}$ or $BC_6F_{2-4}$ lines. Serraj *et al.* (2005) developed near isogenic lines of more drought tolerance in sorghum. $BC_4F_3$ progenies from selected $BC_4F_2$ plants homozygous for various QTLs combination were evaluated under a range of terminal stress environments as done in present study for charcoal rot.

### 5.3.2 Field evaluation of $BC_3F_3$ NILs for charcoal rot resistance

Field evaluation of NILs carry combination of QTLs was carried out in charcoal rot sick plot during *rabi* 2010 at MARS, Dharwad. A set of 16 lines carrying various combinations of charcoal rot resistant QTLs, donor and recurrent parents were sown and the experiment experienced typical *rabi* situation. Lines selection was based upon percentage of recurrent parent genome recovery. Selected lines include, all three QTLs (*cr*1*, cr*2*, cr*3), double QTLs in combinations (*cr*1 and *cr*2, *cr*2 and *cr*3, *cr*1 and *cr*3), single QTL (*cr*1, *cr*2, *cr*3) and null QTLs. Typical to the *rabi* situation, there were no rains during flowering and crop maturity; typical terminal drought was experienced by the crop. The *Macrophomina phaseolina* pathogen, D-4 isolate which was found to be most virulent from our previous studies (Rajkumar *et al*., 2007), was tooth pick inoculated in the form of infected sorghum grain to each and every plant in the experimental plot. Terminal drought stress coupled with artificial inoculation in a sick plot situations manifested charcoal rot disease to its best and genetic worth of the QTLs could be assessed stringently.

Analysis of variance showed significant variation among NILs. Mean lodging per cent, mean number of internodes crossed by the fungus and length of infection was higher in recurrent parents and null QTL lines and E36- 1, the donor parent. The NILs of M35-1, SPV-86 harbouring *cr*1, *cr*2 and *cr*3 recorded best lodging per cent, number of internodes crossed by the fungus and length of infection. Among combinations, *cr*1 + *cr*3 found better compared to other combinations on both backgrounds. None of the QTLs on their own could be of practical use under field conditions. The M35-1 and SPV-86 were highly susceptible to the infection. The sick plot situation is exposed to have most virulent strains of pathogen (Rajkumar *et al*., 2007). The D-4 strain used in artificial inoculation was from Dharwad location and was one of the most virulent one as pathogen evolves rapidly, it is important to test NILs in different locations possibly having different combinations of naturally occurring *Macrophomina phaseolina* pathogen strains.

## Future line of work

1. Deploying the stable QTLs identified for post flowering drought tolerance into recurring backgrounds
2. Fine mapping of these regions and map based cloning of genes within the QTL region
3. Further saturation and use of genetic linkage maps to understand other complex traits in sorghum.
4. Large scale testing of lines introgressed with charcoal rot resistant QTLs

# 6. SUMMARY AND CONCLUSIONS

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop next only to wheat, maize, rice and barley, and has a predominant role in the food and fodder security for millions of rural families and cattle in arid and semi-arid regions of the world. There are numerous factors contributing to yield loss in sorghum. Drought stress is one of the major agronomic problem that limit the attainment of maximum yield more than any other environmental parameters.

Drought stress generally changes plant growth and development pattern by suppressing cell division, organ growth, net photosynthesis, protein synthesis and alters hormonal balance in major plant tissue, which eventually causes severe effects on crop yield. Drought is an important abiotic stress in crop plants, which influences many agronomically important traits. Serious attempts have been made to understand the mechanisms of drought tolerance and to identify and improve genotypes that can tolerate moisture stress in sorghum. Progress in genetic manipulation of economically important traits related to drought tolerance are essential for the sustained improvement of sorghum to meet the future demand. Lack of understanding of inheritance of drought tolerance and complex interrelations have hindered the progress of evolving new varieties tolerant to drought.

Among the characters that may contribute to drought tolerance, delayed senescence or stay-green in sorghum is considered a valuable trait, as it improves adaptation of genotypes to post-flowering drought stress, particularly in environments where the crop depends largely on stored soil moisture during grain filling and maturation. This trait has been characterized by many independent groups using different stay-green sources in sorghum. Further, water use efficiency as a trait in moisture stress tolerance under post-flowering is relevant and this trait has been proved to be useful in practical sense in crops such as wheat, barley, rice and maize. However, identification of genes/ QTLs and their genetic deployment is important and challenging as well. The advent of molecular marker technology in quantitative genetics greatly facilitated the study of complex traits and has made it possible to dissect the complex traits. Different kinds of non-genic and gene based markers such as RFLPs, RAPDs, AFLPs, SSRs, genic SSRs microsatellites and SNPs have been developed in sorghum and other crops and used to construct linkage maps which facilitated QTL mapping of traits of agronomic importance and to determine their map positions in the genome. Identification of QTLs can augment direct genetic manipulation through marker-assisted selection. MAS in backcross programmes aiming at the transfer of quantitative traits from non-adapted resources to elite breeding lines can be practiced at a higher precision. Results of present study are summarized as follows.

1. Two recombinant inbred population (RIPs) each consisting of 226 RILs derived from IS9830 x E36-1(RIP1) and N13 x E36-1(RIP2) crosses were evaluated in post rainy season (*rabi*) for post-flowering drought tolerance and yield related traits over three years at Main Agricultural Research Station (MARS), Dharwad and over two years at Regional Research Station (RRS), Bheemarayanagudi locations. A set of 13 traits in both RIPs besides carbon isotope discrimination as a surrogate trait were monitored and measured at both locations. CID was investigated in RIP2.

2. The data of different seasons, within a location was more consistent than between locations within a season, thus, accordingly the pooling of data was done.

3. In both the RIPs, three seasons pooled analysis of variance at Dharwad and two seasons at Bheemarayanagudi locations revealed highly significant difference among individual RILs and parents for all the traits studied pointing at potential utility of their populations for trait mapping and going through simple situations

4. In RIP1, a higher PCV and GCV was noticed for traits such as per cent GLA 30 DAF, per cent GLA 45 DAF, earhead length, panicle exertion, stem girth, spicklets per plant and yield per plant. On the other hand, in RIP2, higher values of PCV and GCV were recorded for per cent GLA 45 DAF, panicle exertion, yield per plant, and moderate values of PCV and GCV were noticed for GLA 30 DAF, days to 50 per cent flowering, total green leaf area, earhead length, stem girth, plant height, spicklets per head and 100 seed weight.

5. Heritability was on higher side for all the traits, indicating additive genes. In RIP1 and RIP2 higher heritability was recorded for per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, days to 50 per cent flowering panicle exertion, plant height, spicklets per head, 100 seed weight, total green leaf area and stem girth and while moderate heritability was noticed for yield per plant, number of leaves and earhead length.

6. Trait such as per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, panicle exertion, spicklets per head recorded higher genetic advance and traits such as 100 seed weight and yield per plant revealed moderate levels of genetic advance in both RIPs. In RIP1, high genetic advance over per cent mean was recorded for days to 50 per cent flowering, earhead length, plant height, stem girth. However, per cent GLA 30 DAF, per cent GLA 45 DAF, panicle exertion, per cent GLA 15 DAF resulted lower genetic advance in RIP2.

7. In both RIPs, per cent GLA 15 DAF was positively correlated with per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area, number of leaves, stem girth, plant height, spicklets per plant and 100 seed weight but was found negatively correlating with days to 50 per cent flowering, earhead length, panicle exertion. A highly significant and positive association was found between per cent GLA 45 DAF and number of leaves per plant; stem thickness, number of spicklets per head, total green leaf area and yield per plant.

8. Grain yield was highly significant and positively correlated with total green leaf area, earhead length, per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF and 100 seed weight.

9. In RIP2, carbon isotope discrimination found to be positively correlated with total GLA, per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, spicklets per plant, but had negative association with stem girth, panicle exertion, days to 50 per cent flowering and earhead length.

10. A total of 530 EST-SSR and 270 non-genic SSR markers were used to identify the potential polymorphic ones in RIP1 and RIP2 parents. For RIP1, 40 EST-SSR (7.55 %) and 71 non-genic SSR (26.30 %) were available for genotyping. Similarly in RIP2, 68 EST-SSR (12.83 %) and 70 nuclear SSRs (25.93 %) markers showed polymorphism and thus be available for genotyping. The total polymorphic markers detected between RIP1 (33.85 %) were less as compared to RIP2 (38.76%).

11. A set of 23 and 22 SNP polymorphic markers and their genotyped data from another study in laboratory was additionally adopted to facilitate linkage map construction and QTL mapping.

12. Genotyping of individual RILs was carried out either on 2.5 per cent agarose or 6 per cent PAGE and then silver stained to realize the amplicons. The segregation pattern of all the polymorphic marker loci in RILs was compared and checked for their goodness of fitness to expected 1:1 ratio. All marker loci were segregated in 1:1 ratio which is expected in RIL populations.

13. In RIP1, a total of 134 markers were assigned on to the ten linkage group *viz.,* LG-A, LG-B, LG-C, LG-D, LG-E, LG-F, LG-G, LG-H, LG-I and LG-J with respective 15, 23, 16, 17, 15, 6, 16, 7, 8 and 11 markers and the genetic linkage map had a total length of 1661.1 cM.

14. The number of markers mapped per linkage group ranges from 6 in LG-F to 23 in LG-B with average distance coverage of 12.39 cM. The highest and lowest distances observed were between 77.6 cM on LG-D is flanked by Xsnp83 – Xiabt353 and 1.7 cM on LG-A flanked by Xtxp248 – Xtxp340 markers respectively.

15. In RIP2, a total of 160 markers were mapped on to ten linkage groups *viz.,* LG-A, LG-B, LG-C, LG-D, LG-E, LG-F, LG-G, LG-H, LG-I and LG-J with respective 24, 30, 17, 17, 18, 8, 15, 8, 15 and 8 markers with a genetic linkage map distance coverage of 2003.8 cM. The highest distance of 47.9 cM was observed between Xiabt82 – Xtxp73 on LG-B, the lowest distance of 2 cM on LG-E flanked between xisp362-xumc085 markers.

16. QTL analysis by composite interval mapping (CIM) procedure implemented with a LOD score of 2.0 as threshold value for QTL significance was done in QTL cartographer (2.5 b). The threshold values, with permutation times 300 and significance at 0.05 per cent was adopted.

17. Identification of QTLs that occur consistently in most of the environments would be important for practical breeding applications. In the present study, the QTLs commonly detected at *i.e.*, Dharwad for the three years (2007, 2008, 2009) and Bheemarayanagudi for two years (2008 and 2009) were considered as stable.

18. In RIP1, three QTLs were found common over season and across locations for per cent GLA 15 DAF, which were flanked by Xtxp34-Xtxp285 (LG-A), Xtxp38-Xsnp19 (LG-C) and Xiabt227-Xsnp56 (LG-D) with LOD value of 2.24 – 2.85, 2.51 – 2.73 and 2.70 – 3.13, per cent phenotypic variation of 9.05 - 11.04, 7.56 - 8.38 and 7.49 – 9.39 respectively. For per cent GLA 30 DAF, two QTLs were detected as common over season and across locations, bracketed by Xtxp114-Xumc7 on LG-C and Xtxp205-Xtxp231 on LG-E with LOD value of 2.39 – 3.32 and 3.12 – 3.14, phenotypic variation of 8.27 – 10.24 and 8.21 – 10.48 per cent respectively. And for per cent GLA 45 DAF, two QTLs on LG-J (Xtxp298-Xtxp324) and on LG-E (Xtxp295-Xtxp168) were detected as common over season and across locations with LOD value of 2.73 – 3.35 and 4.60 – 4.80, per cent phenotypic variation of 10.34 – 19.49 and 9.00 – 10.46 respectively. In all cases E36-1 contributed the favorable allele.

19. In RIP2, three QTLs were found to be common over seasons and across locations for per cent GLA 15 DAF, flanked by Xtxp34-Xtxp285 (LG-A), Xtxp38-Xsnp19 (LG-C) and Xiabt227-Xsnp56 (LG-D) with LOD score of 3.15 – 3.97, 3.08 – 3.68 and 2.63 – 2.68, per cent phenotypic variation of 13.05 – 14.46, 6.39 – 8.47 and 7.56 – 8.49 respectively. The QTLs were bracketed by Xtxp205-Xtxp231 (LG-E) and Xtxp8-Xtxp1 (LG-B) were found common over season and across locations for per cent GLA 30 DAF with per cent phenotypic variation of 16.0 – 17.37 and 8.00 – 9.94, LOD value of 2.89 – 2.29 and 2.32 – 2.79 respectively and QTLs from LG-J (Xtxp298-Xtxp324), LG-G (Xiabt341-Xiabt178) were found common over seasons and across locations with per cent phenotypic variation of 17.34 – 17.93 and 16.00 – 16.99.

20. QTL for carbon isotope discrimination was found on LG-I, flanked by Xtxp6-Xiabt70 with length of 10.00 – 10.48 and LOD value of 2.69 – 3.10, per cent phenotypic variation of 6.80 – 7.23 with positive additive effect conferring favourable allele from E36-1 parent.

21. Totally three QTLs on linkage groups A (Xtxp34-Xtxp285), C (Xtxp38-Xsnp19) and D (Xiabt227-Xsnp56) were found stable over seasons, across locations and across populations for GLA 15 DAF had their LOD value of 2.24 – 3.97, 2.51 – 3.68 and 2.63 – 3.13.

22. The QTL for per cent GLA 30 DAF found on LG-E flanked by Xtxp205-Xtxp231 at 128.05 – 145.73 position with length of 7.10 – 9.01 recorded LOD value of 2.89 – 3.14. One QTL for per cent GLA 45 DAF was found stable over seasons, across locations and across populations with length of 19.5 – 25.63, LOD value of 2.34 – 3.35.

23. A QTL mapped on LG-B for days to 50% flowering flanked by xumc136-Xsnp28 markers, and for plant height, QTL flanked by Xtxp61-Xtxp37 on LG-A expressing negative effect conferring favourable allele from N13/IS9830 parent were found common over seasons, across locations and across populations.

24. QTL for number of leaves flanked by Xtxp145-Xtxp6 marker was mapped on LG-I and one QTL for panicle exertion bracketed by Xtxp47-Xtxp18 marker was on LG-H.

25. A QTL for stem girth on LG-B flanked by Xtxp201-Xumc136 , one QTL for spicklets per head on LG-H (Xtxp47-Xtxp18). A QTL on LG-D (Xtxp327-Xisp229) for 100 seed weight and two QTLs for seed yield per plant found on LG-I (Xtxp145-Xtxp6) and LG-D (Xsnp36-Xtxp27) were found common over seasons, across locations and across populations with positive additive allele contributed from E36-1 parent.

26. QTL networking algorithm indicated no interactions among the QTLs.

27. For marker assisted introgression three stable QTLs for charcoal rot resistance trait *viz*., number of internode crossed, length of infection and percent lodging were used. These collectively contributed 43 per cent of phenotypic variance for the disease resistance.

28. Foreground and limited background selection during $BC_3F_1$ was employed in M35-1 and SPV86 derived backcross progenies.

29. Foreground selection was employed in $BC_3F_2$ generation in both backgrounds. The progenies were screened with 6 linked markers conditioning the target QTLs. Progenies which showed homozygosity with donor parent allele at different QTL combinations *viz., cr1*+ *cr2*+*cr3*, *cr1*+ *cr2*, *cr2*+*cr3*, *cr1*+ *cr3*, *cr1*, *cr2*, *cr3*, null *cr* were selected and whole genome background selection was exercised to check percentage of recurrent parent genome transferred were selected and selfed to $BC_3F_3$.

30. The $BC_3F_3$ progenies which showed highest percentage of recurrent parent genome recovery were tested for their homozygosity in 10 randomly selected plants and were found to be homozygous at respective QTL combinations in M35-1 and SPV86 backgrounds.

31. Mean lodging per cent, mean number of internodes crossed by the fungus and length of infection was higher in recurrent parents and null QTL lines and E36- 1, the donor parent.

32. The NILs of M35-1, SPV-86 harboring *cr1*, *cr2* and *cr3* recorded best lodging per cent, number of internodes crossed by the fungus and length of infection.

33. Among combinations, *cr1* + *cr3* found better compared to other combinations on both backgrounds.

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**Appendix I: List of stock solutions used in experiment**

| | |
|---|---|
| 1. ExtraCtion buffer | :100 mM Tris HCl, pH 8.0<br>50 mM EDTA, pH 8.0<br>500 mM NaCl |
| 2. 20 % SDS | :20 g of sodium doedycyl sulphate (sodium lauryl sulphate)<br>100 ml of $dH_2O$.<br>Should not be autoclaved |
| 3. 5 M potassium acetate | : 60.0 ml 5 M potassium acetate<br>11.5 ml Glacial acetic acid<br>28.5 ml $H_2O$ |
| 4. Isopropanol Ammonium Acetate mixture | :Three volumes of 10 M Iso-propanol<br>One volume of 10 M ammonium acetate |
| 5. $T_{10}$ $E_1$ | : Tris 10 mM containing 1 mM EDTA |
| 6. RNase (10 mg/ml) | : Dissolve Rnase in water, place in a tube in a boiling water bath for 10 minutes. Allow this to cool on a bench and store at –20 $^0$C |
| 7. Chloroform : isoamyl alcohol (24:1) | : Chloroform 240 ml<br>Isoamyl alcohol 10 ml.<br>Store in a dark room temperature. Make up and dispense the solution in a fumed cupboard. |
| 8. Ethanol (70 %) | : Absolute alcohol (70 ml)<br>Distill water (30 ml) |
| 9. NaCl 5 M | : Dissolve 292.2 g NaCl in 750 ml water.<br>Make up to 1 litre with water, filter and autoclave. |
| 10. Phenol /chloroform | : Mix equal volumes of the buffered phenol and chloroform : isoamyl alcohol (24:1). Store at 4 $^0$C |
| 11. Sodium acetate (2.5 M, pH 5.2) | : Dissolve 340.2 g sodium acetate in 500 ml of water adjust pH to 5.2 with glacial acetic acid and make up the volume to 1 litre and autoclave. |
| 12. Tris HCl (1 M, pH 8.0) | : Dissolve 121.1 g Tris in 800 ml of water. Adjust pH 8.0 with conc. HCl. Make up the volume to 1 L and autoclave |
| 13. Ethidium bromide (10 mg/ml) | : Dissolve 100 mg ethidium bromide in 10 ml of distil water, warp tube in aluminium foil and store at 4 $^0$C.<br>*Caution* : Ethidum bromide is extremely mutagenic |
| 14. 6% Acrylamide solution (1000 ml) | : 450 ml double distilled water<br>100 ml 5× TBE<br>420 g Urea<br>100 ml, 40 % Acrylamide/bisacrylamide (19:1) (w/w) |

*Contd…*

| | |
|---|---|
| | Combine water and TBE buffer in beaker, heat using a microwave, add urea and stir until dissolved. Adjust the volume to 900 ml with water and filter to remove any large particles.<br>Then acrylamide solution is combined with other ingredients in a storage container.<br>*Note :* Acrylamide is a neuro toxic |
| 15. 5× TBE | : 540 g Tris base<br>46 g EDTA<br>276 g boric acid<br>$dH_2O$ to 10 L<br>Store at room temperature, if left for ong-periods of time, some of the salts willprecipitate. It may be advisable to discard this buffer and make fresh buffer |
| 16. 0.5 % Acetic acid in 95% ethanol | : 1 ml glacial acetic acid<br>199 ml 95% ethanol<br>Aliquot and store at room temperature |
| 17. 10 % APS (ammonium persulfate) | : 1 g ammonium persulfate<br>10 ml $H_2O$<br>Should be stored at –20 $^0$C<br>if the APS will be used within the few weeks, store in light-tight bottle at 4 $^0$C |
| 18. TEMED (N, N, N', N' – tetra methyl ethylene diamine) | : Store at 4 $^0$C |
| 19. Binding solution | :Bind silane (γ - methacryloxy profile trimethoxysilane)M-6514 sigma industries<br>4 µl of bind silane + 1000 µl of 0.5 % acetic acid in 95% ethanol |
| 20. Repel solution | : Repel silane (dichloro dimethyl silane 99 %)<br>M-440272 sigma industries<br>250 µl repel silane + 750 µl of 0.5 % acetic acid in 95% ethanol (should be done in fumehood) |
| 21. Loading dye/tracking dye (10×) | : Sucrose 167 mg<br>Bromophenol blue 4.2 mg<br>Water 1 ml |
| 22. 3× SSR dye (3× STR loading solution) | : 5 M NaOH 0.2 ml<br>95 % formamide 95 ml<br>Bromophenol blue 50 mg<br>xylene cyonol 50 mg<br>water make upto 100 ml<br>*Note* : Farmamide is a toxic compound |
| 23. 100 bp DNA ladder | : 10 µl of 100 bp marker (genetics)<br>95 µl of 3× SSR dye<br>95 µl sterile $dH_2O$ |
| 24. Fix/stop solution (10% acetic acid) | : 200 ml glacil acetic acid in 1800 ml $dH_2O$ |

*Contd…*

| | |
|---|---|
| 25. Impregnate solution | : 10 % glycerol + 10 % glacial acetic acid |
| 26. Staining solution | : 2 g silver nitrate<br>3 ml 37 % formaldehyde<br>2000 ml $ddH_2O$<br>Should be stored at room temperature in a cabinet or other dark storage space<br>*Note :* Formaldehyde is a potential carcinogen |
| 27. Developer solution | : 60 g sodium carbonate<br>3 ml 37 % formaldehyde<br>400 µl sodium thiosulphate (10 mg/ml)<br>2000 ml $dH_2O$<br>This solution must be prepared fresh for each use<br>Prepared the solution by dissolving the sodium carbonate in water and chilling to about 10 $^0$C by placing on ice or in freezer<br>Just before use add the formaldehyde and sodium thiosulphate |
| 29. Loading dye (6×) | : 0.25 Bromophenol blue<br>40 % (W/V) sucrose in water<br>Started at 4°C |
| 30. 50× TAE (Tris-Acetate EDTA) (Miniatis *et al.,* 1982**)** | **:** 242 g/l Tris base and 57.1 ml Glacial acetic acid<br>100 ml 0.5 M EDTA (pH 8.0)<br>1000 ml Distilled water |
| 31. Saturated aqueous bromophenol blue solution | : 16.00 |
| EDTA (0.5M; pH: 8.0) | : 80.00 |
| Formeldehyde | : 720.00 |
| Glycerol (100 %) | : 2000.00 |
| Formamide | : 3048.00 |
| 10× FA gel buffer | : 4000.00 |
| Rnase free water | : 100.00 |
| Total | : 10 ml |
| 32. Recipe for 1.2 per cent formaldehyde agarose gel (60 ml) | |
| Agarose | : 720 mg |
| Formaldehyde | : 1 ml |
| Ethidium bromide (10 mg/ml) | : 3 µl |
| 1× MOPs buffer | : 60 ml |

*Contd…*

| | |
|---|---|
| 33. 10x MOPs eleCtrophoresis buffer | |
| MOPs | : 41.8 g |
| Sodium Acetate (DEPC-treated) | : 20 ml |
| EDTA (DEPC-treated; pH: 8.0) | : 20 ml |
| Total volume 1000 ml with DEPC treated water | |
| 34. Recipe for 1 per cent Agarose gel (40 ml) | |
| Agarose | 400 mg |
| 1x TAE | 40 ml |
| EtBr (10 mg/ml) | 2 µl |

**Appendix II: List of genic and nuclear SSR primers used in the present investigation for genetic map construction using RILs derived from the cross IS9830 x E36-1**

| Sl. No | Primer | Forward sequence (5'-3') | Reverse sequence (5'-3') |
|---|---|---|---|
| 1 | Xtxp8 | GCAGTGCCGTCAAAAAGTAG | GCAGCAACCACTTCCGATTC |
| 2 | Xtxp10 | CTGCTCCTACTCATGGAGGC | AAGATGCTCTGGTGGTGAGG |
| 3 | Xtxp47 | GGTGCGAGCTAGTTAAGTGGG | CAATGGCTTGCACATGTCCTA |
| 4 | Xtxp50 | AGCCTATGTATGTGTTCGTCC | TGATGTTGTTACCCTTCTGG |
| 5 | Xtxp65 | AGTAATCGTCTCCGGTGCTG | GCAATTGACAACGCATCTGG |
| 6 | Xtxp6 | AACTGAATCAGGTCATGGGC | GGCAGTAGCAGGCGTTTAAG |
| 7 | Xtxp14 | CTGGAAAGCCGAGCAGTATG | TACATCACAGCAGGGACAGG |
| 8 | Xtxp22 | CGACAGCGCACACAAGTC | CCAGTTCAGTTCAGTCCATACG |
| 9 | Xtxp34 | CTGATGTTTCTGTTCCTGCG | GCCTCAGCTGACTCCAATTC |
| 10 | Xtxp35 | TAGACACATCACACTCCGGC | CTTTTCTTGGGCTGACAAGC |
| 11 | Xtxp58 | TTCCCTTGCTGTTGCTTGTG | CAAAGTGCCCGGTTAAGACCT |
| 12 | Xtxp73 | ATCTTTAGCCGCCACATGAC | GGTTCTGTCGTCATCACCAG |
| 13 | Xtxp100 | TGCCCCAACGCTCACGCTCCC | CCGGCCGGCCAACCAACCAC |
| 14 | Xtxp160 | GGATATGGGATGGTTTGG | AGTCGGCTGGTAAAGTGG |
| 15 | Xtxp196 | CGAAGCTGGCGAAGT | CAGCGAGTGCAAGGA |
| 16 | Xtxp201 | CTCATAAGGCAGGACCAAC | GCGTTTATGGAAGCAAAAT |
| 17 | Xtxp207 | TGATAGACTTGTGAGCAGCTCC | ACACATCTACTACCCTCTCACCCT |
| 18 | Xtxp250 | GAACAGGACGATGTGATAGAT | GCACATCCTCTAAAACTACTTAGT |
| 19 | Xtxp270 | GCGAAATTATTTTGAAATGGAGTTGA | AGCAAGAAGAAGGCAAGAAGAAGG |
| 20 | Xtxp285 | TTGTCATTTCCCCCTTCTTTCTTTT | ATTTGATTCTTCTTGCTTTGCCTTGT |
| 21 | Xtxp297 | GCACAATCTTCGCCTAAATCAACAAT | GACCCATATGTGGTTTAGTCGCAAAG |
| 22 | Xtxp298 | GCTGTTAGCTTCTTCTAATCGTCGGT | GCATGTGTCAGATGATCTGGTGA |
| 23 | Xiabt146 | GTCCAGGCACCTGTCACTCTC | AAAGGAGGCTTGTCGTGCTA |
| 24 | Xiabt195 | GTTTCCGTTGTCGAGGAGAG | AGCTCGTGATTTCCCATGAC |
| 25 | Xiabt302 | GGATTAGATACGGGCGGATTG | CGCCTTTCGAGAAGAAACAG |
| 26 | Xiabt303 | GTGAACGCCTCCTTGTTCTC | CTTTCCCTTTCGCCTTTTTC |
| 27 | Xiabt312 | CGTCAAAGCATAAAGCACCA | GCCAAGTCCATGGTCAAGATG |
| 28 | Xiabt323 | CGAAGCTAACAGCAGCAAGA | GGTTCAAGTGGGCAAAGTGTG |
| 29 | Xiabt370 | AGGAGAACGCCATGCTCA | CCAATCCCCGCTATTTCC |
| 30 | Xiabt377 | GCCCTTGAACGACCCTATG | GAGCACCTGCACCTCAGTC |
| 31 | Xiabt397 | AAGGGAGGAGCGATAAAGGA | GAGCAGTGAGGAAAGGCAAG |
| 32 | Xiabt399 | CATGCCTGCCTGTTTTTCTTA | ACACGCTCCCTGTTTCTCAC |
| 33 | Xiabt422 | TTTTGGTAGCGCACAGACAG | TTCGGCTCTTCCCACAAGTA |
| 34 | Xiabt426 | CCACCAATACGTGCATTAGC | GCCACTGCCCTCAGGTGTA |
| 35 | Xiabt430 | TCTCTGTGGCATCATTCAGC | CGACCGCATGAGTGTTTTTA |
| 36 | Xiabt438 | TGCCCATTTGCCTTATCTTC | GACGAGGTAGATCCCGAAGG |
| 37 | Xiabt454 | TGAAGCTTTTGATTCAACTTGC | CCTTCCAGTGAGGATCAGGA |
| 38 | Xiabt509 | CCAAGAGCTGAACAATCACG | ATTTGCTTCACGGCTTTCAG |
| 39 | Xtxp307 | CCCACTAAACTAAAGCGGACA | GATGCCCATGCCTTGC |
| 40 | Xtxp205 | AGTAATCGTCTCCGGTGCTG | GCAATTGACAACGCATCTGG |
| 41 | Xiabt60 | GGTCCAAGGAATGCTGACATA | GGAAGAGTGCCTGAGTGGAG |
| 42 | Xiabt51 | TGAACAGAGAGATTTTCACATGC | GCTACCACGGTTTTTGCCTA |
| 43 | Xiabt95 | GAGTACCCCTCCGTCGAGA | AAGATTCCCAAAGCTGCTGA |
| 44 | Xiabt111 | CGGTGGTTCAGTGTGTTTTG | GGGGAGGTTCAGGAACAAGA |

**Appendix III : List of genic and nuclear SSR primer pairs used in the present investigation for genetic map construction using RILs derived from the cross N13 x E36-1**

| Sl. No | Primer | Forward sequence (5'-3') | Reverse sequence (5'-3') |
|---|---|---|---|
| 1 | Xtxp1 | TGCAGGAAGGGAGGATGTAG | CATGGGCGGGTTGAAGAC |
| 2 | Xtxp15 | CAATAAAAGAAGGGGGGAGC | ATACACTCCCAAGCCAGCAC |
| 3 | Xtxp31 | ACATACTTGGTGGTTTCGGC | AGTTCCTTCCCCAGCTTCAC |
| 4 | Kaf2 | TACGTAGGCGGTTGGATT | TCGGCGAGCATCTTACA |
| 5 | Xtxp45 | GGTCAAAGCGCTCTCCTCCTC | CCAGTTTCGCGAGAGGAGGAG |
| 6 | Xtxp71 | AGCTTCGTCGTCTCTGGTTC | CCACCTGTTGATGGGTTCC |
| 7 | Xtxp104 | GAATCGCTGCCAAATAAA | TAACCTATGCGGATAAAACAG |
| 8 | Xtxp210 | GATGAGCGATGGAGGAGAG | CGCTTTTCTGAAAATATTAAGGAC |
| 9 | Xtxp213 | GGAAGCAGTGCAGGAT | AACATTTCTTCCAGGCTC |
| 10 | Xtxp296 | ATGCTGTTATGATTTAGAGCCTGTAGAGTT | CAGAAATAACATATAATGATGGGGTGAA |
| 11 | Xtxp298 | GCTGTTAGCTTCTTCTAATCGTCGGT | GCATGTGTCAGATGATCTGGTGA |
| 12 | Xtxp34 | CTGATGTTTCTGTTCCTGCG | GCCTCAGCTGACTCCAATTC |
| 13 | Xtxp73 | ATCTTTAGCCGCCACATGAC | GGTTCTGTCGTCATCACCAG |
| 14 | Xtxp159 | GGGGGAGAAACGGTGAG | ACCCAAAGCCCAAATCAG |
| 15 | Xtxp231 | AGGCAAAGGGTCATCA | GGAAATCCAGGATAGGGT |
| 16 | Xtxp250 | GAACAGGACGATGTGATAGAT | GCACATCCTCTAAAACTACTTAGT |
| 17 | Xtxp285 | TTGTCATTTCCCCCTTCTTTCTTTT | ATTTGATTCTTCTTGCTTTGCCTTGT |
| 18 | Xtxp324 | ACATAACACAAGCAGGGAGAG | GCAAGAGTAACCTGCATTAAATTA |
| 19 | Xiabt143 | AGATGCTAAAGCAGCCTCCA | TGGTGGCATCAAAGTGTCATA |
| 20 | Xiabt146 | GTCCAGGCACCTGTCACTCTC | AAAGGAGGCTTGTCGTGCTA |
| 21 | Xiabt178 | GAGACTAGGCGTCACGGAAC | CATGTCGTTGGTGGAGTACG |
| 22 | Xiabt206 | CAGGAGGCAGGTTTCCACTA | CCCCGACGAACCCTAATAATC |
| 23 | Xiabt207 | TTGGACAAACGCATAAGCTG | TCCTCTTGCTGGAACTTCGTG |
| 24 | Xiabt215 | CAGCATCTGCACTTCTGCTC | CTCCCGCTACAGAATCAAGC |
| 25 | Xiabt227 | CACGTCCTTGAGGAGCTTGTA | AGAAGCGGCAGTACTCTTCG |
| 26 | Xiabt234 | GCGTACTTGCTCCACCTCTC | GCGTCGACTACCTCGTCTTC |
| 27 | Xiabt241 | TTTGTGGCACGAAAACACATA | GCCCACCAAATACAAGAACC |
| 28 | Xiabt263 | CGGTACTCCGACTCGATCTC | CCTTCTCCGATCACACAACC |
| 29 | Xiabt267 | AAGAGATCGGAGAAGGTCTCG | CTCGTTCCCGTAGCTGTCTC |
| 30 | Xiabt294 | GCTGCTTCCTCCTCCTCTTTC | GAAGCAGGCAGAGAGGTGAC |
| 31 | Xiabt310 | GAGAAGCATTTGCCATGGATG | CACAAGACACGCACAAAGGTG |
| 32 | Xiabt341 | GACTGGACTGCAACTGCAAA | TAGCTTTTTGCCCCATCATC |
| 33 | Xiabt350 | GACGAGCAGAGACCAGAACC | CGTCTTCTTCCACATGACCA |
| 34 | Xiabt353 | CCCTCGAGTGGTGTTCTAGC | CCGAATCACAAGTACGCAAA |
| 35 | Xiabt358 | CTCCAACTGTGGAGAGCACA | ACGGCTCAAACACCAACTTTG |
| 36 | Xiabt364 | CAGCAGGCAGAGTGTAGGTG | GCACTAATGCCATGCAAATG |
| 37 | Xiabt370 | AGGAGAACGCCATGCTCA | CCAATCCCCGCTATTTCC |
| 38 | Xiabt377 | GCCCTTGAACGACCCTATG | GAGCACCTGCACCTCAGTC |
| 39 | Xiabt380 | CCCGTCGAAGCACTCACTATA | CTTCCCCATAGCCAGAATCA |
| 40 | Xiabt388 | ACGACGACCTCTGTTGTGC | CCAGCCCTAGCTACATTCCA |

*Contd……*

| Sl. No | Primer | Forward sequence (5'-3') | Reverse sequence (5'-3') |
|---|---|---|---|
| 41 | Xiabt430 | TCTCTGTGGCATCATTCAGC | CGACCGCATGAGTGTTTTTA |
| 42 | Xiabt437 | GAAATTCATGAACCCCAACG | ACCACTACCTGGGGTCTGTG |
| 43 | Xiabt444 | GGGAGAGAGAGAGGGTCCATA | CCTTCTTCCACCTCCGTTCTC |
| 44 | Xiabt445 | CGGTGGTTCAGTGTGTTTTG | GGGGAGGTTCAGGAACAAGA |
| 45 | Xiabt451 | GGGTATGAGCAACAACAAGG | ATCCGCCTGTTTGTATCAGC |
| 46 | Xiabt488 | GCATCGCCATCTCTCTCTTC | AAAAGGCACCACCTTCTCCTC |
| 47 | Xtxp183 | TGTCTGCCCAGTTCCCAC | GATAACTTTGGCCAACTCGC |
| 48 | Xtxp211 | TGGAAGAGTACGGATCGAGG | AGAAGGACGACGAGAAGCAG |
| 49 | Xtxp205 | ACATACTTGGTGGTTTCGGC | AGTTCCTTCCCCAGCTTCAC |
| 50 | Xtxp132 | ACGGGGATTAGCCTTTTAGG | GCGCTGCTGTGTGTTGTTC |
| 51 | Xtxp282 | GGAGAGCCCGTCACTT | TGGCGGACATCCTATT |
| 52 | Xiabt54 | AAACGTCAAATTCTAGCTCCTATCA | GTCGTCGTCTTCCTCCAAGA |
| 53 | Xiabt72 | GCCACCAGAGGAGATTTGAA | CGGTGGTACAGACAGCAAGA |
| 54 | Xiabt80 | GCATCTTCCTCGAAGTCGTC | CCGTCTCTTCCCGGACTTAC |
| 55 | Xiabt73 | CATCTCCTTCTTGGCTTTGC | CGTGTCGATCTTCGTCTTCC |
| 56 | Xiabt82 | TTGAATCGGTTGCATGGATA | CAGAGCATACCTCCCCTGAA |
| 57 | Xiabt51 | CTTTGCGGCACTAAAACACA | GCCCACCAAATACAAGAACC |
| 58 | Xiabt79 | ATGGATGATGATGGGCTTGTG | CTCTAACGCCCTCCCTCTCTC |
| 59 | Xiabt134 | ACCACGACCGTTGGATAGTC | CTGCAGTACGTGCACATCAA |
| 60 | Xiabt32 | GGATTAGATACGGGCGGATTG | CGCCTTTCGAGAAGAAACAG |
| 61 | Xiabt78 | CTGCACACCCTGCTCTACAA | ATGTCCTTGGGTGTCCAGTC |
| 62 | Xiabt70 | GATCAGAAACCACGCTCACA | GAGGACAGCGAGGACGATAG |
| 63 | Xiabt86 | AATCCTTTTTCGTTGCCAGA | ACCTGCAGCCTACCACAGATC |
| 64 | Xiabt29 | CAATCCATCCATGTGCAGAG | AAGTTCACCGGAGGGTTCTTC |
| 65 | Xiabt58 | GGAGGAGCATACAGCCACTG | GTGCCAGCTTATGTTGAACG |

**Appendix IVa: Analysis of variance for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2007 at Dharwad location**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
| 1. | Genotype | 225 | 85.00** | 728.00** | 735.00** | 187.00** | 264771.00** | 41.40** | 4.04** | 122.00** | 26.00** | 3626.00** | 314.00** | 2.26** | 456.00** |
| 2. | Replication | 3 | 130.00 | 351.00 | 43.00 | 575.00 | 604082.00 | 451.90 | 80.60 | 178.00 | 122.40 | 491.00 | 397.00 | 12.70 | 1810.00 |
| 3. | Error | 675 | 4.90 | 9.90 | 17.45 | 0.44 | 6932 | 8.00 | 0.97 | 10.89 | 3.30 | 156.00 | 3.40 | 0.29 | 111.00 |
| 4. | SEm$\pm$ | | 1.11 | 1.58 | 2.08 | 0.33 | 41.63 | 1.41 | 0.49 | 1.65 | 0.91 | 6.25 | 0.92 | 0.27 | 5.28 |
| 5. | CD at 5% | | 3.09 | 4.37 | 5.78 | 0.92 | 115.30 | 3.93 | 1.36 | 4.57 | 2.52 | 17.32 | 2.56 | 0.74 | 14.64 |
| 6. | CD at 1% | | 4.07 | 5.75 | 7.60 | 1.21 | 151.60 | 5.16 | 1.80 | 6.01 | 3.31 | 22.77 | 3.37 | 0.98 | 19.25 |
| 7. | CV (%) | | 12.67 | 14.99 | 9.66 | 4.10 | 7.54 | 15.87 | 14.98 | 20.88 | 17.08 | 7.17 | 5.31 | 12.03 | 20.42 |

* - Significance at 5% probability level ** - Significance at 1% probability level NS – Non-significant

$X_1$: % GLA 15 DAF $X_2$: % GLA 30 DAF $X_3$: % GLA 45 DAF $X_4$: Days to 50 % flowering $X_5$: Total GLA
$X_6$: Earhead length $X_7$: Number of leaves $X_8$: Panicle exertion $X_9$: Stem girth $X_{10}$: Plant height
$X_{11}$: Spicklets per head $X_{12}$: 100 grain weight $X_{13}$: Grain yield per plant

**Appendix IVb: Magnitude of genetic variability parameters for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2007 at Dharwad location**

| Sl. No. | Traits | Range | | Grand mean | GCV (%) | PCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Minimum | Maximum | | | | | |
| 1. | % GLA 15 DAF | 76.00 | 95.00 | 85.50 | 5.72 | 7.01 | 66.77 | 8.02 |
| 2. | % GLA 30 DAF | 22.200 | 89.50 | 55.85 | 20.71 | 21.43 | 93.40 | 75.04 |
| 3. | % GLA 45 DAF | 12.20 | 70.60 | 41.40 | 22.87 | 25.27 | 81.87 | 95.53 |
| 4. | Days to 50 % flowering | 47.00 | 70.00 | 58.50 | 6.39 | 7.49 | 52.78 | 27.01 |
| 5. | Total green leaf area ($cm^2$) | 515.00 | 1913.68 | 1214.00 | 19.56 | 27.24 | 51.55 | 1.84 |
| 6. | Earhead length (cm) | 11.40 | 27.00 | 19.20 | 59.89 | 61.40 | 94.90 | 33.85 |
| 7. | Number of leaves | 3.80 | 9.00 | 6.40 | 14.40 | 16.75 | 73.88 | 35.75 |
| 8. | Panicle exertion (cm) | 3.10 | 29.00 | 16.05 | 33.79 | 36.05 | 87.55 | 36.79 |
| 9. | Stem girth (cm) | 2.20 | 7.20 | 4.70 | 11.96 | 15.10 | 62.75 | 40.91 |
| 10. | Plant height (cm) | 117.00 | 257.00 | 187.00 | 13.74 | 15.16 | 82.87 | 12.76 |
| 11. | Spicklets per head | 19.50 | 59.00 | 39.50 | 22.35 | 22.76 | 95.42 | 11.39 |
| 12. | 100 grain weight (g) | 2.60 | 4.80 | 3.70 | 15.99 | 18.58 | 76.65 | 42.43 |
| 13. | Grain yield per plant (g/plant) | 34.00 | 170.00 | 102.00 | 30.28 | 32.55 | 56.55 | 13.00 |

**Appendix IVc: Phenotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2007 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.135* | 0.362** | -0.015 | 0.089 | -0.049 | 0.069 | -0.057 | 0.126 | 0.032 | 0.109 | 0.124 | 0.004 |
| $X_2$ | | 1 | 0.035 | -0.030 | 0.067 | 0.022 | 0.015 | -0.082 | 0.018 | -0.086 | 0.067 | 0.083 | -0.441** |
| $X_3$ | | | 1 | -0.026 | 0.225** | -0.024 | -0.031 | -0.201** | 0.393** | 0.02 | 0.181* | 0.115 | 0.073 |
| $X_4$ | | | | 1 | -0.248** | 0.003 | 0.077 | 0.059 | 0.205** | 0.083 | 0.036 | 0.207** | -0.008 |
| $X_5$ | | | | | 1 | 0.053 | 0.012 | -0.024 | 0.165* | 0.22* | 0.279** | -0.039 | 0.061 |
| $X_6$ | | | | | | 1 | 0.053 | -0.04 | 0.107 | 0.054 | 0.032 | -0.01 | 0.038 |
| $X_7$ | | | | | | | 1 | -0.009 | -0.045 | 0.042 | 0.009 | 0.024 | 0.029 |
| $X_8$ | | | | | | | | 1 | -0.241** | -0.177* | -0.005 | -0.127 | -0.009 |
| $X_9$ | | | | | | | | | 1 | -0.059 | -0.337** | 0.226** | 0.01 |
| $X_{10}$ | | | | | | | | | | 1 | -0.165* | -0.076 | -0.001 |
| $X_{11}$ | | | | | | | | | | | 1 | 0.117 | 0.017 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.035 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix IVd: Genotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2007 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.158* | 0.408** | -0.015 | 0.106 | -0.078 | 0.126 | -0.08 | 0.172* | 0.043 | 0.124 | 0.186** | 0.012 |
| $X_2$ | | 1 | 0.037 | -0.031 | 0.077 | 0.029 | 0.033 | -0.099 | 0.029 | -0.094 | 0.07 | 0.119 | 0.062 |
| $X_3$ | | | 1 | -0.028 | 0.248* | -0.049 | -0.073 | -0.257** | 0.497** | 0.006 | 0.192** | 0.158* | 0.113 |
| $X_4$ | | | | 1 | 0.262** | 0.007 | 0.123 | 0.067 | 0.255** | 0.088 | 0.041 | 0.271** | -0.006 |
| $X_5$ | | | | | 1 | 0.062 | 0.009 | -0.027 | 0.219** | 0.244* | 0.297** | -0.055 | 0.085 |
| $X_6$ | | | | | | 1 | 0.117 | -0.065 | 0.150* | 0.095 | 0.031 | -0.032 | 0.052 |
| $X_7$ | | | | | | | 1 | -0.005 | -0.075 | 0.037 | 0.016 | 0.019 | 0.071 |
| $X_8$ | | | | | | | | 1 | -0.285** | -0.221** | -0.006 | -0.169* | -0.034 |
| $X_9$ | | | | | | | | | 1 | -0.06 | -0.429** | 0.381** | 0.046 |
| $X_{10}$ | | | | | | | | | | 1 | -0.184* | -0.122 | -0.026 |
| $X_{11}$ | | | | | | | | | | | 1 | 0.155* | 0.031 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.078 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix IVe: Analysis of variance for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2008 at Dharwad location**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
| 1. | Genotype | 225 | 83.00** | 728.00** | 735.00** | 187.00** | 264771.00** | 41.00** | 4.04** | 122.00** | 90.00** | 3638.00** | 314.00** | 2.30** | 899.00** |
| 2. | Replication | 3 | 91.00 | 81.43 | 133.00 | 437.00 | 19238.00 | 341.00 | 43.60 | 155.00 | 93.00 | 674.00 | 106.00 | 33.91 | 1254.00 |
| 3. | Error | 675 | 8.30 | 9.90 | 17.45 | 0.44 | 6932.00 | 8.31 | 0.90 | 10.89 | 4.98 | 156.00 | 3.40 | 0.30 | 116.00 |
| 4. | SEm± | | 1.44 | 1.58 | 2.08 | 0.33 | 41.63 | 1.44 | 0.49 | 1.65 | 1.11 | 6.25 | 0.92 | 0.27 | 5.38 |
| 5. | CD at 5% | | 4.00 | 4.37 | 5.78 | 0.92 | 115.39 | 3.99 | 1.36 | 4.57 | 3.09 | 17.33 | 2.56 | 0.76 | 14.92 |
| 6. | CD at 1% | | 5.26 | 5.75 | 7.60 | 1.21 | 151.70 | 5.25 | 1.80 | 6.01 | 4.06 | 22.78 | 3.37 | 1.00 | 19.62 |
| 7. | CV (%) | | 13.45 | 4.99 | 9.67 | 13.11 | 7.56 | 16.14 | 14.99 | 20.84 | 13.57 | 7.18 | 5.17 | 12.35 | 19.30 |

* - Significance at 5% probability level ** - Significance at 1% probability level

$X_1$: % GLA 15 DAF $X_2$: % GLA 30 DAF $X_3$: % GLA 45 DAF $X_4$: Days to 50 % flowering $X_5$: Total GLA
$X_6$: Earhead length $X_7$: Number of leaves $X_8$: Panicle exertion $X_9$: Stem girth $X_{10}$: Plant height
$X_{11}$: Spicklets per head $X_{12}$: 100 grain weight $X_{13}$: Grain yield per plant

**Appendix IVf: Magnitude of genetic variability parameters for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2008 at Dharwad location**

| Sl. No. | Traits | Range | | Grand mean | GCV (%) | PCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Min | Max | | | | | |
| 1. | % GLA 15 DAF | 68.00 | 95.00 | 81.50 | 5.18 | 6.23 | 69.30 | 10.13 |
| 2. | % GLA 30 DAF | 12.54 | 89.50 | 51.02 | 21.17 | 21.75 | 94.70 | 60.41 |
| 3. | % GLA 45 DAF | 12.00 | 81.93 | 46.96 | 31.00 | 32.48 | 91.10 | 64.05 |
| 4. | Days to 50 % flowering | 45.75 | 73.00 | 59.37 | 11.29 | 11.34 | 99.00 | 27.20 |
| 5. | Total green leaf area ($cm^2$) | 111.37 | 777.20 | 944.28 | 23.05 | 24.26 | 90.30 | 10.17 |
| 6. | Earhead length (cm) | 6.00 | 30.00 | 18.00 | 16.15 | 22.83 | 95.01 | 25.22 |
| 7. | Number of leaves | 4.50 | 10.00 | 7.25 | 13.27 | 20.02 | 44.21 | 17.52 |
| 8. | Panicle exertion (cm) | 4.04 | 32.00 | 18.02 | 33.35 | 39.33 | 71.90 | 57.05 |
| 9. | Stem girth (cm) | 1.61 | 14.92 | 8.26 | 48.80 | 54.19 | 81.12 | 16.95 |
| 10. | Plant height (cm) | 62.67 | 263.00 | 162.83 | 16.93 | 18.39 | 84.81 | 38.92 |
| 11. | Spicklets per head | 14.00 | 60.00 | 37.00 | 24.61 | 25.15 | 95.83 | 55.22 |
| 12. | 100 grain weight (g) | 1.00 | 7.00 | 4.00 | 16.05 | 20.25 | 62.80 | 32.00 |
| 13. | Grain yield per plant (g/plant) | 35.82 | 170.66 | 103.24 | 43.27 | 54.60 | 58.20 | 9.82 |

**Appendix IVg: Phenotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2008 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.138* | 0.345** | -0.014 | 0.085 | -0.047 | 0.058 | -0.058 | 0.141* | 0.036 | 0.116 | 0.142* | 0.005 |
| $X_2$ | | 1 | 0.035 | -0.03 | 0.067 | 0.021 | 0.015 | -0.082 | 0.007 | -0.086 | 0.067 | 0.078 | 0.044 |
| $X_3$ | | | 1 | -0.026 | 0.225** | -0.019 | -0.031 | -0.201** | 0.409** | 0.11 | 0.181* | 0.118 | 0.042 |
| $X_4$ | | | | 1 | -0.248** | 0.120 | 0.077 | 0.059 | 0.262** | 0.083 | 0.036 | -0.211* | -0.011 |
| $X_5$ | | | | | 1 | 0.05 | 0.012 | -0.024 | 0.132* | 0.221** | 0.279** | -0.039 | 0.058 |
| $X_6$ | | | | | | 1 | 0.056 | -0.041 | -0.123* | 0.054 | 0.034 | 0.009 | 0.024 |
| $X_7$ | | | | | | | 1 | -0.009 | -0.047 | -0.042 | -0.009 | 0.026 | -0.001 |
| $X_8$ | | | | | | | | 1 | -0.265** | 0.177* | 0.005 | 0.123 | -0.001 |
| $X_9$ | | | | | | | | | 1 | -0.058 | 0.347** | 0.272** | 0.03 |
| $X_{10}$ | | | | | | | | | | 1 | -0.165* | -0.077 | -0.014 |
| $X_{11}$ | | | | | | | | | | | 1 | 0.122 | 0.014 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.06 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix IVh: Genotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2008 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.172* | 0.415** | -0.012 | 0.11 | -0.082 | 0.112 | -0.088 | 0.178* | 0.05 | 0.142* | 0.221* | 0.011 |
| $X_2$ | | 1 | 0.037 | -0.031 | 0.077 | 0.028 | 0.033 | -0.099 | 0.002 | -0.094 | 0.07 | 0.112 | 0.051 |
| $X_3$ | | | 1 | -0.028 | 0.248* | -0.055 | -0.073 | -0.257** | 0.465** | 0.006 | 0.192* | 0.162* | 0.052 |
| $X_4$ | | | | 1 | -0.262** | 0.003 | 0.123 | 0.067 | 0.289** | 0.088 | 0.04 | -0.276** | -0.01 |
| $X_5$ | | | | | 1 | 0.058 | 0.009 | -0.027 | 0.152* | 0.244** | 0.297** | -0.056 | 0.074 |
| $X_6$ | | | | | | 1 | 0.118 | -0.066 | -0.148* | 0.095 | 0.034 | 0.035 | 0.038 |
| $X_7$ | | | | | | | 1 | -0.005 | -0.061 | -0.037 | -0.016 | 0.029 | -0.092 |
| $X_8$ | | | | | | | | 1 | -0.303** | 0.221** | 0.006 | 0.163* | -0.006 |
| $X_9$ | | | | | | | | | 1 | -0.051 | 0.388** | 0.391** | 0.045 |
| $X_{10}$ | | | | | | | | | | 1 | -0.184** | -0.102 | -0.036 |
| $X_{11}$ | | | | | | | | | | | 1 | 0.161* | 0.02 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.113 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix IVi: Analysis of variance for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2009 at Dharwad location**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
| 1. | Genotype | 225 | 92.00** | 728.00** | 735.00** | 187.14** | 264771.00** | 41.48** | 4.04** | 122.50** | 5.90** | 3638.00** | 314.00** | 2.12** | 314.00** |
| 2. | Replication | 3 | 342.00 | 1002.00 | 334.00 | 865 | 2270594 | 632.00 | 193.60 | 234.60 | 154.00 | 388.00 | 9.15 | 19.02 | 2492.00 |
| 3. | Error | 675 | 4.30 | 9.90 | 17.46 | 0.44 | 69.00 | 8.00 | 0.97 | 10.80 | 4.20 | 156.00 | 3.40 | 0.29 | 121.00 |
| 4. | SEm± | | 1.04 | 1.58 | 2.08 | 0.33 | 41.63 | 1.41 | 0.49 | 1.65 | 1.02 | 6.25 | 0.92 | 0.27 | 5.51 |
| 5. | CD at 5% | | 2.89 | 4.37 | 5.78 | 0.92 | 115.39 | 3.92 | 1.36 | 4.57 | 2.56 | 17.33 | 2.84 | 0.75 | 15.28 |
| 6. | CD at 1% | | 3.80 | 5.75 | 7.60 | 1.22 | 151.65 | 5.16 | 1.80 | 6.01 | 3.74 | 22.78 | 3.37 | 0.98 | 20.09 |
| 7. | CV (%) | | 12.50 | 14.99 | 9.67 | 11.11 | 7.53 | 15.86 | 14.99 | 20.93 | 17.33 | 7.17 | 5.48 | 11.96 | 19.70 |

* - Significance at 5% probability level ** - Significance at 1% probability level

$X_1$: % GLA 15 DAF $X_2$: % GLA 30 DAF $X_3$: % GLA 45 DAF $X_4$: Days to 50 % flowering $X_5$: Total GLA
$X_6$: Earhead length $X_7$: Number of leaves $X_8$: Panicle exertion $X_9$: Stem girth $X_{10}$: Plant height
$X_{11}$: Spicklets per head $X_{12}$: 100 grain weight $X_{13}$: Grain yield per plant

**Appendix IVj: Magnitude of genetic variability parameters for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-evaluated during 2009 at Dharwad location**

| Sl. No. | Traits | Range | | Grand mean | GCV (%) | PCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Min | Max | | | | | |
| 1. | % GLA 15 DAF | 67.75 | 94.80 | 81.27 | 5.64 | 6.17 | 83.50 | 10.84 |
| 2. | % GLA 30 DAF | 32.54 | 89.50 | 61.02 | 21.17 | 21.75 | 94.70 | 44.02 |
| 3. | % GLA 45 DAF | 12.00 | 81.93 | 46.96 | 31.00 | 32.47 | 91.10 | 56.09 |
| 4. | Days to 50 % flowering | 58.50 | 71.08 | 64.79 | 11.28 | 11.34 | 99.00 | 21.61 |
| 5. | Total green leaf area ($cm^2$) | 115.87 | 1781.71 | 948.79 | 22.96 | 24.16 | 90.30 | 52.38 |
| 6. | Earhead length (cm) | 5.97 | 29.90 | 17.93 | 16.18 | 22.66 | 51.00 | 23.70 |
| 7. | Number of leaves | 5.8 | 8.60 | 7.20 | 13.27 | 20.01 | 44.00 | 16.53 |
| 8. | Panicle exertion (cm) | 3.97 | 31.92 | 17.94 | 33.50 | 39.50 | 71.90 | 51.39 |
| 9. | Stem girth (cm) | 2.52 | 85.26 | 5.39 | 5.54 | 18.19 | 9.32 | 7.61 |
| 10. | Plant height (cm) | 62.77 | 263.11 | 162.94 | 16.92 | 18.38 | 84.80 | 34.34 |
| 11. | Spicklets per head | 12.00 | 58.00 | 35.00 | 26.06 | 26.63 | 95.80 | 50.80 |
| 12. | 100 grain weight (g) | 2.07 | 7.09 | 4.58 | 15.30 | 19.41 | 62.10 | 24.45 |
| 13. | Grain yield per plant (g/plant) | 28.00 | 157.00 | 92.50 | 18.66 | 35.08 | 28.30 | 8.22 |

**Appendix IVk: Phenotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2009 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.123* | 0.358** | -0.015 | 0.087 | 0.048 | 0.076 | -0.053 | 0.048 | 0.027 | 0.096 | 0.098 | 0.008 |
| $X_2$ | | 1 | 0.035 | -0.03 | 0.067 | 0.023 | 0.015 | -0.082 | -0.068 | -0.086 | 0.067 | 0.088 | 0.034 |
| $X_3$ | | | 1 | -0.026 | 0.225** | -0.028 | -0.031 | -0.201** | 0.12 | 0.003 | 0.181* | 0.111 | 0.191** |
| $X_4$ | | | | 1 | 0.248** | 0.005 | 0.077 | 0.059 | 0.056 | 0.083 | 0.036 | -0.200** | 0.001 |
| $X_5$ | | | | | 1 | 0.056 | -0.012 | -0.024 | 0.146* | 0.22** | 0.279** | -0.038 | 0.052 |
| $X_6$ | | | | | | 1 | 0.05 | -0.039 | 0.016 | 0.054 | 0.029 | -0.01 | 0.048 |
| $X_7$ | | | | | | | 1 | 0.009 | -0.015 | -0.042 | 0.009 | 0.021 | -0.06 |
| $X_8$ | | | | | | | | 1 | -0.038 | 0.177* | 0.005 | -0.139* | -0.017 |
| $X_9$ | | | | | | | | | 1 | 0.024 | 0.11 | -0.006 | -0.044 |
| $X_{10}$ | | | | | | | | | | 1 | 0.165* | -0.074 | -0.017 |
| $X_{11}$ | | | | | | | | | | | 1 | 0.111 | 0.017 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.001 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix IVI: Genotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2009 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.146* | 0.398** | -0.018 | 0.101 | 0.074 | 0.139* | -0.073 | 0.187** | -0.036 | 0.106 | 0.152* | 0.009 |
| $X_2$ | | 1 | 0.037 | -0.031 | -0.077 | 0.031 | 0.033 | -0.099 | -0.236** | -0.094 | 0.07 | 0.127 | 0.057 |
| $X_3$ | | | 1 | -0.028 | 0.248** | -0.042 | -0.073 | -0.257** | 0.342** | 0.006 | 0.192** | 0.154* | 0.212** |
| $X_4$ | | | | 1 | 0.262** | 0.011 | 0.123 | 0.067 | 0.187** | 0.088 | 0.04 | 0.264** | 0.006 |
| $X_5$ | | | | | 1 | 0.066 | -0.009 | -0.027 | 0.536** | 0.244* | 0.297** | -0.055 | 0.079 |
| $X_6$ | | | | | | 1 | 0.115 | -0.064 | 0.034 | 0.094 | 0.028 | -0.028 | 0.06 |
| $X_7$ | | | | | | | 1 | 0.005 | -0.122 | -0.037 | 0.016 | 0.009 | -0.001 |
| $X_8$ | | | | | | | | 1 | -0.067 | 0.221** | 0.006 | -0.175* | -0.08 |
| $X_9$ | | | | | | | | | 1 | 0.077 | 0.398** | -0.063 | -0.018 |
| $X_{10}$ | | | | | | | | | | 1 | 0.184** | -0.098 | -0.005 |
| $X_{11}$ | | | | | | | | | | | 1 | 0.148* | 0.042 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.002 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix Va: Analysis of variance for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2007 at Dharwad location**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
| 1. | Genotype | 225 | 268.00** | 91.00** | 1241.00** | 102.60** | 233984.00** | 28.00** | 2.90** | 85.01** | 1.27* | 2484.00** | 413.00** | 1.18 | 308.00** | 0.072 |
| 2. | Replication | 3 | 296.00 | 422.00 | 49300 | 1833.00 | 374517.00 | 858.00 | 38.00 | 19.90 | 97.16 | 798.90 | 8156.00 | 73.60 | 1321.00 | 0.00068 |
| 3. | Error | 675 | 9.70 | 21.10 | 26.60 | 3.17 | 1302.00 | 6.30 | 0.56 | 1.26 | 0.52 | 521.40 | 146.00 | 0.54 | 123.00 | 0.064 |
| 4. | SEm± | | 1.55 | 2.29 | 2.28 | 0.89 | 18.04 | 1.25 | 0.37 | 0.56 | 0.36 | 11.41 | 6.05 | 0.36 | 5.56 | 0.17 |
| 5. | CD at 5% | | 4.31 | 6.36 | 7.15 | 2.47 | 50.01 | 3.48 | 1.04 | 1.56 | 1.00 | 31.64 | 16.78 | 1.01 | 15.02 | 0.49 |
| 6. | CD at 1% | | 5.67 | 8.37 | 9.40 | 3.24 | 65.73 | 4.57 | 1.36 | 2.05 | 1.32 | 41.59 | 22.05 | 1.33 | 20.27 | 0.65 |
| 7. | CV (%) | | 13.42 | 5.96 | 8.46 | 12.85 | 19.40 | 13.86 | 9.98 | 9.03 | 14.93 | 12.75 | 20.45 | 2.91 | 20.07 | 6.02 |

* - Significance at 5% probability level ** - Significance at 1% probability level

$X_1$: % GLA 15 DAF $X_2$: % GLA 30 DAF $X_3$: % GLA 45 DAF $X_4$: Days to 50 % flowering $X_5$: Total GLA
$X_6$: Earhead length $X_7$: Number of leaves $X_8$: Panicle exertion $X_9$: Stem girth $X_{10}$: Plant height
$X_{11}$: Spicklets per head $X_{12}$: 100 grain weight $X_{13}$: Grain yield per plant $X_{14}$: Carbon isotope discrimination

**Appendix Vb: Magnitude of genetic variability parameters for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2007 at Dharwad location**

| Sl. No. | Traits | Range | | Grand mean | GCV (%) | PCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Min | Max | | | | | |
| 1. | % GLA 15 DAF | 57.00 | 99.00 | 78.00 | 9.71 | 11.82 | 67.35 | 10.87 |
| 2. | % GLA 30 DAF | 22.00 | 90.00 | 56.00 | 14.62 | 15.50 | 88.93 | 35.71 |
| 3. | % GLA 45 DAF | 7.70 | 81.50 | 44.35 | 30.17 | 31.23 | 93.31 | 46.36 |
| 4. | Days to 50 % flowering | 47.00 | 72.00 | 59.50 | 8.22 | 8.75 | 58.27 | 17.29 |
| 5. | Total green leaf area ($cm^2$) | 675.10 | 1993.40 | 1334.25 | 18.30 | 23.30 | 67.20 | 2.00 |
| 6. | Earhead length (cm) | 9.70 | 27.60 | 18.65 | 60.70 | 62.30 | 94.98 | 33.57 |
| 7. | Number of leaves | 5.20 | 10.22 | 7.71 | 10.46 | 14.95 | 48.99 | 11.28 |
| 8. | Panicle exertion (cm) | 2.70 | 23.40 | 13.05 | 36.72 | 37.90 | 93.86 | 81.99 |
| 9. | Stem girth (cm) | 2.80 | 6.20 | 4.50 | 13.36 | 19.26 | 48.87 | 12.44 |
| 10. | Plant height (cm) | 11.70 | 26.70 | 19.20 | 12.36 | 19.90 | 61.56 | 10.64 |
| 11. | Spicklets per head | 19.00 | 66.00 | 42.50 | 19.69 | 22.88 | 74.06 | 26.38 |
| 12. | 100 grain weight (g) | 2.50 | 4.70 | 3.60 | 9.35 | 18.26 | 86.24 | 22.22 |
| 13. | Grain yield per plant (g/plant) | 35.00 | 170.00 | 102.50 | 20.70 | 29.99 | 47.65 | 8.52 |
| 14. | Carbon isotope discrimination | 3.26 | 5.20 | 4.23 | 1.49 | 6.20 | 5.75 | 73.29 |

**Appendix Vc: Phenotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2007 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.558** | 0.463** | 0.004 | -0.005 | 0.021 | -0.016 | 0.049 | -0.077 | 0.003 | 0.089 | 0.057 | 0.048 | 0.064 |
| $X_2$ | | 1 | 0.667** | 0.003 | -0.021 | 0.051 | -0.006 | 0.104 | -0.038 | 0.043 | 0.127 | 0.054 | 0.013 | 0.158* |
| $X_3$ | | | 1 | 0.05 | -0.055 | 0.077 | -0.013 | 0.045 | -0.071 | 0.007 | 0.064 | 0.141* | 0.012 | 0.148* |
| $X_4$ | | | | 1 | -0.028 | -0.026 | -0.032 | 0.026 | -0.07 | -0.048 | 0.021 | 0.011 | 0.009 | 0.043 |
| $X_5$ | | | | | 1 | -0.116 | -0.015 | 0.093 | 0.027 | 0.053 | 0.026 | -0.049 | -0.002 | 0.014 |
| $X_6$ | | | | | | 1 | 0.018 | 0.059 | -0.028 | 0.105 | 0.120* | 0.117 | 0.028 | 0.056 |
| $X_7$ | | | | | | | 1 | 0.027 | -0.002 | 0.036 | 0.011 | -0.014 | -0.011 | 0.013 |
| $X_8$ | | | | | | | | 1 | -0.047 | -0.081 | 0.001 | -0.007 | -0.025 | 0.026 |
| $X_9$ | | | | | | | | | 1 | -0.123 | 0.304** | -0.038 | -0.048 | -0.069 |
| $X_{10}$ | | | | | | | | | | 1 | -0.06 | 0.064 | 0.020 | 0.173* |
| $X_{11}$ | | | | | | | | | | | 1 | -0.1 | -0.049 | -0.035 |
| $X_{12}$ | | | | | | | | | | | | 1 | -0.023 | 0.291** |
| $X_{13}$ | | | | | | | | | | | | | 1 | 0.039 |
| $X_{14}$ | | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF
$X_2$: % GLA 30 DAF
$X_3$: % GLA 45 DAF
$X_4$: Carbon isotope discrimination
$X_5$: Days to 50 % flowering
$X_6$: GLA
$X_7$: Earhead length
$X_8$: Number of leaves
$X_9$: Panicle exertion
$X_{10}$: Stem girth
$X_{11}$: Plant height
$X_{12}$: Spicklets per head
$X_{13}$: 100 grain weight
$X_{14}$: Grain yield per plant

**Appendix Vd: Genotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2007 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.628** | 0.507** | 0.150* | -0.006 | 0.023 | -0.006 | 0.059 | -0.084 | 0.029 | 0.118 | 0.103 | 0.065 | 0.162* |
| $X_2$ | | 1 | 0.733** | 0.103 | -0.029 | 0.062 | -0.021 | 0.130 | -0.043 | 0.084 | 0.181* | 0.096 | 0.034 | 0.329** |
| $X_3$ | | | 1 | 0.429** | -0.060 | 0.081 | -0.007 | 0.061 | -0.083 | 0.005 | 0.096 | 0.281** | 0.03 | 0.308** |
| $X_4$ | | | | 1 | -0.151* | 0.149* | -0.225** | 0.281** | -0.504** | -0.814** | 0.009 | 0.334** | 0.241** | 0.329** |
| $X_5$ | | | | | 1 | -0.127 | -0.024 | 0.132 | 0.031 | 0.123 | 0.015 | -0.049 | -0.074 | -0.007 |
| $X_6$ | | | | | | 1 | 0.033 | 0.083 | -0.027 | 0.199** | 0.186* | 0.202** | 0.05 | 0.098 |
| $X_7$ | | | | | | | 1 | 0.071 | -0.015 | 0.157* | 0.012 | -0.05 | -0.116 | 0.05 |
| $X_8$ | | | | | | | | 1 | -0.08 | -0.145* | 0.016 | -0.01 | -0.017 | 0.066 |
| $X_9$ | | | | | | | | | 1 | -0.231** | 0.442** | -0.085 | -0.093 | -0.107 |
| $X_{10}$ | | | | | | | | | | 1 | -0.254** | 0.007 | 0.031 | 0.188* |
| $X_{11}$ | | | | | | | | | | | 1 | -0.228* | -0.16* | -0.11 |
| $X_{12}$ | | | | | | | | | | | | 1 | -0.132 | 0.334** |
| $X_{13}$ | | | | | | | | | | | | | 1 | 0.005 |
| $X_{14}$ | | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF
$X_2$: % GLA 30 DAF
$X_3$: % GLA 45 DAF
$X_4$: Carbon isotope discrimination
$X_5$: Days to 50 % flowering
$X_6$: GLA
$X_7$: Earhead length
$X_8$: Number of leaves
$X_9$: Panicle exertion
$X_{10}$: Stem girth
$X_{11}$: Plant height
$X_{12}$: Spicklets per head
$X_{13}$: 100 grain weight
$X_{14}$: Grain yield per plant

**Appendix Ve: Analysis of variance for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2008 at Dharwad location**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
| 1. | Genotype | 225 | 269.00** | 422.00** | 1241.00** | 102.00** | 233984.00** | 28.70** | 2.90** | 84.00** | 1.20* | 2901.00** | 320.00** | $1.60^{NS}$ | 305.00** | 0.14** |
| 2. | Replication | 3 | 101.00 | 642.00 | 680.00 | 1492.00 | 1589.00 | 732.00 | 8.70 | 2.00 | 29.80 | 726.00 | 9068.00 | 42.35 | 1443.00 | 3.29 |
| 3. | Error | 675 | 6.90 | 21.12 | 26.00 | 3.17 | 1302.00 | 6.30 | 0.50 | 1.20 | 0.50 | 971 | 140.00 | 0.54 | 121.00 | 0.13 |
| 4. | SEm$\pm$ | | 1.32 | 2.29 | 2.58 | 0.89 | 18.04 | 1.25 | 0.37 | 0.56 | 0.36 | 15.58 | 5.93 | 0.36 | 5.51 | 0.25 |
| 5. | CD at 5% | | 3.66 | 6.36 | 7.15 | 2.47 | 50.01 | 3.48 | 1.04 | 1.56 | 1.00 | 43.19 | 16.44 | 1.01 | 15.28 | 0.71 |
| 6. | CD at 1% | | 4.81 | 8.37 | 9.40 | 3.24 | 65.75 | 4.57 | 1.36 | 2.05 | 1.32 | 56.77 | 21.62 | 1.33 | 20.09 | 0.93 |
| 7. | CV (%) | | 5.89 | 5.96 | 8.50 | 4.85 | 13.88 | 13.84 | 10.27 | 9.06 | 15.18 | 17.33 | 14.43 | 20.17 | 19.71 | 8.60 |

* - Significance at 5% probability level ** - Significance at 1% probability level

$X_1$: % GLA 15 DAF $X_2$: % GLA 30 DAF $X_3$: % GLA 45 DAF $X_4$: Days to 50 % flowering $X_5$: Total GLA
$X_6$: Earhead length $X_7$: Number of leaves $X_8$: Panicle exertion $X_9$: Stem girth $X_{10}$: Plant height
$X_{11}$: Spicklets per head $X_{12}$: 100 grain weight $X_{13}$: Grain yield per plant $X_{14}$: Carbon isotope discriminatio

**Appendix Vf: Magnitude of genetic variability parameters for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2008 at Dharwad location**

| Sl. No. | Traits | Range | | Grand mean | GCV (%) | PCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Min | Max | | | | | |
| 1. | % GLA 15 DAF | 42.03 | 93.75 | 67.55 | 8.86 | 9.31 | 90.40 | 26.78 |
| 2. | % GLA 30 DAF | 21.03 | 90.25 | 55.64 | 12.99 | 14.29 | 82.60 | 38.07 |
| 3. | % GLA 45 DAF | 11.37 | 83.25 | 47.31 | 28.68 | 29.91 | 91.90 | 83.15 |
| 4. | Days to 50 % flowering | 50.75 | 79.25 | 65.00 | 7.96 | 8.45 | 88.70 | 16.94 |
| 5. | Total green leaf area ($cm^2$) | 578.70 | 1864.52 | 1221.61 | 19.27 | 19.49 | 97.80 | 7.97 |
| 6. | Earhead length (cm) | 5.88 | 25.33 | 15.60 | 13.04 | 19.02 | 8.47 | 23.01 |
| 7. | Number of leaves | 5.15 | 10.15 | 7.65 | 10.54 | 14.72 | 51.30 | 16.08 |
| 8. | Panicle exertion (cm) | 2.73 | 23.41 | 13.07 | 36.82 | 37.91 | 94.30 | 80.26 |
| 9. | Stem girth (cm) | 2.77 | 6.38 | 4.57 | 9.08 | 17.68 | 26.40 | 10.28 |
| 10. | Plant height (cm) | 98.75 | 267.25 | 183.00 | 12.22 | 21.20 | 33.20 | 15.02 |
| 11. | Spicklets per head | 12.87 | 51.00 | 31.93 | 16.63 | 33.80 | 24.20 | 22.11 |
| 12. | 100 grain weight (g) | 2.00 | 5.50 | 3.75 | 11.02 | 22.98 | 90.23 | 10.93 |
| 13. | Grain yield per plant (g/plant) | 24.06 | 161.40 | 92.73 | 18.25 | 34.86 | 27.40 | 8.23 |
| 14. | Carbon isotope discrimination | 3.54 | 5.00 | 4.27 | 1.66 | 8.76 | 3.59 | 63.93 |

**Appendix Vg: Phenotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2008 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.537** | 0.472** | 0.003 | -0.002 | 0.019 | -0.001 | 0.039 | -0.076 | 0.011 | 0.082 | 0.051 | 0.047 | 0.079 |
| $X_2$ | | 1 | 0.667** | 0.003 | -0.021 | 0.051 | -0.006 | 0.104 | -0.038 | -0.043 | 0.122 | 0.055 | 0.013 | 0.154* |
| $X_3$ | | | 1 | 0.05 | -0.055 | 0.077 | -0.013 | 0.045 | -0.07 | 0.007 | 0.062 | 0.133* | 0.012 | 0.152* |
| $X_4$ | | | | 1 | -0.028 | 0.026 | 0.032 | 0.026 | 0.07 | -0.047 | -0.02 | 0.013 | 0.009 | 0.038 |
| $X_5$ | | | | | 1 | 0.116 | -0.015 | -0.093 | 0.027 | 0.053 | 0.023 | -0.054 | -0.002 | -0.018 |
| $X_6$ | | | | | | 1 | -0.018 | 0.058 | -0.028 | 0.105 | -0.098 | 0.124 | 0.028 | 0.059 |
| $X_7$ | | | | | | | 1 | 0.027 | -0.002 | 0.036 | 0.005 | -0.02 | -0.011 | 0.013 |
| $X_8$ | | | | | | | | 1 | -0.047 | 0.081 | 0.005 | 0.006 | 0.025 | 0.024 |
| $X_9$ | | | | | | | | | 1 | -0.123 | 0.254** | -0.024 | -0.048 | -0.07 |
| $X_{10}$ | | | | | | | | | | 1 | -0.064 | 0.075 | -0.02 | 0.171* |
| $X_{11}$ | | | | | | | | | | | 1 | -0.084 | -0.019 | 0.022 |
| $X_{12}$ | | | | | | | | | | | | 1 | -0.036 | 0.305** |
| $X_{13}$ | | | | | | | | | | | | | 1 | 0.039 |
| $X_{14}$ | | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF
$X_2$: % GLA 30 DAF
$X_3$: % GLA 45 DAF
$X_4$: Carbon isotope discrimination
$X_5$: Days to 50 % flowering
$X_6$: GLA
$X_7$: Earhead length
$X_8$: Number of leaves
$X_9$: Panicle exertion
$X_{10}$: Stem girth
$X_{11}$: Plant height
$X_{12}$: Spicklets per head
$X_{13}$: 100 grain weight
$X_{14}$: Grain yield per plant

**Appendix Vh: Genotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2008 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.632** | 0.511** | 0.144* | -0.002 | 0.02 | -0.009 | 0.054 | -0.082 | 0.012 | 0.117 | 0.105 | 0.06 | 0.176 |
| $X_2$ | | 1 | 0.733** | 0.104 | -0.029 | 0.062 | -0.021 | 0.130 | -0.043 | -0.084 | 0.204** | 0.11 | 0.034 | 0.325** |
| $X_3$ | | | 1 | 0.429** | -0.06 | 0.081 | -0.007 | 0.061 | -0.083 | 0.005 | 0.108 | 0.305** | 0.031 | 0.312** |
| $X_4$ | | | | 1 | -0.15 | 0.149* | 0.225** | 0.282** | 0.504** | -0.814** | -0.048 | 0.403** | 0.239** | 0.36** |
| $X_5$ | | | | | 1 | 0.127* | -0.024 | -0.132 | 0.031 | 0.123 | 0.019 | -0.067 | -0.074 | -0.011 |
| $X_6$ | | | | | | 1 | -0.033 | 0.083 | -0.027 | 0.199** | -0.184** | 0.194** | 0.05 | 0.104 |
| $X_7$ | | | | | | | 1 | 0.071 | -0.015 | 0.157* | 0.035 | -0.068 | -0.116 | 0.05 |
| $X_8$ | | | | | | | | 1 | -0.08 | 0.145* | 0.024 | 0.014 | 0.017 | 0.052 |
| $X_9$ | | | | | | | | | 1 | -0.231** | 0.449** | -0.072 | -0.093 | -0.109 |
| $X_{10}$ | | | | | | | | | | 1 | -0.308** | 0.003 | -0.031 | 0.187* |
| $X_{11}$ | | | | | | | | | | | 1 | 0.24** | -0.122 | 0.129 |
| $X_{12}$ | | | | | | | | | | | | 1 | -0.106 | 0.385** |
| $X_{13}$ | | | | | | | | | | | | | 1 | 0.01 |
| $X_{14}$ | | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF
$X_2$: % GLA 30 DAF
$X_3$: % GLA 45 DAF
$X_4$: Carbon isotope discrimination
$X_5$: Days to 50 % flowering
$X_6$: GLA
$X_7$: Earhead length
$X_8$: Number of leaves
$X_9$: Panicle exertion
$X_{10}$: Stem girth
$X_{11}$: Plant height
$X_{12}$: Spicklets per head
$X_{13}$: 100 grain weight
$X_{14}$: Grain yield per plant

**Appendix Vi: Analysis of variance for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2009 at Dharwad location**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
| 1. | Genotype | 225 | 273.00** | 422.00** | 1241.00** | 102.00** | 233984.00** | 28.70** | 2.90** | 84.00** | 1.20* | 2425.00** | 537.00** | 1.18 | 315.00** |
| 2. | Replication | 3 | 693.00 | 224.00 | 648.00 | 2287.00 | 1528070.00 | 1004.00 | 202.00 | 62.00 | 356.00 | 1754.00 | 7552.00 | 168.00 | 1231.00 |
| 3. | Error | 675 | 17.91 | 21.12 | 26.60 | 3.17 | 130.00 | 6.30 | 0.50 | 1.20 | 0.50 | 370.00 | 157.00 | 0.50 | 129.00 |
| 4. | SEm± | | 2.11 | 2.29 | 2.58 | 0.89 | 18.04 | 1.25 | 0.37 | 0.56 | 0.36 | 9.62 | 6.26 | 0.36 | 5.68 |
| 5. | CD at 5% | | 5.86 | 6.36 | 7.15 | 2.47 | 50.01 | 3.48 | 1.04 | 1.56 | 1.00 | 26.67 | 17.37 | 1.01 | 15.76 |
| 6. | CD at 1% | | 7.71 | 8.37 | 9.40 | 3.24 | 65.73 | 4.57 | 1.36 | 2.05 | 1.32 | 35.05 | 22.86 | 1.33 | 20.71 |
| 7. | CV (%) | | 14.67 | 5.96 | 8.40 | 12.87 | 12.96 | 13.89 | 9.72 | 9.02 | 14.73 | 10.79 | 21.99 | 18.86 | 20.83 |

* - Significance at 5% probability level ** - Significance at 1% probability level

$X_1$: % GLA 15 DAF $X_2$: % GLA 30 DAF $X_3$: % GLA 45 DAF $X_4$: Days to 50 % flowering $X_5$: Total GLA
$X_6$: Earhead length $X_7$: Number of leaves $X_8$: Panicle exertion $X_9$: Stem girth $X_{10}$: Plant height
$X_{11}$: Spicklets per head $X_{12}$: 100 grain weight $X_{13}$: Grain yield per plant

**Appendix Vj: Magnitude of genetic variability parameters for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2009 at Dharwad location**

| Sl. No. | Traits | Range | | Grand mean | GCV (%) | PCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Min | Max | | | | | |
| 1. | % GLA 15 DAF | 46.24 | 99.00 | 72.62 | 8.83 | 9.99 | 78.10 | 20.05 |
| 2. | % GLA 30 DAF | 32.03 | 93.26 | 62.64 | 12.99 | 14.29 | 82.60 | 29.93 |
| 3. | % GLA 45 DAF | 11.78 | 83.47 | 46.12 | 28.44 | 29.66 | 91.90 | 74.61 |
| 4. | Days to 50 % flowering | 50.16 | 79.42 | 64.74 | 8.02 | 8.52 | 88.70 | 15.40 |
| 5. | Total green leaf area ($cm^2$) | 578.75 | 1834.28 | 1206.51 | 19.74 | 19.96 | 97.80 | 40.73 |
| 6. | Earhead length (cm) | 5.81 | 25.26 | 15.53 | 13.09 | 19.08 | 47.00 | 21.51 |
| 7. | Number of leaves | 5.58 | 10.58 | 8.08 | 9.96 | 13.91 | 51.30 | 13.99 |
| 8. | Panicle exertion (cm) | 2.78 | 23.47 | 13.12 | 36.65 | 37.74 | 84.30 | 69.74 |
| 9. | Stem girth (cm) | 1.71 | 6.53 | 4.12 | 8.80 | 17.14 | 26.40 | 10.92 |
| 10. | Plant height (cm) | 98.66 | 267.25 | 182.95 | 12.70 | 16.67 | 58.10 | 19.45 |
| 11. | Spicklets per head | 18.00 | 57.92 | 37.96 | 24.89 | 40.53 | 37.70 | 32.52 |
| 12. | 100 grain weight (g) | 2.25 | 5.75 | 4.00 | 10.30 | 21.49 | 23.00 | 9.75 |
| 13. | Grain yield per plant (g/plant) | 24.02 | 161.37 | 92.69 | 18.50 | 35.95 | 3.10 | 7.55 |

**Appendix Vk: Phenotypic correlation coefficient for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2009 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.559** | 0.441** | -0.012 | 0.022 | -0.031 | 0.057 | -0.076 | 0.006 | 0.082 | 0.058 | 0.047 | 0.048 |
| $X_2$ | | 1 | 0.667** | -0.021 | 0.051 | -0.006 | 0.104 | -0.038 | 0.043 | 0.116 | 0.053 | -0.013 | 0.16* |
| $X_3$ | | | 1 | -0.055 | 0.077 | -0.013 | 0.045 | -0.07 | 0.007 | 0.057 | 0.145* | 0.012 | 0.142* |
| $X_4$ | | | | 1 | -0.116 | -0.015 | 0.093 | -0.027 | 0.053 | 0.026 | -0.043 | 0.002 | -0.011 |
| $X_5$ | | | | | 1 | 0.018 | 0.059 | -0.028 | -0.105 | -0.131 | 0.13 | -0.028 | 0.052 |
| $X_6$ | | | | | | 1 | 0.027 | -0.002 | 0.036 | 0.017 | -0.009 | -0.011 | 0.012 |
| $X_7$ | | | | | | | 1 | -0.047 | -0.081 | 0.004 | -0.007 | -0.025 | 0.029 |
| $X_8$ | | | | | | | | 1 | -0.123 | 0.325** | -0.048 | -0.048 | -0.068 |
| $X_9$ | | | | | | | | | 1 | -0.046 | 0.054 | -0.02 | 0.172* |
| $X_{10}$ | | | | | | | | | | 1 | 0.108 | -0.081 | 0.047 |
| $X_{11}$ | | | | | | | | | | | 1 | 0.012 | 0.275** |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.038 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix VI: Genotypic correlation coefficient for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2009 at Dharwad location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.626** | 0.503** | -0.014 | 0.025 | -0.021 | 0.064 | -0.086 | 0.047 | 0.117 | 0.099 | 0.071 | 0.148* |
| $X_2$ | | 1 | 0.733** | -0.029 | 0.062 | -0.021 | 0.134 | -0.043 | 0.084 | 0.156* | 0.085 | 0.034 | 0.332** |
| $X_3$ | | | 1 | -0.06 | 0.081 | -0.007 | 0.061 | -0.083 | 0.005 | 0.083 | 0.261** | 0.031 | 0.304** |
| $X_4$ | | | | 1 | -0.127 | -0.024 | 0.132 | -0.031 | 0.123 | 0.011 | -0.037 | 0.074 | -0.002 |
| $X_5$ | | | | | 1 | 0.033 | 0.083 | -0.027 | -0.199** | -0.185** | 0.206** | -0.05 | 0.092 |
| $X_6$ | | | | | | 1 | 0.071 | -0.015 | 0.157* | 0.01 | -0.037 | -0.116 | 0.05 |
| $X_7$ | | | | | | | 1 | -0.08 | -0.145* | 0.007 | -0.007 | -0.017 | 0.08 |
| $X_8$ | | | | | | | | 1 | -0.231** | 0.430** | -0.093 | -0.093 | -0.106 |
| $X_9$ | | | | | | | | | 1 | -0.198** | 0.012 | -0.031 | 0.189* |
| $X_{10}$ | | | | | | | | | | 1 | 0.221** | -0.195** | 0.09 |
| $X_{11}$ | | | | | | | | | | | 1 | 0.148* | 0.293** |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.001 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix VIa: Analysis of variance for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2008 at Bheemarayanagudi location.**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
| 1. | Genotype | 225 | 93.00** | 490.70** | 919.00** | 148.00** | 300595.00** | 38.60** | 3.90** | 185.00** | 1.18 | 2512.00** | 291.00** | 1.20* | 461.00** |
| 2. | Replication | 3 | 383.00 | 2064.00 | 55.27 | 1426 | 908317.00 | 637.00 | 46.00 | 3.00 | 3.20 | 2511.00 | 279.00 | 31.60 | 24.00 |
| 3. | Error | 675 | 21.30 | 0.79 | 11.60 | 5.50 | 32453.00 | 14.81 | 0.50 | 3.40 | 0.44 | 177.00 | 2.50 | 0.24 | 7.82 |
| 4. | SEm± | | 2.31 | 1.23 | 1.70 | 1.18 | 90.07 | 1.92 | 0.38 | 0.93 | 0.33 | 6.65 | 0.79 | 0.24 | 1.40 |
| 5. | CD at 5% | | 6.41 | 4.10 | 4.72 | 3.26 | 249.67 | 5.33 | 1.05 | 2.59 | 0.92 | 18.44 | 2.20 | 0.68 | 3.88 |
| 6. | CD at 1% | | 8.42 | 1.61 | 6.20 | 4.28 | 328.14 | 7.01 | 1.37 | 3.40 | 1.20 | 24.23 | 2.89 | 0.89 | 5.09 |
| 7. | CV (%) | | 5.17 | 13.36 | 10.11 | 4.39 | 17.08 | 21.46 | 4.06 | 11.19 | 14.86 | 7.52 | 4.37 | 14.47 | 9.20 |

* - Significance at 5% probability level ** - Significance at 1% probability level

$X_1$: % GLA 15 DAF $X_2$: % GLA 30 DAF $X_3$: % GLA 45 DAF $X_4$: Days to 50 % flowering $X_5$: Total GLA
$X_6$: Earhead length $X_7$: Number of leaves $X_8$: Panicle exertion $X_9$: Stem girth $X_{10}$: Plant height
$X_{11}$: Spicklets per head $X_{12}$: 100 grain weight $X_{13}$: Grain yield per plant

**Appendix VIb: Magnitude of genetic variability parameters for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2008 at Bheemarayanagudi location**

| Sl. No. | Traits | Range | | Grand mean | GCV (%) | PCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Min | Max | | | | | |
| 1. | % GLA 15 DAF | 45.78 | 99.50 | 72.64 | 4.70 | 6.90 | 45.46 | 10.27 |
| 2. | % GLA 30 DAF | 24.88 | 90.75 | 57.81 | 16.82 | 16.82 | 99.34 | 49.77 |
| 3. | % GLA 45 DAF | 12.39 | 80.87 | 46.63 | 44.18 | 45.34 | 94.94 | 81.45 |
| 4. | Days to 50 % flowering | 57.67 | 70.42 | 64.04 | 11.09 | 11.91 | 86.67 | 22.74 |
| 5. | Total green leaf area ($cm^2$) | 333.83 | 1772.35 | 1053.09 | 24.22 | 29.66 | 66.67 | 52.11 |
| 6. | Earhead length (cm) | 10.60 | 34.51 | 22.55 | 13.58 | 25.47 | 28.45 | 15.12 |
| 7. | Number of leaves | 5.17 | 13.17 | 9.17 | 13.38 | 17.37 | 59.30 | 20.17 |
| 8. | Panicle exertion (cm) | 1.75 | 32.03 | 16.89 | 40.12 | 41.60 | 93.03 | 10.71 |
| 9. | Stem girth (cm) | 2.91 | 6.78 | 4.84 | 9.36 | 17.54 | 28.48 | 11.98 |
| 10. | Plant height (cm) | 118.33 | 237.33 | 177.83 | 13.68 | 15.62 | 76.72 | 31.34 |
| 11. | Spicklets per head | 18.50 | 60.00 | 39.25 | 23.20 | 28.48 | 97.67 | 55.72 |
| 12. | 100 grain weight (g) | 1.10 | 4.47 | 2.70 | 14.36 | 20.39 | 49.60 | 33.33 |
| 13. | Grain yield per plant (g/plant) | 2.38 | 165.89 | 94.63 | 34.49 | 35.70 | 93.30 | 28.01 |

**Appendix VIc: Phenotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2008 at Bheemarayanagudi location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.312** | 0.123 | -0.174* | 0.162* | 0.028 | 0.128 | -0.082 | 0.034 | -0.092 | 0.143* | 0.132 | 0.037 |
| $X_2$ | | 1 | 0.408** | -0.034 | 0.219** | 0.025 | 0.178* | -0.06 | 0.022 | -0.008 | 0.116 | 0.077 | 0.064 |
| $X_3$ | | | 1 | -0.045 | 0.152* | 0.094 | 0.201* | -0.09 | 0.085 | -0.057 | 0.057 | 0.009 | 0.160* |
| $X_4$ | | | | 1 | 0.052 | -0.026 | 0.017 | 0.08 | -0.084 | 0.001 | -0.088 | -0.125 | 0.001 |
| $X_5$ | | | | | 1 | 0.106 | 0.337** | -0.194** | 0.216* | 0.227* | 0.183** | 0.011 | 0.045 |
| $X_6$ | | | | | | 1 | 0.047 | 0.015 | 0.009 | 0.032 | 0.047 | -0.052 | 0.011 |
| $X_7$ | | | | | | | 1 | -0.138* | -0.111 | 0.122 | 0.148* | 0.032 | 0.044 |
| $X_8$ | | | | | | | | 1 | -0.138* | 0.334** | -0.085 | -0.003 | -0.049 |
| $X_9$ | | | | | | | | | 1 | 0.038 | 0.051 | 0.004 | -0.045 |
| $X_{10}$ | | | | | | | | | | 1 | -0.133 | -0.011 | -0.025 |
| $X_{11}$ | | | | | | | | | | | 1 | -0.046 | 0.023 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.129 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix VId: Genotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2008 at Bheemarayanagudi location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.465** | 0.183** | -0.304** | 0.286** | 0.059 | 0.212** | -0.128 | 0.108 | -0.134 | 0.213** | 0.288** | 0.075 |
| $X_2$ | | 1 | 0.42** | -0.037 | 0.271** | 0.039 | 0.231** | -0.063 | 0.044 | -0.009 | 0.118 | 0.108 | 0.067 |
| $X_3$ | | | 1 | -0.051 | 0.197** | 0.183** | 0.261** | -0.096 | 0.151* | -0.067 | 0.058 | 0.024 | 0.173* |
| $X_4$ | | | | 1 | 0.069 | -0.066 | 0.017 | 0.091 | -0.158* | 0.012 | -0.088 | -0.213** | 0.001 |
| $X_5$ | | | | | 1 | 0.203** | 0.543** | -0.233** | 0.537** | 0.308** | 0.223** | 0.043 | 0.052 |
| $X_6$ | | | | | | 1 | 0.183** | 0.039 | 0.062 | 0.052 | 0.099 | -0.171* | 0.024 |
| $X_7$ | | | | | | | 1 | -0.184** | -0.294** | 0.205** | 0.205** | 0.014 | 0.051 |
| $X_8$ | | | | | | | | 1 | -0.264** | 0.383** | -0.096 | -0.009 | -0.053 |
| $X_9$ | | | | | | | | | 1 | 0.052 | 0.108 | 0.063 | -0.085 |
| $X_{10}$ | | | | | | | | | | 1 | -0.155** | -0.028 | -0.032 |
| $X_{11}$ | | | | | | | | | | | 1 | -0.071 | 0.024 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.182** |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix VIe: Analysis of variance for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2009 at Bheemarayanagudi location**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
| 1. | Genotype | 225 | 93.00** | 486.00** | 1186.61** | 149.00** | 243448.00** | 26.40** | 2.60** | 117.83** | $1.05^{NS}$ | 3119.00** | 299.00** | 1.20* | 462.00** |
| 2. | Replication | 3 | 384.00 | 2262.00 | 2743.00 | 316.00 | 9730366.00 | 177.00 | 4.70 | 1523.10 | 0.90 | 76650.00 | 681.00 | 23.92 | 23.80 |
| 3. | Error | 675 | 21.40 | 1.04 | 99.60 | 10.26 | 76252.00 | 14.60 | 0.70 | 28.70 | 0.70 | 1243.00 | 1.20 | 0.24 | 8.30 |
| 4. | SEm± | | 8.43 | 1.86 | 9.71 | 0.90 | 502.99 | 6.31 | 1.58 | 6.77 | 1.25 | 61.82 | 2.04 | 0.36 | 2.10 |
| 5. | CD at 5% | | 2.31 | 0.51 | 2.66 | 0.25 | 138.07 | 1.73 | 0.43 | 1.86 | 0.34 | 16.97 | 0.56 | 0.92 | 4.30 |
| 6. | CD at 1% | | 8.42 | 1.86 | 18.18 | 5.83 | 502.99 | 7.01 | 1.58 | 9.76 | 1.54 | 64.22 | 2.03 | 0.90 | 5.26 |
| 7. | CV (%) | | 5.17 | 4.67 | 18.99 | 5.90 | 19.94 | 21.67 | 11.26 | 24.22 | 22.52 | 18.24 | 3.08 | 12.77 | 9.00 |

* - Significance at 5% probability level ** - Significance at 1% probability level

$X_1$: % GLA 15 DAF $X_2$: % GLA 30 DAF $X_3$: % GLA 45 DAF $X_4$: Days to 50 % flowering $X_5$: Total GLA
$X_6$: Earhead length $X_7$: Number of leaves $X_8$: Panicle exertion $X_9$: Stem girth $X_{10}$: Plant height
$X_{11}$: Spicklets per head $X_{12}$: 100 grain weight $X_{13}$: Grain yield per plant

**Appendix VIf: Magnitude of genetic variability parameters for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2009 at Bheemarayanagudi location**

| Sl. No. | Traits | Range | | Grand mean | GCV (%) | PCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Min | Max | | | | | |
| 1. | % GLA 15 DAF | 83.12 | 98.72 | 90.92 | 4.74 | 7.01 | 45.14 | 8.29 |
| 2. | % GLA 30 DAF | 40.94 | 76.06 | 58.50 | 17.96 | 18.04 | 99.14 | 49.32 |
| 3. | % GLA 45 DAF | 24.23 | 80.43 | 52.33 | 47.86 | 55.95 | 73.15 | 71.89 |
| 4. | Days to 50 % flowering | 49.92 | 66.58 | 58.25 | 10.86 | 12.36 | 77.18 | 23.38 |
| 5. | Total green leaf area ($cm^2$) | 858.70 | 1845.91 | 1352.50 | 14.76 | 24.81 | 35.40 | 23.66 |
| 6. | Earhead length (cm) | 14.82 | 22.15 | 18.48 | 10.12 | 24.23 | 17.46 | 6.17 |
| 7. | Number of leaves | 7.00 | 8.33 | 7.66 | 9.00 | 14.41 | 39.00 | 14.75 |
| 8. | Panicle exertion (cm) | 4.90 | 18.50 | 11.70 | 32.14 | 45.60 | 43.68 | 70.17 |
| 9. | Stem girth (cm) | 3.38 | 5.98 | 4.68 | 7.66 | 23.75 | 10.42 | 5.13 |
| 10. | Plant height (cm) | 185.00 | 249.58 | 217.29 | 11.20 | 21.41 | 27.38 | 13.71 |
| 11. | Spicklets per head | 26.42 | 62.58 | 44.50 | 23.79 | 23.99 | 98.35 | 50.63 |
| 12. | 100 grain weight (g) | 2.10 | 3.80 | 2.95 | 13.13 | 18.32 | 51.91 | 32.54 |
| 13. | Grain yield per plant (g/plant) | 24.20 | 165.01 | 94.60 | 33.13 | 34.34 | 93.13 | 28.57 |

**Appendix VIg: Phenotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2009 at Bheemarayanagudi location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.302** | 0.192** | -0.153* | 0.122 | 0.002 | 0.094 | -0.034 | 0.017 | 0.076 | 0.145* | 0.129 | 0.036 |
| $X_2$ | | 1 | 0.427** | -0.010 | 0.085 | -0.028 | 0.174* | -0.039 | 0.001 | 0.045 | 0.122 | 0.008 | 0.055 |
| $X_3$ | | | 1 | -0.016 | 0.130 | 0.015 | 0.114 | -0.080 | 0.050 | 0.026 | 0.071 | 0.015 | 0.090 |
| $X_4$ | | | | 1 | -0.073 | 0.056 | -0.014 | 0.076 | -0.060 | -0.032 | -0.056 | -0.145* | -0.007 |
| $X_5$ | | | | | 1 | 0.065 | 0.239** | -0.075 | 0.222** | -0.084 | 0.181** | 0.030 | 0.045 |
| $X_6$ | | | | | | 1 | 0.039 | 0.055 | -0.018 | 0.060 | 0.03 | 0.070 | 0.027 |
| $X_7$ | | | | | | | 1 | -0.102 | 0.106 | -0.079 | 0.128 | 0.021 | 0.054 |
| $X_8$ | | | | | | | | 1 | -0.097 | 0.215** | -0.052 | 0.033 | -0.033 |
| $X_9$ | | | | | | | | | 1 | -0.012 | 0.081 | -0.018 | -0.048 |
| $X_{10}$ | | | | | | | | | | 1 | 0.081 | -0.030 | -0.043 |
| $X_{11}$ | | | | | | | | | | | 1 | -0.035 | 0.033 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.122 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix VIh: Genotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross IS9830 x E36-1 evaluated during 2009 at Bheemarayanagudi location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.449** | 0.301** | -0.282** | 0.191** | 0.016 | 0.238** | -0.04 | 0.156* | 0.096 | 0.213** | 0.327** | 0.072 |
| $X_2$ | | 1 | 0.506** | -0.013 | 0.142* | -0.066 | 0.288** | -0.058 | 0.006 | 0.077 | 0.124 | -0.029 | 0.058 |
| $X_3$ | | | 1 | -0.030 | 0.235** | 0.055 | 0.231** | -0.174* | 0.111 | 0.043 | 0.079 | 0.023 | 0.112 |
| $X_4$ | | | | 1 | -0.118 | 0.011 | -0.002 | 0.131 | -0.139* | -0.057 | -0.065 | -0.253** | -0.007 |
| $X_5$ | | | | | 1 | 0.381** | 0.473** | -0.157* | 0.721** | -0.306** | 0.311** | 0.046 | 0.089 |
| $X_6$ | | | | | | 1 | 0.044 | 0.175* | -0.193** | 0.320** | 0.079 | 0.271** | 0.088 |
| $X_7$ | | | | | | | 1 | -0.127 | 0.595** | -0.155* | 0.203** | -0.017 | 0.102 |
| $X_8$ | | | | | | | | 1 | -0.155* | 0.535** | -0.080 | 0.118 | -0.037 |
| $X_9$ | | | | | | | | | 1 | -0.239** | 0.258** | -0.175* | -0.137 |
| $X_{10}$ | | | | | | | | | | 1 | 0.149* | -0.114 | -0.070 |
| $X_{11}$ | | | | | | | | | | | 1 | 0.047 | 0.034 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.169* |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix VIIa: Analysis of variance for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2008 at Bheemarayanagudi location**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
| 1. | Genotype | 225 | 387.00** | 1000.00** | 1299.00** | 144.78** | 243195.00** | 54.60** | 4.20** | 119.00** | 1.60** | 2141.00** | 354.00** | $0.90^{NS}$ | 494.00** | 0.13** |
| 2. | Replication | 3 | 6669.00 | 519.00 | 621.00 | 18986.00 | 1763439.00 | 81.00 | 3.50 | 3.70 | 30.40 | 1476.00 | 746.00 | 16.14 | 152.00 | 0.40 |
| 3. | Error | 675 | 126.00 | 32.00 | 30.65 | 2.01 | 59653.00 | 9.20 | 0.40 | 1.70 | 0.50 | 4.08 | 20.00 | 0.39 | 13.00 | 0.13 |
| 4. | SEm± | | 5.63 | 2.49 | 2.77 | 0.71 | 122.12 | 1.52 | 0.34 | 0.67 | 0.36 | 28.02 | 2.25 | 0.31 | 1.84 | 0.26 |
| 5. | CD at 5% | | 15.60 | 6.91 | 7.67 | 1.96 | 338.50 | 4.22 | 0.93 | 1.84 | 1.00 | 28.02 | 6.24 | 0.87 | 5.10 | 1.73 |
| 6. | CD at 1% | | 20.50 | 2.47 | 10.80 | 2.58 | 444.88 | 5.54 | 1.22 | 2.42 | 1.30 | 36.82 | 8.20 | 1.14 | 6.69 | 0.96 |
| 7. | CV (%) | | 14.07 | 8.30 | 16.35 | 3.19 | 18.04 | 17.47 | 9.09 | 11.59 | 16.38 | 11.10 | 12.21 | 15.58 | 8.96 | 8.85 |

* - Significance at 5% probability level ** - Significance at 1% probability level

$X_1$: % GLA 15 DAF $X_2$: % GLA 30 DAF $X_3$: % GLA 45 DAF $X_4$: Days to 50 % flowering $X_5$: Total GLA
$X_6$: Earhead length $X_7$: Number of leaves $X_8$: Panicle exertion $X_9$: Stem girth $X_{10}$: Plant height
$X_{11}$: Spicklets per head $X_{12}$: 100 grain weight $X_{13}$: Grain yield per plant $X_{14}$: Carbon isotope discrimination

**Appendix VIIb: Magnitude of genetic variability parameters for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2008 at Bheemarayanagudi location**

| Sl. No. | Traits | Range | | Grand mean | GCV (%) | PCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Min | Max | | | | | |
| 1. | % GLA 15 DAF | 66.30 | 100.00 | 83.15 | 10.10 | 17.32 | 33.99 | 14.89 |
| 2. | % GLA 30 DAF | 27.73 | 88.81 | 58.27 | 14.83 | 25.22 | 80.01 | 71.39 |
| 3. | % GLA 45 DAF | 11.57 | 70.93 | 46.25 | 52.60 | 55.08 | 91.19 | 96.69 |
| 4. | Days to 50 % flowering | 59.80 | 71.96 | 65.88 | 9.23 | 9.49 | 94.67 | 23.19 |
| 5. | Total green leaf area ($cm^2$) | 462.39 | 2043.78 | 1253.08 | 15.82 | 24.00 | 43.48 | 29.64 |
| 6. | Earhead length (cm) | 10.13 | 25.92 | 18.02 | 19.31 | 26.04 | 54.99 | 36.40 |
| 7. | Number of leaves | 5.00 | 10.17 | 7.58 | 13.18 | 16.02 | 67.71 | 27.70 |
| 8. | Panicle exertion (cm) | 1.50 | 24.33 | 12.91 | 47.30 | 48.70 | 94.34 | 10.51 |
| 9. | Stem girth (cm) | 1.63 | 6.45 | 4.04 | 12.25 | 20.45 | 35.85 | 20.79 |
| 10. | Plant height (cm) | 123.67 | 267.42 | 195.54 | 11.43 | 15.94 | 51.46 | 20.08 |
| 11. | Spicklets per head | 12.50 | 59.00 | 35.75 | 24.77 | 27.62 | 80.47 | 60.31 |
| 12. | 100 grain weight (g) | 2.70 | 5.15 | 3.92 | 9.75 | 18.41 | 28.03 | 13.78 |
| 13. | Grain yield per plant (g/plant) | 23.57 | 184.28 | 103.52 | 26.75 | 28.21 | 89.90 | 26.42 |
| 14. | Carbon isotope discrimination | 3.19 | 5.74 | 4.46 | 3.32 | 8.73 | 1.02 | 17.26 |

**Appendix VIIc: Phenotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2008 at Bheemarayanagudi location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.383** | 0.336** | 0.019 | -0.007 | 0.1 | -0.009 | 0.007 | -0.053 | 0.031 | -0.021 | 0.076 | 0.101 | 0.022 |
| $X_2$ | | 1 | 0.443** | 0.003 | -0.161* | 0.009 | -0.015 | 0.040 | -0.045 | 0.053 | -0.020 | 0.008 | 0.083 | 0.060 |
| $X_3$ | | | 1 | 0.041 | -0.07 | 0.01 | -0.035 | 0.085 | -0.094 | 0.059 | -0-.012 | 0.100 | 0.083 | 0.034 |
| $X_4$ | | | | 1 | -0.054 | 0.086 | 0.068 | 0.032 | 0.086 | -0.088 | 0.009 | -0.037 | 0.023 | 0.014 |
| $X_5$ | | | | | 1 | -0.06 | 0.029 | 0.068 | 0.119 | 0.005 | 0.045 | 0.001 | 0.004 | -0.019 |
| $X_6$ | | | | | | 1 | -0.047 | 0.103 | -0.006 | 0.167* | 0.077 | 0.007 | -0.045 | 0.167* |
| $X_7$ | | | | | | | 1 | -0.007 | 0.146* | -0.076 | 0.124 | 0.048 | 0.078 | 0.008 |
| $X_8$ | | | | | | | | 1 | -0.075 | 0.006 | 0.085 | 0.055 | -0.023 | 0.285** |
| $X_9$ | | | | | | | | | 1 | -0.045 | 0.382** | -0.164* | -0.03 | -0.154* |
| $X_{10}$ | | | | | | | | | | 1 | -0.003 | -0.180* | 0.028 | 0.054 |
| $X_{11}$ | | | | | | | | | | | 1 | -0.062 | -0.019 | 0.019 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.005 | 0.161* |
| $X_{13}$ | | | | | | | | | | | | | 1 | -0.083 |
| $X_{14}$ | | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF
$X_2$: % GLA 30 DAF
$X_3$: % GLA 45 DAF
$X_4$: Carbon isotope discrimination
$X_5$: Days to 50 % flowering
$X_6$: GLA
$X_7$: Earhead length
$X_8$: Number of leaves
$X_9$: Panicle exertion
$X_{10}$: Stem girth
$X_{11}$: Plant height
$X_{12}$: Spicklets per head
$X_{13}$: 100 grain weight
$X_{14}$: Grain yield per plant

**Appendix VIId: Genotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2008 at Bheemarayanagudi location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.661** | 0.558** | 0.066 | -0.025 | 0.114 | -0.067 | 0.039 | -0.106 | 0.175* | -0.044 | 0.130 | 0.254* | 0.026 |
| $X_2$ | | 1 | 0.464** | 0.018 | -0.165* | 0.014 | -0.021 | 0.048 | -0.046 | 0.091 | -0.028 | 0.009 | 0.162* | 0.063 |
| $X_3$ | | | 1 | 0.250** | -0.076 | 0.019 | -0.047 | 0.108 | -0.102 | 0.121 | -0.005 | 0.113 | 0.199* | 0.040 |
| $X_4$ | | | | 1 | -0.322** | 0.734** | 0.293* | 0.415** | 0.486** | -0.633** | 0.057 | -0.277* | 0.037 | 0.191** |
| $X_5$ | | | | | 1 | -0.108 | 0.043 | 0.082 | 0.116 | 0.021 | 0.055 | 0.005 | 0.006 | -0.021 |
| $X_6$ | | | | | | 1 | -0.197** | 0.219* | -0.002 | 0.323** | 0.163* | 0.009 | -0.01 | 0.257** |
| $X_7$ | | | | | | | 1 | -0.037 | 0.194** | -0.181** | 0.204* | 0.078 | 0.044 | 0.007 |
| $X_8$ | | | | | | | | 1 | -0.094 | 0.024 | 0.149* | 0.039 | -0.004 | 0.355** |
| $X_9$ | | | | | | | | | 1 | -0.076 | 0.551** | -0.179* | -0.07 | -0.170* |
| $X_{10}$ | | | | | | | | | | 1 | -0.023 | -0.301** | 0.225** | 0.106 |
| $X_{11}$ | | | | | | | | | | | 1 | -0.087 | -0.134 | 0.021 |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.015 | 0.185** |
| $X_{13}$ | | | | | | | | | | | | | 1 | 0.159* |
| $X_{14}$ | | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF
$X_2$: % GLA 30 DAF
$X_3$: % GLA 45 DAF
$X_4$: Carbon isotope discrimination
$X_5$: Days to 50 % flowering
$X_6$: GLA
$X_7$: Earhead length
$X_8$: Number of leaves
$X_9$: Panicle exertion
$X_{10}$: Stem girth
$X_{11}$: Plant height
$X_{12}$: Spicklets per head
$X_{13}$: 100 grain weight
$X_{14}$: Grain yield per plant

**Appendix VIIe: Analysis of variance for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2009 at Bheemarayanagudi location**

| Sl. No. | Source of variation | | Mean sum of squares | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components | d.f. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
| 1. | Genotype | 225 | 379.00** | 984.50** | 1274.00** | 144.40** | 260282.00** | 53.80** | 2.20** | 108.10** | 1.03.00$^{NS}$ | 5995.00** | 351.30** | 1.04$^{NS}$ | 515.00** |
| 2. | Replication | 3 | 8702.00 | 1661.00 | 390.00 | 1891.00 | 2991214.00 | 324.80 | 44.66 | 201.20 | 28.50 | 29000.00 | 1063.00 | 7.54 | 9.10 |
| 3. | Error | 675 | 124.00 | 1.90 | 33.10 | 2.07 | 718.71 | 13.96 | 1.10 | 11.40 | 0.77 | 3534.00 | 20.17 | 0.25 | 5.50 |
| 4. | SEm$\pm$ | | 20.32 | 2.54 | 10.49 | 0.93 | 488.32 | 6.81 | 1.91 | 6.16 | 1.60 | 108.29 | 8.18 | 0.23 | 2.50 |
| 5. | CD at 5% | | 5.58 | 0.70 | 2.88 | 0.25 | 134.04 | 1.87 | 0.52 | 1.69 | 0.44 | 29.73 | 2.25 | 0.72 | 4.17 |
| 6. | CD at 1% | | 20.32 | 2.54 | 10.48 | 2.58 | 488.32 | 6.80 | 1.91 | 6.16 | 1.60 | 108.29 | 1.18 | 0.92 | 4.31 |
| 7. | CV (%) | | 13.96 | 12.60 | 19.12 | 2.29 | 18.47 | 20.04 | 12.79 | 18.70 | 22.73 | 20.17 | 12.17 | 11.56 | 5.59 |

* - Significance at 5% probability level ** - Significance at 1% probability level

$X_1$: % GLA 15 DAF $X_2$: % GLA 30 DAF $X_3$: % GLA 45 DAF $X_4$: Days to 50 % flowering $X_5$: Total GLA
$X_6$: Earhead length $X_7$: Number of leaves $X_8$: Panicle exertion $X_9$: Stem girth $X_{10}$: Plant height
$X_{11}$: Spicklets per head $X_{12}$: 100 grain weight $X_{13}$: Grain yield per plant

**Appendix VIIf: Magnitude of genetic variability parameters for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2009 at Bheemarayanagudi location**

| Sl. No. | Traits | Range | | Grand mean | GCV (%) | PCV (%) | Heritability (%) | Genetic advance as % of mean |
|---|---|---|---|---|---|---|---|---|
| | | Min | Max | | | | | |
| 1. | % GLA 15 DAF | 72.71 | 96.41 | 84.56 | 10.00 | 17.17 | 33.88 | 14.45 |
| 2. | % GLA 30 DAF | 45.49 | 84.31 | 64.90 | 29.16 | 29.28 | 99.21 | 63.25 |
| 3. | % GLA 45 DAF | 34.44 | 80.30 | 57.37 | 58.50 | 61.55 | 90.34 | 76.76 |
| 4. | Days to 50 % flowering | 58.25 | 75.00 | 66.62 | 9.49 | 9.75 | 94.63 | 22.89 |
| 5. | Total green leaf area ($cm^2$) | 1439.43 | 2090.82 | 1765.12 | 14.95 | 23.77 | 39.59 | 20.35 |
| 6. | Earhead length (cm) | 10.78 | 21.58 | 16.18 | 16.94 | 26.25 | 41.65 | 33.07 |
| 7. | Number of leaves | 6.50 | 10.50 | 8.50 | 6.37 | 14.27 | 19.93 | 7.18 |
| 8. | Panicle exertion (cm) | 4.00 | 21.00 | 12.50 | 37.40 | 45.39 | 67.87 | 85.20 |
| 9. | Stem girth (cm) | 3.41 | 6.05 | 4.73 | 6.60 | 23.66 | 7.78 | 3.81 |
| 10. | Plant height (cm) | 124.33 | 206.00 | 165.16 | 12.59 | 32.69 | 14.82 | 15.20 |
| 11. | Spicklets per head | 24.50 | 58.40 | 41.45 | 24.66 | 27.50 | 80.40 | 51.75 |
| 12. | 100 grain weight (g) | 3.20 | 6.12 | 4.66 | 10.08 | 15.25 | 43.21 | 16.31 |
| 13. | Grain yield per plant (g/plant) | 22.41 | 190.48 | 106.44 | 26.63 | 27.21 | 95.76 | 27.22 |

**Appendix VIIg: Phenotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2009 at Bheemarayanagudi location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.376** | 0.318** | -0.015 | 0.006 | 0.013 | 0.078 | 0.048 | 0.025 | 0.016 | 0.070 | 0.105 | 0.015 |
| $X_2$ | | 1 | 0.439** | -0.152* | 0.003 | 0.047 | 0.045 | 0.008 | 0.029 | 0.036 | 0.009 | 0.980** | 0.053 |
| $X_3$ | | | 1 | -0.069 | 0.015 | 0.023 | 0.080 | 0.028 | 0.066 | 0.016 | 0.087 | 0.101 | 0.034 |
| $X_4$ | | | | 1 | -0.008 | 0.005 | -0.054 | -0.034 | -0.013 | 0.005 | 0.008 | -0.003 | -0.020 |
| $X_5$ | | | | | 1 | 0.092 | 0.019 | -0.023 | -0.121 | -0.065 | 0.030 | 0.028 | 0.110 |
| $X_6$ | | | | | | 1 | -0.004 | -0.191** | -0.032 | -0.007 | 0.034 | 0.002 | 0.004 |
| $X_7$ | | | | | | | 1 | 0.003 | -0.118 | 0.012 | -0.035 | 0.003 | 0.156* |
| $X_8$ | | | | | | | | 1 | -0.023 | 0.098 | -0.122 | -0.043 | -0.107 |
| $X_9$ | | | | | | | | | 1 | -0.030 | -0.061 | 0.069 | -0.116 |
| $X_{10}$ | | | | | | | | | | 1 | -0.015 | 0.051 | 0.021 |
| $X_{11}$ | | | | | | | | | | | 1 | -0.007 | 0.159* |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.058 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

**Appendix VIIh: Genotypic correlation coefficients for stay-green and yield related traits in the recombinant inbred lines derived from the cross N13 x E36-1 evaluated during 2009 at Bheemarayanagudi location**

| Traits | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $X_1$ | 1 | 0.652** | 0.528** | -0.039 | 0.054 | 0.055 | 0.262* | 0.115 | -0.140* | 0.128 | 0.121 | 0.280** | 0.017 |
| $X_2$ | | 1 | 0.462** | -0.156* | 0.005 | 0.069 | 0.112 | 0.009 | 0.069 | 0.088 | 0.012 | 0.151* | 0.055 |
| $X_3$ | | | 1 | -0.074 | 0.016 | 0.036 | 0.225** | 0.034 | 0.054 | -0.043 | 0.104 | 0.173* | 0.043 |
| $X_4$ | | | | 1 | -0.015 | 0.001 | -0.040 | -0.042 | -0.008 | 0.013 | 0.013 | -0.008 | -0.022 |
| $X_5$ | | | | | 1 | 0.184** | 0.110 | -0.022 | -0.390** | -0.096 | 0.005 | 0.136 | 0.182** |
| $X_6$ | | | | | | 1 | -0.020 | -0.408** | -0.021 | -0.147* | 0.071 | 0.083 | 0.014 |
| $X_7$ | | | | | | | 1 | 0.107 | -0.311** | 0.121 | -0.013 | 0.013 | 0.376** |
| $X_8$ | | | | | | | | 1 | -0.064 | 0.274** | -0.155* | -0.013 | -0.125 |
| $X_9$ | | | | | | | | | 1 | -0.203** | -0.22** | 0.317** | -0.423** |
| $X_{10}$ | | | | | | | | | | 1 | -0.043 | 0.056 | 0.001 |
| $X_{11}$ | | | | | | | | | | | 1 | -0.022 | 0.187** |
| $X_{12}$ | | | | | | | | | | | | 1 | 0.102 |
| $X_{13}$ | | | | | | | | | | | | | 1 |

** - Significant at 1% level of probability  * - Significant at 5% level of probability

$X_1$: % GLA 15 DAF  $X_2$: % GLA 30 DAF  $X_3$: % GLA 45 DAF  $X_4$: Days to 50 % flowering  $X_5$: Total GLA
$X_6$: Earhead length  $X_7$: Number of leaves  $X_8$: Panicle exertion  $X_9$: Stem girth  $X_{10}$: Plant height
$X_{11}$: Spicklets per head  $X_{12}$: 100 grain weight  $X_{13}$: Grain yield per plant

# GENOME-WIDE QTL MAPPING FOR POST-FLOWERING DROUGHT TOLERANCE AND VALIDATION OF CHARCOAL ROT RESISTANCE QTLs IN NILs OF SORGHUM

**SUVARNA PATIL** **2011** **DR. B. FAKRUDIN**
**MAJOR ADVISOR**

## ABSTRACT

Two Recombinant Inbred Line Populations (RIP) derived from the cross IS9830 x E36-1 (RIP1) and N13 x E36-1 (RIP2) were field evaluated for stay-green and yield related traits during *rabi* season at two locations over three years. Parents IS9830 and N13 were non-stay-green and E36-1 was a stay-green donor. Analysis of variance showed positive significant association in both RIPs. A total of 530 genic and 270 nuclear SSR markers were screened to finally genotype 40 genic, 71 nuclear SSR markers for RIP1 and 68 genic and 70 nuclear SSR for RIP2 to construct genetic linkage maps together with 46 anchor markers. The genetic linkage maps spanned over 1661.1 cM and 2003.8 cM for RIP1 and RIP2, respectively. Same were used for mapping the stable QTLs for stay-green components: *stg1* (GLA15DAF) flanked by Xtxp34-Xtxp285 on LG-A, *stg2* (GLA30DAF) flanked by Xtxp205-Xtxp231 on LG-E, *stg3* (GLA45DAF) flanked by Xtxp298-Xtxp324 on LG-J and δC flanked by Xtxp6-Xiabt73 on LG-I, together accounting for 40.91 and 48.76 per cent of phenotypic variance in RIP1 and RIP2, respectively; the positive additive effects at all the loci was contributed by E36-1. The stable QTLs for other yield related traits were also mapped.

The stable QTLs for component traits of charcoal rot resistance *viz*., *cr1* (Xtxp176-Xiabt312) for lodging per cent on LG-I, *cr2* (Xtxp297-Xiabt173) for number of internodes crossed by the fungus on LG-B and *cr3* (Xiabt275-Xiabt241) for length of infection on LG-I were introgressed from E36-1 into M35-1 and SPV86 susceptible backgrounds with recurrent foreground and background screening across $BC_1F_1$, $BC_2F_1$, $BC_3F_1$ generations and the $BC_3F_2$ progenies with various QTLs combinations in homozygous condition pinned down. The $BC_3G_3$ near isogenic lines (NILs) were evaluated: NILs with all the 3 QTLs recorded lodging per cent of 0.9 and 1.2 against 41.46 and 53.38 per cent incidence in controls, respectively in M35-1 and SPV86 backgrounds. The next best NIL in both backgrounds carried *cr1* and *cr2*. These NILs have immediate practical utility as resistant versions.