

MOLECULAR MAPPING OF QTLs FOR COMPONENT TRAITS OF DROUGHT TOLERANCE IN SORGHUM

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I. INTRODUCTION

Sorghum is one of the important food crops for a large section of people in South East Asia and Africa and also a good source of fodder for cattle. It is well adapted to low input, high temperature and low moisture situations. Global cultivation of sorghum covers an area of 43.73 m ha with annual production of 58.8 mt (Anon., 2004). India has the largest area in sorghum (9.90 m ha) and ranks seventh in production with 8.0 mt.

Sorghum is grown both during southwest monsoon (*khari*) and post monsoon seasons (*Rabi*) in India. Rabi crop produces high quality grains as they mature during winter season under clear dry and rain-free climate during maturity. In these areas rainfall is erratic and drought is a major constraint. Dry spells can occur at any time during the growing season, hence especially during terminal stages of the sorghum crop.

There are several factors of drought, which include precipitation, evapotranspiration, temperature, humidity and others, individually or in combination (Renu and Suresh, 1998). Although selection for genotypes with increased productivity in drought environments has been an important goal of many plant breeding programs, the biological basis for drought tolerance is still poorly understood. Further, drought stress is highly complex, varies in time and space and is unpredictable. This makes it difficult to evaluate reaction of genotypes to drought in a consistent manner. Crop productivity in a water-limited environment depends on mechanisms that either permit tolerance of episodes of cellular dehydration or that minimize water loss and there by maintaining a favorable water status for leaf development. Identification of genetic factors involved in plant responses to drought stress will provide a definite foundation to improve drought tolerance. Drought resistance *per se* 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. This trait is considered as the most difficult to improve through conventional plant breeding. In the recent years extensive research has lead to identification of component traits, sources of genes and field management practices to approach this complex problem.

Plants perceive water loss by triggering a cellular signal transduction pathway where physical stress is converted into a biochemical response (Ingram and Bartels, 1996). 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 (Shinozaki and Yamaguchi-Schinozaki, 1997) besides playing other important roles. 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. Further, stomatal closure during drought, however, leads to a limitation of carbon uptake and a subsequent reduction in leaf photosynthesis (Chaves, 1991). The accumulation of soluble sugars (sucrose, glucose and fructose) is strongly correlated with the acquisition of drought tolerance in plants (Hoekstra and Buitink, 2001). Generally, sugars function as osmoprotectants, as substrates for growth and play an important role in regulation of gene expression (Koch, 1996). Thus, response to drought in plants is a complex quantitative trait, which is difficult to phenotype.

Modern tools of molecular biology have been useful in genetic dissection of complex quantitative trait through the identification of genes, gene products and quantitative trait loci (QTLs) which dealing with drought tolerance in sorghum, the term staygreen has been used to describe an important component of post-flowering drought response. It is a drought resistance mechanism that delays premature senescence under soil moisture stress during grain filling and maturity and it is also associated with stalk rot resistance (Rosenow 1984). It

is considered as a valuable trait to improve the adaptation of genotypes to post flowering drought stress, particularly in environment where crop mainly depends on stored soil moisture to fill and mature grains (Rosenow *et al.*, 1977). In all these contexts, the stomatal frequency and its functional dynamics in terms of opening and closure are important in manifesting drought tolerance response by plants.

The use of DNA based markers for genetic analysis and manipulation of important traits has become an increasingly useful tool in plant breeding. However, their greatest potential appears to be in accelerating the rate of gain from selection for desirable QTLs that govern complex traits. Marker Assisted Selection (MAS) has the potential to greatly improve the efficiency of selection of quantitative traits. Since, the components of drought resistance are quantitative in nature, it is important to identify DNA markers linked to genomic regions harbouring desired QTLs from the point of view of genetics and breeding. The ultimate goal of QTL analysis is to develop tools that are useful for MAS in practical plant breeding programme for achieving higher level of resistance in agronomically elite genetic background or for gene pyramiding.

Keeping the above points in view, following objectives were set for the present investigation

1. Field evaluation for drought and yield component traits in recombinant inbred lines of IS22380 x E36-1.
2. Construction of genetic linkage map and Identification of genomic regions having QTLs for drought and yield components.

II. REVIEW OF LITERATURE

Drought at best could be defined as a persistent and abnormal moisture deficiency, that has an adverse impact on plants, which one of the most important factor that limits crop productivity around the world. It is more severe in semi-arid tropics, where rainfall is generally low and erratically. Tolerance to drought is complex trait from both genetics and physiological points of view. In fact, sorghum is one of the most drought-tolerant grain crops and its rich genetic diversity for stress tolerance makes it an excellent model crop for studying the genetic and physiological mechanisms of drought tolerance (Doggett 1988). Susceptibility to drought can occur when moisture stress is imposed at different stages of growth. Sorghum genotypes with good tolerance during one of the developmental stages might be susceptible to drought during the other growth stages (Rosenow and Clark 1981), making it a complex character work with use of molecular markers and analysis quantitative trait loci (QTLs) has the potential to alleviate the problems associated with the phenotype evaluation of response to moisture stress. Information on various aspects of drought, mapping and QTL analysis is reviewed here.

2.1 PHENOTYPIC TRAITS ASSOCIATED WITH DROUGHT TOLERANCE

2.1.1 Plant height

The plant height is often influenced by the soil fertility, moisture stress and other environmental factors. Several studies have revealed that, moisture stress generally decreases the plant height. Sutro and Tirtoum, (1989) reported that plant height reduces as a result of water stress and the recovery of sorghum crop is slower than corn. The decrease in plant height due to water stress at boot leaf stage was because of decreased peduncle extension (Eck and Musick, 1979). Terminal drought stress reduces plant height and biomass (Anon, 1989). Average height of the non-senescent sorghum genotype was 3 to 4 cm shorter than the senescent genotype (Duncan, et al 1981). In a study with 57 sorghum Recombinant inbred lines (RILs) of a cross, K886 x CS3541, Menkir and Ejeta (2003) found that plant height and days to flowering in non-stress environments were slightly higher than those in the respective stress environments through the differences were not significant statistically. Heritability estimates for each trait, in contrasting environments followed a similar trend as the genetic variance estimates.

2.1.2 Stomatal frequency

Stomata are a tiny openings or pore, found mostly on the undersurface of the leaf used for gas exchange. Air containing CO₂ and O₂ enter the plant through these openings where they get used in photosynthesis and respiration considerable amount if moisture is also lost due to transpiration. (Wikipedia, 2006). Drought stress known to inhibit photosynthesis by decreasing stomatal aperture but not through reduced ATP synthesis (Cornic, 2000). Stomatal closure is a common response to water stress in sorghum, which is reflected by increasing stomatal resistance (Ackerson and Krieg, 1977). Stomatal closure under drought could be attributed to increased delivery of ABA from roots to the leaves (Blanke and Cooke, 2004). Stomatal closure leads to decrease in photosynthesis and inhibition of metabolism (Downton *et al.*, 1988; Cornic, 2000). Density and size of stomata are positively related to transpiration (Wilson, 1975a; Anderson and Briske, 1990) and in smooth brome grass, stomatal density is also related to yield (Walton, 1974).

2.1.3 Stem thickness

Stem stature defines plant responses to abiotic stress in a number of ways. Duncan *et al.*, (1981) reported higher basal stem sugar content in stay-green compared with non stay-green genotypes, especially during post anthesis drought in sorghum. The stem reserves and grain yield are affected by water regime. The nonscenescent lines had significantly larger stem diameter and maintained a higher sugar concentration in the basal part of the stem at the time of harvest (Duncan, 1981).

2.1.4 Days to 50 per cent flowering

The studies on changes in the phenological and physiological characters of genotypes under drought stress have indicated that drought tolerant genotypes generally tend to flower early under drought conditions (Omany *et al.*, 1997). The non-scenescent genotypes require approximately 2 days longer to reach 50% anthesis in sorghum (Duncan, 1981). Sorghum is known to be sensitive to water stress during flowering and early grain filling stages as compared to early growth period (Seetarama, 1987). As the number of days from 50 per cent flowering to physiological maturity increased, the yield also increased in hybrids and this increase in yield was due to increased number of days for dry matter accumulation in the grain (Reddy and Rao, 1978). Early flowering, fewer tillers (or no tillers) and deeper roots are known to result in escaping late-season stress (Blum, 2005). Phenotype, that avoid dehydration are characterized by the maintenance of high plant water status under stress show any of the following features; early flowering, lower plant height, low leaf area, or limited tillering in cereals (Blum, 1988, Haussmann *et al.*, 2002).

2.1.5 CARBON ISOTOPE AND ITS DISCRIMINATION

There are two naturally occurring stable isotopes of carbon, ^{12}C and ^{13}C . Most of the carbon in CO_2 is ^{12}C (98.9%) and the remaining with 1.1% is ^{13}C in atmosphere. The chemical properties of $^{13}\text{CO}_2$ are identical to those of $^{12}\text{CO}_2$, but because of the slight difference in mass (2.3%) most plants assimilate less $^{13}\text{CO}_2$ than $^{12}\text{CO}_2$. The isotopes are unevenly distributed among and within different compounds and this isotopic distribution can reveal information about the physical, chemical and metabolic processes involved in carbon transformations. The overall abundance of ^{13}C relative to ^{12}C in plant tissue is usually less than that in atmospheric CO_2 , indicating that carbon isotope discrimination occurs during the incorporation of CO_2 into plant biomass. As the isotopes are stable, the tissues retain information inherent in the ratio of abundance of carbon isotopes, presented by convention as $^{13}\text{C}/^{12}\text{C}$. In plant samples, $\Delta^{13}\text{C}$ is measured by using a Isotope Ratio Mass Spectrometer (IRMS), specially designed for high precision measurements of the ratio R, defined as:

$$R = ^{13}\text{CO}_2/^{12}\text{CO}_2$$

The plant material is converted to CO_2 by combustion to determine the isotope composition. In general R is low in organic sample. The atmosphere has a relatively higher fractionation value of around -7.8 per mil (‰), which is in comparison with a standard PDB (Pee Dee Belemnite, from North Carolina, USA). The R in this standard is 0.0124 and in many of the plant material it is approximately 0.012, suggesting a very minor changes in the R value, and hence R in a sample can be compared with that of standard and expressed as ^{13}C in units per mil or parts per thousand (‰)

$$\Delta^{13}\text{C} = (R\text{-sample} - R\text{-standard})/R\text{-standard} \times 1000$$

Since the organic sample has low R value than the standard, $\Delta^{13}\text{C}$ of organic material is more negative, *i.e.*, less $\Delta^{13}\text{C}$ content, (O'Leary, 1984).

Importance and biochemical basis of $\Delta^{13}\text{C}$ and its relationship with water use efficiency (WUE) has been extensively studied (Farquhar *et al.*, 1982; Hubick, *et al.*, 1988; Condon *et al.*, 1990; Read, *et al.*, 1991). The range of $\Delta^{13}\text{C}$ values across crop types with different photosynthetic pathways is given below:

Range of $\Delta^{13}\text{C}$ composition

1. Atmosphere (air)	= -6.4 to -7.0 %
2. C ₃ plants	= -22.0 to -44.0 %
3. C ₄ plants	= -9.0 to -19.0 %
4. CAM plants	= -11.0% (approximate)

Carbon isotope discrimination provides an integrated measure of WUE. Samples are easily collected and processed, permitting analysis of a large numbers of samples from diverse environments (Cregg and Zhang 2000). In several crops, including cereals, carbon isotope discrimination ($\Delta^{13}\text{C}$) has been associated with water-use efficiency and yield stability in drought-prone environments (Teulat, 2002).

$\Delta^{13}\text{C}$ and WUE

Plants discriminate against the heavy isotope of carbon ($\Delta^{13}\text{C}$) during the process of photosynthesis. However the extent of discrimination depends on the P_i and hence ^{13}C content in the plant samples has a potential to quantify P_i and WUE. Several reports confirm the close relationship between P_i and ^{13}C , therefore it could be a time integrated estimate of P_i . Since, P_i/P_a ratios predominantly determine the variation in WUE and $\Delta^{13}\text{C}$, a strong relationship between $\Delta^{13}\text{C}$ and WUE can be expected and explained by the following equation (Farquhar *et al.*, 1989). Carbon dioxide fixation in C₄ species is characterized by a CO_2 concentration mechanism that requires the coordinated function of mesophyll.

2.1.6 Stay-green

The term stay-green has been used to describe a condition in post flowering drought response in sorghum (Rosenow and Clark, 1981), where the plant retains significant green leaf surface that gives sorghum to premature senescence during grain filling. The stay-green trait results in greater functional photosynthetic leaf area during grain filling and even after physiological maturity. It is a valuable trait as it improves genotype adaptation to post flowering drought stress, particularly in environments where the crop depends on stored soil moisture to fill the grains (Rosenow *et al.*, 1977). Greater green leaf area duration during grain filling appears to be a product of a combination of three distinct factors; green leaf area at flowering time, onset of senescence and subsequent rate of senescence (Vanoosterom *et al.*, 1996). Sources expressing different components can be combined easily in breeding programs (Borrell *et al.*, 2000a). Stay-green hybrids were shown to produce significantly greater total biomass after anthesis, retain greater stem carbohydrate reserves, maintain higher grain growth rates, and to have significantly higher grain yields under terminal drought stress than related but senescent hybrids (Borrell *et al.*, 2000b). Stay-green genotypes also appear to have higher leaf-nitrogen concentration (specific leaf nitrogen) at flowering and maintain it during grain filling (Borrell and Hammer, 2000), which is possibly associated with a higher transpiration efficiency in the best stay-green hybrids (Borrell *et al.*, 2000c). Conventional breeding for stay-green has been based primarily on two genetic stocks; sources for this trait, B 35 and E36-1 (Borrell *et al.*, 2000a).

Inheritance of stay-green

E 36-1 and B35 staygreen types, which are well adapted to tropical climate. 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 (Richard *et al.*, 1994). It was suggested that environment had a strong influence on the mode of expression of the gene(s) controlling this 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 nuclear gene controlled quantitative trait.

Stay-green and yield

In a study of nine genotypes varying in stay-green feature, the grain yield was correlated positively with green leaf area at maturity and negatively with rate of leaf senescence suggesting that sorghum genotypes possessing the stay-green trait have a significant yield advantage under post-anthesis drought compared to genotypes not possessing this trait (Borrell *et al.*, 1999b). Sorghum genotypes with this trait continue to fill their grain normally under drought conditions (Rosenow and Clark, 1981) and display increased resistance to charcoal rot (Rosenow, 1984) and lodging resistance (Henzell *et al.*, 1984;). Stay-green genotypes have also been reported to contain more cytokinins (McBee, 1984) and basal stem sugars (Duncan, 1984) compared to senescent genotypes. In an experiment Borell *et al.*, (2000) evaluated i6 with varying stay-green levels and found that stay-green hybrids produced 47 per cent more biomass between anthesis and maturity than their senescent counterparts under water-limited conditions. Thus, sorghum hybrids possessing this trait had a significant yield advantage under drought conditions. Stay-green genotype under different moisture regimes interacted favourably in 4 out of 7 features; length of the grain filling period, grain size, rate of leaf senescence, and green leaf area at maturity. No genotype x water regime interaction was observed for the four indicators of pre- and post-anthesis drought resistance viz., grain yield, biomass at maturity, crop growth rate and harvest index.

2.2 MOLECULAR MARKERS

2.2.1 DNA markers

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. Marker assisted selection is one of the practical utility of markers in plant breeding. DNA markers also permit plant breeders to correctly map various interacting genes that condition complex agronomic traits. 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, 1998), striga tolerance (Hausmann *et al.*, 2004) and charcoal rot resistance (Punnuri, 2004 and Rajkumar, 2004).

Several types of DNA markers have been used widely (Mohan *et al.*, 1997; Gupta and Varshney, 2000; Babu *et al.*, 2004) for linkage mapping viz., RFLPs (Botstein *et al.*, 1980), RAPDs (Williams *et al.*, 1990), SSRs or microsatellites (Litt and Luty, 1989), AFLPs (Vos *et al.*, 1995), SNPs in combination with DNA chip technology (Wang *et al.*, 1998; Kanazin *et al.*, 2002) and EST-derived SSR markers (Yu J.K. *et al.*, 2004; Varshney, R.K. *et al.*, 2005). Recently several derivatives of already existing markers have been in use (Rafalski and Tingley, 1999) including genetic elements such as retrovirus as markers (Long *et al.*, 1995).

2.2.2 Random Amplified Polymorphic DNA (RAPD)

In this technique, a single short oligonucleotide sequence is used for cyclic amplification of DNA, which exposes the polymorphism distributed throughout the genome. These amplified fragments were called as random amplified polymorphic DNAs (Williams *et al.*, 1990). This technique has been used in genome mapping and gene tagging in several crop plants (Martin *et al.*, 1991; Paran *et al.*, 1995; Reiter *et al.*, 1992).

RAPD markers genetically linked to plant height (*DW2* gene) (Zhang *et al.*, 2000) and fertility restoration (*Rf1* gene) were identified in sorghum using bulked segregant analysis. RAPD markers along with RFLP were used for the identification of QTLs associated with pre-flowering drought tolerance in sorghum (Tuinstra *et al.*, 1996). Recently, RAPD markers were also linked to anthracnose disease resistance in sorghum were identified (Panday *et al.*,

2002). Despite simple to use in absence of any genomic sequence, RAPD markers are not preferred due to problems associated with consistency of results.

2.2.3 SSR and genic SSR markers

Simple sequence repeats (SSRs) or microsatellites are highly variable short sequences of nucleotides. They are highly variable even between related individuals and distributed are well throughout the genome (Karp *et al.*, 1996).

SSRs happen to be the most reliable and highly reproducible molecular markers, for developing framework linkage maps and also for high density linkage maps (Bell and Ecker, 1994 and Akkaya *et al.*, 1995). SSRs are co-dominant, occur in high frequency and appear to be distributed throughout the genome of most higher plants and animals. In plant genomes, the frequency of microsatellite repeats appears to be lower than in animals genomes (Morgante and Olivieri, 1993) and it is also estimated that plants have about 10 times less SSRs than humans (Mohan *et al.*, 1997). They also display high levels of polymorphism even among closely related accessions (Brown *et al.*, 1996). Though they are expensive to develop, SSRs are relatively less expensive to use in mapping and MAS.

Simple repeat motifs, though rare, could be present in functional genes (Morgante and Olivieri, 1993) called expressed sequence tags (ESTs), they have been scanned for the presence of SSRs in *Arabidopsis* (Morgante *et al.*, 2002), cotton (Saha *et al.*, 2003), and sorghum (Kantety *et al.*, 2002). 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, 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).

2.2.4 Genomic and genic SSRs in sorghum

SSR containing clones isolated from both BAC and enriched DNA libraries were the sources for sorghum SSRs mapped by Bhatramakki *et al.*, (2000). Identification of SSR *loci* in BAC clones is one of the most efficient method so far (Cregan *et al.*, 1999).

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, (Bhatramakki *et al.*, 2000) have been reported to date in sorghum. SSR makers have been integrated with their existing RFLP based map of Xu *et al.* (1994), Kong *et al.* (1997), Peng *et al.* (1999) and of Bhatramakki *et al.* (2000). Majority of SSRs contain (AG/TC)₂ and AC/TG)_n repeats at 52 per cent and 91 per cent respectively (Bhatramakki *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).

In sorghum most of SSR *loci* are sufficiently polymorphic to be useful in MAS programs (Kong *et al.*, 2000). The probability that two accessions in any working groups will have different alleles at a locus ranged from 0.67 to 0.88 depending upon the working group to which the accession belonged. In addition, the number of alleles per locus was positively correlated ($r=0.6$) with the number of repeated units at the *loci* in BTx623, the strain from which the SSRs were originally isolated.

In a study with 15 SSRs markers on three subsets of the sorghum landrace collection at ICRISAT Grenier *et al.*, (2000) found that the homogeneity rate varied between 46 and 100 per cent, depending on the subset and locus considered. In another study, fifty genetically diverse elite sorghum inbreds were used to compare the discrimination ability of 15 SSR primers with 104 RFLPs and genetic analysis was assessed by molecular data and by pedigrees (Smith *et al.*, 2000).

2.3 LINKAGE MAPS AND MAPPING POPULATIONS

Linkage between a marker and a genomic region (QTL) that influences a quantitative traits permits detection and mapping of QTLs in relation the markers. Hence, construction of

linkage maps is the most fundamental step required for a detailed genetic study of quantitative characters in any crop. High-density map facilitates MAS especially between closely related types as it provides information on many potential polymorphic markers in any genomic region (Chittenden *et al.*, 1994).

The SSR markers are ideal in such efforts as they exhibit high levels of polymorphism, even in narrow crosses (Rongwen *et al.*, 1995, Young *et al.*, 2001).

Back cross and F₂ populations are suitable for DNA marker based mapping, but recombinant inbred populations and double haploids (DH) provide a permanent mapping resource because of homozygosity of the lines and that they can be distributed, replicated and evaluated for experiments in different environments, which is essential for more accurate measurement for any quantitative trait (Burr, 2001; Zamir *et al.*, 1993; Goldman *et al.*, 1995 and Paran *et al.*, 1995). Different linkage blocks in each individual RILs arise is due to more opportunity for meiotic recombination and provide an opportunity for distinguishing more closely linked QTLs (Tanksley, 1993). The only limitation of RILs is the time required for their development. Double Haploid Lines (DHLs) can also be used for linkage mapping (Huen *et al.*, 1991). 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, 2000).

2.3.1 Linkage maps in sorghum

Sorghum genome mapping based on DNA markers began in early 1990s and since then, several 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).

Most of the early sorghum molecular marker based genetic maps used maize RFLP probes in the context of comparative mapping (Binneli *et al.*, 1992, Whitkus *et al.*, 1992; Melake Berhan *et al.*, 1993; Pereira *et al.*, 1994). In 1994, three different groups (Chittenden *et al.*, 1994, Ragab *et al.*, 1994, Xu *et al.*, 1994) developed RFLP maps using mainly sorghum DNA probes. Dufour *et al.* (1997) published a map based on maize, sugarcane and cereal anchor probes. This composite linkage map was further saturated with the addition of more heterologous probes and AFLP markers (Biovin *et al.*, 1999). A few cDNA clones of sorghum and maize related to photosynthesis and drought stress were assigned to the map of Subudhi and Nguyen (2000). Similarly, the RFLP maps developed by Xu *et al.* (1994) and Peng *et al.* (1999) have been improved with the addition of over 100 SSR markers (Kong *et al.*, 1997; and Bhatramakki *et al.*, 2000). Recently, more than 500 AFLP markers have been added to the map of Xu *et al.*, (1994), which is currently being used to generate a high quality physical map of the sorghum genome (Subudhi and Nguyen, 2000). Sorghum genetic linkage map was constructed by using two RIPs one derived from IS9830 x E36-1 and another derived from N13 x E36-1. A total of 235 marker loci in RIP1 and 297 marker loci in RIP2 were mapped (Haussmann *et al.*, 2004).

The maps have also been developed from interspecific crosses (Chittenden *et al.*, 1994 and Lin *et al.*, 1995), an inter-subspecific cross (Pereira *et al.*, 1994) and rest of the maps from intra-specific crosses. Both F₂ (Hulbert *et al.*, 1990; Chittenden *et al.*, 1994, Pereira *et al.*, 1994, Ragab *et al.*, 1994; Xu *et al.*, 1994) and RIL populations (Dufour *et al.*, 1997; Tao *et al.*, 1998; Biovin *et al.*, 1999; Crasta *et al.*, 1999; Peng *et al.*, 1999) have been used in map development. Only the of Chittenden *et al.* (1994) and Pereira *et al.* (1994) are complete, containing all the ten linkage groups. The crosses, types of populations, number of linkage groups, number of markers and type of markers used thus far in sorghum maps are summarized in Subudhi and Nguyen (2000).

To have a genetic, physical, and cytological perspectives of the *Sorghum bicolor* genome, Kim *et al.*, 2005 selected 40 BAC clones that had different linkage group markers, 21 from linkage group 2 (LG-02) and 19 from linkage group 8 (LG-08). Multi-BAC probe cocktails were constructed for each chromosome from the landed BACs, which were also preevaluated for Fluorescence In-Situ Hybridization (FISH) signal quality, relative position, and collective chromosome coverage. Comparison to the corresponding linkage map revealed full concordance of locus order between cytological and genetic analyses.

Linkage relationships among 85 maize low copy number nuclear DNA probes and seven isozyme *loci* in an F₂ population derived from a cross of *S. bicolor* ssp. *Bicolor* x *S. bicolor* ssp. *Arundinaceum* lead to a total of 13 linkage groups, three more than 10 chromosomes of sorghum. Use of maize probes to produce linkage maps in sorghum allowed to make several inferences concerning processes involved in the evolutionary divergence of maize and sorghum genomes. The results show that many linkage groups are conserved between these two genomes (Whitkus *et al.*, 1992).

Levels of genetic variation and linkage disequilibrium (LD) are critical factors in association mapping methods as well as in identification of loci that have been the target of selection. In a study by Hamblin *et al.* (2004) population genetic parameters in sorghum were estimated by surveying 27 diverse *S. bicolor* accessions for sequence variations in 29,186 bp from 95 short regions derived from RFLP map. Consistent with its higher level of inbreeding, the extent of LD was several fold greater in sorghum than in maize, but the total sequence variation in sorghum was about four fold lower than that in maize. Use of mapped maize genomic clones provided a basis for comparative linkage analysis. Approximately 67 per cent of the maize genomic probes produced a strong hybridization signal with sorghum (Pereira *et al.*, 1994).

2.4 QTL MAPPING

Quantitative inheritance of complex phenotypic characters is a common feature of several traits in eukaryotes. These characters are controlled by many genes with small individual effects; exhibit high G X E variance and low heritability. Under these situations, progress through selection is rather low. Marker assisted selection has been suggested as a strategy for increasing the probability of selecting superior genotypes. It has been shown that MAS subsequently decrease the resources needed to accomplish a selection goal for a low to moderate heritability trait when the selection goal and the selection intensity is high Knapp (1989). The basic concept of associating genetic markers with quantitative traits was first proposed by Sax (1923). Geneticists have recognized the potential use of linkages between qualitative genes and QTL for studying the nature of quantitative genetic variation (Sax, 1923; Lindstrom, 1926; Waxelson, 1923; Smith, 1937; Everson and Schaller, 1955 and Thoday, 1961). Unfortunately, the availability of relatively small number and some times-deleterious nature of qualitative marker genes limited their utility in the past (Bubeck *et al.*, 1993). The advent of DNA based molecular markers have changed the situation dramatically.

High-density genetic maps are necessary for marker-QTL association and subsequent utility. The need for saturated linkage maps in a wide range of crop species, has accelerated development of newer and simpler DNA marker systems like RAPDs (Williams *et al.*, 1990), AFLPs (Vos *et al.*, 1995) SSRs, (Akkaya *et al.*, 1992), SNP in combination with DNA chip technology (Wang *et al.*, 1998) and genic repeats (Yu, J.K., *et al.*, 2004). Analysing plants at the seedling stage, screening multiple characters that would normally be epistatic with one another, minimizing linkage drag and rapidly recovering recurrent parent genotype were considered as some of the potential advantages of MAS (Tanksley *et al.*, 1989). The success of MAS will depend on identifying polymorphic marker(s) as close to the gene as possible to ensure its/their utility across many breeding populations (Mohan *et al.*, 1997).

The first attempt to link a quantitative trait to a major gene locus in plants was study of seed weight and color in an F₂ cross of bean by Sax (1923). The term QTL has come to referne or a group of closely linked genes with a significant contribution to a gpolygenes underlying a quantitative trait. Numerous studies have been reported on identifying QTL for various traits in plants, animals and humans. Statistical techniques for using a marker map to detect QTL have reached a fairly high level of sophistication, but improvements are still being made (Kearsey and Farquhar, 1998). The QTL x E is commonly seen when growing a mapping population under a range of environments. Quantitative trait locus mapping is considered as a highly effective approach for studying genetically complex forms of plant disease resistance and other traits (Young., 1996). Conserved efforts on QTL mapping for quantitative traits such as yield, quality, maturity, and resistance to biotic and abiotic stress has been initiated in many crop species (Lin 1995).

Portyanko *et al.*, (2005) identified four consistently detected major QTLs for partial rust resistance in oat. A number of QTLs controlling agronomic traits (Siripoonwiwat *et al.*,

1996; Holland *et al.*, 1997; 2002; Jin *et al.*, 1998; Kianian *et al.*, 1999; Groh *et al.*, 2001b; De Koeyer *et al.*, 2001; 2004) including some QTLs for crown rust resistance (Bush and Wise, 1996; Zhu and Kaeppler, 2003; Zhu *et al.*, 2003) have been detected in oat.

2.5.1 QTL mapping in sorghum

In sorghum many efforts have been made to tag genes or link them with molecular markers. RAPD markers were used for single gene traits such as resistance to downy mildew, sorghum head smut, anthracnose, and leaf blight in bulk segregant analysis (Magill *et al.*, 1997). Most economically important characters in crop plants are polygenic in nature and phenotypic selection for such trait is difficult. Theoretically Selection based on markers could facilitate manipulation of such trait without affecting others (Tuinstra *et al.*, 1997).

Extensive studies have been carried out to map QTLs that control the stay-green and chlorophyll content in sorghum, by using RFLP map information from RIL populations. Xu *et al.* (2000) identified 4 stay-green QTLs located on 3 linkage groups. The QTLs (*stg1* and *stg2*) were on linkage group A with other two *stg3* and *stg4* on linkage groups D and J, respectively. Two stay-green QTLs, *Stg1* and *Stg2*, accounting for 13-20 and 20-30 per cent of the phenotypic variability, respectively, were consistently identified in all trials at different locations over two years. Similarly, QTLs affecting flooding tolerance and other physiological and morphological traits in *Echinochloa crusgalli* were analyzed (Fukao *et al.*, 2003). As a part of comparative mapping study between sugarcane and sorghum, a sugarcane cDNA clone with homology to maize *Rp1-D* rust resistance gene was mapped in sorghum (McIntyre *et al.*, 2005).

The genetic maps of RIP-1 and RIP-2 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 sets 1 and 2 of RIP-1 explaining 77.6 and 80.0 per cent of genetic variance respectively (Haussmann *et al.*, 2004).

QTLs conferring resistance to sorghum downy mildew (*Peronosclerospora sorghi*) and Rajasthan downy mildew (*P. heteropogoni*) have been mapped (Nair *et al.*, 2005). Sorghum midge resistance and green bug resistance mechanisms have been identified through QTL (Tao *et al.*, 2003 and Agrama *et al.*, 2002). Comparative mapping between sorghum and sugarcane led to the identification of useful markers for stalk number and suckering in sugarcane, which was facilitated by focusing on sorghum QTLs associated with related traits (Jordan *et al.*, 2004). Similarly, disease resistance in sugarcane and sorghum were studied and QTLs for rust resistance were identified (McIntyre *et al.*, 2005).

Plant genes conferring resistance to diseases have been isolated from many plants and categorized into four classes based on their predicted protein products. The majority of the R genes belong either to a class encoding a stretch of leucine rich repeats (LRRs) and a putative Nucleotide Binding Site (NBS) (Ori *et al.*, 1997; Yoshimura *et al.*, 1998), or to a class that encodes an LRR domain but no NBS (Dixon *et al.*, 1996). Mapping and establishing the relationship between individual resistant gene analogue (RGA) and different diseases would go a long way on the functional genomics of plant disease resistance. There are reports of mapping plant RGAs on to linkage map and determine their position in sorghum (McIntyre *et al.*, 2005), maize (Collins *et al.*, 1998) and barley (Collins *et al.*, 2001). Some RFLPs were shown to map to genomic regions containing virus and fungus resistance genes (Collins *et al.*, 1998). Therefore it is important that homologous RGAs have to be characterized before mapping. A set of 13 RGAs were characterized from sorghum at IABT previously (Anand *et al.*, 2004). McIntyre *et al.* (2005) characterized 54 different sugarcane RGAs sequences, the map location of 31 of the RGAs was determined in sugarcane and compared with the location of QTL for brown rust resistance. Three RGAs were shown to generate markers that were significantly associated with resistance to this disease.

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 $\delta^{13}\text{C}$ were identified on chromosomes 2, 4, 8, 9, 11, and 12 across plant parts, stages, and years.

Differential expression of QTL for $\delta^{13}\text{C}$ among suggested that each QTL a stage specific role. Some QTLs for $\delta^{13}\text{C}$ were associated with or collocated with QTLs for leaf traits, which indicating that their physiological role may be complex. Values of $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ found in this study in irrigated land in rice (Laza *et al.*, 2006).

2.6 STATISTICAL TECHNIQUES FOR QTL ANALYSIS

QTL analysis looks for association between a quantitative trait and a set marker alleles segregating in a population. It has three essential stages: mapping of the markers (genotyping), scoring of the trait (phenotyping) and association of trait with markers (QTL-mapping).

The traditional approach for detecting a QTL in the vicinity of a marker, one at a time (Soller and Brody, 1976, Tanksley *et al.*, 1982; Edwards *et al.*, 1987). However, if the QTL does not potion at the marker locus the chance of detecting it is reduced as the distance (recombination frequency) between the marker locus and the QTL increases (Edwards *et al.*, 1987; Lander and Botstein, 1989). To overcome this, Knapp (1989) developed an approach, which utilizes pairs of markers in a sequential manner and estimates the phenotypic effect of the QTL and its significance in the region, bracketed by the two markers in each pair. Lander and Botstein (1989) reported development of interval mapping method for QTLs, using 'Log of Odd ratios' (LOD) scores. An alternative approach using multiple regressions was developed by Haley (1992). It often produces very similar results and employs simple programming. Tests of significance and confidence intervals can be obtained by boot strapping approaches (Visscher *et al.*, 1996). However, it is reported by Tanksely and Nelson (1996) that the statistical detection of QTLs is likely to depend not only on the type of population utilized, but also be on the intralocus and interlocus interactions of the segregating QTL.

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. MAPMAKER/EXP performs full multipoint linkage analysis for dominant, recessive (such as qualitative phenotypic marker) and co-dominant (such as RFLP) markers in BC, F₂ and F₃ (self) intercroses, 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 experimental 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 TeasdaleKao *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 types gene action, QTL-environment interactions, and close linkage. QTL Cartographer mapping programs can handle data from backcrosses, intercroses, 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; Zeng 1994). Compared to simple interval mapping (SIM; without cofactors), 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 ($LOD \geq 3.0$). A total of 21 QTLs were identified for fiber yield and yield components in cotton, which were located on 15 linkage groups.

PLABQTL software, as described by Rami *et al.*, (1998) can also perform CIM, but it differs from QTL Cartographer in that it uses regression-based methods for estimating QTL effects. QTL Cartographer uses a combination of maximum likelihood estimation similar to interval mapping and regression to control genetic background (Beavis, 1994).

To use linkage information for subsequently in marker for subsequently assisted breeding, a program like Mapmanager (Manley and Cudmore, 1998) helps to keep the marker data in a population of interest. While, Hyper gene helps to display graphical genotypes (Young and Tanksley, 1989). The program qGENE seeks to bring all of these important DNA marker tools together into a single package (Nelson, 1997). In spite of these developments, all methods of analysis yield essentially similar QTL locations and gene effects, while there is some variation in the confidence intervals.

III. MATERIAL AND METHODS

The present investigations were carried out at the Institute of Agri-Biotechnology (IABT), Main Agricultural Research Station (MARS), Dharwad and Agricultural Research Station (ARS), Bijapur. The details of material used and the methodologies adopted in the present investigation are described here.

3.1 EXPERIMENTAL MATERIALS

The experimental material consisted of a set of 93 Recombinant Inbred Lines (RILs) and two parents. The mapping population was obtained from International Crops Research Institute for the Semi Arid Tropics (ICRISAT) Patancheru, India. The two parents were IS22380, a Sudanese dwarf line, sensitive to moisture stress, susceptible to charcoal rot and non stay-green and E36-1, a stay-green type well adapted to tropics and also resistant to charcoal rot. The mapping population including two parents was phenotyped for terminal stay-green and other drought related traits. The individuals were genotyped with selected SSRs, genic SSRs and RAPD markers. The pedigree information of mapping population is given in Appendix I.

3.2 EVALUATION OF RILs FOR STAYGREEN AND OTHER TRAITS

3.2.1 Location, season and experimental design

A set of 100 entries was taken up for phenotyping under field conditions, comprising of 93 RILs and two parents (repeated 3 times) and a check (RS29). The material was sown in the month of October 2004 as *rabi* crop with a spacing of 45 x 15 cm and other recommended package of practices at ARS, Bijapur and MARS, Dharwad

Simple lattice design was adopted with three replications. Each replication consisted of 10 blocks each with 10 entries planted randomly. Each entry had a single row with 15 plants. To eliminate the border effect, three rows of M35-1 was sown on all sides of the field. Plots were thinned 10 days after seedling emergence (DAE) to a spacing of 15 cm between plants within rows.

3.3 OBSERVATIONS ON COMPONENT TRAITS OF DROUGHT RESISTANCE

3.3.1 Estimation of senescence

At the time of emergence of flag leaf, five representative plants in each entry (line) were tagged to measure length and breadth of upper six leaves. Area of each upper six leaves was estimated as leaf length x leaf breadth x 0.7 (factor). The part (percentage) of the leaf remaining green was estimated at 15 days intervals from date of flowering. For each fortnight, it was calculated by dividing the estimated green leaf area for that fortnight by its measured leaf area at flowering as described in Haussmann *et al.*, 2002 and Mahalakshmi *et al.*, 2002.

3.3.2 Stomatal frequency

The stomatal frequency (number of stomata per unit leaf area) was calculated from leaf area surface impression of xylene + thermocol paste. The paste was smeared on abaxial surface of third leaf from top. About 2-3 minutes later, the solidified layer was peeled off and mounted on a slide with cover slip and observed under 40x magnification. The number of stomata was counted and expressed in terms of stomata per cm² leaf area.

3.3.3 Carbon isotope discrimination

Quantification of $\Delta^{13}\text{C}$ in leaf samples: The Carbon isotope discrimination ($\Delta^{13}\text{C}$) was determined with an objective to study how far $\Delta^{13}\text{C}$ is associated with drought tolerance in sorghum RILs.

1. Flag leaf at post flowering stage of crop was collected (5-6 for each genotype)
2. Dried at 80°C for 3 days.
3. Dried samples were powdered in a mortar and pestle. Care was taken to prevent any mixing of different samples by washing the pestle and mortar with alcohol after grinding each sample.
4. 1 g of powdered leaf sample was put in glass vial and properly labeled.

$\Delta^{13}\text{C}$ was measured at the National Facility for quantification of stable isotopes in the Department of Crop Physiology, UAS, Bangalore.

Mass spectrometric analysis: Carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$) in comparison with the PDB (Pee Dee Belemnite, 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. Finely 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 are 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 cobaltous-cobaltic-oxide heated to 1050°C. 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°C. In this reaction, the N_2O is reduced to N_2 and the excess O_2 is absorbed. The resultant gases are then flushed through scrubbers to trap CO_2 and water. The pure CO_2 and N_2 gas after passing through a GC column (5Å molecular sieve) and a thermal conductivity detector (TCD) into the ion source of the IRMS. At the source, CO_2 is ionized by electron impacts ionization to produce molecular radicals (CO^+). When accelerated radicals pass through a strong magnetic field, they are deflected with the radius of deflection being proportional to the molecular mass of the radicals. These deflecting $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ are collected Faraday cups and the signal is amplified and transmitted to the computer.

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

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

3.3.4 Observations on other traits

Observations on early seedling vigour, plant height, days to 50 per cent flowering, stem thickness and seed yield per plant were recorded as follows:

Early seedling vigour: Recorded at 15 days after emergence of seedlings on a following visual scale

Score	Phenotype
1	Very good
2	Good
3	Average
4	Below average
5	Poor

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

Days to 50 % flowering: Number of day required from sowing to anthesis in atleast least 50 % of the plants.

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

Seed yield per plant: Weight of seed in grams (g) from each plant was recorded at the time of harvest.

3.4 MOLECULAR MARKER ANALYSIS

3.4.1 Genomic DNA preparation from sorghum leaves

The details on preparation of solutions and buffers are presented in Appendix II. DNA was extracted from a bulk of 6 to 7 individual plants of each entry. Three to four week old seedlings were taken from field and shoots were frozen in liquid nitrogen and at -80°C till use. DNA was prepared following Krishna and Jawali (1997) method with minor modifications as follows:

- 1 About 2 g of frozen tissue sample was ground into fine powder in liquid nitrogen, using autoclaved mortar and pestle.
- 2 The ground tissue was transferred to 2.0 ml eppendorf tubes containing 900 μl of extraction buffer, then 90 μl of 20 per cent SDS were added to each tube.
- 3 The contents were mixed well and incubated at 65°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°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 the 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 % 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 and mixed by tapping and incubated at room temperature (37°C) for 30 min.
- 13 The DNA was precipitated by adding 1/10th volume of 3M sodium acetate and 2.5 volumes of absolute ethanol.
- 14 The contents were mixed gently and incubated at 4°C for 30 min.
- 15 The contents were centrifuged for 1 min at 3000 rpm.
- 16 DNA pellet was rinsed with 70 % ethanol twice and centrifuged at 3000 rpm for 1 min.

- 17 Supernatant was discarded and tubes were put upside down on paper towel to get rid of excess ethanol.
- 18 Pellet was dissolved in 100 µl of T₁₀E₁.

3.4.2 Purification of 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 Steps 1 and 2 were repeated
- 4 To the supernatant above, 10 µl of 3 M NaOAc, (1/10 volume of aqueous layer) and 2.5 volumes of chilled absolute ethanol was added, mixed gently and incubated at – 20 °C for 15-20 min.
- 5 The DNA was spooled in 1.5 ml eppendorf tubes using a glass hook, washed with 70 % alcohol and dried in vacuum.
- 6 DNA was dissolved in 100 µl T₁₀E₁ and kept at –20 °C till further use.

3.4.3 Determination of quantity and quality of isolated DNA

The amount of DNA following extraction was quantified and purification and quality checked using a spectrophotometer at 260 nm and 280 nm in 1:1000 dilution of the extract .The concentration of the DNA was calculated as

$$\text{DNA } (\mu\text{g/ ml}) = \text{OD}_{260 \text{ nm}} \times 50 \times \text{dilution factor}$$

The ratio between the readings at 260 nm and 280 nm ($\text{OD}_{260 \text{ nm}} / \text{OD}_{280 \text{ nm}}$) was used as an estimate of the purity of the DNA samples. Pure preparations of DNA have $\text{OD}_{260 \text{ nm}} / \text{OD}_{280 \text{ nm}}$ ratio between 1.7 and 1.8 (Sambrook et al., 2001). Computed OD values were used to dilute the DNA samples to working concentrations.

3.5 PARENTAL POLYMORPHISM USING NUCLEAR SSRs, GENIC SSRs AND RAPD MARKERS

Initial screening of parents was carried out for polymorphism before genotyping RILs. For this, parental DNA from IS22380 (P1) and E36-1 (P2) were subjected to PCR amplification with a total of 281 SSR, 30 genic SSRs, 4 sRGAs and 240 RAPD markers of these markers, 64 SSR, 8 genic SSRs, 2RGA and 19 RAPD markers have showed polymorphism between parents. The information source for SSR markers were from the work of Taramino *et al.* (1997), Bhattaramakki *et al.*, (2000) and Cornell set of SSR markers for sorghum. Genic SSRs used in the present investigation are a subset of sorghum expressed sequence tag (ESTs) database recently developed in our laboratory. Further an in-house database of annotated ESTs containing SSRs database was searched for the ESTs having similarity to the known drought and resistance related genes. Ten such sequences were available in the database and the primers were designed for the repeats present in them with an optimal T_m of 60°C with a range of 57-65°C, GC content of 30-70%, with a low chance of dimer or hair loop formation and with 100-300bp amplicon size. These primers were used for parental polymorphism and then to genotype to RILs.

The sequence information of both forward and reverse primer of nuclear SSR, genic SSRs, RAPD and RGA markers used is given in Appendix III ,Appendix IV and Appendix V. The experimental protocol used for these studies are

A. PCR reaction conditions for the amplification of marker loci

Amplification reaction mixture for RAPD, genic and genomic SSRs was prepared in 0.2 ml thin walled PCR tubes (Tarson Pvt Ltd, India) containing following components in a total volume of 25 µl.

Components	Quantity (µl/reaction)
Genomic DNA	2
dNTPs mix (2.5 mM each)	1
Primer (10 pM/ml)	1
Mgcl ₂ (25 mM)	1
10x assay buffer	2.5
Taq DNA polymerase (in-house)	0.34
Sterile water	17.16

PCR reactions were carried out using Master Cycler gradient 5331-Eppendorf version 2.30 31-09, Germany. The cycler was programmed as follows,

Genomic and genic SSRs:

Step	Temperature (°C)	Duration (min)	No of cycles
Initial denaturation	94	5	1
Denaturation	94	1	44
Primer annealing	42-62	1	
Primer extension	72	2	
Complete primer extension	72	5	1
Store	4	until removed	1

The annealing temperature was adjusted depending on the requirement for each primer or primer pair. For RAPD, except for annealing (36° C, 2 min) and final extension (72° C, 8 min), all the other conditions were as described above. After the completion of PCR, the products were stored at 4° C until the gel electrophoresis was done.

Polyacrylamide gel electrophoresis

Six per cent polyacrylamide gels were used for separation and visualization of PCR amplified microsatellite products. Denaturing gels were casted in Sequi-Gen GT nucleic acid electrophoresis cell (Biorad Ltd) as per the protocol in manual published by MRF, Hyderabad.

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 in chemical fumebood by adding 4 µl of bindsilane (Sigma-Aldrich, U.S.A) 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 entire surface of the plate. Treated side was marked. Repel silane (Sigma-Aldrich, U.S.A) 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 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 faced each other. Care was taken to correctly fit the spacers 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 acrylamide gel was prepared. Just prior to pouring, 60 μ l of N, N, N'-tetramethylethylenediamine (TEMED) and 600 μ l of 10 per cent ammonium per sulphate (APS) was added to initiate polymerisation. The contents were mixed gently by swirling and bubbles were avoided. Before pouring, the assembly was placed on such that it made a 45-degree angle with the bench top. Assembly was tilted so that one of the bottom corners is raised above the other and then carefully the solution was poured carefully into the space between glass plates, starting at the lower corner. As the acrylamide solution filled the space, gel assembly was lowered so that both bottom corners were on the bench, parallel to the bench top.

Shark toothcomb of 0.4 mm thickness (49 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. If bubbles formed during pouring, they were dislodged by tapping. Gel was left for 20 to 40 minutes for complete polymerization.

Electrophoresis of polyacrylamide gels

After the polymerization process, assembly was detached from the clamp and precision caster base and was placed in universal base against the back wall. IPC was locked to the base in vertical position by fitting stabilizer bar.

1. Tris free base-boric acid-EDTA (TBE) (5x) was poured in upper tank with IPC unit and the rest was poured in bottom chamber (1.8 L of buffer was prepared fresh each time).
2. Comb was removed and then excess polyacrylamide gel was removed with razor blade. Tissue paper was used to clean the glass plates with buffer.
3. Air bubbles and unpolymerised acrylamide on the top of gel were removed by squirting with 5% TBE.
4. Pre-run was given to achieve gel surface temperature of approximately 45 to 50 °C with following conditions. Temperature 50 °C, power 2000 V, 50 mA, constant watt of 75 W.
5. SSR loading dye (3x STR dye) was added to PCR products to a final of 1% and samples were denatured by heating to 95°C for 4 min and immediately cooled on ice.
6. After the pre-run, urea from the well area was flushed using a transfer pipette and carefully inserted the sharktooth comb into the gel so that teeth were just touching the surface of the gel. Care was taken to avoid the piercing of gel too deeply.
7. 6 μ l of each sample was loaded into the sample wells and DNA ladder of 100 bp size was loaded into the first or last well after denaturing.
8. Gel was run using the same conditions as in step4 and continued till the tracking dye reached bottom of the gel.

Visualization of SSR bands: 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 small glass plate.

DNA fragments were visualized following silver staining. 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 during the staining process.

Silver staining:

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 the 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.

All steps were done with constant shaking conditions.

3.4.6 Separation of PCR products for RAPD

The amplified products along with 2 µl of loading dye (Bromophenol blue) were separated on 1.2 per cent agarose gel at 80 volts (45 volts per cm of gel) using IX TAE buffer of pH 8.0 containing ethidium bromide (0.5 l/10 ml of gel). Lambda DNA-EcoR I/Hind III double digest was used as DNA molecular weight marker. The gels were photographed using gel documentation system (UVI Tech, England).

3.4.7 Genotyping RILs with SSR, genic SSRs and RAPD markers

Mapping population consisting of 93 RILs and parents were screened with polymorphic SSR and RAPD markers based on parental survey.

Scoring of nuclear SSR, genic SSRs and RAPD alleles and genotyping

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

1. Homozygote for the allele from parent P1 (IS22380) at the locus as A=1
2. Homozygote for the allele for parent P2 (E36-1) at the locus as B=3
3. Heterozygote carrying the alleles from both P1 and P2 as H=2
4. Off types showing banding pattern different from the parents and missing data for the individual at the locus=0

After scoring the individual progeny, the data was entered in Microsoft Excel spreadsheet in a format suitable for linkage analysis by MAPMAKER/EXP (3.0b) and MAPMAKER/QTL (1.1b) software.

3.5 SORGHUM RGAs

Previously about 450 RGAs representing NBS-LRR, P motif and Kinases classes of R genes were isolated and a set of 13 SRGAs were characterised by sequencing in our laboratory (Anand et al., 2005). Specific primers, were designed for 4 SRGAs that were similar to already known R genes, targeting about 400 to 600 bp region were designed. The polymorphism was determined by restriction analysis and sequencing each of them, separately, as follows;

PCR amplification of SRGAs

A PCR condition used for amplification of SRGAs is as follows. The annealing temperature was primer specific.

Step	Temperature (°C)	Duration (min)	No of cycles
Initial denaturation	94	5	1
Denaturation	94	2	29
Primer annealing	Primer specific	1	
Primer extension	72	2	
Complete primer extension	72	30	1
Store	4	Until use	1

The product obtained after the PCR reactions were kept at 4 °C till they were used further.

Restriction analysis

The amplification fragments corresponding to 400 to 600 bp were excised from the 0.8 per cent low melting agarose gel using a sharp sterile scalpel blade by keeping the gels at low intensity UV transilluminator. The Qiagen Gel extraction kit (QIAGEN sciences, Maryland) was used to elute the DNA from the agarose pieces. Gel purified PCR fragments were subjected for restriction analysis using some common 4 bp and 6 bp recognizing restriction enzymes viz; *Alu I*, *Hae III*, *Xba I*, *Sac I*, *Eco RI*, *Sau3A I* and *Taq I* and visualised on 1.8 per cent agarose gel for cleaved amplified fragment polymorphism for both parents. Genes that did not show any restriction polymorphism between parents were considered for sequencing and further analysis. Sequence information of the primers used is;

SRGAMF5'CGATGTCGAGGAAGCACGTC3'

SRGAMR5'CCGTGCCGCCATCGGAATG3'

SRGA212F5'ACGTAGTGGCAATCCCTGGCAC3'

SRGA212R5'TGGCGGTGAACAGCAGGTGC3'

SRGA114F5'GGTTGGGAAGACAACGTTCC3'

SRGA114R5'ACGGTAGTGGCAATCCCTGGCA3'

SRGA113F5'TGGGAAGACAACGTTCCCCATG3'

SRGA113R5'ACGCTAGTGGCAATCCCTG3'

Primers were designed using Primer3 software (www.genome.wi.mit.edu/cgi-bin/primer/primer3). Polymorphism between E36-1 and IS22380 was tested by size fractionation both on agarose gel and 6 % sequencing gel as described earlier.

Separation and elution of PCR products: A large-scale amplification was done to elute the PCR fragment. The major amplification fragment corresponding to 500 to 600 bp region was excised from the 0.8 per cent low melting agarose gel using a sharp sterile scalpel blade after keeping the gel at low intensity UV transilluminator. The agarose gel piece containing the band was collected in a sterile pre-weighed 1.5 ml micro centrifuge tube. The Qiagen Gel extraction kit (QIAGEN Sciences, Maryland) was used to elute the DNA from the agarose piece.

Cloning of PCR products: Eluted and purified PCR fragments were ligated to pTZ57R/T vector as described in InsT/A clone™ PCR product cloning kit (InsT/Aclone™, MBI Fermentas Life Sciences, USA). The vector supplied after cleaving with Eco321 (an Isoschizomer of *EcoRV*) and treated with terminal deoxynucleotidyl transferase to create 3' ddT overhangs at both ends. When PCR fragment with 3'-A overhang is ligated into the vector, a circular molecule with two nicks is produced. The circular plasmid with insert was directly transformed in *E. coli* DH5α. The same procedure was followed for all PCR amplified; gel eluted and purified RGA fragments. Looking into the conversion table for the amount of PCR fragment required per ligation reaction, an optimal molar ratio of ends of 1:3 of vector: insert was calculated. The components of the ligation mix (Appendix VI) were mixed into a 0.5 ml micro-centrifuge tube and were incubated at 22°C overnight in circulating water bath. A control ligation reaction was performed using control PCR fragment provided in the kit by adding components

Transformation was carried out using transform Aid™ bacterial transformation system (InsT/A clone™ PCR product cloning Kit, MBI Fermentas Life Sciences, USA). The *E. coli* strain DH 5α cells were made competent for transformation by following kit protocol supplied by manufacturer.

Selection of recombinant clones: After overnight incubation, the recombinant clones were identified by the blue-white assay X-gal plates and induce IPTG. About 100-150 white colonies (recombinants) were randomly picked up and streaked on fresh LA plates containing Ampicillin @ 100 µg/100 ml, X-gal and IPTG. White colonies picked up from blue white assay were further analyzed for the presence of desired DNA fragment in the transformed bacteria by restriction and PCR analysis. Three independent colonies for each gene were sequenced at Bangalore GENEI pvt ltd. The quality of the sequences was taken care by comparing three independent sequences in each case. Presence of cleaved amplicon polymorphic sequences (CAPS) was identified by comparing restriction maps between parents desired from REBASE (www.rebase.net.com). Further, the presence of SNP was detected by simple alignment.

3.6 STATISTICAL ANALYSIS

3.6.1 Phenotypic characters

Analysis of variance (ANOVA): The analysis of variance for different characters was performed as in 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 formula (Singh and Chaudhary, 1977).

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

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

Where,

PCV	Phenotypic coefficient of variation
V_p	Phenotypic variance
GCV	Genotypic coefficient of variation
V_g	Genotypic variance
$\bar{X}$	Mean of RILs

GCV and PCV values were categorized as low, moderate and high values as indicated by Sivas Subramanian 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).

$$r_p = \frac{\text{Cov}_p(x, y)}{\sqrt{\text{Var}_p(x) \text{Var}_p(y)}}$$

Where,

r_p	Phenotypic correlation coefficient
$\text{Cov}_p(x, y)$	Phenotypic covariance between characters x, y
$\text{Var}_p(x)$	Phenotypic variance in character x
$\text{Var}_p(y)$	Phenotypic variance in character y

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

Heritability

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

$$h^2 =$$

Where,

$$h^2 = \frac{V_g}{V_p}$$

h^2	heritability coefficient
V_g	Genotypic variance
V_p	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)

$$GA\% = h \times p \times K$$

h = Heritability

p = Phenotypic standard deviation

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

a. Genetic advance as percent mean

$$GA \text{ as per cent mean} = (GA / X) \times 100$$

Where,

GA =

— Genetic advance

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

3.6 MARKER DATA ANALYSIS

3.6.1 Marker segregation

For checking the goodness of fit for segregation of marker loci, χ^2 values were compared with table values were calculated and at one degree of freedom for each marker locus. The marker status of any of the 93 RILs and parents for a locus was scored either as P1 or P2 allele.

3.6.2 Construction of genetic linkage map

A computer package, 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 3.0 and a maximum distance of 30 % 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.

Previously a set of SSR markers were mapped in IS22380 x E36-I RIL population in our laboratory by punnuri (2004). They were used as reference for assigning markers to definite linkage groups for six SSR markers and 18 RAPD markers were assigned to linkage groups using computer package MAPMAKER/EXP version 3.0 b, as described above. In all, data for a total of 64 SSRs, 8 genic SSRs and 18 RAPD markers were used to construct a genetic map for the RIL population IS22380 x E36-I.

Linkage distance in terms of centimorgan (cM) values was calculated using Kosambi's mapping function accordingly, interval-mapping technique was adopted for the construction of linkage groups and QTL analysis.

3.6.3 QTL analysis

MAPMAKER/EXP performs full multipoint linkage analysis for dominant and co-dominant markers in a mapping population. The output of MAPMAKER/EXP contains information on linkage groups and linkage distances between the markers on each linkage group are included as input file for QTL Cartographer (2.5b). QTL Cartographer is a suite of programs for mapping quantitative trait loci (QTLs) onto a genetic linkage map. The QTLs identified for components of resistance were assigned to the linkage groups based on position of markers on respective linkage maps.

IV. EXPERIMENTAL RESULTS

The results of present investigation are presented under the following headings.

- 4.1 Estimation of variability and character association between yield and component traits of stay-green character.
- 4.2 Correlation between pairs of characters.
- 4.2 Molecular mapping and QTL analysis.

4.1 ESTIMATION OF VARIABILITY AND CHARACTER ASSOCIATION

Pooled analysis for variance revealed highly significant difference among genotypes (RILs) for all the 9 traits at both Dharwad and Bijapur location and single location analysis for carbon isotope discrimination. Variability and other genetic parameters were also estimated. Range, mean, phenotypic and genotypic coefficient of variation (PCV and GCV), heritability estimates and predicted genetic advance as per cent of mean of characters studied are presented in Table 2.

The details of frequency distribution patterns of RIL phenotypes for each trait are presented in Fig. 1, 2 and 3.

4.1.1 Early seedling vigour

The mean value of early seedling vigour was 2.73, ranging from 1.50 to 3.63. The percent PCV and GCV for this trait were 28.84 and 18.64 respectively. Heritability of 41.77 and genetic gain of 24.81 per cent were recorded for this trait.

4.1.2 Plant height

A large variation was observed for plant height where the mean value among the RILs ranged from 74.82 to 145.90 with an overall mean of 131.40 cm. The phenotypic and genotypic coefficients of variability were 12.31 and 8.35 per cent respectively. The estimate of heritability was 46.01 per cent with an expected genetic advance of 11.66 per cent.

4.1.3 Day to 50 per cent flowering

The mean of days to 50 per cent flowering was 67.80 with a range of 60.13 to 73.10. The phenotypic and genotypic coefficients of variability were 8.30 and 6.20 per cent respectively. The heritability estimate of 55.79 per cent with an expected genetic advance of 9.50 per cent was observed for this trait.

4.1.4 Stem thickness

The mean value for this trait was 3.52 cm ranging from 1.37 to 5.75 cm. The PCV and GCV for this trait were 37.25 and 33.42 respectively. Both heritability (80.49%) and GAM (61.10%) were rather high for this trait.

4.1.5 Grain yield per plant

Grain yield per plant significantly varied from 14.37 to 71.46 g across RILs with a mean of 41.52 g. The per cent phenotypic and genotypic coefficients of variability for this trait were 38.51 and 31.24 respectively. High heritability of 65.80 per cent with an expected genetic advance of 52.20 per cent was recorded.

4.1.6 Stomatal frequency (cm²)

The mean number of stomata per cm² was 16214, ranging from 13078 to 20724. The PCV and GCV for this trait were 18.50 and 16.50 respectively. Both heritability (79.54 %) and GAM (30.39 %) were moderate for stomatal frequency.

Table 1. Pooled analysis of variance for different traits in RIP derived from IS22380 x E36-1

Source of variation	d.f	X1	X2	X3	X4	X5	X6	X7	X8	X9
Environment	1	1.51**	1717.00**	391.25**	0.21	81.0*	4571	258.75*	254.35*	255.32**
Replication	2	0.24	9.50	9.00	0.13	0.21	260.50	10.62	5.12	4.53
Genotypes	92	0.98**	206.95**	38.35*	11.74**	1645.10**	18714**	916.25**	682.43**	476.32**
G X E	92	0.12	316.75**	40.82**	0.09	177.50**	9290678**	87.34*	185.87**	154.24**
Error	368	0.09	112.40	28.21	0.11	20.27	4089804.40	63.23	48.24	28.64
S.Em.±		2.73	6.12	3.06	0.19	2.59	1167.59	4.591	4.01	3.09
CD at 5 %		0.17	17.02	8.52	0.53	7.21	3246.52	12.77	11.15	8.59
CD at 1 %		0.64	22.41	11.29	0.70	9.49	4274.80	16.81	14.68	11.31
CV (%)		11.12	8.07	7.83	9.41	10.8	12.47	11.38	13.29	14.20

* Significance at 5 % level

** Significance at 1 % level

X1 : Early seeding vigour

X2 : Plant height (cm)

X3 : Days to 50 % flowering

X4 : Stem thickness (cm)

X5: Seed yield / plant (g)

X6 : stomatal frequency (cm²)

X7 : % GL 15

X8 : % GL 30

X9 : % GL 45

Table 2. Magnitude of variability parameters in 10 traits (pooled) of RIP derived from IS22380 x E36-1

Trait	Range		Grand mean	PCV (%)	GCV (%)	Heritability (%)	Genetic advance/mean (%)
	Min	Max					
X1	1.5	3.63	2.729	28.84	18.64	41.77	24.81
X2	74.82	145.9	131.4	12.31	8.35	46.01	11.66
X3	60.13	73.1	67.8	8.3	6.2	55.79	9.5
X4	1.375	5.75	3.538	37.25	33.42	80.49	61.1
X5	14.37	71.46	41.52	38.51	31.24	65.8	52.2
X6	13078	20724	16214	18.5	16.5	79.54	30.39
X7	55.28	97.62	69.85	20.32	15.37	57.21	23.94
X8	37.76	89.58	52.25	21.54	18.32	72.33	32.09
X9	13.25	55.28	37.69	32.14	23.12	51.74	34.26
X10	3.75	5.985	4.866	22.5	21.2	88.77	41.14

X1 : Early seeding vigour
 X2 : Plant height (cm)
 X3 : Days to 50 % flowering
 X4 : Stem thickness (cm)
 X5 : Seed yield / plant (g)
 X6 : stomatal frequency (cm²)
 X7 : % GL 15
 X8 : % GL 30
 X9 : % GL 45
 X10 : carbon isotope discrimination

Note: Values for $\Delta C(x10)$ were analysed for single location(Dharwad).

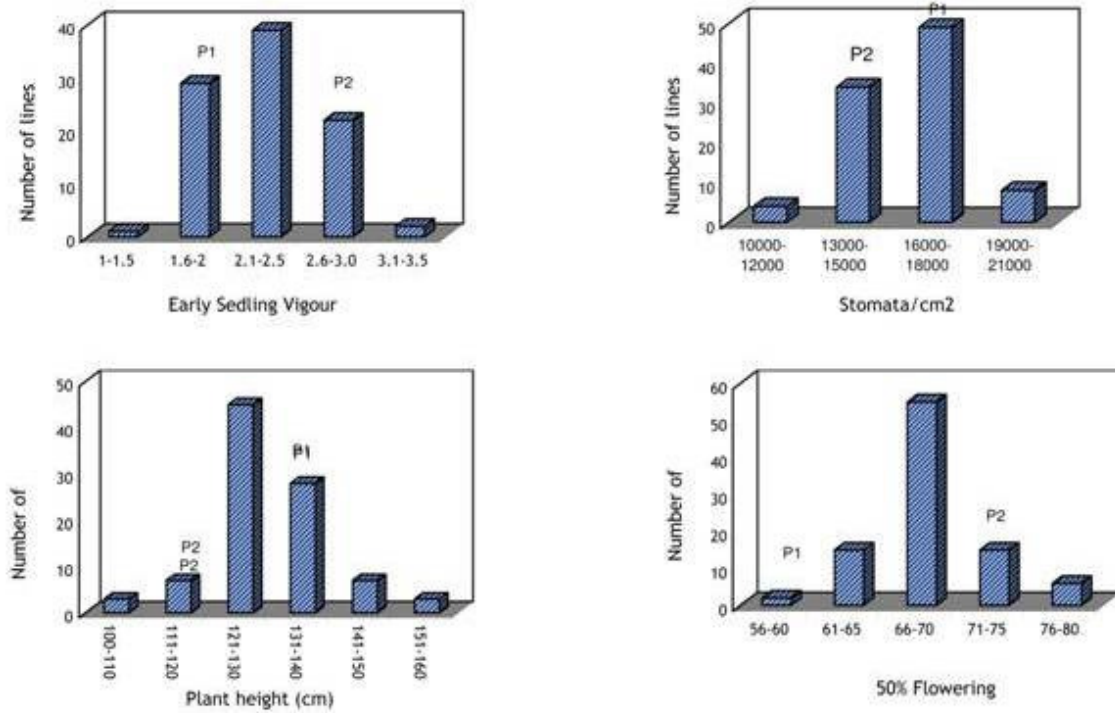


Fig. 1 Frequency distribution of phenotypes for different quantitative traits (pooled) in 93 RILs derived from IS22380 x E36 - 1

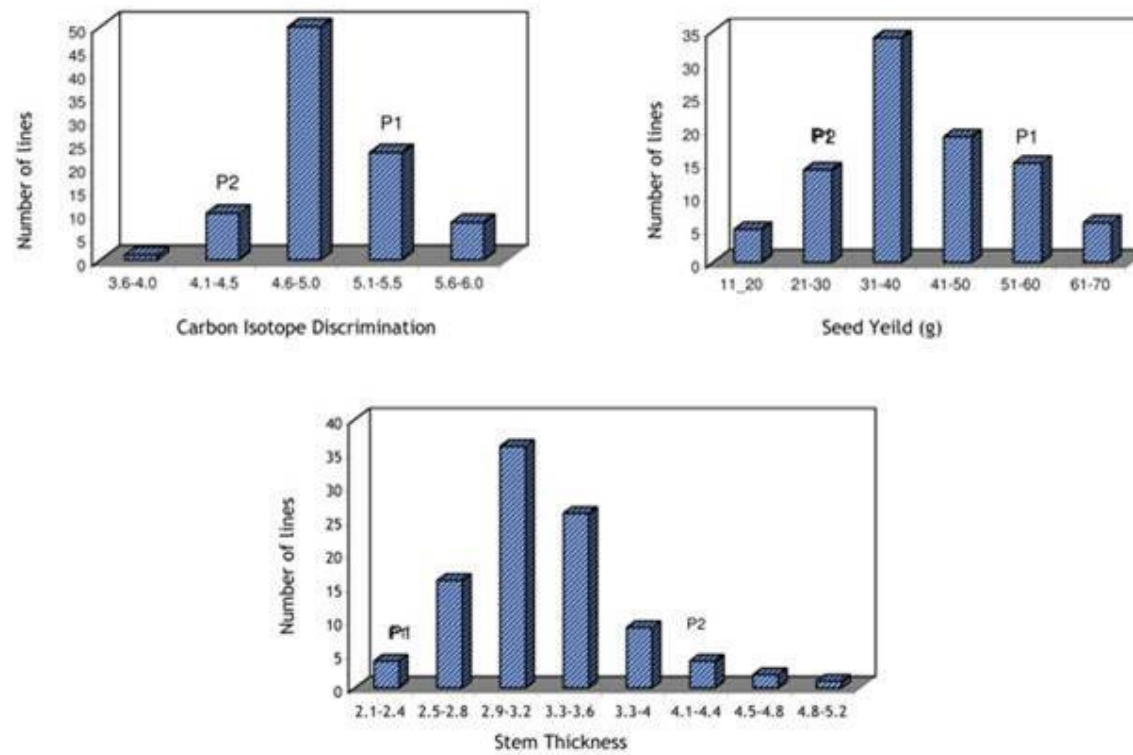


Fig. 2 Frequency distribution of phenotypes for different quantitative traits (pooled) in 93 RILs derived from IS22380 X E36-1

4.1.7 Carbon isotope discrimination ($\Delta^{13}\text{C}$)

The mean value 4.86 for this trait ranged from 3.75 to 5.98. The PCV and GCV for this trait were 22.50 and 21.20 per cent respectively. Both heritability (88.77 %) and GAM (41.14 %) were significant for carbon isotope discrimination.

4.1.8 Stay-green

Frequency distribution for RIP for stay-green trait was unimodal and approximately normal.

4.1.8.1 Per cent green leaf at 15 days after flowering (% 15 GL)

A range of variation between 55.28 and 97.62 with a mean value of 69.85 for leaf senescence was observed. The phenotypic and genotypic coefficients of variation for this trait were 20.32 and 15.37 per cent respectively, with a high heritability of 57.21 per cent and an expected genetic advance of 23.94 per cent over mean.

4.1.8.2 Per cent green leaf at 30 days after flowering (% 30 GL)

A wide range of mean values between 37.76 and 89.58 with a grand mean of 52.25 was recorded for this trait. The PCV and GCV recorded for this trait were 21.54 and 18.32 per cent respectively. High heritability of 72.33 per cent with an expected genetic advance of 32.09 per cent was recorded.

4.1.8.3 Per cent green leaf at 45 days after flowering (% 45 GL)

A wide range of variation from 13.25 to 55.28 per cent among RILs with a mean of 37.69 per cent green leaf area was recorded. The PCV of 32.14 per cent, GCV of 23.12 per cent and a high heritability 51.74 per cent with an associated genetic advance of 34.26 per cent over the mean were recorded for this trait.

4.2 Correlation analysis

Genotypic and phenotypic correlation co-efficient were computed to know the nature of association among different characters. Based on significance, the association of the most important traits with all other traits was investigated. Nature of association for $\Delta^{13}\text{C}$ with other traits was estimated for Dharwad location alone. Details of phenotypic and genotypic correlations are presented in the Table 3 and 4.

4.2.1 Association among yield components

Early seedling vigour showed negative correlation with stomatal frequency, while its association was positive with all other traits *viz.*, plant height, days to 50 per cent flowering, stem thickness and seed yield per plant at both genotypic and phenotypic level.

At genotypic level, plant height had significant negative association with days to 50% flowering. The phenotypic and genotypic correlation was positive for plant height with seed yield per plant and stomatal frequency. Days to 50 % flowering showed negative correlation with seed yield per plant and stomatal frequency while its association was positive with stem thickness at both genotypic and phenotypic level.

A highly significant and negative correlation was observed between stem thickness and stomatal frequency at genotypic and phenotypic level. While positive correlation was observed between stem thickness and seed yield per plant. Negative phenotypic and genotypic correlation was observed between seed yield per plant and stomatal frequency.

4.2.2 Correlation of carbon isotope discrimination ($\Delta^{13}\text{C}$) with other traits

Correlation of carbon isotope discrimination with other traits was done only for Dharwad location. At both genotypic and phenotypic level, highly significant positive association was observed for carbon isotope discrimination value with stem thickness, while the relationship was negative with early seedling vigour, plant height, days to 50 % flowering and stomatal frequency.

Table 3. Phenotypic correlation coefficients among 10 traits (pooled) in RIP derived from IS22380 x E36-1

Traits	X1	X2	X3	X4	X5	X6	X7	X8	X9
X1	1	0.11	0.10	0.10	0.16	-0.10	0.01	0.03	0.02
X2		1	-0.11	-0.07	0.11	0.06	0.12	0.20	0.11
X3			1	0.01	-0.10	-0.09	0.10	-0.29**	-0.17
X4				1	0.02	-0.19	0.16	0.12	0.20
X5					1	-0.13	0.19	0.27**	0.30**
X6						1	-0.03	-0.19	-0.09
X7							1	0.27**	0.18
X8								1	0.20
X9									1

* Significance at 5 % possibility level

** Significance at 1 % possibility level

X1 : Early seeding vigour X6 : stomatal frequency (cm²)

X2 : Plant height (cm) X7 : % GL 15

X3 : Days to 50 % flowering X8 : % GL 30

X4 : Stem thickness (cm) X9 : % GL 45

X5 : Seed yield / plant (g)

Table 4. Genotypic correlation coefficients among 10 traits in RIP derived from IS22380 x E36-1

Traits	X1	X2	X3	X4	X5	X6	X7	X8	X9
X1	1	0.16	0.12	0.16	0.17	-0.11	0.02	0.06	0.03
X2		1	-0.29**	-0.08	0.20	0.08	0.17	0.20	0.20
X3			1	0.05	-0.11	-0.11	0.12	-0.32**	0.21
X4				1	0.04	-0.27**	0.19	0.19	0.31**
X5					1	-0.17	0.20	0.38**	0.41**
X6						1	-0.07	-0.27	-0.11
X7							1	0.36**	0.20
X8								1	0.27**
X9									1

* Significance at 5 % possibility level

** Significance at 1 % possibility level

X1 : Early seeding vigour

X2 : Plant height (cm)

X3 : Days to 50 % flowering

X4 : Stem thickness (cm)

X5 : Seed yield / plant (g)

X6 : stomatal frequency (cm²)

X7 : % GL 15

X8 : % GL 30

X9 : % GL 45

4.2.3 Correlation of green leaf area with other traits

Green leaf area at 15 days after flowering showed positive phenotypic and genotypic correlation with early seedling vigour, plant height, days to 50 % flowering, stem thickness, seed yield per plant and carbon isotope discrimination. In contrast its association was negative with stomatal frequency Table 3 and Table 4.

A highly significant and positive association at both genotypic and phenotypic level was recorded for green leaf at 30 days with seed yield per plant, carbon isotope discrimination and green leaf area at 15 days. At genotypic level green leaf area at 38 days was significantly negatively correlated with days to 50 % per cent flowering and stomatal frequency.

The genotypic and phenotypic correlation for green leaf area at 45 days was significant and positively correlated with stem thickness, seed yield per plant and green leaf area at 30 days interval.

4.3 MOLECULAR MAPPING AND QTL ANALYSIS

4.3.1 Parental polymorphism

The parental lines, IS22380 and E36-1 were surveyed against nuclear SSR, genic SSR and RAPD markers to detect polymorphism and to identify useful markers for genotyping RILs. A Data for 213 nuclear SSRs, 30 genic SSRs and 240 RAPD markers was available, of which 45 SSRs (22 per cent), 8 genic SSRs (26 per cent) and 19 RAPD (7.91 per cent) markers detected polymorphism between parents (plate 1). All the polymorphic markers were used to genotype individual RILs of mapping population (Plate 2).

4.3.2 Marker segregation

The segregation pattern of all the 89 marker loci in RILs was checked for the expected 1:1 ratio. Calculated χ^2 values at 1 degree of freedom were compared with table value $P = 0.05$ for each marker locus (Table 5). All 89-marker loci segregated in 1:1 ratio.

Restriction analysis of all the four RGAs using selected 4 and 6 base cutters did not reveal any polymorphism between parents on the agarose gels. The nucleotide sequence of individual genes separately from IS22380 and E36-1 were subjected to in silico restriction mapping and SNP analysis. In SRGA214, has BbvI, Fnu4HI, ApeKI, TseI, NspI, CviAI, AfIII and FatI restriction sites were different from that in IS22380 parent (Fig 8). Difference in restriction pattern is due to the presence of a single nucleotide polymorphism (SNP) at 265th bp. Further, one SNP at 183th bp in SRGA212 was noticed, which however did not result in any CAPS (Fig 4). Sequence alignment and restriction maps of SRGAs between both parents are presented in Fig 4 to 9.

4.3.3 Construction of linkage map

The marker data from all the 89 markers was used to construct a linkage map. Mapping of markers was accomplished using MAPMAKER/EXP (3.0b) software (Lander *et al.*, 1987). Linkage distance in terms of centimorgan (cM) values was calculated using Kosambi mapping function. A total of 89 markers were found to be associated among themselves with varied distances on ten linkage groups. The details of linkage groups to which these 89 markers belong and linkage distances in cM are given in Table 9. The remaining 5 markers were independent. Linkage map for drought in is given in plate 3, and linkage map for bijapur and dharwad location show in plate 4 and 5.

All the markers were assigned to different linkage groups. Based on the anchor markers and that of the order of SSR markers in the linkage map of Bhatramakki *et al.* (2000), Haussmann *et al.* (2004). The linked markers in the present study were assigned to all the ten linkage groups viz., A, B, C, D, E, F, G, H, I and J, (Plate 4). The number of markers mapped per linkage group ranged from three (G) to eighteen (B). The linkage map thus constructed had a total coverage 764.66 cM. The markers distributed unevenly through the linkage groups with an average distance of 10.76 cM.

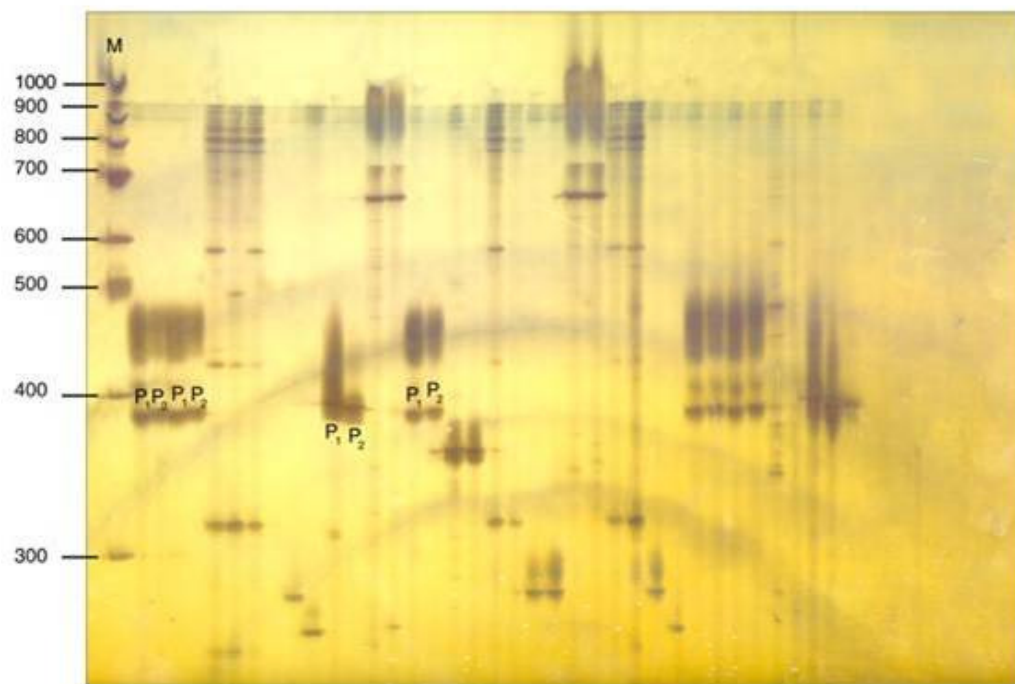


Plate 1. Parental survey to identified polymorphic gSSR marker on 6% PAGE

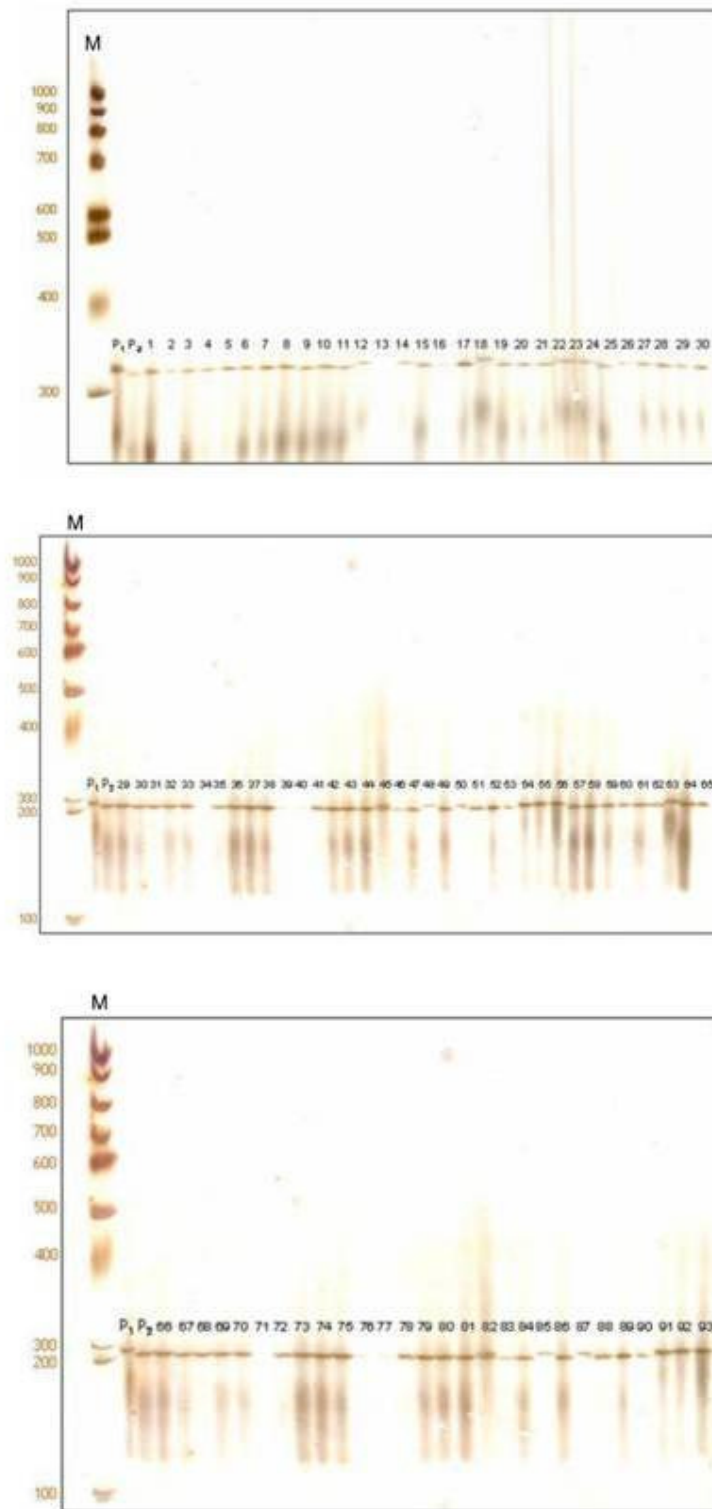


Plate 2. Segregation of iabtgs 155 marker across the RILs and parents on 6% PAGE

Table 5. Test of significance for segregation of RAPD, nuclear SSR and genic SSR marker in RILs of IS22380 x E36-1

Sl. No.	Marker	P1 allele	P2 allele	χ^2
1	Xtxp9	44	46	0.044
2	SbAGB02	49	44	0.268
3	Xtxp318	47	45	0.043
4	Xtxp63	47	45	0.043
5	Xtxp211	43	49	0.392
6	X txp8	45	47	0.043
7	X txp343	45	46	0.011
8	Xtxp12	40	53	1.816
9	Xtxp295	39	49	1.136
10	SbAGE03	49	42	0.538
11	Xtxp67	38	50	1.942
12	Xtxp230	44	49	0.263
13	Xtxp258	37	50	1.942
14	SbAGH04	46	47	0.01
15	Xtxp354	41	49	0.711
16	SbAGA01	48	47	0.01
17	Xtxp274	42	47	0.281
18	Xcup9	44	50	0.382
19	SvPEPCAA	44	49	0.268
20	AB4	54	41	1.778
21	AC12	43	52	0.852
22	AC13	46	49	0.094
23	AC19	48	47	0.01
24	AC20	45	50	0.263
25	AK10	51	44	0.515
26	AT5	40	55	2.041
27	AT9	51	44	0.19
28	BH13	41	54	0.085
29	G16	40	55	2.368
30	G17	43	50	0.526
31	G19	44	51	0.515
32	H3	48	47	0.01
33	H4	40	55	2.368
34	L18	43	52	0.852
35	M3	50	45	0.263
36	M7	48	47	0.01
37	M10	55	40	2.368
38	corn61	47	46	0.011
39	txtp267	42	49	0.538
40	txtp250	40	50	1.111
41	txtp316	40	49	0.910
42	corn74	39	53	2.130
43	txtp287	40	47	0.563
44	txtp201	44	46	0.044
45	txtp348	42	50	0.696

Contd...

Sl. No.	Marker	P1 allele	P2 allele	χ^2
46	xtxp303	46	47	0.011
47	corn59	49	44	0.269
48	corn24	45	45	0.000
49	xtxp210	40	51	1.330
50	xtxp353	44	48	0.174
51	xtxp159	41	52	1.301
52	xtxp358	38	53	2.473
53	xtxp204	39	51	1.600
54	xtxp284	43	50	0.527
55	pepC	39	52	1.857
56	xtxp254	43	48	0.275
57	xtxp217	40	50	1.111
58	xtxp329	39	54	2.419
59	xtxp248	44	49	0.269
60	xtxp96	41	46	0.287
61	xtxp176	38	51	1.899
62	xtxp312	47	44	0.099
63	xtxp325	40	50	1.111
64	xtxp324	42	46	0.182
65	xtxp338	44	49	0.269
66	xtxp273	43	49	0.391
67	xtxp327	41	52	1.301
68	xtxp48	40	53	1.817
69	corn73	40	47	0.563
70	xtxp71	46	44	0.044
71	xtxp92	47	44	0.099
72	xtxp160	37	52	2.528
73	xtxp213	51	41	1.087
74	xtxp297	42	45	0.103
75	xtxp302	47	45	0.043
76	iabtgs2	37	51	2.227
77	iabtgs9	43	49	0.391
78	xtxp298	42	50	0.696
79	iabtgs20	39	54	2.419
80	xtxp183	41	52	1.301
81	xtxp45	39	54	2.419
82	iabtgs1	47	46	0.011
83	M9	37	54	3.176
84	xtxp290	38	55	3.108
85	xtxp350	41	52	1.301
86	iabt155	44	49	0.268
87	iabt477a	47	46	0.010
88	iabt477b	47	46	0.010
89	iabt83a	53	40	1.8172
90	iabt83b	53	40	1.8172
91	iabt259	39	54	2.4193

* 0.05<P>0.01 : 3.81

** P<0.01> : 6.63 Expected ratio : 1:1

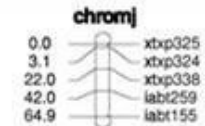
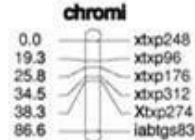
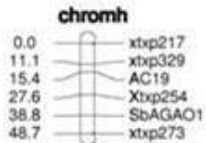
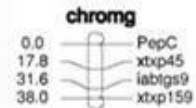
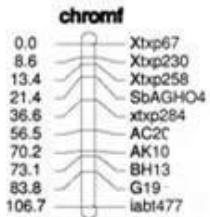
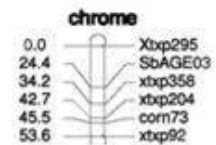
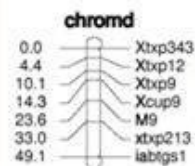
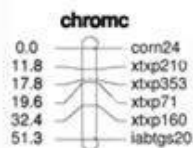
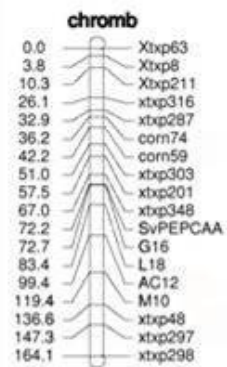
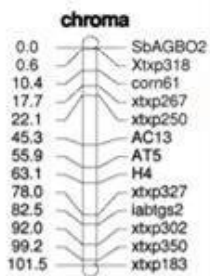


Plate 3. Linkage map for drought in sorghum RIL population derived from IS22380 X E36-1

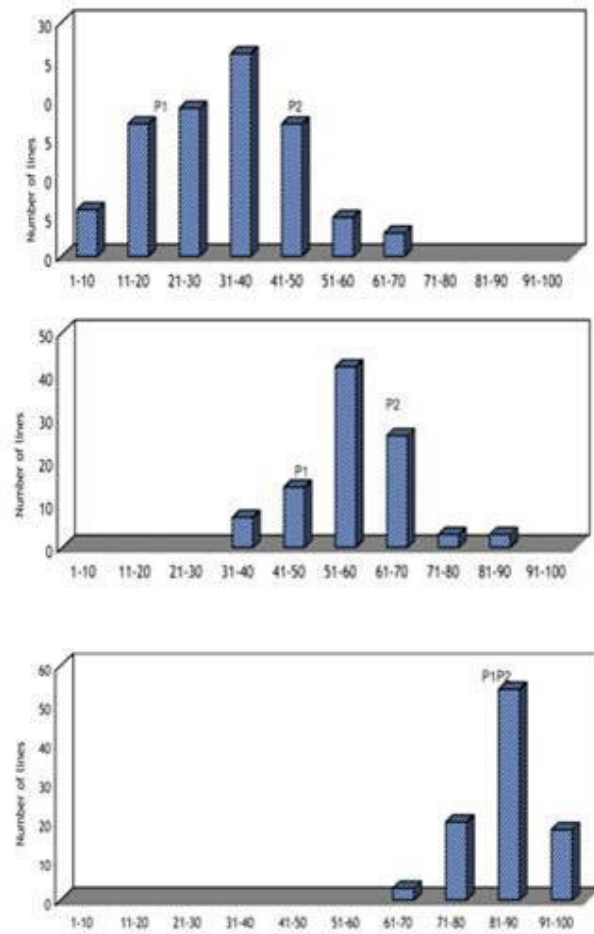


Fig . 3 Frequency distrubution of phenotypes for green leaf area (pooled) in 93 RILs derived from IS22380 X E36-1

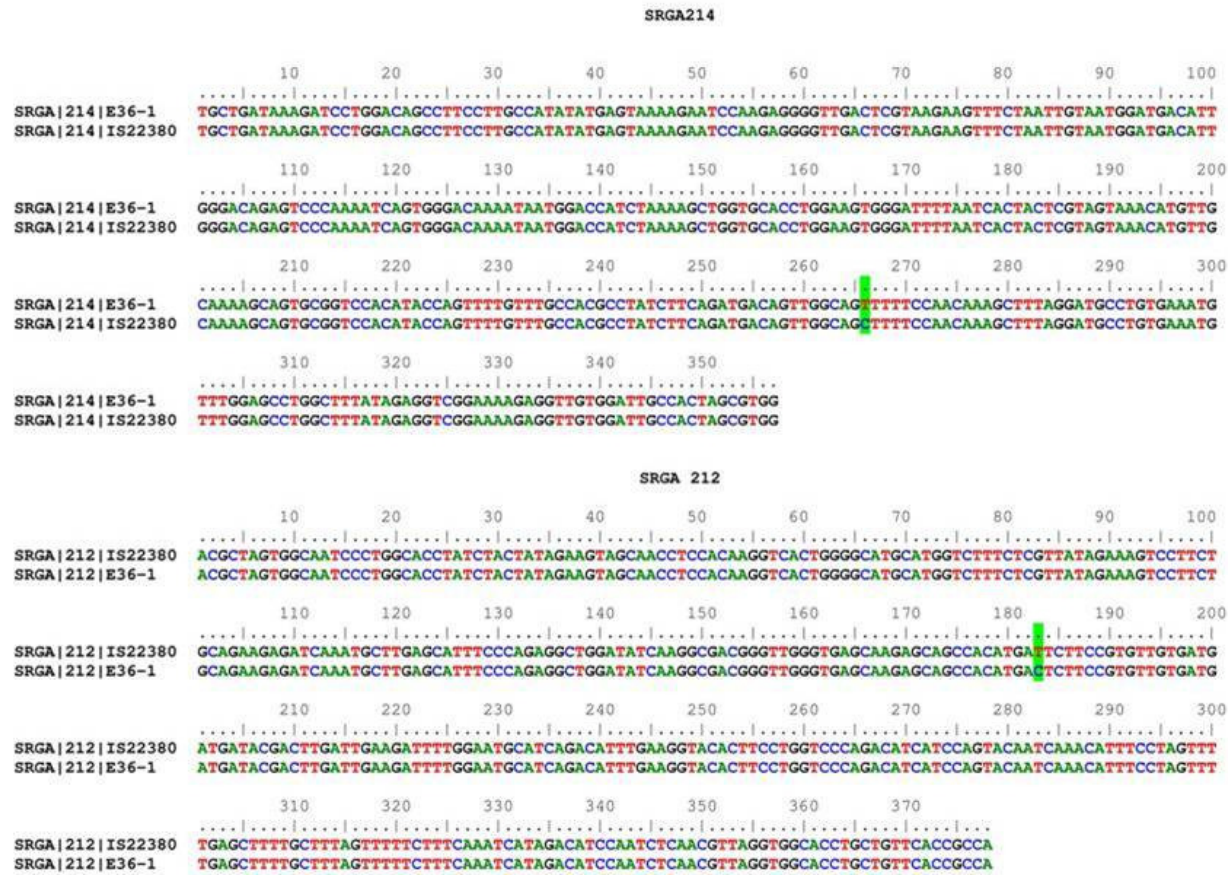


Fig. 4 Alignment of SRGA214 and SRGA212 from IS22380 and E36-1 parental lines

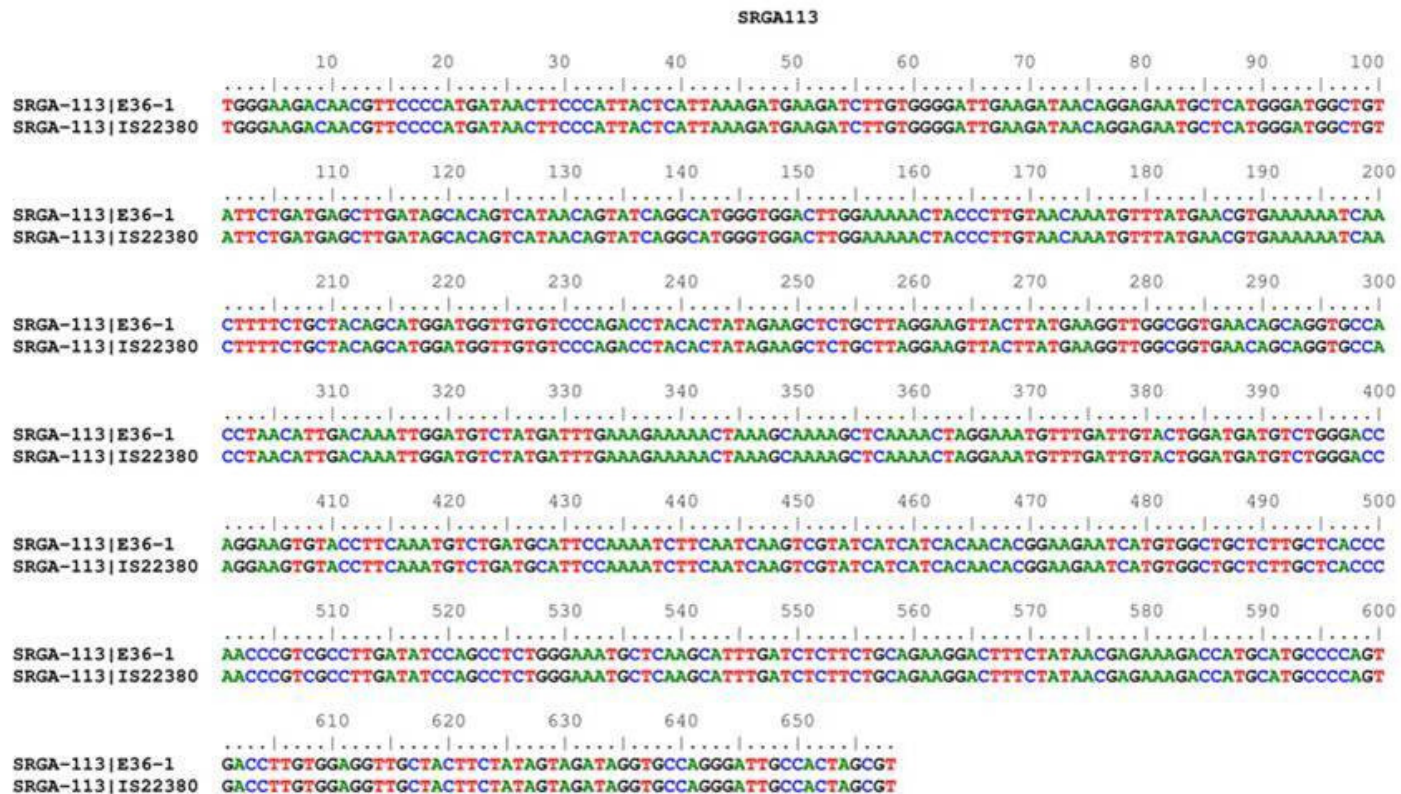


Fig. 5 Alignment of SRGA113 isolated from IS22380 and E36-1 parental lines.

A

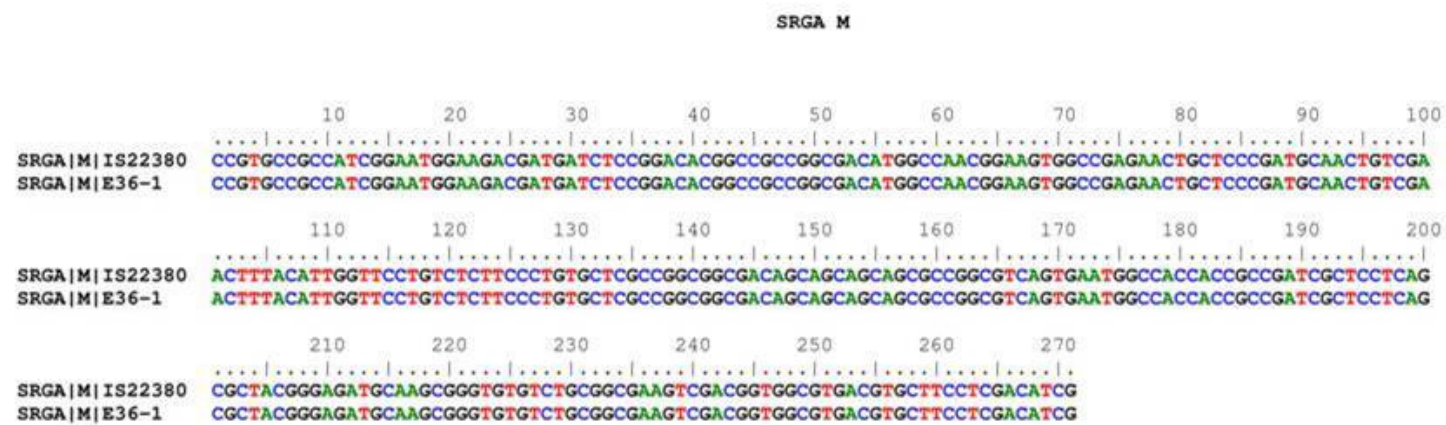


Fig. 6 Alignment of SRGA113 isolated from IS22380 and E36-1 parental lines.

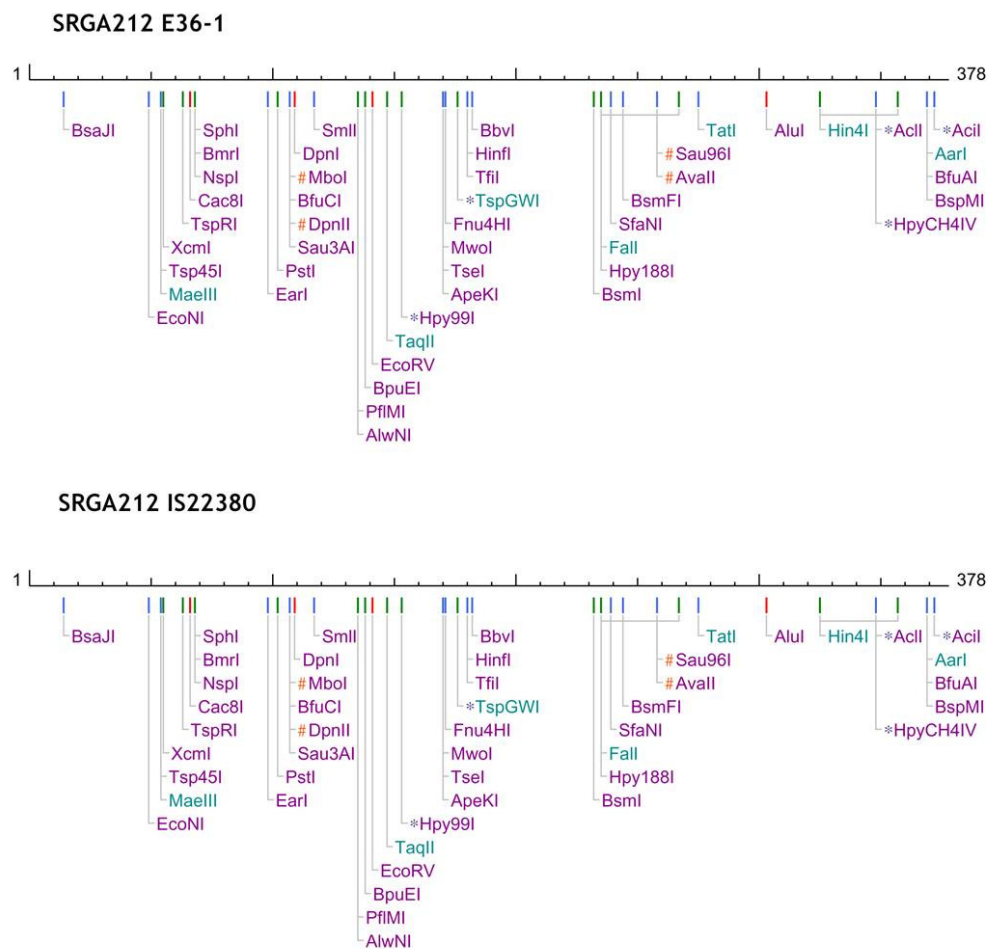


Fig. 7 Restriction map of SRGA212 between IS22380 and E36-1

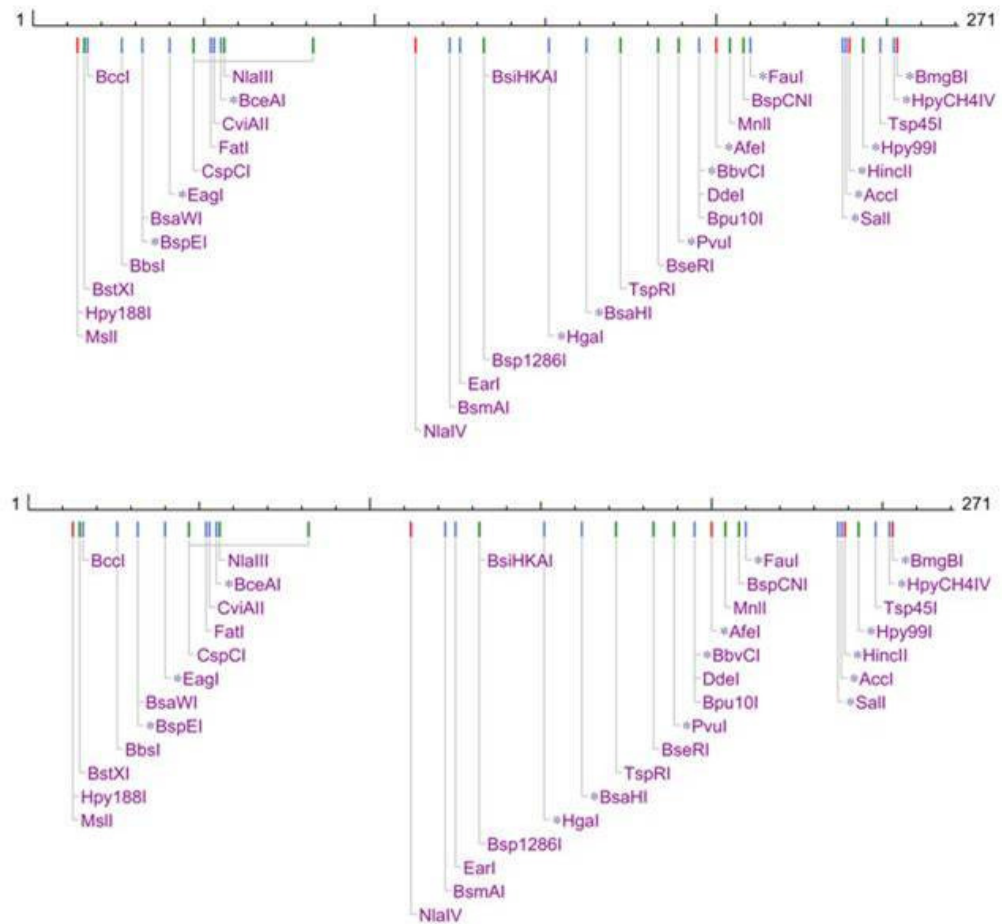


Fig. 9 Restriction map of SRGA M between IS22380 and E36-1

Table 9. Linkage distance between the pairs of SSR, genic SSRs and RAPD markers mapped onto linkage map of RILs derived from IS22380 x E36-1

Sl. No.	Linkage group	Pairs of markers	Distance
1	A	sbAGB02-XtxP318	0.6
2	A	xtxP318-corn61	9.8
3	A	corn61-xtxp267	7.3
4	A	xtxp267-xtxp250	4.4
5	A	xtxp250-AC13	23.2
6	A	AC13-AT5	10.6
7	A	AT5-H4	7.2
8	A	H4-xtxP327	14.9
9	A	xtxP327-iabtqS2	4.5
10	A	iabtgs2-xtxP302	9.5
11	A	xtxP302-xtxP350	7.2
12	A	xtxP350-xtxP183	2.3
13	B	xtxPb3-xtxP8	3.8
14	B	xtxP8-xtxP211	6.5
15	B	xtxP-211-xtxP316	15.8
16	B	xtxP316-xtxP287	6.8
17	B	xtxP287-corn74	3.36
18	B	corn74-corn59	6.0
19	B	corn59-xtxP303	8.8
20	B	xtxP303-xtxP201	6.5
21	B	xtxP201-xtxP348	9.5
22	B	xtxP348-SVPEpCAA	5.2
23	B	SVPEpCAA-G16	0.5
24	B	G16-L18	10.7
25	B	L18-AC12	16.0
26	B	AC12-M10	20.0
27	B	M10-xtxP48	17.2
28	B	xtxP48- xtxP297	10.7
29	B	xtxP297-xtxP296	16.8
30	C	corn24-xtxP210	11.8
31	C	xtxP210-xtxP353	6.0
32	C	xtxP353-xtxP71	1.8

Sl. No.	Linkage group	Pairs of markers	Distance
33	C	xtxP71-xtxP160	12.8
34	C	xtxP166-iabtgs20	18.9
35	D	xtxP343-xtxP12	4.4
36	D	xtxP12-xtxP9	5.7
37	D	xtxP9-xcup9	4.2
38	D	xcnp9-M9	9.3
39	D	M9-xtxp213	9.4
40	D	xtxp213-iabtgs1	16.1
41	E	Xtxp295-SbAG803	24.4
42	E	SbAG803-xtxP358	9.8
43	E	xtxP358-xtxP204	8.5
44	E	xtxP204-corn73	2.8
45	E	corn73-xtxP92	8.1
46	F	xtxP67-xtxP230	8.6
47	F	xtxP230-xtxP258	4.8
48	F	xtxP258-SbAGH04	8.0
49	F	SbAGH04-xtxP284	15.2
50	F	XtxP284-AC20	19.9
51	F	AC20-AK10	13.7
52	F	AK10-BH13	2.9
53	F	BH13-G19	10.7
54	f	G19-iabt477	23.1
55	G	Pepc-xtxP45	17.8
56	G	XtxP45-iabtgs9	13.8
57	G	iabtgs9-xtxP159	6.4
58	H	XtxP217-xtxP329	11.1
59	H	XtxP329-AC19	4.3
60	H	AC19-xtxP254	12.2
61	H	xtxP254-SbAGA01	11.2
62	H	SbAGA01-xtxP273	9.9
63	I	xtxP273-xtxP96	19.3
64	I	xtxP96-xtxP176	6.5
65	I	XtxP171-xtxP312	8.7
66	I	XtxP312-xtxP274	3.7
67	I	Xtxp274-iabtgs83	48.3
68	J	XtxP325-xtxP324	3.1
69	J	XtxP324-xtxP338	18.9
70	J	Xtxp338-iabt259	20
71	J	iabt259-iabtgs155	22.9

Table 6. Characteristics of QTLs associated with traits based on composite interval mapping (QTL cartographer 2.5b) using RILs derived from IS22380 x E-36-1 at Bijapur location

Traits	Marker	Linkage group	QTL position	Peak lod2	Additive effect	Phenotypic variance
Early seedling vigour	corn61	A	14.45	2.14	-0.25	11.33
	xtxp250	A	28.07	2.25	-0.25	17.59
	Corn59	B	42.19	2.97	-0.20	10.48
	xtxp297	B	163.30	3.03	0.20	11.70
	xtxp12	D	6.36	4.96	0.34	22.62
	xcup9	D	18.30	2.73	0.28	17.90
Plant Height	SbAG02	A	0.01	2.61	4.24	10.04
	M10	B	129.36	2.50	6.51	21.02
	Ac19	H	19.40	3.51	7.05	31.47
	xtxp274	I	40.47	2.21	7.16	19.57
Days to 50% flowering	AT5	A	55.95	4.46	-1.58	15.56
Stem thickness	AT5	A	55.95	4.25	0.57	13.39
	Xtxp297	B	151.30	2.13	0.60	8.88
	AK10	F	70.23	3.88	0.60	12.10
	xtxp176	I	25.85	2.71	-0.66	8.79
Seed yield / plant (g)	corn24	C	0.01	2.66	5.74	10.71
	xtxp45	G	29.84	2.66	-6.12	13.03
	xtxp325	J	0.01	2.10	7.54	8.34
Stomata (cm 2)	Xtxp287	B	32.95	3.08	-1086.09	9.26
	corn74	B	40.25	2.06	-812.58	8.01
	corn59	B	44.19	2.11	-817.87	8.10
	xtxp254	H	27.61	2.53	961.69	7.26
% GL 15	xtxp213	D	49.01	2.33	7.81	10.40
% GL 30	xtxp297	B	163.30	3.08	5.50	12.76
% GL 45	Xtxp48	B	146.60	2.93	9.85	15.11
	Xtxp297	B	149.30	3.19	11.10	23.42

Table 7. Characteristics of QTLs associated with traits based on composite interval mapping (QTL cartographer 2.5 b) using RILs derived from IS22380 x E36-1 at Dharwad Location

Traits	Marker	Linkage group	QTL position	Peak LOD2	Additive effect	Phenotypic variance
Early seedling vigour	Xtxp267	A	17.75	2.2032	-0.166	8.08
	Xtxp250	A	30.07	2.0592	-0.18	19.43
	Corn59	B	44.1	2.1930	-0.18	10.56
	Xtxp12	D	4.36	2.5916	0.18	9.52
Plant height	G19	F	115.84	2.9531	-6.99	12.53
	SbAGA01	H	46.79	2.1815	8.60	10.49
Days to 50% flowering	corn74	B	36.25	2.1117	-1.48	7.5
	AC12	B	117.44	3.4793	2.41	18.37
	M10	B	121.36	3.2788	2.29	16.31
	Xtxp248	I	0.01	2.4149	1.33	8.58
Stem thickness	AK10	F	70.23	2.4196	0.54	7.75
	iabt477a	F	118.18	2.952	0.60	11.36
	SBAGA01	H	38.79	2.6375	-0.67	8.5
Seed yield / plant (g)	corn24	C	0.01	2.8395	5.92	11.4
	txtp45	G	29.84	2.7112	-6.19	13.19
	txtp325	J	0.01	2.0878	7.49	8.23
Stomata (cm ²)	L18	B	85.43	3.3362	827.50	9.76
Carbon isotope discrimination	SBAGA01	H	38.7900	2.8768	0.049	10.38
% GL 15	txtp213	D	49.01	2.3463	7.827	10.44
% GL 30	txtp48	B	146.6	2.8977	9.805	15
% GL 45	txtp48	B	146.6	2.9367	9.84	15.11
	txtp297	B	149.3	3.1929	11.10	23.42

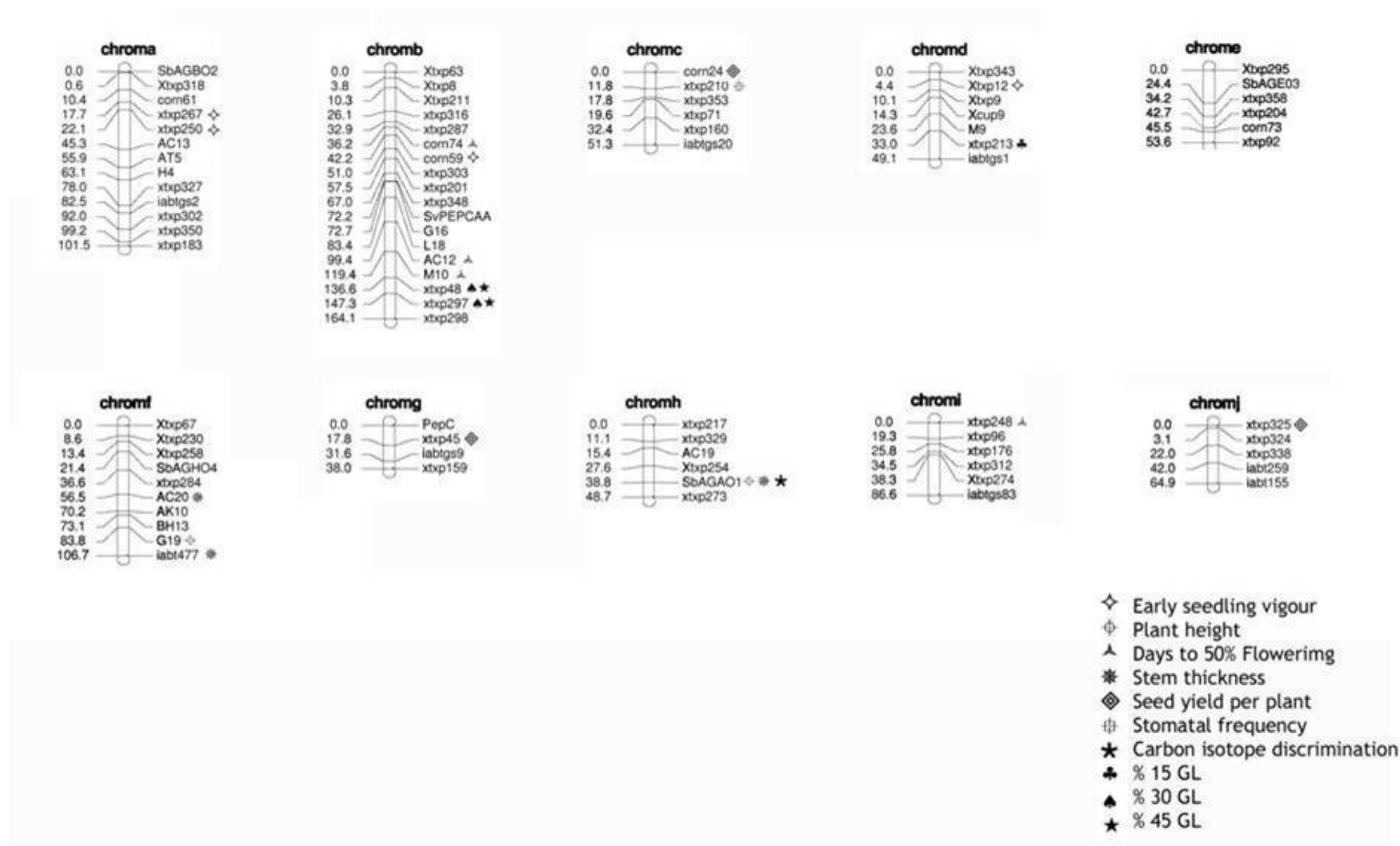


Plate. 4. Localisation of QTLs associated with drought and yield attributes in sorghum RIL population of IS22380 X E36-1 (threshold LOD>2.00) at Dharwad.

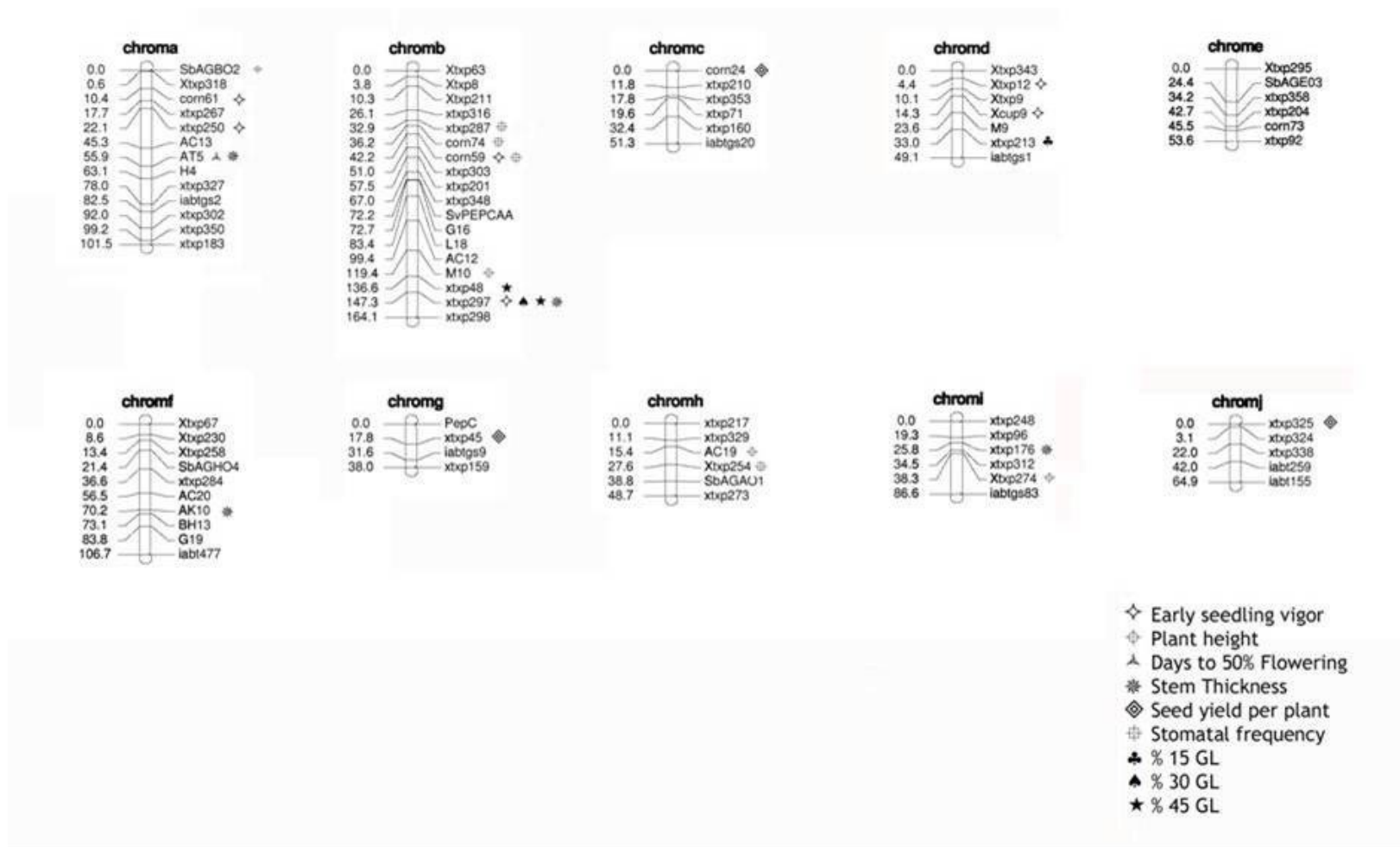


Plate.5. Localisation of QTLs associated with drought and yield attributes in sorghum RIL population of IS22380 X E36-1 (threshold LOD>2.00) at Bijapur

The highest and lowest distances observed were between *txp295-iabtgs83* (24.4 cM) on LG (E) and *SvPEPCAA-G16* (0.5 cM) on LG (B), respectively. As many as 13, 18, 6, 7, 6, 10, 4, 6, 6 and 5 markers were got assigned to linkage groups A, B, C, D, E, F, G, H, I and J, respectively. Four genic SSRs viz., *iabtgs83* (Pyruvate dehydrogenase kinase), *iabtgs155* (thinopyrum intermedium isolate/resistance gene analog), *iabtgs259* (dehydration responsive element binding protein (DREB1)), *iabtgs477* (dehydrin-like protein (*dhy-l*) gene) and *iabtgs83* (dehydrogenase kinase) were assigned to J, F and I linkage group respectively.

4.3.3.1 QTL localization in Dharwad and Bijapur locations

The software QTL Cartographer (2.5v) was used to analyze data 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 portion of phenotypic variation explained by individual QTL were computed. The QTLs associated with 10 phenotypes recorded at Bijapur and Dharwad locations are presented in Table6 and Table7. Graphical representation of chromosomal positions of QTLs and flanking markers is presented in plate 5 and plate6 respectively for Dharwad and Bijapur locations.

In this mapping population, the female parent (marker allele A) IS22380 is non-stay green and male parent (marker allele B) E36-1 is stay green. The results are described below from CIM analysis for identifications of QTLs for yield and charcoal rot disease components.

4.3.3.2 QTL for early seedling vigour

At Dharwad four QTLs for early seedling we detected on linkage group A, B and D, bracketed by *txp267-txp250*, *txp250-AC13*, *corn59-txp304* and *txp343-txp12* at a respective map distance of 17.75, 30.07, 44.10 and 4.36cM. At Bijapur location six QTLs on linkage groups A, B, and D were identified bracketed by *corn61-txp267*, *txp250-AC13*, *corn59-txp303*, *txp297-txp298*, *txp12-txp9* and *txp9-xcup9* markers at a distance of 14.45, 28.07, 42.19, 163.30, 6.36 and 18.30 cM respectively, together covering 91.61 per cent of the phenotypic variance.

4.3.3.2 QTLs for plant height

At Dharwad two QTLs for plant height on linkage groups F and H were identified bracketed by *G19-iabt477* and *SBAGA01-txp273* markers at distances of 84.1 and 46.79 cM, respectively. At Bijapur, four major QTLs were identified for plant height on linkage groups A, B, H and I bracketed by *SBAG02-txp318*, *M10-txp48*, *AC19-txp254* and *txp274-iabtgs83* markers at a distances of 0.01, 129.36 19.40 and 40.47cM respectively, which explained a total phenotypic variance of 62.75 per cent.

4.3.3.4 QTLs for days to 50 per cent flowering

Three major and one minor QTL each for days to 50 per cent flowering were detected. They were on linkage group B and I bracketed by *corn74-corn59*, *AC12-M10* and *M10-txp248* markers at a distance of 36.25, 117.44, 121.36 and 0.01 cM, respectively which explained 7.50, 18.37, 16.31 and 8.58 phenotypic variance per cent respectively were detected at Dharwad location. At Bijapur, one major QTL for days to 50 per cent flowering on linkage group A was flanked by the marker *AT5-H4* at a distance of 55.95 cM, which explained a phenotypic variance of 15.56 per cent.

4.3.3.5 QTLs for stem thickness

At Dharwad location one major and two minor QTLs for stem thickness were detected on linkage groups F and H bracketed by *iabt477*, *AK10-BH13* and *SBAGA01* markers at a distances of 107.01, 7.75, and 8.5 cM with LOD value of 2.42, 2.95 and 2.64 respectively, which accounted for total phenotypic variance of 27.61 per cent. At Bijapur location two major and minor QTLs were detected on the linkage groups A, B, F and I flanked by the SSR markers *AT5-H4*, *AK10-BH13*, *txp297-txp298* and *txp176* at a distances of 55.95, 70.23, 151.30 and 25.85 cM respectively, which accounted for a total phenotypic variance of 43.16 per cent.

4.3.3.6 QTLs for seed yield

Two major and one minor QTLs on linkage groups C, G and J bracketed by *corn24-xtxp210*, *xtxp45-iabtgs9* and *xtxp325-xtxp324* markers at a distances of 0.01, 29.84 and 0.01 cM, respectively, which accounted for a total phenotypic variance of 32.82 per cent were identified for the seed yield at Dharwad location. The LOD scores for these QTLs ranged between 2.8, 2.7 and 2.0. At Bijapur also the same set of two major and one minor QTLs were detected for this trait on same linkage groups, explaining a total phenotypic variance of 32.08 per cent at a LOD score ranging between 2.66, 2.66 and 2.10.

4.3.3.7 QTL for stomata frequency

One minor QTL on linkage group B flanked by markers L18 at a distance of 85.43 cM, but bijapur four minor QTLs were identified on linkage group B and H, flanked by marker *xtxp287-corn 74*, *corn 74-corn 59*, *corn 59-xtxp303* and *xtxp303-xtxp254* with an LOD score of 3.08, 2.06, 2.11 and 2.53, covering with a phenotypic variance of 32.63 per cent at Bijapur location.

4.3.3.8 QTLs for carbon isotope discrimination

4.3.3.9 QTLs for styagreen

QTLs for percent green leaf at 15 days after anthesis (% 15GL)

At Dharwad location one major QTL was detected for this trait on linkage group D bracketed by *xtxp213-iabtgs1*, at a distance of 49.01cM explaining a phenotypic variance of 10.44 per cent. At Bijapur location, also the same of QTL was detected on the same linkage group explaining a total phenotypic variance 10.40 per cent with additive effects of an allele from E36-1.

QTLs for percent green leaf at 30 days after anthesis (% 30GL)

At Dharwad location, two major QTLs on linkage groups B were detected. They explained 15.00 and 23.21 per cent of phenotypic variance respectively with additive effect of an allele from E36-1. *Xtxp49-xtxp297* and *xtxp297-xtxp298* markers bracketed qTLs detected on linkage group B. These QTLs were detected at a LOD threshold of 2.89 and 3.16, respectively. At Bijapur location, one major QTL on linkage group B bracketed by *xtxp297-xtxp298* marker was detected with a LOD value of 3.08. This QTL explained a total phenotypic variance of 12.17 per cent with additive effect of an allele from E36-1.

QTLs for 30 GL overlapped with QTLs for other traits like stem thickness, ear head length and 45 GL on linkage group B in the vicinity of the marker *xtxp297* at a distance of 149.3 cM, at Bijapur location. At Dharwad location QTL for percent 30 GL was overlapping with the QTLs of percent 45 GL linkage group B in the vicinity of the marker *xtxp297* at a distance of 147.3 cM.

QTLs for green leaf percent at 45 after anthesis (% 45 GL)

At both Dharwad and Bijapur location, two major QTLs were detected on linkage group B bracketed by *xtxp48-xtxp297* and *xtxp 297-xtxp298* marker with the phenotypic variance 15.11 and 23.42 per cent.

4.4 Stable QTLs across locations

Out of total 23 QTLs identified, 10 were common to both locations. The QTLs for the traits like early seedling vigour, stem thickness, seed yield per plant, per cent 15 GL, 30 GL and 45 GL were found to be stable across locations. The characteristics of these common QTLs are presented in Table 8.

4.4.1 Stable QTLs for yield and its components

On linkage group B and D two stable QTLs were bracketed by *xtxp343-xtxp12*, *corn59-xtxp303* for early seedling vigour at a distance of 4.36 and 44.10 cM. For stem thickness, one stable QTL on linkage F bracketed by *AK10* at distance of 70.23 cM respectively. Three QTLs on linkage group C, G and J for yield bracketed by *corn24-xtxp210*,

Table 8. Map position and statistical details of common (stable) QTLs across Bijapur and Dharwad locations

Traits	Linkage group	Marker	QTL position	Peak LOD'S	Additive effect		Phenotypic variance	
					Bij	Dwd	Bij	Dwd
Early seedling vigour	txxp12	D	4.36	2.5916	0.34	0.1831	22.62	9.52
	Corn59	B	44.1	2.1930	-0.20	-0.1786	10.48	10.56
Stem thickness	AK10	F	70.23	2.4196	0.60	0.545	12.10	7.75
Seed yield / plant (g)	corn24	C	0.01	2.66	5.74	5.9235	10.71	11.4
	txxp45	G	29.84	2.66	-6.12	13.03	-6.16	13.19
	txxp325	J	0.01	2.10	7.54	8.34	7.49	8.34
%GL 15	txxp213	D	49.01	2.35	7.81	7.83	10.40	10.44
% GL 30	txxp297	B	163.30	3.08	5.49	11.05	12.76	23.21
% GL 45	txxp48	B	146.60	2.93	9.84	9.84	23.42	15.11
	txxp297	B	149.30	3.18	11.10	11.10	15.11	23.42

txp45-iabtgs9 and *txp325-txp324* markers at a distance of 0.01, 29.84 and 0.01 cM were common to both location.

4.4.2 Stable QTLs for staygreen

One QTL each for per cent 15 GL, 30 GL and two QTLs for per cent 45 each were detected at both locations on linkage groups D and B bracketed by *txp213-iabtgs1*, *txp297-txp298*, *txp48-txp297* and *txp297-txp298* markers at a distance of 49.01, 163.30, 146.60 and 149.30 cM respectively.

V. DISCUSSION

Drought tolerance is an important agronomic trait in many field crop field crops, but the genetic and physiological mechanisms that condition its expression are complex and not well understood. Sorghum is one of the drought tolerant crops and it can serve as model system for studying mechanisms of drought tolerance in higher plants. For sorghum grown in post rainy season under stored soil moisture drought tolerance is important attribute. Drought and charcoal resistance are known to be positively associated and the cultivars that are drought tolerant are also resistant to charcoal rot. Both charcoal rot and drought resistance are polygenic in nature, involving a number of components traits. The elasticity of genotypes to adjust with the moisture stress condition mainly depends on genetic, biophysical and physiological processes. The involvement of many genes in controlling polygenic traits, each with very little individual effect, is highly influenced by environment. Phenotypic selection for such traits will be generally difficult. The genotypes possessing stay-green trait is known have yield advantage under receding soil moisture condition.

Selection based on markers could facilitate manipulation of quantitative traits without affecting other important agronomic characters by allowing evaluation of breeding progeny populations and for the presence of target gene/genes. Despite concerted efforts over the last three decades of utilizing the existing genepool, the level of resistance achieved in popular varieties and hybrids is limited for both drought and charcoal rot in sorghum. Marker assisted selection under such situations can increase the efficiency of breeding efforts and gain finer understanding of the complex nature of such traits. Mapping and identification of genomic regions associated with drought and yield would serve as the basis to initiate marker assisted transfer of such genome regions into elite cultivars. A saturated genetic map and QTL analysis efforts would also pave a way for positional cloning of desired gene in sorghum.

In the present investigation, RILs derived from IS22380 (non stay green, drought susceptible) x E36-1 (staygreen source, drought tolerant) were characterized for the components of drought resistance and yield parameters. The parental polymorphism for both phenotypic traits and markers (RAPD, nuclear SSRs, RGAs and genic SSRs) was done first and later the genotypic data for individual RILs was generated to construct a genetic linkage map for this population. The phenotypic evaluation of RILs and parents at Dharwad and Bijapur locations, construction of genetic linkage map and QTL analysis was done to understand the genetic architecture of drought, and yield parameters to identify genome regions harbouring QTLs.

5.1 GENETIC VARIABILITY AND CHARACTER ASSOCIATION FOR DROUGHT AND OTHER TRAITS

For understanding the genetic nature of drought resistance, as it is for any quantitative trait, the phenotypic characterization of inbred lines and parents across locations is a prerequisite. Manifestation of any quantitative trait is brought about by the interaction of environment and often it is difficult to distinguish the individual effects. Through quantitative genetic theory, analysis of such traits for separating genetic effect from the environmental effects is possible, it is important that the test population have sufficient variability to make proper inference.

RILs displayed considerable differences in their mean phenotypic value for all the traits studied. Genetic variability among the RILs is expected, as they were phenotypically distinct for a number of characters, including drought tolerance, stay green and charcoal rot resistance. Wide range of variation among the lines indicates the utility of RILs for further genetic investigation. Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits studied is indicative of their polygenic inheritance and influence of environment.

High values of PCV and GCV were evident for early seedling vigour, stem thickness, seed yield per plant, carbon isotope discrimination ($\Delta^{13}\text{C}$) and stay-green parameters. The parental lines differed significantly for per cent 30 and 45 days, post flowering. The ability to maintain green leaf area is positively associated with yield. Extended greenness of leaves

allow continued photosynthesis and better translocation from source to sink. This eventually leads to better grain fill and increased yield. In sorghum similar trend between stay green and yield was noticed by Nimbalkar *et al.* (1988), Khannure (1993), Birdar *et al.* (1996), Rao and Patil (1996), Reddy *et al.* (1996), Borrel *et al.* (2000b) and Haussmann *et al.* (2002). Further, seed yield, plant height, days to 50% flowering, stem thickness the findings with respect to variability per se are in accordance with the results of Punhuri (2004) and Rajkumar (2004) who investigated the same population. Low phenotypic and genotypic variance was observed for plant height and days to 50 per cent flowering in the present study.

The range of variability was comparatively low for days to 50 per cent flowering. Similar observation were also made by Patel *et al.* (1980), Nimbalkar *et al.* (1988) and Biradar *et al.* (1996) in sorghum germplasms. GCV is necessary for improvement through selection. Published reports, with reference to this RIL population are not available. However, one of the parents, E36-1 has been used both in breeding and mapping efforts for staygreen, striga resistance and charcoal resistance (Haussmann *et al.*, 2004, 2002, Punhuri, 2004 and Rajkumar, 2004) and drought tolerance both in India and elsewhere, where as, the behaviour of IS22380 is not known on records.

In the present study, high estimates of broad sense heritability for four characters viz., stem thickness, seed yield per plant, staygreen, $\Delta^{13}\text{C}$, and stomata frequency was noticed. Several workers (Rao and Patil 1996) have also made similar observations in sorghum germplasm, though not in this population, for some of these traits. The high heritability observed for these traits indicates that it is less subjected to environmental influences. Genetic advance and other feature of the population is less relevant to this study.

Among the drought parameters, green Leaf was positively correlated with yield under post anthesis drought. Similar study was made by Monneveux *et al.* (2005) in a set of sorghum germplasm. Significant negative association was found between days to 50 per cent flowering and per cent 30 GL which is also analogue to the observation of Haussmann *et al.* (2002) in two RILs of sorghum involving E36-1 as one of the parents. In present study, at both genotypic and phenotypic level negative association was observed between $\Delta^{13}\text{C}$ with early seedling vigour, plant height and days to 50 per cent flowering. Further, significant negative association was found between plant height and days to 50 per cent flowering at genotypic level. Stem thickness, showed significant negative association with stomatal frequency both at genotypic and phenotypic level and positive association with $\Delta^{13}\text{C}$. On the contrary, stem thickness had negative association with plant height at genotypic level. Similar observations were made by Esehia *et al.* (1977). Significant negative association was observed between 50 per cent flowering and per cent 30 GL, similar study was made by Kebede, (2001) for this trait.

Among the yield components, relationship of plant height was negative with days to 50 per cent flowering at genotypic level. Similar results were obtained by Rao and Gouda (1979), Wenzel (1990) and Lin *et al.* (1995) in sorghum.

At both genotypic and phenotypic level, per cent 30 GL had significant negative association with plant height, while significant positive association with yield, $\Delta^{13}\text{C}$, per cent 15 GL and 45 GL. Similarly significant positive association of per cent 30 GL with yield at both genotypic and phenotypic level was made by Haussmann *et al.* (2002) in two sorghum RILs involving E36-1 as one of the parents.

5.2 CONSTRUCTION OF LINKAGE MAP

Rapid advances in molecular biology and in associated sciences have helped in the development of saturated molecular maps in several crops. The availability of a good permanent mapping population such as RILs is a useful resource. RILs can be genotyped with respect to any available marker, their position genetically mapped and updated with more markers as they become available. Any trait for which the parents of the mapping population vary can be accurately phenotyped and related to the genetic data.

Most of SSR markers available at present in sorghum have been mapped previously. Selection of some of the SSR markers in the present study was based on the linkage map published by Bhattaramakki *et al.* (2000), Taramino *et al.* (1997) and Haussman *et al.* (2002a,

2002b) and from Cornell set of SSR markers for sorghum. Further, through in-house efforts several genic SSR were identified in EST database of sorghum and some of them were also used in the present study. Out of four SRGA used to find polymorphism between parents, SRGA214 showed SNP, which was also associated with CAPS and SRGA212 had only SNP between parents. These SNP/CAPS can be used to map these two SRGAs onto the linkage map. A linkage distance of 20 cM was considered as limit of resolution.

Linkage maps are available in sorghum for striga resistant, staygreen (Hausmann *et al.*, 2004, 2002) and drought (Bhatramakki *et al.*, 2000) targeted populations. It has been reported that the QTLs for stay-green, The stay-green trait that makes plant to remain green for extended period and impart terminal drought tolerance and charcoal rot resistance is an important feature. It has been reported that the QTLs for stay-green located in linkage groups A, B, D, E, G and H (Tuinstra *et al.*, 1997). It was considered logical to use the information on stay green and RGAs related to diseases (as a component of charcoal rot resistance) as well. Linkage map for population specifically developed for studying inheritance of charcoal rot incidence was available with 37 markers on six linkage groups viz., A, B, D, E, F and H earlier developed by Punhuri (2004) and Rajkumar (2004) at IABT.

The RAPDs have been used for QTL mapping by Hausmann *et al.* (2002) and Tuinstra *et al.* (1997) and reported six QTLs associated with pre-flowering drought tolerance. These QTLs were also found to be associated with staygreen in linkage groups A, E, F, G and H.

A total of 89 (19 RAPD, 62 nuclear SSR and 8 genic SSR) markers were used for linkage map construction with the help of MAPMAKER / EXP (3.0b). Out of 89 markers, 83 could be mapped on to linkage groups which we named as A, B, C, D, E, F, G, H, I and J (spanned a length of 64.66 cM) in comparison with already available maps. The map was constructed using the same order of markers as in the map of Bhatramakki *et al.* (2000), Tuinstra *et al.* (1997) and Hausmann *et al.* (2004) spanned a distance of 1406 k, 1580 R and 1599 H respectively. Non-association of five markers may be because of more linkage distance, between thus and other markers concerned in this study.

The selected markers on linkage group A in case of Bhatramakki *et al.* (2000) and Tuinstra *et al.* (1997) were far apart (>89 cM), but the same markers, SbAGB02 and xtxp318 in present investigation were located very close to each other on linkage group A at a distance of 0.6 cM. The map distance between xtxp302 and xtxp350 was 7.9 cM on linkage group A as also seen in Bhatramakki *et al.* (2000). In this population the map distance between the same markers was 9.2 cM. The map distance between the markers, xtxp63, xtxp211, xtxp8, and SvPEPCAA on linkage groups B in case Bhatramakki *et al.* (2000) was also large, (180 cm), while in the present study they are on the same linkage group, but 72.2 cM apart. Similarly distance between xtxp201 and xtxp348 on linkage group B was 70 cM in map of Hausmann *et al.* (2004), but in the present study they were much closer to each other 9.5 cM on linkage group B. These variations in the distances between markers may be because of chromosomal aberrations, which need to be investigated further.

The markers xtxp343 and xtxp12 located in D linkage group was more (4.4 cM) than that observed by Bhatramakki *et al.* (2000). The markers Xtxp67, xtxp230 and xtxp258 mapped on to linkage group F, over a total length of 13.4 cM. The same markers were mapped on linkage group F over a total length of 6.0 cM in the map of Bhatramakki *et al.* (2000). The markers xtxp295, SbAGEo3, xtxp204, corn73 and xtxp92 were mapped on to linkage group E over a total length of 53.6 cM and while xtxp325, xtxp324 and xtxp338 were mapped on linkage group J over a total length of 22 cM. The relative order of marker in linkage groups, A, B, C, D, E, F, G, H, I and J constructed in the present study were same as in the map of Bhatramakki *et al.* (2000), Taramino *et al.* (1997) and Hausmann *et al.*, (2004). In the present investigation eight genic SSR markers viz., iabtgs1, iabtgs2, iabtgs9, iabtgs256, iabtgs477, iabtgs155, iabtgs20 and iabtgs83 (developed at IABT) were mapped on linkage groups D, A, G, F, I, J and C respectively.

Thus, staygreen QTLs are likely to be detected when the alleles are in association than comparison with other genetic linkage maps of sorghum (Bovin *et al.*, 1999, Peng *et al.*, 1999, Bhatramakki *et al.* 2000, Kong *et al.* 2000, Hausmann *et al.*, 2002,) aligned staygreen studies. In present study, we detected QTL for per cent 15 GL, 30 GL and 45 GL on B and D

Linkage group. Similar staygreen QTLs were detected on B and D linkage group Tuinstra *et al.* (1997), Subudhi *et al.* (2000) and Crasta *et al.*, (1999).

The minimum and maximum distance between two individual markers were 0.5 cM (SvPEPCAA and G16 on linkage group B) and 48.3 cM (Xtxp274 and iabtgs83 on linkage group I) respectively. Majority of the markers mapped closely indicated that the QTLs detected were generally tightly linked. It was not possible to establish relationship for four RAPD markers viz., AB4, AT9, G17 and M7 and one SSR marker Xtxp274.

5.3 QTL MAPPING

Analysis of co-segregation pattern of the markers based on recombination frequency of marker loci and phenotypic data from field gives the information on the location of a QTL between these flanking markers. If the proportion of recombinants with higher or lower phenotypic values is less, tight linkage of between flanking markers and QTL is indicated. Theoretically, 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. Besides, QTL analysis is the only molecular tool available to dissect a complex quantitative character, governed by many genes with small individual effects, into recognizable genetic units or blocks. Xu *et al.* (2000) reported that the extreme phenotypes of quantitative traits comes from the association of favorable or unfavorable 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 allelic status in breeding population and the genetic 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 constructed in the present study for the 93 individuals of the RIL population of cross IS22380 x E36-1 was used for QTL mapping. The software QTL cartographer (2.5b) 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.

5.4 QTLs for stomatal frequency

At Dharwad location one major QTL was detected for this trait on linkage group B. The QTL on linkage group B located at a distance of 85.43 cM bracketed by the markers L18-AC12 had a LOD value of 3.33 and accounted for 9.76 per cent phenotypic variation.

At Bijapur location four major QTLs were detected on linkage group B and H with a LOD threshold of 3.08, 2.06, 2.11 and 2.53 accounted for 32.63 per cent phenotypic variation. These QTLs were bracketed by the markers xtxp287-corn 24, corn74-corn59, corn59-xtxp303 and xtxp254-SBAGA01 respectively.

5.5 QTLs for carbon isotope discrimination ($\Delta^{13}\text{C}$)

One major QTL for carbon isotope discrimination was detected on linkage group H bracketed by the markers xtxp254-SbAGA01 at Dharwad location. This QTL had LOD threshold of 2.99 explaining 10.38 per cent phenotypic variation at Dharwad location. This QTL had an additive effect of 0.048. It is also indicative that this QTL has an additive effect of an allele from parent E36-1, probably having a minor gene for drought. Teulat *et al.* (2003) have identified ten QTLs for grain carbon isotope discrimination in Barley.

5.6 QTL FOR STAY - GREEN

One major QTL was detected for per cent green leaf at 15 days interval on linkage group D bracketed by marker xtxp213-iabtgs1 with per cent phenotypic variance of 10.44 in both locations, indicating stability of its QTL and its action as minor gene.

Two QTL were detected for per cent 30 days after flowering this trait on linkage group B bracketed by markers xtxp48-xtxp297 and xtxp297-xtxp298 with per cent phenotypic variance 38.27 with LOD threshold 2.80 and 3.10 respectively. Additive effect was 9.81 and 11.05 respectively. For per cent green leaf at 45 days after flowering two QTLs were detected for on linkage group B bracketed by xtxp48 and xtxp297 in both locations. It indicates that this QTL has an additive effect of an allele from parent E36-1, probably having a minor gene for drought. Association between RFLP markers and staygreen character were found in several regions of linkage groups B, D, F, G and H in RILs of B35 x QLD. Xu *et al.* (1989) identified four staygreen QTLs located on three linkage groups viz., A, D and J explaining 20-30 per cent of phenotypic variance. Tuinstra *et al.* (1997) also detected QTLs for staygreen on linkage group B, E, F, G and H (using 150 RAPDs and 20 RFLP markers). Haussmann *et al.* (2002) reported three QTLs located on linkage groups A, E and G for the same character. Since staygreen and charcoal rot are associated, previous study of Rajkumar lead to detection of QTLs for this trait in the same RIP.

5.7 QTLs FOR YIELD AND ITS COMPONENTS

Four QTLs for early seedling vigour on four linkage groups A, B, and D which explained 47.59 per cent total phenotypic variation respectively with an additive effect from E36-1 at Dharwad location were detected.

At Bijapur location six and three QTLs respectively for early seedling vigour and were detected on five linkage groups A, B, and D. These QTLs explained 91.32 per cent total phenotypic variation respectively for early seedling vigour. Similar QTLs were detected by Rajkumar (2004) in the same region and in this mapping population in the previous year and this year.

At Dharwad, two QTLs for plant height on linkage group F and H bracketed by the markers G19-iabt477 and SBAGA01-xtxp273 were identified. QTL for plant height accounting for 23.02 per cent phenotypic variation with the additive effect of an allele from IS22380 was noted. At Bijapur, four major QTLs were identified for plant height on linkage groups A, B, H, and I that explained a total phenotypic variation 82.1 per cent.

A common feature of QTLs is that their effects depend on environment. If the QTLs detected location specific that it is called location specific, those are called location specific QTLs. For practical breeding application, identification of QTLs that pop-up in most environments would be important. In present investigation, the QTLs commonly detected at both Dharwad and Bijapur locations were considered as stable.

Since the QTLs detected for staygreen were for per cent green leaf area at 15 days, 30 days, and 45 days were similar to Reddy 2006 were found on linkage group B and D for number of internode crossed, per cent lodging in same population. This indicates staygreen and charcoal rot are positively associated.

Two stable QTLs were identified common in both the locations for early seedling vigour on linkage group D and B and flanked by marker xtxp12 and corn 59 and one QTL for stem thickness on linkage group F flanked by AK10. Two QTLs for seed yield were detected on linkage group C, G and J.

For per cent GL for 15 and 30 days one QTL was detected as common in both the locations and two QTL for 45 days GL on B linkage group.

Future line work

1. Further saturation of linkage map with additional and informative functional markers.
2. Fine mapping of chosen region of the map for better resolution to facilitate map based cloning.
3. Identification of genes involved in regulating stay-green trait, which can be manipulated for high yield.

VI. SUMMARY

Sorghum is being important staple food crop for million's of people and good cattle feed throughout Africa Asia. It is well adapted to high temperature and low moisture situation in semi arid tropics and being regarded as model plant for drought research; it has been considered for whole genome sequencing recently. Drought is a major abiotic stresses limiting crop production throughout world. Drought resistance is a function of several component traits, which are controlled by several genes each with small but cumulative effect. Nonetheless, several early and late responses genes have been isolated and characterized in model species such as Arabidopsis. Among several traits related to drought tolerance, stay-green, $\Delta^{13}\text{C}$, and stomatal frequency are regarded as important. Stay-green is an important component of post-flowering drought tolerance, which give resistance to premature senescence under soil moisture stress during post-flowering period. The molecular genetic dissection of the QTLs affecting drought will provide opportunity to elucidate the underlying physiological mechanism involved in drought tolerance in sorghum besides their precise transfer to desired background by MAB process. The summary of the investigation on title of this is as follows:

1. A set of 93 RILs derived from the cross IS22380 x E36-1 were evaluated under rainfed condition at Dharwad and Bijapur locations for drought tolerance and yield components.
2. In Dharwad and Bijapur locations analysis of variance revealed significant difference between individual RILs and parents for all the traits investigated.
3. High PCV and GCV were observed for stem thickness, seed yield, per cent green leaf at 30 days after flowering (% 15 GL) and per cent green leaf at 45 days after flowering (% 45 GL). However days to 50 % flowering had a low GCV and PCV value.
4. High heritability coupled with genetic advance was observed for stem thickness, seed yield, stomatal frequency, $\Delta^{13}\text{C}$ and per cent green leaf at 30 days after flowering.
5. Correlation revealed highly significant and negative association between days to 50 per cent flowering and per cent green leaf at 30 days after flowering.
6. Parental screening with 240 RAPD, 262 nuclear SSRs, 30 genic SSRs markers lead to identification of 62 nuclear SSRs, 8 genic SSR and 19 RAPDs as polymorphic between the parents. All polymorphic markers showed 1:1 segregation ratio in the RIL population, indicating its variability for mapping work.
7. MAPMAKER/ EXP, assigned 89 markers to 10 linkage groups A, B, C, D, E, F, G, H, I and J in relation to earlier maps. The number of markers mapped per linkage group ranged from four to eighteen. The linkage thus constructed covered 746.66 cM covered. The relative order of marker remained the same as that of reported maps in sorghum.
8. QTL analysis indicated the presence of two major QTLs for per cent green leaf at 45 days after flowering in both the location with marker *txtp48* and *txtp294* on B linkage group for stay green trait.
9. QTL for per cent green leaf at 30 days after flowering detected one major and minor QTL at Dharwad location with marker *txtp48*, *txtp297* were noticed. At Bijapur one QTL for per cent green leaf at 30 days after flowering was noticed flanked by markers on *txtp297* on linkage group B.
10. One QTL for $\Delta^{13}\text{C}$ was detected on linkage group H flanked by marker *SBAGA01* was observed.
11. Stomatal frequency had one minor QTL on linkage group B flanked by marker L18 were detected in Dharwad. At Bijapur four QTLs for stomatal frequency was

detected on B and H linkage group flanked by marker *txp287*, *corn 74*, *corn59* and *txp254* were noticed.

12. Stable QTLs were detected for early seedling vigour, stem thickness, seed yield per plant, per cent green leaf at 15, 30 and 45 days after flowering at both the locations.
13. In house 4 SRGA were used to find polymorphism in order to map of which SRGA214 showed CAPS and SNP and SRGA212 showed only CAP marker.

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Appendix I:

Pedigree information of the RIL population derived from IS22380 × E 36-1

RIL No.	Plant No.	Pedigree	RIL No.	Plant No.	Pedigree
1.	2422	E 36-1 × IS 22380	50.	2471	IS 22380 × E 36-1
2.	2423	E 36-1 × IS 22380	51.	2472	IS 22380 × E 36-1
3.	2424	E 36-1 × IS 22380	52.	2473	IS 22380 × E 36-1
4.	2425	E 36-1 × IS 22380	53.	2474	IS 22380 × E 36-1
5.	2426	E 36-1 × IS 22380	54.	2475	IS 22380 × E 36-1
6.	2427	E 36-1 × IS 22380	55.	2476-1	IS 22380 × E 36-1
7.	2428	E 36-1 × IS 22380	56.	2476-2	IS 22380 × E 36-1
8.	2429	E 36-1 × IS 22380	57.	2476-3	IS 22380 × E 36-1
9.	2430	E 36-1 × IS 22380	58.	2477	IS 22380 × E 36-1
10.	2431	E 36-1 × IS 22380	59.	2478	IS 22380 × E 36-1
11.	2432	E 36-1 × IS 22380	60.	2479	IS 22380 × E 36-1
12.	2433	E 36-1 × IS 22380	61.	2480	IS 22380 × E 36-1
13.	2434	E 36-1 × IS 22380	62.	2481	IS 22380 × E 36-1
14.	2435	E 36-1 × IS 22380	63.	2482	IS 22380 × E 36-1
15.	2436	E 36-1 × IS 22380	64.	2483	IS 22380 × E 36-1
16.	2437	E 36-1 × IS 22380	65.	2484	IS 22380 × E 36-1
17.	2438	E 36-1 × IS 22380	66.	2485	IS 22380 × E 36-1
18.	2439	E 36-1 × IS 22380	67.	2486	IS 22380 × E 36-1
19.	2440	E 36-1 × IS 22380	68.	2487	IS 22380 × E 36-1
20.	2441	E 36-1 × IS 22380	69.	2488	IS 22380 × E 36-1
21.	2442	E 36-1 × IS 22380	70.	2489	IS 22380 × E 36-1
22.	2443	E 36-1 × IS 22380	71.	2490	IS 22380 × E 36-1
23.	2444	E 36-1 × IS 22380	72.	2492	IS 22380 × E 36-1
24.	2445	E 36-1 × IS 22380	73.	2493	IS 22380 × E 36-1
25.	2446	E 36-1 × IS 22380	74.	2494	IS 22380 × E 36-1
26.	2447	E 36-1 × IS 22380	75.	2495	IS 22380 × E 36-1
27.	2448	E 36-1 × IS 22380	76.	2496	IS 22380 × E 36-1
28.	2449	IS 22380 × E 36-1	77.	2497	IS 22380 × E 36-1
29.	2450	IS 22380 × E 36-1	78.	2498	IS 22380 × E 36-1
30.	2451	IS 22380 × E 36-1	79.	2499	IS 22380 × E 36-1
31.	2452	IS 22380 × E 36-1	80.	2500	IS 22380 × E 36-1
32.	2453	IS 22380 × E 36-1	81.	2501	IS 22380 × E 36-1
33.	2454	IS 22380 × E 36-1	82.	2502	IS 22380 × E 36-1
34.	2455	IS 22380 × E 36-1	83.	2503	IS 22380 × E 36-1
35.	2456	IS 22380 × E 36-1	84.	2504	IS 22380 × E 36-1
36.	2457	IS 22380 × E 36-1	85.	2505	IS 22380 × E 36-1
37.	2458	IS 22380 × E 36-1	86.	2506	RS-29 (susceptible check)
38.	2459	IS 22380 × E 36-1	87.	2507	IS 22380 × E 36-1
39.	2460	IS 22380 × E 36-1	88.	2508	IS 22380 × E 36-1
40.	2461	IS 22380 × E 36-1	89.	2509	IS 22380 × E 36-1
41.	2462	IS 22380 × E 36-1	90.	2510	IS 22380 × E 36-1
42.	2463	E 36-1 (Parent-2)	91.	2511	IS 22380 × E 36-1
43.	2464	IS 22380 × E 36-1	92.	2512	IS 22380 × E 36-1
44.	2465	IS 22380 × E 36-1	93.	2513	IS 22380 × E 36-1
45.	2466	IS 22380 × E 36-1	94.	2514	IS 22380 × E 36-1
46.	2467	IS 22380 × E 36-1	95.	2515	IS 22380 × E 36-1
47.	2468	IS 22380 × E 36-1	96.	Parent-1	IS 22380
48.	2469	IS 22380 × E 36-1	97.	Parent-2	E 36-1
49.	2470	IS 22380 × E 36-1			

Appendix II:

Preparation of stock solutions

Extraction buffer	Tris HCl, pH 8.0	100 mM
	EDTA, pH 8.0	50 mM
	NaCl	500 mM
20% SDS	20 g of sodium doedecyl sulphate (sodium lauryl sulphate) dissolved in 100 ml of dH ₂ O. Should not be autoclaved	
5 M potassium acetate	5M potassium acetate	60.0 ml
	Glacial acetic acid	11.5 ml
	H ₂ O	28.5 ml
Isopropanol Ammonium Acetate mixture	Three volumes of 10 M Iso-propanol and 1 volume of 10 M ammonium acetate	
T ₁₀ E ₁	Tris 10 mM containing 1 mM EDTA	
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°C	
Chloroform isoamyl alcohol (24:1)	Chloroform 240 ml Isoamyl alcohol 10 ml. Store in a dark room temperature. Make up and dispense the solution in a fumed cupboard.	
Ethanol (70%)	Absolute alcohol	70 ml
	Distill water	30 ml
NaCl 5 M	Dissolve 292.2 g NaCl in 750 ml water. Make up to 1 litre with water, filter and autoclave.	
Phenol chloroform (24:1)	Mix equal volumes of the buffered phenol and chloroform isoamyl alcohol (24:1). Store at 4°C	
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.	
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	

Ethidium bromide (10 mg/ml)	Dissolve 100 mg ethidium bromide in 10 ml of distil water, wrap tube in aluminium foil and store at 4 ⁰ C.	
4% acrylamide solution (1000 ml)	double distilled water	450 ml
	5x TBE	100 ml
	Urea	420 g
	40% Acrylamide/ bisacrylamide (19:1) (w/ w)	100 ml
	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. Then acrylamide solution is combined with other ingredients in a storage container.	
5x TBE	Tris base	540 g
	EDTA	46 g
	boric acid	276 g
	dH ₂ O to 10 L (store at room temperature, if left for long-periods of time, some of the salts will precipitate. It may be advisable to discard this buffer and make fresh buffer	
0.5% acetic acid in 95% ethanol	glacial acetic acid	1 ml
	95% ethanol	199 ml
	Aliquot and store at room temperature	
10% APS (ammonium persulfate)	ammonium persulfate	1 g
	dH ₂ O	10 ml
	Should be stored at –20 ⁰ C. If the APS will be used within the few weeks, store in light-tight bottle at 4 ⁰ C	
TEMED (N, N, N', N' – tetra methyl ethylene diamine)	Store at 4 ⁰ C	
Binding solution	Bind silane (γ - methacryloxy profile trimethoxy silane) M-6514 sigma industries	
	4 µl of bind silane + 1000 µl of 0.5% acetic acid in 95% ethanol	

Repel solution	Repel silane (dichloro dimethyl silane 99%) M-440272 sigma industries 250 µl repel silane + 750 µl of 0.5% acetic acid in 95% ethanol (should be done in fumehood)	
Loading dye/tracking dye (10x)	Sucrose	167 mg
	Bromophenol blue	4.2 mg
	Water	1 ml
3x SSR dye (3x STR loading solution)	5 M NaOH	0.2 ml
	95% formamide	95 ml
	Bromophenol blue	50 mg
	Xylene cyonol	50 mg
	Sd water make upto	100 ml
100 bp DNA ladder	100 bp marker (Genetix)	10 µl of
	3x SSR dye	95 µl
	sterile dH ₂ O	95 µl
Fix/stop solution (10% acetic acid)	glacil acetic acid in 1800 ml dH ₂ O	200 ml
Impregnate solution	glycerol	10%
	glacial acetic acid	10%
Staining solution	silver nitrate	2 g
	37% formaldehyde	3 ml
	ddH ₂ O	2000 ml
	Should be stored at room temperature in a cabinet or other dark storage space	
Developer solution	sodium carbonate	60 g
	37% formaldehyde	3 ml
	sodium thiosulphate (10 mg/ml)	400 µl
	dH ₂ O	2000 ml
	sodium carbonate	60 g
	This solution must be prepared fresh for each use. Prepare the solution by dissolving sodium carbonate in water and chilling to about 10 ⁰ C by placing on ice or in freezer. Just before use add the formaldehyde and sodium thiosulphate	

Loading dye (6x)	Bromophenol blue	0.25 g
	Sucrose in water	40% (W/V)
	Stored at 4°C	
50x TAE (Tris-Acetate EDTA)	Tris base	242 g/l
	Glacial acetic acid	57.1 ml
	0.5 M EDTA (pH 8.0)	100 ml
	Distilled water	1000 ml

APPENDIX III

List of genomic SSRs used in the present investigation

SSR No.	LG	Locus name	Type of SSR's	Sequence of the forward primer (5' to 3')	Sequence of the reverse primer (5' to 3')	Amplicon size	Annealing temp. (°C)
1	A	Xtxp318*	(AGA) ₁₂	CCA GCT TCA CTT ACG AGG AGA TG	ATG CCC GTT TTC TAA TTC TTC TAC T	331	55
2	B	Xtxp63*	(GA) ₂₄	CCA ACC GCG TCG CTG ATG	GTG GAC TCT GTC GGG GCA CTG	204	55
3	D	Xtxp343*	(AGT) ₂₁	CGA TTG GAC ATA AGT GTT C	TAT AAA CAT CAG CAG AGG TG	234	55
4	F	Xtxp230*	(GA) ₂₈	CGT ACC GCT GCT GCT CT	AGG GGG CAT CCA AGA AAT	165	55
5	D	Xtxp9*	(TG) ₁₂ TT(TG) ₁₄ (AG) ₁₃	AAT AGC ACC GCC GCG CG	CAT TGT GGA GTC CCT GAT AC	150	55
6	B	Xtxp8*	(TG) ₃₁	ATA TGG AAG GAA GAA GCC GG	AAC ACA ACA TGC ACG CAT G	148	60
7	B	Xtxp211*	(CT) ₂₃	TCA ACG GCC AAT GAT TTC TAA C	AGG TTG CGA ATA AAA GGT AAT GTG	216	55
8	D	Xtxp12*	(CT) ₂₂	AGA TCT GGC GGC AAC G	AGT CAC CCA TCG ATC ATC	193	55
9	F	Xtxp67*	(GA) ₂₈	CCT GAC GCT CGT GGC TAC C	TCC ACA CAA GAT TCA GGC TCC	175	55
10	F	Xtxp258*	(AAC) ₁₉	CAC CAA GTG TCG CGA ACT GAA	GCT TAG TGT GAG CGC TGA CCA G	222	55
11	E	Xtxp295*	(CTT) ₁₆ + (AGG) ₆	AAG TGG GGT GAA GAG ATA	CTG CCT TTC CGA CTC	290	55
12	H	Xtxp354*	(GA) ₂₁ + (AAG) ₃	TGG GCA GGG TAT CTA ACT GA	GCC TTT TTC TGA GCC TTG A	157	55
13	I	Xtxp274*	(TTC) ₁₉	GAA ATT ACA ATG CTA CCC CTA AAA GT	ACT CTA CTC CTT CCG TCC ACA T	331	55
14	-	SvHPRGPG	(CGC) ₈	ACTCCGACGCACCCTAAG	CTCCATTCTTGTCACGTA	246	54
15	D	Xcup9*		CTGGTGAGGACAGCACAATG	CTTCTTGCTATCTCTGCC		62
16	-	Xcup66		GGCTTTAGCGATCGAGCTTC	AGGGTACGACGTGGAGATTG	145	58
17	-	Xcup19		CCGAGTTCTCACTCCCTCTC	GACCTTGTCGAACCTGCTTC	128	58
18	-	Xcup43		GCCTAACTCCCTTGATGC	GTCAGTGGATGTGGATGTGC	161	50.2
19	-	Xcup42		CACACCTGTCTCTCTCTCTCCG	AGATCATCTTCGCCCTTCTC	145	62
20	-	Xcup52		CTCCTCGCCGTCATCATC	TAAAGAGAAACGCAGGCAGG	125	62
21	-	Xcup70		GGAGGAACACGCACAAAAAG	CACTCTAGCTATGGCCTGGG	138	58
22	-	Xcup68		TACCTCACCCACTCCTACCG	AACCTCACCTGCAATCAACC	108	58
23	-	Xcup13		TCTCCTCCACCTTGCAACC	CCTTGCCATCGACCACTC	190	54
24	-	Xcup49		TCCACCTCCATCATCTTTCC	CTCCACCACCTCCATGACTC	126	58
25	-	Xcup57		CTGCAGAGAGCTAATTGTGC	TCTTGGAAGAGACGGACCTG	210	58
26	-	Xcup16		TGCAGTGCTAGCTCATGGTC	CTTTCCAGCCTCCCATATCC	230	62
27	-	Xcup17		CTGAGGAGTGTTTCATCCC	CATCACCGTTCCCCTCTTC	145	58
28	H	SbAGA01*	(AG) ₁₃	CGAACCATGATAAATGACTG	ATCCGTTTCACAAAAAAGT	106	55
29	-	SbAGG02	(AG) ₄₁	ATCCATGCATATATTCCGAC	TTCGTACCCACAACATAC	190	55
30	-	SbAGD02	(AG) ₃₂	AGCACTGCTTGACACTCC	CTTTGAAACCCTGAACATCAT	148	62
31	B	SvPEPCAA*	(AT) ₁₀	GCAGCTCAGGGACAAATAC	CTGCTTCAGGTAAGGATCG	255	54
32		Xcup50		TGATTGATTGAGGCAGGCAC	TTCCGGTCTGTCCATTTT	110	58
33	F	SbAGB03	(AG) ₄₁	GTGTGTGTAGCTTCTTGGG	ACGTAGGAGTAGTTTCTAGGATT	158	53

Appendix III. Contd.....

SSR No.	LG	Locus name	Type of SSR's	Sequence of the forward primer (5' to 3')	Sequence of the reverse primer (5' to 3')	Amplicon size	Annealing temp. (°C)
34	F	SbAGH04*	(AG) ₃₉	GGCACTCATGGAGTCACA	TTTATCCAAATCAAACCGG	170	54
35	E	SbAGE03*	(AG) ₃₄ +GA(CA)	AGCTCTCAGCCTTTCACAAT	GGAAGAAAGGAATGACTTGA	151	58
36	A	SbAGBO2*	(AG) ₃₅	CTCTGATATGTCGTTGTGCT	ATAGAGAGGATAGCTTATAGCTCA	121	50
37	A	SbAGFO6	(AG) ₃₄	ATGGCTGTCTGTCCAGGT	CAGTTGCTAATCTTTGACCG	176	54
38	C	1	(GA) ₈	CATGGGCGGGTTGAAGAC	TGCAGGAAGGGAGGATGTAG	197	56
39	B	59	(TGG) ₅	TAGAGCTGATCGAGGGATGG	TTACTTGCACTGGCCTACCG	228	56
40	B	58	(GATC) ₄	TAGAGCTGATCGAGGGATGG	AGCTAGCCGACACCAACATC	170	55
41	G	2	(GCA) ₆	GACGCAGCTTTGCTCCTATC	GTCCAACCAACCCACGTATC	198	55
42	G	3	(GAG) ₅ (AG) ₃	GATACGTGGGTTGGTTGGAC	CTCACACTGCTAGGTGCTCG	111	55
43	B	4	(TA) ₉	ATTACGCATCCAGACCTGC	TTCTGGATCTTCTGGAACCG	-	55
44	F	5	(GA) ₈	GGAAGGTTTGCAAGAACAGG	CCAGCCCCAACAAGTGCTATC	208	55
45	C	6	(CTGC) ₄	GGCAGTAGCAGGCGTTTAAG	AACTGAATCAGGTCATGGGC	202	55
46	I	7	(CAA) ₈	CTAGAGGATTGCTGGAAGCG	CTGCTCTGCTGTGCTGTGAG	271	55
47	?	8	(TG) ₆	GCAGCAACCACTTCCGATTC	GCAGTGCCGTCAAAAAGTAG	121	56
48	I	10	(GCT) ₅	AAGATGCTCTGGTGGTGAGG	CTGCTCCTACTCATGGAGGC		55
49	A	51	(GA) ₇	AACCTTCGCTGTGCTTTTAC	CCATATGCATGTGTGCCTTC	250	55
50	C	53	(TTTA) ₅	GCAGGAGTATAGGCAGAGGC	CGACATGACAAGCTCAAACG	197	55
51	I	54	(TA) ₆	CGATGTCAATCAAAGGGTGC	TATCCACATTCACAGGCAG	104	55
52	A	55	(CGC) ₅	AGCTGCTCTGCTTCCAGTTC	TCTTCGTCAACGTGCTCATC	245	55
53	J	56	(GGAT) ₅	GTGGCACCATACCAGCCTAC	GTGAGGTACAGGCACGCAC	245	55
54	C	60	(CGGT) ₄	GTATGCATGGATGCCTGATG	GCGAGGGTATGTAGCTCGAC	155	55
55	A	61	(CAG) ₇	TTAGCATGTCCACCACAACC	AAAGCAACTCGTCTGATCCC	198	54
56	C	62	(GAA) ₆	CGAGAAGATCGAGAGAACCC	TGAAGACGACGACGACAGAC	189	55
57	B	63	(GGATGC) ₄	GTAAAGGGCAAGGCAACAAG	GCCCTACAAAATCTGCAAGC	148	55
58	B	64	(TA) ₉	TATTGACACGCAGGTAACGC	GAGGACGAGTGCATGATGAG	223	55
59	A	65	(AAAC) ₄	GCAATTGACAACGCATCTGG	AGTAATCGTCTCCGGTGCTG	193	56
60	I	67	(TA) ₆	GGTCAGTGCTTACACAGATTCC	GGGGATTGCAGGTGTCATAG	245	55
61	B	69	(ATGCG) ₄	ACAGCACCAAGGTGAAGGAC	ATGTAGGGCACCAAGCTTCAC	254	55
62	F	71	(CA) ₇	CCACCTGTTGATGGGTTCC	AGCTTCGTGCTCTCTGGTTC	120	55
63	?B,H	73	(TA) ₁₀	GGTTCTGTGTCATCACACAG	ATCTTTAGCCGCCACATGAC	206	55
64	?B,H	72	(ATT) ₆	GGGAGTGCAGTGACCTTCTC	GAACACACAGCAGGAACACG	252	55
65	B	74	(TG) ₉	GTCCGCCATTGTGATGAAGAG	CAGTAGTCCAGCAAAACGGC	231	55
66	E	21	(GAT)	ATACCATCCACCTCACCAGC	GAAACGTACATGGGTTTGGG	262	55
67	C	22	(AGTAC) ₄	CCAGTTCAAGTTCAGTCCATACG	CGACAGCGCACACAAGTC	248	55
68	F	23	(GCT) ₅	GATAACTTTGGCCAACCTCGC	TGTCTGCCAGTTCCAC	163	55
69	C	24	(TA) ₉	AAACTGGATGCCACACCAAG	AGCTATACCAACACGGGCAG	219	56
70	D	25	(ACG) ₅	GACACCGTGCAAGGATAGC	GCACCAAAGCAGTTCAGTG	112	56

Appendix III. Contd.....

SSR No.	LG	Locus name	Type of SSR's	Sequence of the forward primer (5' to 3')	Sequence of the reverse primer (5' to 3')	Amplicon size	Annealing temp. (°C)
71	B	26	(CT)6	CGATCATCAGATCATGGGAG	CACTTGGGAAGTTGGGATTG	223	55
72	C	27	(CT)6	AGAAGGACGACGAGAAGCAG	TGGAAGAGTACGGATCGAGG	190	55
73	F	28	(TGAG)5	GGTGTGAGACTGTGAGCAGC	TATAGCACGGTTGTTGTGCC	167	55
74	B	29	(AT)6	CTTTCTCGATTCTGGTGCC	TTTACCTTGCCTATGCCTGC	192	55
75	C	30	(CCTG)5	ACGTATATACAGGCGAGGGC	CGCAGATCGCATCAACTAAC	229	54
76	A	31	(GAC)7	AGTTCCTTCCCAGCTTCAC	ACATACTTGGTGGTTTCGGC	243	55
77	A	32	(AAAAT)4	ACTACCACCAGGCACCACTC	GTACTTTTTCCCTGCCCTCC	156	55
78	?C,G	33	(AT)7	GCGCTGCTGTGTGTTGTTT	ACGGGGATTAGCCTTTTAGG	268	56
79	A	34	(TTC)5	GCCTCAGCTGACTCCAATTC	CTGATGTTTCTGTTCTGCG	125	55
80	D	35	(CT)7	CTTTTCTTGGGCTGACAAGC	TAGACACATCACACTCCGGC	214	55
81	D	36	(CA)8	TGAGCTGATAATGGCTGCTG	GCGTCACGGAAGTTGGAC	172	55
82	D	37	(AG)9	CCCAGCCTTCTCCTGATAC	GTACCGACTCCAATCCAACG	209	56
83	C	38	(ACT)5	CTCTCACGGAAGGAAGCAC	TACCGAAGCGGAAGCTACTC	161	55
84	C	39	(CGCT)4	GAGAGCGCTCCTCGATCTG	GAGGATTACCGAAGCGGAAG		56
85	B	40	(TG)7	ACGGAGAATAGAAAGTGGCG	TTGAGCATGCAACCACTAC	196	55
86	B	41	(CAA)5	AACACGAAAAGGTTAGGGGG	TCGAATGGTCCAGTAGTCCC	232	55
87	C	44	(AC)6	CATGCATGCGTGACCTGAG	TAGCTGTGTCCGTCGATGTC	221	56
88	C	45	(TG)9	CTCTCACTGCTCTGCCATTG	GGTGAAACTGCGAAAGCAAC	193	55
89	C	46	(CGA)5	CATTCCCATGGCTGCTAATC	GGTAGTTGCATTGCCTTGTG	228	55
90	E	47	(GA)21	TGAGCAATGAAGTTAGGGGG	CTACCCTTTGATGGCAGTACC	251	54
91	F	48	(AT)7	TCACTAGCGCCTCCAAAATC	TCCAATCCTTCCTGTGCTTC	270	55
92	F	20	(AT)6	TGGGTGTTGCACTGGGAG	ACTGAAAGCACCGTCTCTGG	222	56
93	G	18	(CAAG)4	GCCTCTACATATCCACAAGCC	CAGATCAGTCATGCCACCTG	231	55
94	C	15	(CA)4(TCCCC)4	ATACACTCCCAAGCCAGCAC	CAATAAAAGAAGGGGGGAGC	236	55
95	A	14	(AG)10	TACATCACAGCAGGGACAGG	CTGGAAGCCGAGCAGTATG	208	55
96	D	12	(TG)7	TGTTACAGAGACGCGCAGAG	GGCTGGTTGCTACCTTGTTT	123	55
97	A	11	(GCTA)4	TACCGCCATGTATCATCAG	CGTATCGCAAGCTGTGTTTG	165	56
98	C	Cha	(TA) ₁₈	AAAGCTCGGCGTTAGAAATA	CGCTTAACAACCTCTACCATC	217	55
99	J	Kaf2	(CAA) ₉	TCGGCGAGCATCTTACA	TACGTAGGCGGTTGGATT	279	55
100	J	Kaf3	(GCA) ₅	GCGAATAGCATCTTACAACA	ACTGATCATTGGCAGGAC	243	55
101	G	PepC	(AT) ₁₀	TGGGAAGCAGCTCAGG	AGGGTGGTGATGTAGGGA	247	55
102	.	Xtxp45	(CT) ₅ + (GA) ₂₅	CTCGGCGGCTCCTCTC	GGTCAAAGCGCTCTCCTCCTC	202	55
103	A	Xtxp46	(GT) ₁₀	GGGCAATCTTGATGGCGACAT	AGGTGTGGCTCGGGGAGAAC	253	55
104	H	Xtxp47	(GT) ₈ (GC) ₅ + (GT) ₆	CAATGGCTTGACATGTCCTA	GGTGCGAGCTAGTTAAGTGGG	264	55
105	.	Xtxp48	(GTCT) ₃ + (ACG) ₃	AATAACACGCGTCTAGTTGTC	CCATCATCGTCCATCC	109	55

Appendix III. Contd.....

SSR No.	LG	Locus name	Type of SSR's	Sequence of the forward primer (5' to 3')	Sequence of the reverse primer (5' to 3')	Amplicon size	Annealing temp. (0C)
106	B	Xtxp50	(CT) ₁₃ (CA) ₉	TGATGTTGTTACCTTCTGG	AGCCTATGTATGTGTTCTGTC	299	55
107	D	Xtxp51	(TG) ₁₁	TCTCGGACTCAAGAGCAGAGG	GGACAGCAGCGGCTTCAG	234	55
108	.	Xtxp53	(TG) ₆ GGG(TG) ₈ (AG) ₉	ATCTATTGCTCCATCTCCCTT	GCATTGTTGCATGTTGGTTAT	365	55
109	B	Xtxp55	(CA) ₁₅ GA(CA) ₆	TCATGGCATGGGACTATTG	AAGGTTGGCGTAGAAATGTGT	209	55
110	B	Xtxp56	(GA) ₃₉	TGTCTTCGTAGTTGCGTGTTG	CCGAAGGAGTGCTTTGGAC	349	55
111	I	Xtxp57	(GT) ₂₁	GGAACCTTTTGACGGGTAGTGC	CGATCGTGATGTCCCAATC	251	55
112	A	Xtxp58	(AG) ₁₃ + (GA) ₁₆	CAAAGTGCCCGGTTAAGACCT	TTCCCTTGCTGTTGCTTG	160	55
113	C	Xtxp59	(GGA) ₅	GAAATCCACGATAGGGTAAGG	GACCCAGAATAGAAGAGAGG	207	55
114	D	Xtxp60	(GT) ₄ GC(GT) ₅	GCTAGCTGACGCACGTCTCTG	TGCAACCGAGCGGTGACTA	224	55
115	A	Xtxp61	(GA) ₁₃	GATGCCCCATGCCTTGC	CCCACTAACTAAAGCGGACA	130	55
116	C	Xtxp69	(TC) ₁₂	ACACGCATGTTTGACTG	TTGATAATCTGACGCAACTG	188	50
117	.	Xtxp70	(CT) ₂₅	AGTGACCTTAGCACCAAGCTC	TCAGGTAGCACTAGAG	215	50
118	.	Xtxp71(Mads)	(GT) ₉ (GA) ₂₈	CATAACGGCCGGAAGCAGTG	TTGTCCCATGACCCTCCACAG	202	60
119	B	Xtxp72	(GA) ₃₆	TTATGGAAGCAAAATGAC	CGAATCCTAATTGAGGTAAGC	123	52
120	A	Xtxp75	(TG) ₁₀	CGATGCCTCGAAAAAAAACG	CCGATCAGAGCGTGCCAGG	172	50
121	.	Xtxp77	(TA) ₈ (GA) ₈	ACTGGACGTATGGCATTATTG	TGCAGGACAGTAGCGTAGG	244	53
122	.	Xtxp78	(GT) ₁₅	GCCAACACAAATTGAAGATG	TGCGATTAATCAGCGACC	179	50
123	.	Xtxp79(Mads)	(CT) ₂₇ (CA) ₁₀	CTCCACAGCCAGAACATT	GAAGCAGTGCAGGATTCACT	178	47
124	.	Xtxp80	(CT) ₁₃ + (ACG) ₃	GCTGCACTGTCTCCACAA	CAGCAGGCGATATGGATGAGC	287	53
125	.	Xtxp81	(TG) ₉	TCGATATTGTTGGCAAGATTC	CATTGGCTCAGCATTGG	251	47
126	.	Xtxp82	(GA) ₁₂ (GC) ₆	GCAGCGGTTCTCTGTGCC	ATTATACTCGGCTTCCTTTAG	185	60
127	.	Xtxp83(Kaf)	(GT) ₉ (AT) ₁₂	CGCTAGTCAAAATTGAGGAG	CAAACCGATAAACAAGTATGA	274	55
128	B	Xtxp84	(AG) ₉	CCGATCAGCACACCAG	GTAAGGTCCAATCCAGC	210	50
129	.	Xtxp85	(CT) ₁₃	TCCTCTTGCTGCCTTGC	TTTGAACCTTCGTTGACTACGC	157	50
130	E	Xtxp90(Prt)	(AC) ₉	GCGTGCAAGATGCGGAAACA	GACGCGCGGTAAAGTGGAATG	172	55
131	.	Xtxp91	(AG) ₁₉	GGTCTCTGGATACTAGGTG	AAGTAATAGACGAGTGAAAGC	170	50
132	.	Xtxp92	(GAA) ₅	ACTTGCAGGTTAATTTCTGTC	GGCGAGCTTGCGGTAG	170	50
133	.	Xtxp93	(AC) ₉	CAGGGCTCACGACTAAGACA	ATGCCGTTTGCTACTGTATGA	109	50
134	.	Xtxp94	(TC) ₁₆	TTTCACAGTCTGCTCTCTG	AGGAGAGTTGTTCTGTTA	232	50
135	E	Xtxp95	(GA) ₁₈ (GC) ₄	TCTCCGTTTGCCCGCCAG	CACCGTACCGCTCCCGAATC	90	53
136	I	Xtxp96	(GA) ₂₄	GCTGATGTCATGTTCCCTCAC	CATTCTGTTGACTCTGTCCG	198	52
137	D	Xtxp97	(CA) ₈ + (GCC) ₆	CAAATAAACGGTGACACTCA	GTATGATTGGAGACGAGACGG	130	53
138	B	Xtxp98	(CTGT) ₅	GCCAGCTTATCGGAAACAAG	GTGTCCACTAGAGCGGCTATG	215	60
139	C	Xtxp99	(GGA) ₈	CACAAAGGCACCGAACAAC	CAGAGGCCGAGGACGAG	287	50
140	.	Xtxp100(Kaf)	(CT) ₁₉	CCGCGCGGCCAACCAACCAC	TGCCCCAACGCTCAGCTCCC	117	55
141	B	Xtxp101	(GA) ₁₀	CCCAGAGGCCAGACTACAAC	CCAACCCATCTTCGTTCC	183	50

Appendix III. Contd.....

SSR No.	LG	Locus name	Type of SSR's	Sequence of the forward primer (5' to 3')	Sequence of the reverse primer (5' to 3')	Amplicon size	Annealing temp. (0C)
142	.	<i>Xtxp104</i>	(GGC) ₆ + (GT) ₇	TAACCTATGCGGATAAAACAG	GAATCGCTGCCAAATAAA	188	50
143	B	<i>Xtxp105</i>	(TG) ₅ + (CT) ₆ GTCT(GT) ₇	TGGTATGGGACTGGACGG	TGTTGACGAAGCAACTCCAAT	291	55
144	.	<i>Xtxp106(Kaf)</i>	(GGA) ₄ AGAAG(AGG) ₄	ACAGCACGGGCACAAA	CCGCTAACAAAGCTAGAAAAGG	336	53
145	C	<i>Xtxp107</i>	(TG) ₉	CAAAGTGAGCGTGGTC	GGACAGGGATAACATAACATA	281	42
146	B	<i>Xtxp109</i>	(TG) ₁₂	CTTGTGTATGATGATGATGTG	CTCGCATTTTGCAGTAG	139	47
147	A	<i>Xtxp112</i>	(GT) ₆ (CT) ₁₁ C(AT) ₂₁ ACA(CG) ₁₀	TCTACGACCGCATCAACGAC	GCTCTCGCTCTAATCGCACAC	209	50
148	H	<i>Xtxp113</i>	(GA) ₁₁	CTCAGCTAATTTAGCCATAG	CAAGTAATAGACGAGTGAAAG	198	50
149	B	<i>Xtxp114</i>	(AGG) ₈	CGTCTTCTACCGCGTCCT	CATAATCCCACTCAACAATCC	234	50
150	D	<i>Xtxp115</i>	(GT) ₈	TTGTTTCGGTGACCAC	TATCTTTAAATTGCCTTTGTT	318	60
151	.	<i>Xtxp116</i>	(CAGA) ₅	TGGTGGCAATGCAAGCTACAG	AGCGAGACGATCGACAGGG	148	55
152	.	<i>Xtxp127</i>	(ACAT) ₅	TACGTGCTACAGAATGG	GCTGGCTAGGACACAA	154	55
153	C	<i>Xtxp129</i>	(AT) ₆	TCCTCGACATCCTCCA	GACACCTCGTAGCACTCC	173	55
154	.	<i>Xtxp131</i>	(CT) ₇	AAGCCCCGATCACATCAA	AGCCGAGCCTCATCCC	161	55
155	G	<i>Xtxp134</i>	(GGC) ₅	CTCACTGCATCCCAAAGC	ACGGGCGTAGGACAGG	218	55
156	C	<i>Xtxp135</i>	(GGC) ₅	AGGCCAACCTGAAAGC	GACCGGCATCTCCAC	218	55
157	I	<i>Xtxp139</i>	(AC) ₁₁	AACGCTCCTTTAGGTGATG	TTTCTAAAGGACCTGTTAACTGTT	163	55
158	.	<i>Xtxp144</i>	(AG) ₅	GCTACTAACC GCACACAGACAC	CTGCCTGCAATCGAAGAAC	326	55
159	J	<i>Xtxp145</i>	(AG) ₂₂	GTTCTCCTGCCATTACT	CTTCGCGACATCCAC	238	55
160	E	<i>Xtxp149</i>	(CT) ₁₀	AGCCTTGATGATGTTCC	GCTATGCTTGGTGTGGG	169	55
161	C	<i>Xtxp150</i>	(GT) ₁₄	CATGATTGCCTTTCCTTC	TTGGCATTGGACAGATTT	209	55
162	A	<i>Xtxp156</i>	(GGA) ₇	GATCTTGCCCGTGGACAT	GGCCAAAGTTTCGGACCT	234	55
163	F	<i>Xtxp157</i>	(GGA) ₆	TTGTACGGTGGGCACTA	AAAGGATGCGGCAGGAT	179	55
164	.	<i>Xtxp159</i>	(CT) ₂₁	ACCCAAAGCCCAAATCAG	GGGGGAGAAACGGTGAG	169	55
165	.	<i>Xtxp160</i>	(TG) ₁₄	AGTCGGCTGGTAAAGTGG	GGATATGGGATGGTTTGG	158	55
166	A	<i>Xtxp162</i>	(GGA) ₆	CCCGATATCTCTCTGAC	CCCCAGTTTAGCCAAGT	213	55
167	H	<i>Xtxp164</i>	(CA) ₁₀	TATTCTACCCGTCCAAC	GACTACATGGCGACAGA	185	55
168	F	<i>Xtxp165</i>	(AT) ₄ AGCC(CA) ₈	GGATCCTTTCATAATAT	CGATAGGGAAGAAAC	182	55
169	J	<i>Xtxp168</i>	(AC) ₁₀	AGTCAAAACCGCCACAT	GAGAAGGGGAGAGGAGAA	178	55
170	I	<i>Xtxp176</i>	(AG) ₄ AAC(GA) ₄	TGGCGGACATCCTATT	GGAGAGCCCGTCACTT	161	55
171	C	<i>Xtxp177</i>	(CT) ₇ (GT) ₈	GCCGGTTGTGACTTG	TTAAAGCGATGGGTGTAG	169	55
172	.	<i>Xtxp179</i>	(GT) ₈ AC(GT) ₅	TAAGCTGATTTGGGGGACAT	GGTGATCGAGTGCGGAGTA	319	55
173	.	<i>Xtxp183</i>	(TG) ₉	AAGTTGTAATGGGGCTATTG	TTAAGAGGTGGGATATTGGT	190	55
174	G	<i>Xtxp196</i>	(GGA) ₄	CAGCGAGTGCAAGGA	CGAAGCTGGCGAAGT	261	55
175	H	<i>Xtxp197</i>	(AC) ₁₀	GCGTCAATTAATCCAAACAGCCTC	GAGTTCCTATTCCCGTTCATGGTGAT	154	55
176	I	<i>Xtxp198</i>	(AG) ₁₂	CCCTTGGTTGCCGGAAGA	TTCGTATGTTGTGGGGATTGTGAG	222	55

Appendix III. Contd.....

SSR No.	LG	Locus name	Type of SSR's	Sequence of the forward primer (5' to 3')	Sequence of the reverse primer (5' to 3')	Amplicon size	Annealing temp. (°C)
177	.	Xtxp201	(GA) ₃₆	GCGTTTATGGAAGCAAAAT	CTCATAAGGCAGGACCAAC	222	55
178	E	Xtxp204	(GT) ₇	TCGTGCGTCATCGTGT	GCATGTGCCACAAGATTCAA	161	55
179	B	Xtxp207	(CT) ₁₄	ACACATCTACTACCCTCTCACCT	TGATAGACTTGTGAGCAGCTCC	185	55
180	A	Xtxp208	(GGA) ₈	AAGGCCGTGAGGATG	AAGCAGCCAAGAGCAG	257	55
181	C	Xtxp210	(CT) ₁₀	CGCTTTTCTGAAAATATTAAGGAC	GATGAGCGATGGAGGAGAG	188	55
182	F	Xtxp212	(GT) ₁₀	TTTCCCTCTTTCTTGTGTC	CTCGGCGTCGTCGTA	150	55
183	.	Xtxp213	(CT) ₂₇	AACATTTCTCCAGGCTC	GGAAGCAGTGCAGGAT	168	50
184	F	Xtxp214	(TC) ₁₄	TCCCTCCGTAAGAAAAACAAA	TGGAGGACAAAACGTTGAGAAG	222	55
185	.	Xtxp215	(CA) ₉	CCCAAAGCCAAGAAAAAG	CGGCGGAAGCAGAC	174	50
186	H	Xtxp216	(TG) ₅ C(TG) ₄	GTTCAATTTGCCACTCATTC	TATATTGGATAGGCGTGTG	184	55
187	H	Xtxp217	(GA) ₂₃	GGCCTCGACTACGGAGTT	TCGGCATATTGATTTGGTTT	175	55
188	E	Xtxp218	(CA) ₁₀	CCGGAACCTGCTACTG	ACGCCGAAGGAGAAG	213	55
189	B	Xtxp219	(CGG) ₆ + (GT) ₇	AACCTATGCGGATAAAACAGA	GGATCGGTGCCAAATAAA	187	55
190	B	Xtxp221	(TCC) ₆	GCAACGGGAACCAAAA	TGACGACGGCAGAG	170	55
191	B	Xtxp224	(GT) ₁₀	TTTTCGCTGGGACTG	CTTTCGGAGCCATTTT	196	50
192	J	Xtxp225	(CT) ₉ (CA) ₈ CCC(CA) ₆	TTGTTGCATGTTGGTTATAG	CAAACAAGTTCAGAAGCTC	165	55
193	A	Xtxp227	(GT) ₅ CCCTC(GT) ₅ + (TG) ₄	TGAAAGTTTTGGCATTGA	TGTAGGATAGCCCAGGTT	178	50
194	J	Xtxp228	(TC) ₁₂	ACAGGTTGGCGATGTTTCTCT	TTCTTTTTCGAATTCATTCTTTT	246	55
195	E	Xtxp231	(GGA) ₄ + (GGA) ₅	GGAAATCCAGGATAGGGT	AGGCAAAGGGTCATCA	188	55
196	B	Xtxp240	(TG) ₃₁ (AG) ₆	AGTAATATGTGGGTTGGTCGTTGG	TAGAGCCGCCGGATGATTTGT	184	50
197	A	Xtxp243(Kaf)	(AT) ₄ + (TAA) ₃	ATCTCTGCCACCTACTAGCTACGTCAA	AATTAGGCAGAGCAAGTATTCATCATATCCAT	270	50
198	I	Xtxp248	(AG) ₅ (GA) ₂₈	GGGTGTCCAATGTTGTCTGC	GGCCGTTACTGTCCCTTACTCA	235	50
199	A	Xtxp250	(AAG) ₁₇ AAT(AAG) ₄ AAA(ACA) ₉	GCACATCCTCTAAAACTACTTAGT	GAACAGGACGATGTGATAGAT	283	50
200	H	Xtxp262	(GT) ₅	TGCCTGCCCCGACCTG	TTGCTGTCTCCGCTTTCC	167	55
201	.	Xtxp266	(GT) ₈	GTTGTCTAGTATAGCAAGGTGGG	ATAATAGTAGATGCGTGTCAAAAGAA	197	55
202	A	Xtxp267	(TCT) ₂₀	TTTTTGGGAAGCGTCGTC	GCGATTTGGCTGAGCAGT	150	55
203	D	Xtxp268	(CAA) ₇	TGGATAAGGCATACCAATTAAGCAG	TTTCTCTTGAGTATTTCTTTCCATTTCTT	189	55
204	.	Xtxp270	(GAA) ₁₂ (GAAA) ₆ + (GAA) ₂₁ +	AGCAAGAAGAAGGCCAAGAAGAAGG	GCGAAATTATTTTGAAATGGAGTTGA	279	55
205	.	Xtxp273(Pbbf)	(TTG) ₂₀	GTACCCATTTAAATTGTTTGCAGTAG	CAGAGGAGGAGGAAGAGAAGG	223	55
206	A	Xtxp276(Kaf)	(CAA) ₉ + (AAT) ₅	ATGACTTGTGAGAAGTGTGGAGATACTG	ATTACCTGAGTAGTTAGGCCTCTGTTGATT	171	55
207	.	Xtxp279	(CTT) ₁₀ + (CTT) ₃ + (CTT) ₆	ATTCTGACTTAACCCACCCCTAAA	AGCTCATCAATGTCCCAAACC	276	55
208	F	Xtxp284	(AAG) ₁₉	CCAGATTGGCTGATGCATACACACT	AAGGGTAATTTATGCACTCCAAGGTAGGAC	219	55
209	A	Xtxp285	(CTT) ₁₁ CTC(CTT) ₁₆	ATTTGATTCTTCTTGCTTTGCCCTTGT	TTGTCATTTCCCCCTTCTTTCTTTT	249	55

SSR No.	LG	Locus name	Type of SSR's	Sequence of the forward primer (5' to 3')	Sequence of the reverse primer (5' to 3')	Amplicon size	Annealing temp. (0C)
210	B	<i>Xtxp287</i>	(AAC) ₂₁	GCAAGCGAGCTGACTTATGTAAACGAGA	CAAAGTGCTACTAAACCTATGCAGGGTGAA	367	55
211	A	<i>Xtxp288</i>	(AC) ₁₂	ATTCACTTTTGTAGGTTCTATTGCTAGAT	ATTTAACACCATTTTATACCTGTCAGACAC	217	50
212	.	<i>Xtxp289</i>	(CTT) ₁₆ + (AGG) ₆	AAGTGGGGTGAAGAGATA	CTGCCCTTCCGACTC	290	55
213	.	<i>Xtxp290</i>	(CT) ₁₇	CGCCGTTCTCCTCTC	TAAACCAGTGGCAAACTA	268	55
214	H	<i>Xtxp292</i>	(AC) ₁₂	CATTTGCGAAGTTACAACATTGCT	CATTCTGACTGCCCTCTCC	332	55
215	.	<i>Xtxp294</i>	(TG) ₁₀ (GT) ₄	GCTGGGGCTCGAGGGTTTTTCATT	AGCTTCCCAAGGACAACTAGCAAGGACA	289	50
216		<i>Xtxp296</i>	(CA) ₁₈	CAGAAATAACATATAATGATGGGGTGAA	ATGCTGTTATGATTTAGAGCCTGTAGAGTT	171	55
217		<i>Xtxp297</i>	(AAG) ₂₄	GACCCATATGTGGTTTAGTCGCAAG	GCACAATCTTCGCCTAAATCAACAAT	220	55
218		<i>Xtxp298</i>	(AGA) ₂₃	GCATGTGTCAGATGATCTGGTGA	GCTGTTAGCTTCTTCTAATCGTCGGT	202	55
219		<i>Xtxp299</i>	(CAT) ₁₉	CTCTCCCCTTTTGTCAATCCATC	TCTTGCCCAACGAGACTTCTC	210	50
220		<i>Xtxp302</i>	(TGT) ₈	TAGGTTCTGGACCACTTTTCTTTTGTGTT	GAATCAACTATGTGCTTGCATTGTGCT	180	55
221	B	<i>Xtxp303</i>	(GT) ₁₃	AATGAGGAAAATATGAAACAAGTACCAA	AATAACAAGCGCAACTATATGAACAATAAA	160	55
222	B	<i>Xtxp304</i>	(TCT) ₄₂	ACATAAAAGCCCCCTTTC	CTTTCACACCCCTTTATTCA	206	55
223	B	<i>Xtxp309</i>	(ACT) ₅ + (ACT) ₃ + (CTT) ₃	TGCCCTTCAGGAATGATTGCACTACTAC	TGCAAAATGCCACAAATAGGAGAAAAA	321	50
224	I	<i>Xtxp312</i>	(CAA) ₂₆	CAGGAAAATACGATCCGTGCCAAGT	GTGAACTATTGCGAAGAAAGTTTGGAGGAAA	192	55
225	B	<i>Xtxp315</i>	(AGA) ₁₂	AACCTACCCTAGCCCGCGCAACTG	CAACATGTCCGCAAGATTGTGATGTGAC	302	55
226	B	<i>Xtxp316</i>	(AGA) ₁₂	CCAGCTTCACTTACGAGGAGATG	ATGCCCGTTTCTAATTCTTCTACT	331	55
227	Unk6,7	<i>Xtxp317</i>	(CCT) ₅ (CAT) ₁₁	CCTCCTTTTCTCCTCCTCCC	TCAGAATCCTAGCCACCGTTG	162	55
228	F	<i>Xtxp319</i>	(TC) ₁₇	TAGACATCTGAATTAAGGAGC	CATGCCCTGAAAAGAGA	160	55
229	Unk7	<i>Xtxp320(PhyB)</i>	(AAG) ₂₀	TAAACTAGACCATATACTGCCATGATAA	GTGCAAATAAGGGCTAGAGTGTT	290	55
230	B	<i>Xtxp323</i>	(CT) ₂₁	TATATGCATGTTTTAGGTGCG	CTGTTGTTCCTTTCTTCC	155	50
231	J	<i>Xtxp324</i>	(TA) ₁₈	GCAAGAGTAACCTGCATTAAATTA	ACATAACACAAGCAGGGAGAG	202	55
232	J	<i>Xtxp325(Kaf)</i>	(AAT) ₂₃	GGGGCGACTCTTTGTA AACATA	ATCGTGTCACTATCTACATCTAAATCGG	247	55
233	Unk8	<i>Xtxp327</i>	(TAG) ₃ + (GA) ₂₂	ACCACTGCTCACGCTCAC	GCGGTGTACAGCTTCGTC	157	55
234	I	<i>Xtxp328</i>	(CT) ₆ + (GGAC) ₆	ACGACGACGAGGTGG	TTCAACAAAGGAAAGGATTC	245	55
235	H	<i>Xtxp329</i>	(ATC) ₈ + (CTT) ₂₂	ACTACGAAGGTGTTTAGTTAAGGG	CATTCAATAAACTAAACGAAAAACG	162	55
236	B	<i>Xtxp331</i>	(GAT) ₃₂	AACGGTTATTAGAGAGGGAGA	AGTATAATAACATTTTGACACCCA	226	55
237	G	<i>Xtxp335</i>	(GT) ₁₂	TATTTCTCTTGAAAGAATCAGGG	TATTCATCGAGCAAAAGGCA	174	55
238	D	<i>Xtxp336</i>	(CGG) ₄ + (GAG) ₆	CAGCGAGCACCGACGAC	CCACCCAACCTGACCTTCT	167	55
239	Unk7	<i>Xtxp337</i>	(AG) ₁₉	GGGGATCAACTATTG	CCAACTATAGCTGAGAG	150	50
240	J	<i>Xtxp338</i>	(GAA) ₁₁	CTTTTTGCTGTAGCACAAATCTG	ATCCTTTGGTACACGACTCACT	197	50
241	D	<i>Xtxp339</i>	(GGA) ₇	CCGCACTCTCCACTCT	CGGAACACAGGGGAAGG	202	55
242	B	<i>Xtxp340</i>	(TAC) ₁₅	AGAAGTGTGCATGTATTCGTCA	AGAAACTCCAATTATCATCCATCA	198	55
243	D	<i>Xtxp344</i>	(TAG) ₁₄ (CAG) ₅	ACAGAGAAGTAGGTTGAAGA	CTTCTATTTTCTACTCTCATCC	177	55
244	Unk7	<i>Xtxp348</i>	(TAA) ₃₇	CGACATCAGCGTTGTCTTTCTA	GCTTACGAATAGGGCAAAAGAACT	290	55
245	Unk7	<i>Xtxp350</i>	(AAT) ₂₃	GCTTCCTTCGTCTTTGC	CTACATCTAAATCGGGAGTATG	290	55
246	J	<i>Xtxp351</i>	(AAC) ₈	ATGCCGATGACATACCAATG	GTTTTCCAGTCACGAACG	292	50
247	C	<i>Xtxp353</i>	(CT) ₁₉	CTCCAACATGCTCTGCTG	GGATCAACTTATTGGGGATG	168	55
248	C	<i>Xtxp355</i>	(CT) ₇ CG(CT) ₁₅ (CTT) ₁₂	TGGTTGGAAAGATAATCAAG	GCCCTAATGAGTCCTCAC	289	55
249	C	<i>Xtxp357</i>	(GT) ₁₀	CGCAGAAATACGATTG	GCTATCTGGAGTAACTGTGT	273	55
250	E	<i>Xtxp358</i>	(ATT) ₂₂	CAAGGACAAGATTCATTTTAAGGG	TCACACCTCACAAAATAAAGTGC	267	55

Appendix IV

List of gSSR primers used in present investigation(RIP derived from IS22380 x E36-1)

New name	Forward (5'→3')	Reverse (5'→3')
iabtgs1	TTTCAAACCTTATTTGTGCGATAGG	GATGCACAATTCATATGTTGATTC
iabtgs2	GTGTGCGCTCGGGATAAATA	GCCCTTTTGCAACGTCTAAA
iabtgs3	GAAGTAGGAGTGTGCCGTAGC	CACACCTGGCTCTGTTTTCA
iabtgs4	CATGGAGCTGCCTTAGATCC	TGAAAAATATCATGGAATAATACGC
iabtgs5	TCGTCGTGATCCTATGTCC	GAAAGAAGCAAGCAGTGACG
iabtgs6	TCCCTTTACCTCGCTGTCTG	TCTCTTTTAGTCTGTTTGAAAATTGC
iabtgs7	GCGGGATTTCAGTTACCG	CCTTGTCTAGCCTCCCCTTG
iabtgs8	CCGACATGCTGCTCTAGCTC	AAAGGTGCTTGCTGTCTGTG
iabtgs9	CATGTCGATCGATCAGTGCTG	ACCGCGTCCTTCAGGTAGTAC
iabtgs10	GCTTTAACGGAACCAAACCA	GAAGCGGTTTTGAGCGTTC
iabtgs11	GCTCGCTATACGGAGACAGC	CCCTCCTGATCCTTCTCCAC
iabtgs12	TGCCTATGGTGCATGTCAATC	TGGGTGGAGATCATGCAATA
iabtgs13	CGCACTCGATCGACTAGCTTAA	CGAGAGAGGCGATAAGGATG
iabtgs14	TTGCTTCTTCGATCGATCTTC	AAATAATGCTGCATGTCTCGTTA
iabtgs15	GAGTACGCATGGAACGAAGG	TGGGTGTATGCAGCAACTTG
iabtgs16	GATCGCACAGAACACAGACC	GCAGGCAAAGCTCCTAACTC
iabtgs17	GACACACAGGCAACCATGAC	TTCCCCAGCTGTACACATCA
iabtgs18	CGGGTATGTAGGCCAGAATC	GCCAGGTTCAAGCTCAGTGTA
iabtgs19	CGAGTCGCTTCAAATGCTCTG	CAGCTACACTGGCCATCTCC
iabtgs20	CCATGCCATTGCTCATCTCA	TGGTTTAGATCGTCGCAACA
iabtgs155	CCAGAAACCCATCAAGGAGA	AGCACATCGAAGCTGGAATC
iabtgs83	GCTTCCGTCACACCTCCTC	TTGTGGGAAATTGGGATGATA
iabtgs83	GCTTCCGTCACACCTCCTC	TTGTGGGAAATTGGGATGATA
iabtgs477	GTTGCTGGTTGGCTTGCTAC	CGAAGAGGCGGTAGATCTTG
iabtgs477	GTTGCTGGTTGGCTTGCTAC	CGAAGAGGCGGTAGATCTTG
iabtgs201	CTCCTCAGGTTTGAACGAG	TCACCGTCACACACACACAC
iabtgs201	CTCCTCAGGTTTGAACGAG	TCACCGTCACACACACACAC
iabtgs341	CACGAGGCTGTTCCCTACTC	AGCATCCAAGAGCCACTACC
iabtgs254	CAACTGGGAGAAGGTGAAGG	GGTGAACCAGTCGTTGTCCTC
iabtgs259	AAACTGCTGCTGCCGAAC	GTAGGAACCCAGCCAGATCC

Appendix V : List of RAPD markers used for parental survey and mapping (RI Population derived from cross IS22380 X E36-1).

Sl. No.	Primer	Sequence of primer (5' to 3')	Sl. No.	Primer	Sequence of primer (5' to 3')
1	H1	GGTCGGAGAA	61	M1	GTTGGTGGCT
2	H2	TCGGACGTGA	62	M2	ACAACGCCTC
3	H3*	AGACGTCCAC	63	M3*	GGGGGATGAG
4	H4*	GGAAGTCGGC	64	M4	GGCGGTTGTC
5	H5	AGTCCTCCCC	65	M5	GGGAACGTGT
6	H6	ACGCATCGCA	66	M6	CTGGGCAACT
7	H7	CTGCATCGTG	67	M7*	CCGTGACTCA
8	H8	GAAACACCCC	68	M8	TCTGTTCCCC
9	H9	TGTAGCTGGG	69	M9	GTCTTGCGGA
10	H10	CCTACGTCAG	70	M10*	TCTGGCGCAC
11	H11	CTTCCGCACT	71	M11	GTCCACTGTG
12	H12	ACGCGCATGT	72	M12	GGGACGTTGG
13	H13	GACGCCACAC	73	M13	GGTGGTCAAG
14	H14	ACCAGGTTGG	74	M14	AGGGTCGTTC
15	H15	AATGGCGCAG	75	M15	GACCTACCAC
16	H16	TCTCAGCTGG	76	M16	GTAACCAGCC
17	H17	CACTCTCCTC	77	M17	TCAGTCCGGG
18	H18	GAATCGGCCA	78	M18	CACCATCCGT
19	H19	CTGACCAGCC	79	M19	CCTTCAGGCA
20	H20	GGGAGACATC	80	M20	AGGTCTTGGG
21	I1	ACCTGGACAC	81	N1	CTCACGTTGG
22	I2	GGAGGAGAGG	82	N2	ACCAGGGGCA
23	I3	CAGAAGCCCA	83	N3	GGTACTCCCC
24	I4	CCGCCTAGT	84	N4	GACCGACCCA
25	I5	TGTTCCACGG	85	N5	ACTGAACGCC
26	I6	AAGGCGGCAG	86	N6	GAGACGCACA
27	I7	CAGCGACAAG	87	N7	CAGCCCAGAG
28	I8	TTTGCCCGGT	88	N8	ACCTCAGCTC
29	I9	TGGAGAGCAG	89	N9	TGCCGGCTTG
30	I10	ACAACGCGAG	90	N10	ACAACGCGG
31	I11	ACATGCCGTG	91	N11	TCGCCGCAAA
32	I12	AGAGGGCACA	92	N12	CACAGACACC
33	I13	CTGGGGCTGA	93	N13	AGCGTCACTC
34	I14	TGACGGCGGT	94	N14	TCGTGCGGGT
35	I15	TCATCCGAGG	95	N15	CAGCGACTGT
36	I16	TCTCCGCCCT	96	N16	AAGCGACCTG
37	I17	GGTGGTGATG	97	N17	CATTGGGGAG
38	I18	TGCCCAGCCT	98	N18	GGTGAGGTCA
39	I19	AATGCGGGAG	99	N19	GTCCGTA CTG
40	I20	AAAGTGCGGG	100	N20	GGTGCTCCGT
41	L1	GGCATGACCT	101	AB1	CCGTCGCTAG
42	L2	TGGGCGTCAA	102	AB2	GGAAACCCCT
43	L3	CCAGCAGCTT	103	AB3	TGGCGCACAC
44	L4	GACTGCACAC	104	AB4*	GGCACGCGTT
45	L5	ACGCAGGCAC	105	AB5	CCCGAAGCGA
46	L6	GAGGGAAGAG	106	AB6	GTGGCTTGGA
47	L7	AGGCGGGAAC	107	AB7	GTAAACCGCC
48	L8	AGCAGGTGGA	108	AB8	GTTACGGACC
49	L9	TGCGAGAGTC	109	AB9	GGGCGACTAC
50	L10	TGGGAGATGG	110	AB10	TTCCCTCCCA
51	L11	ACGATGAGCC	111	AB11	GTGCGCAATG
52	L12	GGGCGGTACT	112	AB12	CCTGTACCGA
53	L13	ACCGCCTGCT	113	AB13	CCTACCGTGG
54	L14	GTGACAGGCT	114	AB14	AAGTGCAGCC
55	L15	AAGAGAGGGG	115	AB15	CCTCCTTCTC
56	L16	AGGTTGCAGG	116	AB16	CCCGGATGGT
57	L17	AGCCTGAGCC	117	AB17	TCGCATCCAG
58	L18*	ACCACCCACC	118	AB18	CTGGCGTGTG
59	L19	GAGTGGTGAC	119	AB19	ACACCGATGG
60	L20	TGGTGGACCA	120	AB20	CTTCTCGGAC

Sl. No.	Primer	Sequence of primer (5' to 3')	Sl. No.	Primer	Sequence of primer (5' to 3')
121	AC1	TCCCAGCAGA	181	BH1	CCGACTCTGG
122	AC2	GTCGTCGTCT	182	BH2	GTAAGCCGAG
123	AC3	CACTGGCCCA	183	BH3	GGAGCAGCAA
124	AC4	ACGGGACCTG	184	BH4	ACCTGCCAAC
125	AC5	GTTAGTGCGG	185	BH5	GTAGGTCGCA
126	AC6	CCAGAACGGA	186	BH6	TCGTGGCACA
127	AC7	GTGGCCGATG	187	BH7	TGTACGGCAC
128	AC8	TTTGGGTGCC	188	BH8	ACGGAGGCAG
129	AC9	AGAGCGTACC	189	BH9	GTCTTCCGTC
130	AC10	AGCAGCGAGG	190	BH10	GTGTGCCCTGG
131	AC11	CCTGGGTCAG	191	BH11	AGCCCAAAGG
132	AC12*	GGCGAGTGTG	192	BH12	TCGCCTTGTC
133	AC13*	GACCCGATTG	193	BH13*	AGTTGGGCAG
134	AC14	GTCGGTTGTC	194	BH14	ACCGTGGGTG
135	AC15	TGCCGTGAGA	195	BH15	GAGAACGCTG
136	AC16	CCTCCTACGG	196	BH16	CTGCGGGTTC
137	AC17	CCTGGAGCTT	197	BH17	CTCTTACGGG
138	AC18	TTGGGGGAGA	198	BH18	GACGCTTGTC
139	AC19*	AGTCCGCCTG	199	BH19	GTCGTGCGGA
140	AC20*	ACGGAAGTGG	200	BH20	CACCGACATC
141	AK1	TCTGCTACGG	201	G1	51CTA CGG AGG A
142	AK2	CCATCGGAGG	202	G2	51GGC ACT GAG G
143	AK3	GGTCCTACCA	203	G3	GAG CCC TCC A
144	AK4	AGGGTCGGTC	204	G4	AGC GTG TCT G
145	AK5	GATGGCAGTC	205	G5	CTG AGA CGG A
146	AK6	TCACGTCCCT	206	G6	GTG CCT AAC C
147	AK7	CTTCCCGGAC	207	G7	GAA CCT GCG G
148	AK8	CCGAAGGGTG	208	G8	TCA CGT CCA C
149	AK9	AGGTGAGCGT	209	G9	CTG ACC TCA C
150	AK10*	CAAGCGTCAC	210	G10	AGG GCC GTC T
151	AK11	CAGTGTGCTC	211	G11	TGC CCG TCG T
152	AK12	AGTGTAGCCC	212	G12	CAG CTC ACG A
153	AK13	TCCCACGAGT	213	G13	CTC TCC GCC A
154	AK14	CTGTCATGCC	214	G14	GGA TGA GAC C
155	AK15	ACCTGCCGTT	215	G15	ACT GGG ACT C
156	AK16	CTGCGTGCTC	216	G16*	AGC GTC CTC C
157	AK17	CAGCGGTCAC	217	G17*	ACG ACC GAC A
158	AK18	ACCCGGA AAC	218	G18	GGC TCA TGT G
159	AK19	TCGCAGCGAG	219	G19*	GTC AGG GCA A
160	AK20	TGATGGCGTC	220	G20	TCT CCC TCA G
161	AT1	CAGTGGTTCC	221	A-01	CAGGCCCTTC
162	AT2	CAGGTCTAGG	222	A-02	TGCCGAGCTG
163	AT3	GA CTGGGAGG	223	A-03	AGTCAGCCAC
164	AT4	TTGCCTCGCC	224	A-04	AATCGGGCTG
165	AT5*	ACACCTGCCA	225	A-05	AGGGGTCTTG
166	AT6	CCGTCCCTGA	226	A-06	GGTCCCTGAC
167	AT7	ACTGCGACCA	227	A-07	GAAACGGGTG
168	AT8	TCCTCGTGGG	228	A-08	GTGACGTAGG
169	AT9*	CCGTTAGCGT	229	A-09	GGGTAACGCC
170	AT10	ACCTCCGGTC	230	A-10	GTGATCGCAG
171	AT11	CCAGATCTCC	231	A-11	CAATCGCCGT
172	AT12	CTGCCTAGCC	232	A-12	TCGGCGATAG
173	AT13	CTGGTGGAAG	233	A-13	TTGATCGCAC
174	AT14	GTGCCGCACT	234	A-14	TCTGTGCTGG
175	AT15	TGACGCACGG	235	A-15	TTCCGAACCC
176	AT16	CTCTCCGTAG	236	A-16	AGCCAGCGAA
177	AT17	AGCGACTGCT	237	A-17	GACCGCTTGT
178	AT18	CCAGCTGTGA	238	A-18	AGGTGACCGT
179	AT19	ACCAAGGCAC	239	A-19	CAAACGTCGG
180	AT20	ACATCAGCCC	240	A-20	GTTGCGATCC

Appendix VI

Components of cloning

Master mix for PCR ($25\ \mu\text{l tube}^{-1}$) for each RGA amplification

PCR mix components	Volume
10× assay buffer with MgCl_2	2.5 μl
dNTPs (2.5 mM)	2.5 μl
Primer (sense)	1.0 μl
Primer (antisense)	1.0 μl
Template DNA (100 ng)	1.0 μl
Taq DNA polymerase (3U/ μl)	0.5 μl
MgCl_2 (25mM)	1.0 μl
Sterile double distilled water	15.5 μl
Total volume	25 μl

Conversion table for the amount of a PCR fragment required per ligation reaction

Length of DNA fragment (bp)	pmoles of ends per 1 μg of DNA	Quantity of PCR fragments for ligation reacting in μg (0.54 pmol ends)
100	30.0	0.018
300	10.0	0.054
500	6.0	0.090
1000	3.0	0.180
2000	1.5	0.360
3000	1.0	0.540

Luria agar

Ingredients	Concentration (g/l)
Tryptone	10.0
Yeast extract	5.0
Sodium chloride	5.0
Agar	18.0
pH 7.2	

I. Ligation reaction recipe

Plasmid vector pTZ57R/T DNA (0.165 µg, 0.18 pmol ends)	3.0 µl
Purified PCR fragment, (Approx. 0.54 pmol ends)	12.5 µl
10× ligation buffer	3.0 µl
PEG 4000 solution	3.0 µl
Deionized water up to	6.5 µl
T4 DNA ligase, 5U	1.0 µl

II : Control ligation reaction recipe

PTZ57R/T DNA (0.165 µg, 0.18 pmol ends)	3.0 µl
Purified PCR fragment, (Approx. 0.54 pmol ends)	12.3 µl
10× ligation buffer	3.0 µl
PEG 4000 solution	3.0 µl
Deionized water up to	29 µl
T4 DNA ligase, 5U	1.0 µl

Chemicals for transformation

1. IPTG (isopropyl-B-D thiogalctopyranoside) 0.1M

IPTG	:	0.24 g
Water	:	8.00 ml
Total volume	:	10.00ml

Filter sterilize and store at 4 °C . 40 µl per plate was used

2. X-gal solution (2% W/V):

Dissolve X-gal at concentration of 20 mg/ml in N, N-dimethylformamide. 40 µl per plate was used

3. Ampicillin (100 µg/ml) –Amp₁₀₀

100 µg of Ampicillin antibiotic dissolved in 1 ml sterile water, filter sterilized and stored at 4 °C, 25 µl per plate was used.

4. Glycerol (25% v/v)

25ml of glicerol was added with sterile ware and volume made upto 100ml with sterile water.

5. Reagents for Plasmid isolation

STE buffer

Tris-cl (pH. 8.0)	:	10 mm
NaCl	:	0.1 M
EDTA (pH 8.0)	:	1.0 mM

Autoclave and store at 4°C

Alkaline lysis solution 1

Glucose	:	50 mM
<i>Tris Cl (pH 8.0)</i>	:	<i>25 mM</i>
EDTA (pH 8.0)	:	10 mm

Autoclave and store at 4°C

Alkaline lysis solution II

NaOH	:	0.2 N
SDS	:	1% (w/v)

(Prepare fresh and use at room temperature)

Alkaline lysis solution III

5 M potassium acetate	:	50 ml
Glacial acetic acid	:	11.5 ml
Double distilled water	:	28.5 ml

Autoclave and store at 4°C

Molecular mapping of QTLs for component traits of
drought tolerance in sorghum
[*Sorghum bicolor* (L.) Moench.]

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ABSTRACT

Drought is the most important of abiotic stresses limiting crop productivity around the world. Stay-green trait is a visible attribute of drought resistance in sorghum. Being a quantitative character with low heritability, detection of major and stable QTLs, flanked by closely linked genomic markers, would facilitate selection for this trait. Phenotypic data for stay-green trait and other characters was collected from replicated field trials involving 93 recombinant inbred lines (RILs) derived from IS22380 (non stay-green) and E36-1 (stay-green) along with other characters during *rabi* season of 2005, in two locations; Dharwad and Bijapur. RILs displayed significant differences in mean performance for all these characters. The Data for 213 nuclear SSRs, 30 genic SSRs and 240 RAPD markers was available, of which 19 RAPD, 62 nuclear SSR and 8 genic SSR markers detected polymorphism. Genetic linkage map was constructed and 83 markers were assigned to A, B, C, E, F, G, H, I and J linkage groups by simple interval mapping [MAPMAKER/EXP and MAPMAKER/QTL]. Stable QTLs were detected for early seedling vigour (txp213, corn50; group D and B), stem thickness (AK10; group F), seed yield per plant (corn24, txp45, txp325; group C, G, J), per cent green leaf area at 15 (txp213; group B) 30 (txp48; group B) and at 45 days after flowering (txp294; group B) at both the locations. Markers used here detected stay-green QTLs on the same linkage group B and D as in earlier studies.