

**GENOME WIDE MOLECULAR MAPPING, INTROGRESSION
OF STABLE QTLs AND EXPRESSIONAL QUANTITATION OF
TRANSCRIPTION FACTOR GENES IN CHARCOAL ROT
MANIFESTATION IN *Sorghum bicolor* (L.) MOENCH.**

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AYYANAGOUDA M. PATIL

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

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4.

(RAMESH BHAT)

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1. INTRODUCTION

India, with an area of 8.40 m ha is the largest sorghum growing country in the world and it ranks seventh in production with 7.4 mt (FAO, 2007). In India, sorghum is an important crop well adapted to low input and low moisture situations. Sorghum is an important component in the diet of millions of people in arid and semi-arid tropics, including India. It is an important cattle feed and also being used in a number of traditional preparation of cultural importance. Sorghum is grown both during south west monsoon (*kharif*) and post monsoon season (*rabi*) in India. Generally, *rabi* sorghum crop produces high quality grains as they mature during winter season which is a clear, dry and rain free climate. Area under *rabi* sorghum is increasing unlike *kharif* sorghum.

Both in India and worldwide, diseases caused by fungal pathogens have been a greatest concern next only to moisture stress in sorghum. However, low levels of productivity in *rabi* sorghum has been due to charcoal rot disease. This disease has a potential to cause cent per cent crop lodging especially when there is a dry spell during grain filling and maturation stage. *Rabi* sorghum generally experiences a receding moisture situation during maturation in India, which indeed is congenial for charcoal rot.

Charcoal rot caused by *Macrophomina phaseolina* Tassi (Goid) reported as early as 1940, is an important disease of sorghum in Asia, Africa and Mediterranean Europe. Important factors limiting sorghum production are drought, photoperiod sensitivity, and lodging (Tenkouano, 1995; Trouche *et al.*, 2001). Lodging may be indirectly related to fungal infections, which weaken the plant eventually leading to lodging (Waniska *et al.*, 2001). This disease is the most important especially under *rabi* situation. A variety of symptoms are associated with charcoal rot. These includes root rot, soft stalks, lodging of plants, premature drying stalks and poorly developed panicles with small and inferior quality grains (His, 1956; Uppal *et al.*, 1936). The most striking and usually first indication of the disease is lodging of plants as they approach maturity. This is caused by the disintegration of pith and cortex by the pathogen and the vascular bundles are profusely covered with tiny black sclerotia of pathogen, giving a charcoal rot appearance. Thus, the name "charcoal rot" describes the appearance of the disease inside the infected roots and stalks. Generally the plants senescence and lodge due to stem breaking at or just above ground level (Reed *et al.*, 1980).

Information on the potentiality of fungicides to control charcoal rot in adult sorghum is scanty and virtually not known. Considering the stage at which the disease normally occurs and additional to cost of production, application of fungicide is not encouraged and practically not followed by the farmers (Anahosur and Patil, 1981).

Although, inheritance of charcoal rot resistance is complex, much progress has been made in development of efficient screening techniques. Breeding and selection for charcoal rot resistance has been suggested to be done in a holistic approach, with a strong emphasis on yield potential, adaptation, maturity and other disease resistance traits (Rosenow, 1983). Selection for stiff-stalk and drought tolerance, especially of the non-senescent type (stay green), combined with high yield, will be more productive than breeding for charcoal rot resistance alone (Maunder, 1993). On the contrary, excellent progress in drought resistance has been possible by screening for charcoal rot under the appropriate late season stress conditions. Deeper understanding of the genetic divergence and virulence in pathogen populations would go a long way in breeding resistance to charcoal rot in sorghum

A number of physiological and biological traits have been proposed to be associated with disease resistance. Analysis of biochemical factors of disease resistance and DNA markers in crosses between parents that differ for definite component traits of a quantitatively inherited complex traits can be used to dissect the genetic mechanism governing such complex phenomenon and there on facilitating mapping genes controlling the trait *per se* (Gale and Witcombe, 1992). The production of plant phenolic compounds which are thought to be part of the chemical defense mechanism of plant is under the control of both genetic and environmental factors. For instance, an increase in the activity of phenolic related enzymes and the accumulation of phenolic compounds have been correlated with resistance of cereals to biotic stresses (Mohammadi and Kazemi, 2002). Leaves of sorghum varieties resistant to

fungi contained a higher level of total phenolics than the leaves of susceptible ones, upon pathogen challenge (Luthra *et al.*, 1988). Insect damage will generally increase the synthesis of these compounds (Woodhead and Bernays, 1978; Guinn and Eidenbock, 1982). These results have usually been obtained by determining the concentrations of total phenols or of different classes of compounds (eg. lignin, tannins and phenolic acids) in plant tissues. However, the interactions of genes involved in lignin metabolism with genetic background and the environment in which the low- lignin crop is cultivated are substantial (Pedersen *et al.*, 2005).

Genetic markers are heritable entities that are associated with economically important traits and have been used by plant breeders as selection tools. Phenotypic evaluation is the primary justification for constructing genetic maps in plants. Closely linked DNA markers facilitate the indirect selection of traits that are difficult to measure and provide a starting point for map based cloning of genes of economic importance. Availability of tightly linked and easily scorable genetic markers for charcoal rot resistance genes can assist breeding efforts to produce and deploy sorghum cultivars resistant to charcoal rot. Such markers can further prove useful in understanding the mechanism of charcoal rot resistance. Marker Assisted Selection (MAS) has the potential to greatly improve the efficiency of selection of quantitative traits. Since the components of charcoal rot resistance are quantitative, it is important to analyse Quantitative Trait Loci (QTL), to develop tools that are useful for MAS in practical plant breeding programmes for increasing the level of resistance or in gene pyramiding. Quantitative genetic analysis of charcoal rot resistance requires replicated, multi-environmental testing under a wide spectrum of *M. phaseolina* and drought pressure for assessment of G x E interactions. Recombinant inbred lines (RILs) are being employed increasingly as they are more efficient and permanent population than other populations for QTL mapping and allow better estimates of genotypic variance.

In sorghum molecular genetics maps have been developed and positions of various DNA markers have been reported. Genetic linkage maps of sorghum harbour RFLP markers (Peng *et al.*, 1999; Xu *et al.*, 2000), others are based on AFLP (Boivin *et al.*, 1999), SSR (Taramino *et al.*, 1997; Bhatramakki, 2000), RAPD (Tuinstra *et al.*, 1996; 1997) and EST-SSR (Reddy *et al.*, 2008) markers. Although recent maps show tremendous improvement in marker density, lack of sufficient marker density for high resolution in genomic regions of interest is a continuing problem. Hence, Current efforts were focussed on mapping new EST-SSR markers. As saturated linkage map is essential for effective MAS, many markers need to be associated with maps to facilitate MAS and marker based cloning of genes. SSRs have several advantages over other markers like high repeatability, even distribution throughout the genome and they allow identification of many alleles at a single locus. Furthermore, genic SSRs are much more repeatable than SSRs and serve as functional markers besides having potential for candidate gene mapping. In a genetic view point identification and mapping of chromosome regions harbouring cascades of genes responsible for resistance to this disease is important both for a basic understanding of genetic regulation and practical plant breeding purpose. While the mapping efforts continue, linkage between mapped markers and various plant characters can be established in terms of genomic regions harboring QTLs for quantitative characters and genes for simply inherited traits.

Molecular markers have been used to identify and characterize QTL associated with several different traits in sorghum including plant height and maturity (Pereira and Lee, 1995), characters concerned with plant domestication (Patterson *et al.*, 1995), disease resistance (Gowda *et al.*, 1995), and drought tolerance (Tuinstra *et al.*, 1998). In sorghum, many efforts have been made for QTL detection and mapping. Inheritance of resistance to charcoal rot disease and drought are polygenic in nature and simple phenotypic selection for such traits is difficult. Selection based on markers would assist in evaluating breeding lines for the presence of genes conditioning drought and charcoal rot tolerance and other quantitative traits in sorghum (Tuinstra *et al.*, 1997). Plant maturity and the amount of inoculum influence the severity of the disease in a susceptible genotype. As a consequence, multiple evaluations made in different locations over years are essential to get an accurate account of the reaction of the genetic material and to compute the genetic contributions towards resistance. Hence, it is essential to assess the stability of QTLs across environments and years to identify the stable QTLs influencing the disease resistance. Developed linkage map with DNA markers and stable QTLs for charcoal rot resistance would lead to initiation of marker assisted

selection for pyramiding genes for these characters into elite *rabi* sorghum genotypes. By using QTL mapping, it is possible to identify specific parental alleles that make a positive contribution and to transfer better alleles from one genetic background to another using MAS strategy (Tanksley and Nelson, 1996).

Backcrossing is often the chosen method to introduce a new trait into a breeding programme and molecular methods can very efficiently increase the efficiency of backcrossing by facilitating early selection of donor allele in the background of the recurrent parent (foreground selection) and selection of background genotype of recurrent parent (background selection). This increases the efficiency in introgression backcross programmes by permitting simultaneous foreground selection for introgression of an exotic allele with the potential to improve performance for the trait of interest and background selection for desired recurrent parent genotype in other genomic regions. Advancing only the selected lines will also have the effect of reducing linkage drag (Vischer, 1996). Single copy or low copy markers with defined map locations, such as genic SSR and SSR are very much ideal for this process (McCouch *et al.*, 1997).

Plants are continually exposed to a vast array of pathogens, and generally succeed in defending themselves. An important part of this process is the induction of defence gene products that hinder pathogen growth and delay or prevent disease development. The way in which plants do this is multi-factorial, and includes regulatory proteins known as transcription factors (TFs) which directly regulate the expression of defence-related genes.

A paramount feature of the inducible defence responses upon pathogenic infection is the transcriptional regulation of a large number of plant defence related genes (Eulgem, 2005). Activation of defence responses towards pathogen infection is associated with fine-tuned transcriptional regulation of transcription factor, which can globally alter gene expression patterns (Eulgem and Somssich, 2007). Quantitative real-time reverse transcription PCR (qRT-PCR) has been a robust and useful tool to quantitative the expression of any gene in biological samples. Identification of TF families and their specific members is important in gaining a deeper understanding of charcoal rot resistance mechanism in sorghum besides to identify the candidate genes that are to be targeted for mapping and further use in breeding. An understanding of folds of expression of TF genes in resistant and susceptible cultivars at crucial time point would be more important. With these issues in background the following objectives were set for the present investigation.

Objectives

1. Functional analysis of selected transcription factor genes in charcoal rot disease manifestation through quantitative real time PCR.
2. Development of genetic linkage map using gene based and nuclear microsatellite markers.
 - a) Validation of gene based markers developed through *in silico* mining of sorghum EST data sets.
 - b) Map localisation of gene based markers along nuclear SSR markers to developed linkage map for IS 2380 x E 36-1 derived RIP.
3. Field evaluation of Recombinant Inbred Lines (RILs) derived from the cross IS 22380 x E 36-1 across three locations and seasons for charcoal rot resistance component traits and mapping of QTLs for morphological and biochemical traits of charcoal rot disease resistance.
4. Marker assisted transfer of stable QTLs for their further validation in susceptible genotypes.

2. REVIEW OF LITERATURE

Biotic and abiotic stresses are the major factors limiting crop production. Sorghum is one of the major cereal crops in semi arid regions of the world, where prolonged periods of drought and wets are frequent. Charcoal rot, which is influenced by drought stress causes a more yield loss in these situations. Genetic factors involved in plant responses to drought stress and charcoal rot incidence will provide a foundation for breeding sorghum with improved drought and charcoal rot tolerance. Breeding for disease resistance is hindered by its quantitative inheritance and interaction with host, pathogen and environment.

With the advent of molecular markers, identification of genomic regions associated with specific traits has been possible. Use of DNA based markers and linkage maps help us to understand the genetic architecture of complex quantitative characters, which are governed by many genes with small cumulative effects. Quantitative Trait Loci (QTL) analysis provides a powerful tool for identifying marker defined genomic regions for any quantitative character. QTLs are the genomic regions harbouring genes with major effect on quantitative traits which forms the basis for marker assisted introgression programme. On the other hand study of transcriptional factor (TF) genes, which forms the early responsive genes for biotic and abiotic stress response may give some clue about members of the TF families involved in the downstream defence signalling pathway leading to charcoal rot resistance.

Review of relevant literature on charcoal rot disease of sorghum and marker technology, including reports on molecular markers, mapping, statistical techniques for QTL mapping, marker assisted selection and transcriptional profiling of transcription factor genes in disease resistance are presented here.

2.1 *Macrophomina phaseolina* (Tassi) Goid.

Macrophomina phaseolina (Tassi) Goid. is soil borne *deuteromycete* phytopathogenic fungus causes charcoal rot in sorghum. This fungus has one of the wide host range, readily invading over 500 different species of monocotyledonous and dicotyledonous plants (Wyllie *et al.*, 1989). Major cultivated hosts of this fungus include: sorghum, groundnut, cabbage, pepper, chickpea, soybean, sunflower, sweet potato, alfalfa, sesame, potato, bean, and corn. Charcoal rot is an important disease during hot, dry weather or when unfavourable environmental condition prevail. In sorghum, the fungus causes stalk rot during hot, dry conditions. Lodging of grain sorghum, can be a serious problem under post rainy conditions. Lodging at either the base of the panicle or stalk is often attributed to a complex of stalk-rotting organisms. Microscopic observations of host cell penetration by *M. phaseolina* have revealed that penetration of the cell wall is very precise process (Bower and Russin, 1999).

a) Disease cycle and manifestation

M. phaseolina survives as microsclerotia in the soil and on infected plant debris, which serve as the primary source of inoculum and have been found to persist within the soil up to three years (Dhingra and Sinclair, 1977). The microsclerotia are black, spherical to oblong structures that are produced in the host tissue and released in to the soil as the infected plant decays. These multi-celled structures allow the persistence of the fungus under adverse conditions such as low soil nutrient levels and temperature above 30 °C. Infected seed do not germinate or produce seedlings that die soon after emergence. Germination of the microsclerotia occurs throughout the growing season when temperatures are between 28 °C and 35 °C. Microsclerotia germinate on the root surface, germ tubes form appresoria that penetrate the host epidermal cell walls by mechanical pressure and enzymatic digestion or through natural openings (Bowers and Russin, 1999). The hyphae grow first intercellularly in the cortex and then intracellularly through the xylem colonizing the vascular tissue.

In sorghum charcoal rot is a greater problem after anthesis and often occurs when the plant is under drought stress. *M. phaseolina* can grow and produce large amounts of microsclerotia under relatively low water potentials allowing this disease to be recognized as favouring drought (Olaya and Abawi, 1996). The mechanical plugging of the xylem vessels by

microsclerotia, toxin production, enzymatic action, and mechanical pressure during penetration lead to disease development. The population of *M. phaseolina* in soil will increase when susceptible hosts are cropped in successive years and can be redistributed by tillage practices (Wyllie, 1988).

Post rainy (*rabi*) sorghum in India typically matures under receding moisture situation. Generally couple of rains during sowing and early crop period drive the completion of *rabi* sorghum crop. Typically *rabi* sorghum is severely affected by the charcoal rot disease. Owing to clean, rainfree conditions of *rabi*, results in superior pearly sorghum grains which is preferred for human consumption in sorghum traditional belts of India. Despite the alternative commercial crop, area under sorghum is not affected. Hence, charcoal rot disease condition is a breeding challenge as no chemical works effectively besides there higher cost and environmental concern.

Symptoms

Charcoal rot in sorghum produces symptoms similar to other fungal stalk rots. The characteristic sign of charcoal rot is the production of black microsclerotia in the vascular tissue and inside the rind of the stalk. In susceptible genotypes fungus crosses as many internodes as possible and brings about stalk lodging. The stalk will appear gray to black in colour. The pith region disintegrates into suspended fibres with charcoal powder like appearance resulting in weakened stalk and there by lodging of plants.

2.2 Genetic variability, inheritance and character association

The range of reaction to charcoal rot by inbreds points to the fact that it is possible to get genetic gain through selection. But in hybrids, dominance or over dominance for susceptibility is the norm. Progress in selection for charcoal rot and lodging resistance or for any character is dependent upon the presence of genetic variation for that character. Fortunately, large genetic differences exist in sorghum for lodging caused by water deficits. Variation in charcoal rot severity has been found, but the presence of heritable differences in resistance to the pathogen(s) has not been proved. Variation may have been caused by differences in the predisposing physiological stress due to differences in maturity (Henzell *et al.*, 1984). But, Keeta and Mayer (1984) reported a positive evidence of the existence of *M. phaseolina* resistance genes since differences in disease incidence could be demonstrated across maturity classes.

Resistance of charcoal rot was reported to be controlled by recessive genes (Rosenow and Frederiksen, 1980). This was further confirmed by the analysis of F₂ generation of an inter-varietal cross, 1202A × CSV-5 by Shinde *et al.* (1981) who noticed dominance of susceptibility, it was governed by three major, one basic complementary and two duplicate complementary genes, fitting to a ratio of 45 susceptible to 19 resistant in F₂ generation.

Reports of partial dominance (Indira and Rane, 1983), non-additive with a high degree of dominance (Indira *et al.*, 1984), polygenic threshold character governed by partial dominance, duplicate epistasis and low heritability in six generation mean analysis of five crosses involving resistant and susceptible parents have been made. Contrary to this, Venkata rao and Shinde (1988) in a study of six generations of a single cross of sorghum, reported polygenic nature of susceptibility, which was dominant in the F₁ generation. They further indicated that non-allelic interactions, like additive × dominance and dominance × dominance played a major role in an inheritance of this trait. Dominant and recessive epistatic interactions between two loci with a third modifying locus appear to provide resistance (Tenkouano *et al.*, 1993).

In a study at ICRISAT, 55 direct hybrids and their 11 parental lines were evaluated for charcoal rot resistance by measuring lodging, stalk infection and susceptibility index. Susceptibility was partially dominant for the number of nodes crossed by the pathogen. All the three parameters were reported to be governed by high degree of dominance and low heritability. Garud and Borikar (1985) in diallel crosses involving four resistant and three susceptible varieties for charcoal rot, observed predominance of dominance for resistance

with over-dominance and low heritability values for number of nodes crossed and length of stem infected by the pathogen.

Resistance to *M. phaseolina* in each environment and across environments, related to maturity and the stalk disintegration scores were significantly negatively correlated both phenotypically and genotypically (Bramel *et al.*, 1988). Reddy *et al.* (2008) showed that per cent lodging had significant positive correlation with plant height. Similar observations were made in sorghum (Esechie *et al.*, 1977; Rajkumar *et al.*, 2007). Negative and significant association was observed between length of infection and seed weight at both genotypic and phenotypic level (Rajkumar *et al.*, 2007). This trait also showed negative and significant association with early seedling vigour and days to flowering at genotypic level (Punnuri, 2004; Gururaj Rao, 1992). There was a negative correlation between polyphenol oxidase activity and percentage of dead hearts at 14 and 28 days after emergence (DAE) among parents and crosses.

Total phenols were measured in internodes of three tolerant and three susceptible sorghum genotypes naturally infected by *Macrophomina phaseolina*. In tolerant genotypes phenol concentration was higher (Patil *et al.*, 1985). High levels of sugars and phenols are considered to be attributable to the resistance mechanism, and their measurement considered useful in identifying sources of resistance. Similarly, Anahosur and Naik (1986) showed total sugars and phenols in the root and first internode were greater in a resistant genotype than in a susceptible one at physiological maturity. These quantities decreased considerably in a susceptible genotype from the full flowering stage, the plants thus succumbing to infection by *M. phaseolina*, leading to 100% lodging. In resistant plants, there were more sugars and phenols from the flowering stage onwards and consequently less infection and least lodging. Ghongale and Shivankar (2003) also observed high phenol content in resistant genotype for resistance to *Gloeocercospora sorghi* in sorghum. Sorghum phenolic compounds can be divided into five basic groups, phenolic acids, lignins, quinines, flavinoids and tannins. Phenolic acids, flavinoids and tannins are the most common groups in sorghum. Sorghum plant produces large amount of phenolic compounds, which constitute a defensive mechanism against fungi, insects and also have anti nutritional effects (Butle, 1990). Many of these phenolic determine the plant colour, appearance, nutritional quality and host defence. The amount of total phenols in the first internode of stem was estimated to test if it were correlated with tolerance to diseases and pests. Such Genotypic difference between sorghum accession of Indian origin (breeding lines) have been reported earlier. In sorghum, first internode is known to have the higher phenol content compared to seven internodes tested. Phenols are known to inhibit the invasion and spread of fungus during dry spell making the sorghum plant tolerant to charcoal rot. Secondary metabolites such as phenols are known to play significant role in improving resistance to insect and diseases (Anahosur and Naik, 1986).

Inheritance of stay green trait in sorghum has been well studied stay green trait in B35 has been reported to be influenced by a major gene that exhibited varied levels of dominant gene action, depending on the environment (Richard *et al.*, 1994). Reports also indicate that the gene(s) controlling stay green are additive or partially dominant depending on the specific environment. Studies of back crossed progenies showed the dominant nature of the gene(s) influencing the inheritance of the staygreen 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 the stay green trait in B35. Under the field environment, behaviour of the character was more dominant than under the extreme water stress environment of the rain-out shelter, where the gene action was described as additive to partial dominance. Stay green trait is a nuclear gene controlled quantitative trait (Walulu *et al.*, 1994). Literature in respect of genetic variability, heritability and genetic advance for different traits have been reviewed and summarised in Table 1.

2.3 Molecular markers

The use of DNA-based markers for the genetic analyses and manipulation of important agronomic traits has become an increasingly useful tool in crop improvement. DNA markers have the potential to enhance the operation of a plant breeding program through a

number of ways, ranging from fingerprinting of elite genetic stocks, assessment of genetic diversity, increasing the efficiency of selection for difficult traits, to making environment-neutral selection possible etc. However, their greatest potential appears to be in accelerating the rate of gain from selection for desirable genotypes and in mapping QTL that condition complex economic traits and also permit to correctly map or place the various interacting genes that govern the complex agronomic traits. Genetic mapping is essential for effective manipulation of important genes. Effective use of marker based selection or marker assisted introgression should permit genetic recombination beyond the range possible in traditional crop improvement. At present many marker are in use that include morphological, biochemical and molecular marker system. Markers that are genetically informative and amenable for high through-put assays have been highly useful in comparative and structural genome biology besides in molecular breeding. Functional markers have opened up new vistas in genomics assisted crop improvement.

2.3.1 DNA markers

The introduction of DNA based markers during the second half of 20th century has changed the pace and precision of genetic analysis (Dodgson *et al.*, 1997). During the last two decades DNA based markers have led to the construction of whole genome linkage maps in many plant and animal genomes, a crucial step for several downstream applications such as gene cloning, genome analyses and marker assisted selection of agricultural crops (Cullis, 2002; Dodgson *et al.*, 1997). A DNA marker is considered good or powerful if it is easy to detect, highly polymorphic and distributed across genome at random. There are different DNA markers such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Sequence Tagged Sites (STS), Simple Sequence Repeats (SSRs), Single Nucleotide Polymorphism (SNPs). The earliest DNA marker system, the RFLP have proved to be very useful, but their development and utilization is laborious, time-consuming, expensive and not suitable for high-throughput automation. For these reasons, PCR-based markers such as RAPD, AFLP, ISSRs, SSRs, CAPs, SNPs and their derivatives have become popular for molecular genetic studies (Paterson, 1996). Out of the PCR based markers, SSR markers quickly became the markers of choice for plant and animal genomes during the last decade because of the small sample size (genomic DNA) requirement for their analysis and their suitability for automation and high-throughput (Hearne *et al.*, 1992; Gutierrez *et al.*, 2005).

2.3.1.1 Microsatellite/Simple Sequence Repeats (SSRs)

Microsatellites contain 1 to 6 bp DNA sequence motifs repeated several times (Tautz and Renz, 1994). Popularly described as simple sequence repeats (SSRs) in plants (Morgante and Oliveri, 1993) and as short tandem repeats (STRs) in animal systems (Edwards *et al.*, 1991). These motifs, known as di-, tri-, tetranucleotide repeats, etc., accordingly. Microsatellites are abundant and occur randomly in all eukaryotic DNA examined so far (Gupta *et al.*, 1996; Gupta and Varshney, 2000). Microsatellites can be termed simple ("perfect" or "pure") if they contain several repeats of two or more nucleotides *i.e.*, (N₁N₂...N_x)_n or they may be more complicated ("imperfect" or "compound") where two or more repeat motifs are present, *e.g.*, (CA)_n(GT)_n, or (CA)_n(GT)_n. Some may have spacers between motifs repeated several times *e.g.*, (GA)_n(N)_n(CT)_n (Chambers and MacAvoy, 2000).

SSRs are thought to arise and develop due to replication slippage and a mutations could expand or contract them. It is also suggested that SSRs undergo a life cycle, having birth, growth and death with life span ranging from tens to even hundreds of millions of years (Messier *et al.*, 1996; Primmer and Ellegren, 1998). In humans about three per cent of the genome is occupied by SSRs, distributed throughout the genome in both coding and non-coding regions (Tothet *et al.*, 2000), respectively termed as genomic and genic SSRs (Varshney *et al.*, 2005). The study of repeat density and its distribution pattern in the genome is expected to help in understanding their significance. There is accumulating evidence to suggest that SSRs might as well regulate gene expression (Kunzler *et al.*, 1995; Moxon and Wills, 1999). Genic SSRs have high proportion of high-quality markers than genomic SSRs and they show prominent bands and distinct allelic peaks (Thiel *et al.*, 2003; Kota *et al.*, 2001; Yu *et al.*, 2004; Saha *et al.*, 2004; Nicot *et al.*, 2004; Cho *et al.*, 2000; Eujayl *et al.*, 2001). High quality and robustness of amplification patterns, along with other merits associated with

Table 1: Summary of review on heritability, variability and genetic advance for various traits in sorghum

Sl. No.	Characters	Class	References		
			Variability	Heritability	Genetic advance (GA)
1.	Days to 50 per cent flowering	Moderate		Raja and Parik, 1980; Dinakar, 1985; Reddy 2006; Punhuri 2004; Rajkumar <i>et al.</i> 2007.	
		Low	Nimbalkar <i>et al.</i> , 1988; Prabhakar, 2001; Reddy, 2006; Rajkumar <i>et al.</i> , 2007	Kanaka and Goud, 1982; Kulkarni and Shinde, 1987	Khanure, 1993; Prabhakar, 2001; Reddy 2006; Rajkumar <i>et al.</i> , 2007.
		High	Khanure, 1993; Reddy <i>et al.</i> , 1996	Khanure, 1993, Reddy <i>et al.</i> , 1996, Prabhakar, 2001, Bello <i>et al.</i> , 2007	Reddy <i>et al.</i> , 1996; Prabhakar, 2001; Bello <i>et al.</i> , 2007
2	Plant height	Moderate	Dhimer and Desai, 1978	Jan-orn, 1974; Ekebil <i>et al.</i> , 1977; Punhuri, 2004; Reddy, 2006.	Reddy, 2006; Rajkumar <i>et al.</i> , 2007
		Low	Lonc, 1969 ; Reddy, 2006 ; Rajkumar <i>et al.</i> , 2007		Amirthadvarathinam <i>et al.</i> , 1994.
		High	Berenji, 1990; Lata <i>et al.</i> , 2001; Bello <i>et al.</i> , 2007	Biradar <i>et al.</i> , 1996; Rao and Patil, 1996; Nguyen <i>et al.</i> , 1999; Lata <i>et al.</i> , 2001; Bello <i>et al.</i> , 2007.	Khanure, 1993; Biradar <i>et al.</i> , 1996; Sankarapandian <i>et al.</i> , 1996; Nguyen <i>et al.</i> , 1999; Bello <i>et al.</i> , 2007

Contd....

Sl. No.	Characters	Class	References		
			Variability	Heritability	Genetic advance (GA)
3	Panicle length	Moderate	Punnuri 2004 ; Reddy 2006 ; Rajkumar <i>et al.</i> 2007 ; Bello <i>et al.</i> , 2007 ; Kachapur and Salimath, 2009	Harare and Bapat, 1982; Dinakar, 1985; Rajkumar <i>et al.</i> , 2007; Bello <i>et al.</i> , 2007; Kachapur and Salimath, 2009	Punnuri, 2004; Reddy 2006; Rajkumar <i>et al.</i> 2007; Bello <i>et al.</i> . 2007; Kachapur and Salimath, 2009
		Low	Aristarkova, 1976; Naphade and Ailwar, 1977	Rao and Goud, 1979	Khanure, 1993
		High	Biradar <i>et al.</i> , 1996; Lata <i>et al.</i> , 2001	Biradar <i>et al.</i> , 1996; Lata <i>et al.</i> , 2001	Biradar <i>et al.</i> , 1996
4	1000 seed weight	Moderate			Punnuri, 2004; Reddy, 2006; Kachapur and Salimath, 2009
		Low	Punnuri, 2004; Reddy; 2006 Kachapur and Salimath, 2009	Bello <i>et al.</i> , 2007	
		High	Singh and Makne, 1980; Prabhakar, 2001	Nimbalkar <i>et al.</i> , 1988; Naguyen <i>et al.</i> , 1999; Prabhakar, 2001; Rajkumar <i>et al.</i> , 2007	Naguyen <i>et al.</i> , 1999; Prabhakar, 2001
5	Grain yield per plant	Moderate			Phul and Allah Rang, 1986
		Low	Wenzel <i>et al.</i> , 1998	Obilana and Okoh, 1984, Bello <i>et al.</i> , 2007	Goud <i>et al.</i> , 1980
		High	Biradar <i>et al.</i> , 1986; Rao and Patil, 1996; Reddy <i>et al.</i> , 1996; Can and Yashoda, 1999; Prabhakar, 2001; Punnuri, 2004; Reddy 2006; Rajkumar <i>et al.</i> , 2007; Bello <i>et al.</i> , 2007; Kachapur and Salimath, 2009	Rao and Patil, 1996; Punnuri 2004; Reddy, 2006; Rajkumar <i>et al.</i> , 2007; Kachapur and Salimath, 2009	Phul and Allah Rang 1996, Prabhakar, 2001; Punnuri, 2004; Reddy, 2006; Rajkumar <i>et al.</i> 2007; Kachapur and Salimath, 2009

Contd....

Sl. No.	Characters	Class	References		
			Variability	Heritability	Genetic advance (GA)
6	Early seedling vigour	Moderate	Reddy, 2006; Rajkumar, <i>et al.</i> 2007	Reddy, 2006; Rajkumar, <i>et al.</i> 2007	Reddy, 2006; Rajkumar, <i>et al.</i> 2007
		Low			
		High			
7	Stem thickness	Moderate			
		Low			
		High	Punnuri, 2004; Reddy, 2006; Rajkumar, <i>et al.</i> , 2007; Kachapur and Salimath, 2009	Punnuri, 2004; Reddy, 2006; Rajkumar <i>et al.</i> , 2007; Kachapur and Salimath, 2009	Punnuri, 2004, Reddy, 2006, Rajkumar <i>et al.</i> , 2007, Kachapur and Salimath, 2009
8	Threshability	Moderate			
		Low			
		High	Punnuri, 2004 ; Reddy, 2006 ; Rajkumar <i>et al.</i> , 2007	Punnuri, 2004 ; Reddy, 2006 ; Rajkumar <i>et al.</i> , 2007	Punnuri, 2004 ; Reddy, 2006 ; Rajkumar <i>et al.</i> , 2007

EST-SSR markers enhance their value, especially for germplasm characterization (Varshney *et al.*, 2005).

SSRs are considered as the most efficient markers, but their use is still limited because of the long and laborious steps needed to develop them. Two general strategies are employed to develop SSR markers: by searching for sequences containing microsatellites in the available databases and screening the genomic or any other library with probes complementary to microsatellite sequences. Exceptionally, some strategies without library construction have also been developed recently (Trojanowska *et al.*, 2004).

2.3.1.2 EST-SSRs or genic SSRs

One of the possible strategies would be to exploit publicly available genomic resources for the development of gene-based SSR markers that are more likely to be transferable across taxonomic boundaries. In fact, the generation of EST-SSRs is rapid as they are obtained by electronic sorting and inexpensive development of SSRs from expressed sequence tag (EST) databases that has been shown to be a feasible option for obtaining high quality nuclear markers (Gupta *et al.*, 2003). Moreover, the National Center for Biotechnology Information (NCBI) EST database (dbEST) contains an ever-increasing number of these 'single-pass' cDNA sequences, meaning that the resources necessary for the efficient development of large numbers of so-called EST-SSRs already exist for a wide variety of taxa (Ellies and Burke, 2007). The EST-SSRs are also referred to as genic-SSRs on the other hand the *de novo* generated SSRs are referred to as genomic SSRs (Varshney *et al.*, 2005) or anonymous SSRs (Ellies and Burke, 2007). These also form a class of functional markers as a putative function can be deduced for an EST-SSR based on corresponding ESTs. Thus, EST-SSR markers have advantages of both functional markers such as complete linkage with trait locus (Anderson and Lubberstedt, 2003) and of SSRs such as highly polymorphic, multiallelic, codominant and random distribution (Powell *et al.*, 1996).

With the establishment of EST sequencing projects for gene discovery programmes in several plant species, a wealth of DNA sequence information has been generated and deposited in online databases. In addition, sequence data for many fully characterized genes and full-length cDNA clones have been generated for some plant species such as rice (Kikuchi *et al.*, 2003). The sequence data for ESTs, genes and cDNA clones can be downloaded from GenBank and scanned for identification of SSRs, which are typically referred to as EST-SSRs or genic- microsatellites. Subsequently, locus-specific primers flanking EST or genic-SSRs can be designed to amplify the microsatellite loci present in the genes (Varshney *et al.*, 2005).

Thus, EST databases provide a novel source for developing SSR markers, which not only preclude the need for DNA sequencing but also such SSR markers will be related to transcribed genes directly, thereby enhancing the usefulness of these genetic markers. For these reasons, many researchers embarked on a project with the aim to generate a large number of SSR markers by exploiting the publicly available EST database (Gao *et al.*, 2003).

Application of genic SSRs

Molecular markers have proven useful to assess and characterize genetic variation within natural populations and among breeding lines for effective conservation and exploitation of genetic resources in crop improvement programs (Mohammadi and Prasanna, 2003). Evaluation of germplasm with SSRs derived from genes, genic ESTs, might enhance the role of genetic markers by assaying the variation in transcribed genes, especially those with known function. Genic SSRs have the potential of being functional markers in cases where polymorphism in the repeat motifs affect the function of the gene (Anderson and Lubberstedt, 2003), permitting direct allele selection if they are shown to be associated or responsible for the target trait (Sorrells and Wilson, 1997). Recently, a Dof homolog (*DAG1* gene) that showed a strong effect on seed germination in *Arabidopsis* (Papi *et al.*, 2000) was mapped on chromosome 1B of wheat by using wheat EST-SSR primers (Gao *et al.*, 2004). Similarly, Yu *et al.* (2004) identified two EST-SSR markers linked to photoperiod response gene (*ppd*) in wheat. Mapping candidate genes and genic SSRs will also facilitate genome alignment across related species (Yu *et al.*, 2004; Varshney *et al.*, 2005). EST-SSRs have

been integrated and genome-wide genetic maps have been prepared in many crops viz., wheat (Yu *et al.*, 2004; Nicot *et al.*, 2004; Holton *et al.*, 2002; Gao *et al.*, 2004), barley (Thiel *et al.*, 2003), rye (Khlestkina *et al.*, 2004) and rye grass (Warnke *et al.*, 2004), cotton (Zhiguo *et al.*, 2006; Han *et al.*, 2004), soybean (Zhang *et al.*, 2004), potato (Feingold *et al.*, 2005), raspberry (Graham *et al.*, 2004). Genic SSRs show a characteristic distribution throughout the genome and get concentrated in gene rich regions, unlike genomic SSRs, which are clustered around the centromere (Thiel *et al.*, 2003; Yu *et al.*, 2004; Gao *et al.*, 2004).

Distribution of genic SSRs on the genetic map will show the distribution of genes in the genome. Another important feature of the genic SSR markers is that, unlike genomic SSRs, they are transferable among related species and genera (Yu *et al.*, 2004; Varshney *et al.*, 2005). Thus, EST-SSR markers could be used in related plant species for which little information is available on SSRs or ESTs. In addition, the genic SSRs are good candidates for the development of conserved orthologous markers for genetic analysis and breeding of different species, for instance, Varshney *et al.* (2005) showed that a set of 12 barley EST-SSR markers had significant homology with the ESTs of four monocotyledonous species (wheat, maize, sorghum and rice) and two dicotyledonous species (*Arabidopsis* and *Medicago*).

2.4 Application of molecular markers in sorghum crop improvement

Molecular markers have proven to be robust and cost-effective for assessment of sorghum genetic diversity (Oliveira *et al.*, 1996; Yang *et al.*, 1996; Dje *et al.*, 2000). Genetic diversity in sorghum has been estimated utilizing several types of molecular markers (Tao *et al.*, 1993; Vierling *et al.*, 1994; Brown *et al.*, 1996; Taramino *et al.*, 1997; Uptmoor *et al.*, 2003). A set of 15 microsatellite or SSR markers has been developed for sorghum that allows a very high rate of discrimination in sorghum genetic diversity assessment (Dean *et al.*, 1999; Dje *et al.*, 2000; Grenier *et al.*, 2000; Smith *et al.*, 2000). Ahnert *et al.* (1996) used a set of 104 RFLP probes to evaluate the genetic diversity among a large set of elite proprietary sorghum inbred lines. Repeated reports clearly indicated that SSR loci give good discrimination between closely related individuals in some cases even when only a few loci were employed (Powell *et al.*, 1996; Kong *et al.*, 2000). The analysis of SSRs has been automated (Saghai Maroof *et al.*, 1984; Powell *et al.*, 1996). Pereira *et al.* (1994) performed segregation analysis on F₂ population using 7 SSR loci in order to verify the reliability of SSR-derived polymorphism for sorghum genetic mapping.

Brown *et al.* (1996) developed 15 SSR markers and were able to identify polymorphic loci among 17 temperately and tropically adopted lines of sorghum. Moreover the 15 SSR marker loci are widely spread on the sorghum genome and 14 of them have mapped to nine of ten sorghum linkage groups (Dean *et al.*, 1999), they also assessed the genetic diversity among 95 'Orange' accessions of sorghum in the USDA germplasm collection and found 3 to 11 alleles per locus by using same set (15) of SSR markers. Taramino *et al.* (1997), Tao *et al.* (1998), and Kong *et al.* (2000) have reported 46 sorghum SSR loci. Kong *et al.* (2000) characterized 38 sorghum SSR loci. They were followed by Bhatramakki *et al.* (2000), who reported primer sequences for 147 sorghum SSR loci, as well as genetic linkage map locations for 113 of these. More recently, Schloss *et al.* (2002) reported nearly 70 additional sorghum SSR primer sequences derived from sorghum cDNA clones that had previously been mapped as RFLP markers.

Ghebru *et al.* (2003) carried out an analysis to assess genetic diversity of 28 Eritrean landraces of sorghum, using a high through put SSR-based strategy developed by Kresovich and coworkers (Dean *et al.*, 1999; Smith *et al.*, 2000). Uptmoor *et al.* (2003) carried out comparative analysis on the genetic relatedness of 46 sorghum accessions from Southern Africa by using molecular markers like AFLPs, RAPDs, SSRs and they concluded that all of these sorghum accessions were uniquely fingerprinted by all three marker systems. Casa *et al.* (2005) assayed 98 simple sequence repeat (SSR) loci distributed throughout the genome in a panel of 104 accessions comprising 73 landraces and 31 wild sorghums. Evaluation of SSR polymorphisms indicated that landraces retained 86% of the diversity observed in the wild sorghums. Folkertsma *et al.* (2005) reported use of 21 SSR

markers to assess genetic diversity in the Guinea-race of sorghum in support of a breeding program aiming to develop F₁ hybrid cultivars within this race as a means of increasing food security in the more humid regions of Western and Central Africa.

2.4.1 Construction of genetic linkage map

Genetic linkage maps are fundamental for the localization of genes conferring biotic and abiotic tolerance. Linkage maps of organisms are constructed to map genomic regions controlling qualitative and quantitative traits, to exercise permit indirect selection for several agronomic traits, and to isolate the genes involved, based on their map position. Nearly every agronomic trait of importance has been subjected to DNA marker mapping and QTL analyses. Till now over 200 morphological and agronomically important marker traits have been identified in nine linkage groups with a density of 2-10 loci per linkage group (Berhan *et al.*, 1993; Pereira *et al.*, 1994). Sorghum genome mapping based on DNA markers began in early 1990s and since then, many genetic maps have been developed with a large number of DNA-based markers including RFLPs, AFLPs and SSRs (Botstein *et al.*, 1980; Vos *et al.*, 1995; Litt and Luty, 1989; Haussmann *et al.*, 2004; Punhuri, 2004; Reddy *et al.* 2008).

The construction of first sorghum linkage map was done using heterologous maize probes (Hulbert *et al.*, 1990; Bennetzen *et al.*, 2001). Later many RFLP linkage maps of *S. bicolor* were constructed (Binelli *et al.*, 1992; Whitkus *et al.*, 1992; Berhan *et al.*, 1993; Chittenden *et al.*, 1994; Pereira *et al.*, 1994; Ragab *et al.*, 1994; Xu *et al.*, 1994; Dufour *et al.*, 1996,1997; Peng *et al.*, 1999; Haussmann *et al.*, 2004; and Punhuri, 2004). All combined, these maps include over 800 markers. Five major RFLP maps independently developed in this species were successfully aligned to produce a consensus maps (Chittenden *et al.*, 1994; Pereira *et al.*, 1994; Ragab *et al.*, 1994; Xu, *et al.*, 1994; Boivin *et al.*, 1999).

Most of the early sorghum genetic maps used maize RFLP probes in the context of comparative mapping (Binelli *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 (Boivin *et al.*, 1999). A few cDNA clones of sorghum and maize related to photosynthesis and drought stress were also assigned to the map of Subudhi and Nguyen (2000). Similarly, the RFLP and AFLP maps developed by Xu *et al.* (1994) and Peng *et al.* (1999) have been improved with the addition of many markers (Kong *et al.*, 1997; Bhatramakki *et al.*, 2000; Haussmann *et al.*, 2004).

The maps have been developed from interspecific (Chittenden *et al.*, 1994; Lin *et al.*, 1995), inter-subspecific (Pereira *et al.*, 1994) and intra-specific crosses. Both F₂ (Hulbert *et al.*, 1990; Binelli *et al.*, 1992; Melake-Berhan *et al.*, 1993; 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; Boivin *et al.*, 1999; Crasta *et al.*, 1999; Peng *et al.*, 1999) have been used. Of the early studies, only the maps of Chittenden *et al.* (1994) and Pereira *et al.* (1994) covered all the ten linkage groups.

A comprehensive reference map of sorghum genome would be an essential research tool for functional and comparative genomics. In this direction a combined sorghum linkage map from two recombinant inbred populations was constructed using SSR, AFLP and RAPD markers. The two recombinant inbred populations analyzed consisted of 225 (RIP1) and 226 (RIP2) of F₃₋₅ lines developed from the crosses IS9830 x E36-1 (RIP1) and N13 X E 36-1 (RIP2), respectively (Haussmann *et al.*, 2002).

To integrate genetic, physical, and cytological perspectives of the *Sorghum bicolor* genome, Kim *et al.* (2004) selected 40 Bacterial Artificial Chromosome (BAC) clones that contained different markers, 21 from linkage group 2 (LG-02) and 19 from group 8 (LG-08). Multi-BAC probe cocktails were constructed for each chromosome from the BACs, which were pre evaluated 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 maps.

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 the 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 are critical factors in association mapping methods as well as in identification of loci that have been targets of selection. In the study by Hamblin *et al.* (2004) population genetic parameters in sorghum were estimated by surveying 27 diverse sorghum accessions for sequence variations at a total of 29,186 bp in 95 short regions derived from RFLP map. Consistent with its higher level of inbreeding, the extent of LD was several fold greater in sorghum than in maize. 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). Recently, Reddy *et al.* (2008), reported the linkage map with segregating markers on 10 linkage group with total length of the genetic map of 650 cM. An overview of published sorghum genetic linkage maps is given in Table 2.

2.5 QTL analysis and mapping

For QTL mapping a segregating population is required, derived from two contrasting parents. Preferably but not necessarily the parents of such a population should be divergent for the trait of interest. Since parental lines are taken from naturally occurring populations (named accessions or ecotypes for wild species, and cultivars, breeding lines, or landraces in crop species), they are likely to differ for many loci. QTL analysis implies the discovery of the association between trait values and the allelic status of the loci of all individuals in the segregating population. The genotype of individuals (or lines) can easily be determined using molecular markers. QTL analysis reveals the genetic region(s) where a gene (or a set of closely linked genes) are located, which have a significant quantitative effect on the trait, and it describes the magnitude of the effect. This implies that a QTL is not a gene, but only a region on a chromosome, and hence it will require further effort to identify the gene(s) responsible for the variation in trait values within the QTL region

2.5.1 Principles of QTL analysis

QTL analysis is based on the natural allelic variation present within a species. This allelic variation gives rise to smaller or larger quantitative effects on the trait under investigation. Natural allelic variation differs from induced variation (mutants), since the alleles occur in natural populations and are supposed to give selective advantage in certain conditions, or at least to be neutral.

QTL analysis has three essential stages: mapping of markers (genotyping), scoring of the trait (phenotyping), and association of the trait with the markers (QTL mapping). QTL analysis can be done by using statistical procedures like maximum likelihood ratio (Lander and Botstein, 1989), non-linear regression (Knapp *et al.*, 1990), linear regression (Bridges *et al.*, 1991), or a combination of maximum likelihood and multiple regression (Jensen and Stam, 1994; Zeng, 1994). Also to carry out the data analysis, the software packages like Mapmaker/QTL (Lander *et al.*, 1987), MAPQTL (Van Oolijon and Maliapaard, 1996) Map manager QTL (Manley and Cudmore, 1998) and QGENE (Tanksley and Nelson, 1996), QTL Cartographer (Basten *et al.*, 1997), and PlabQTL (Utz, 1995; Utz and Melchinger 1996) are available.

Most of the important agronomic traits show quantitative inheritance. They are controlled by several genes and are often considerably influenced by environment. Positions in the genome that have been found to be associated with variation in quantitatively inherited traits are referred to as quantitative trait loci (QTLs). The number of genes and their interactive effects controlling gene expression of quantitative traits are poorly understood. The first report of linkage between a marker gene and a gene that influences a

Table 2: Review of published sorghum genetic linkage map

Reference	Size and type of mapping population	Parent lines	Mapped markers	Linkage groups	Genome length (cM)
Tuinstra <i>et al.</i> , 1996, 1997	98 RIL (F _{5:7-8})	TX 7078 B 35 (durra sorghum from Ethiopia)	150 RAPD 20 RFLP probes from maixer or sorghum	17	Ca 1,580 R
Taramino <i>et al.</i> , 1997	68 F ₂ plants	Same parents as Pereira <i>et al.</i> 1994	7 SSR added to map of Pereira <i>et al.</i> 1994	10	1,575 U
Tao <i>et al.</i> , 1998	120 RIL (F ₅)	QL39 QL41 (both elite lines from Australia)	68 RFLP sorghum probes 87 RFLP of maize, sugarcane, rice, oat, barley,	21	1,400 U
Kong <i>et al.</i> , 2000	137 RIL (F ₆₋₈)	Same parents as Xu <i>et al.</i> 1994	33 SSR	10	1,287 K
Tao <i>et al.</i> , 2000	152 RIL (F ₇)	Same parents as Tao <i>et al.</i> , 1998	111 RFLP probes from Peng <i>et al.</i> 1999 101 RFLP	14	1,871 U
Hulbert <i>et al.</i> , 1990	55 F ₂ plants	Shanqui Red (koaliang from China) M91051 (zera zera from East Africa)	37 RFLP maize probes or cloned genes	8	283 R
Binelli <i>et al.</i> , 1992	149 F ₂ plants	IS 18729 (<i>caudatum bicolor</i> from Texas) IS 24756 (<i>durra caudatum</i> from Nigeria)	21 RFLP maize probes	5	440 U
Whitkus <i>et al.</i> , 1992	81 F ₂ plants	IS 2482C (<i>S. bicolor</i> spp. <i>arundinaceum</i>) IS 18809 (<i>S. bicolor</i>) Unnamed accession of <i>S. propinquum</i>	85 RFLP maize probes 7 Isozymes	13	949 H
Berhan <i>et al.</i> , 1993	55 F ₂ plants	Same parents as Hulbert <i>et al.</i> 1990	96 RFLP maize probes or cloned genes	15	709 R
Chittenden <i>et al.</i> , 1994	56 F ₂ plants	BTx623 (<i>S. bicolor</i>) Unnamed accession of <i>S. propinquum</i>	256 RFLP sorghum probes 20 RFLP probes of maize, rice and oat	10	1,445 U

Contd.....

Reference	Size and type of mapping population	Parent lines	Mapped markers	Linkage groups	Genome length (cM) ^a
Pereira <i>et al.</i> , 1994	78 F ₂ plants	CK 60 (<i>S. bicolor</i>) PI229828 (<i>S. bicolor ssp. rummondii</i>)	191 RFLP maize probes 10 RFLP sorghum probes	10	1,530 U
Xu <i>et al.</i> , 1994	50 F ₂ plants	BTs 623 (zera zera x kafir) IS 3620C (guinea line)	179 RFLP sorghum probes 11 RFLP maize probes	14	1,789 K
Ragab <i>et al.</i> , 1994	93 F _{2:3} plants	BSC 35 BTx 631	38 RFLP sorghum probes 33 RFLP maize probes	15	633 H
Lin <i>et al.</i> , 1995	370 F ₂ plants	Same parents as Chittenden <i>et al.</i> , 1994	78 RFLP sorghum probes	11	935 K
Dufour <i>et al.</i> , 1997	110 RIL (F ₅)	IS 2807 (caudatum from Zimbabwe) 379 (guinea from South Africa)	126 RFLP maize probes 19 RFLP sugarcane probes	13	977 H
	91 RIL (F ₅)	IS 2807 (caudatum from Zimbabwe) 249 (guinea from Burkina Faso)	4 cloned genes, 2 morphological markers 115 RFLP maize probes 8 RFLP sugarcane probes	12	
	Composite map of the two populations		4 cloned genes, 1 morphological markers 164 RFLP maize probes 19 RFLP sugarcane probes 3 cloned genes, 2 morphological markers.		

Contd.....

Reference	Size and type of mapping population	Parent lines	Mapped markers	Linkage groups	Genome length (cM)
Ming <i>et al.</i> , 1998	56 F ₂ plants	Same parents as Chittenden <i>et al.</i> , 1994	328 RFLP probes of sorghum and other cereals	10	1,750 K
Boivin <i>et al.</i> , 1999	Composite map with populations as Dufour <i>et al.</i> 1997 110 RIL (F ₅)0	First population of Dufour <i>et al.</i> , 1997	8 SSR, 3 morphological traits 343 RFLP and morphological markers 128 RFLP sorghum, rice, oat and barley probes 176 AFLP	11	1,352 H
Boivin <i>et al.</i> , 1999	-		151 RFLP loci mapped by Dufour <i>et al.</i> , 1997	11	1,899 H
Crasta <i>et al.</i> , 1999	96 RIL (F _{6:7})	B35 (Durra from Ethiopia) Tx 430	142 RFLP sorghum, maize, rice and wheat clones	14	1,602 K
Peng <i>et al.</i> , 1999	137 RIL (F ₆₋₈)	Same parents as Xu <i>et al.</i> , 1994	323 RFLP from sorghum, maize, rice, oat, barley	10	1,347 K
Bowers <i>et al.</i> , 2000	65 F ₂ plants	Same parents as Chittenden <i>et al.</i> , 1994	2,399 loci based on 1925 RFLP probes	10	1,200 U
Xu <i>et al.</i> , 2000	98 RIL (F ₇)	B 35 (Durra from Ethiopia) Tx 7000 (elite public line used in USA)	162 RFLP sorghum and maize probes, cloned genes or sequenced DNA probes	10	837 H
Bhatramakki <i>et al.</i> , 2000	137 RIL (F ₆₋₈)	Same parents as Xu <i>et al.</i> , 1994	116 SSR 354 SSR, 10 morphological markers	10	1,406 K

Contd.....

Reference	Size and type of mapping population	Parent lines	Mapped markers	Linkage groups	Genome length (cM)
Bennetzen <i>et al.</i> , 2001	Framework map derived from comparison of the maps of Kong <i>et al.</i> , 2000; Peng <i>et al.</i> , 1999; Pereira <i>et al.</i> , 1994; Berhan <i>et al.</i> , 1993.		154 RFLP 34 SSR, 10 morphological markers	10	1,450 U
Klein <i>et al.</i> , 2001	125 RIL(F ₅)	RTx 430 (elite line from USA) Sureno	44 SSR 85 AFLP, 1 morphological trait	10	970 K
Hausmann <i>et al.</i> , 2002	225 RIL(F _{3:5})	IS 9830 (caudatum line from Sudam) E 36-1 (Ethiopian guinea-caudatum)	125 AFLP 45 SSR, 14 RFLP, 3 RAPD	10	1,265 H
	226 RIL (F _{3:5})	N 13 (Indian durra) E 36-1 (Ethiopian guinea-caudatum)	158 AFLP 54 SSR, 16 RFLP	12	1,410 H
	Composite map of the two populations.		339 AFLP, SSR, RFLP and RAPD markers	11	1,424 H
Sajjanar, 2002	92 RIL (F ₇)	BT x 623 <i>S. bicolor</i> unnamed accession of <i>S. propiniquum</i> x IS 18551	44 SSR	5	188.23K
Punnuri, 2004	93 RIL (F ₇)	IS 22380 (dwarf Sudanean variety) x E36-1 durra sorghum Ethiopia	13 SSR	4	24.70k
Rajkumar, 2004	93RILs(F ₇)	IS 22380 (dwarf Sudanean variety) x E36-1 durra sorghum Ethiopia	6 SSR and 18 RAPD markers	6	490.10 K
Reddy <i>et al.</i> , 2008	93 RILs(F ₈)	IS 22380 (dwarf Sudanean variety) x E36-1 durra sorghum Ethiopia	19 RAPD, 4 EST-SSR, 62 SSR	10	650cM

quantitative trait was made by Sax *et al.* (1923). Subsequently, an important attempt to map “polygenes” contributing to quantitative trait variation was reported by Thoday (1961). These early efforts contributed to the subsequent development of the concept of QTLs which when combined with the modern molecular marker technology and genetic linkage maps allow mapping of QTLs (Soller and Beckmann, 1983). QTL mapping studies have now been reported in most of crops for traits related to yield (Bezant *et al.*, 1997), disease and insect resistance (Agrama *et al.*, 2002; Nair *et al.*, 2005), and environmental adaptation (Laurie *et al.*, 1995). Putative map locations and DNA markers linked to QTLs have opened up opportunities for isolation of QTLs via map-based cloning (Alpert and Tanksley, 1996), introgression of QTLs into elite lines or improved germplasm (Bernacchi *et al.*, 1998), and marker-assisted selection (MAS) for QTLs in applied breeding (Lande and Thompson, 1990; Tanksley, 1993). These studies help not only to elucidate the function, regulation, and expression of quantitative traits, but also provide important tools for improving crop plants.

2.5.2 QTL mapping approaches

The first step in QTL mapping studies is to detect QTL, while minimizing the occurrence of false positives (type I errors, that is, declaring an association between a marker and QTL when in fact one does not exist). Two distinct methods are used to detect QTL. The single marker approach, sometimes referred to as the one-way analysis of variance (ANOVA), has been used extensively, especially with isozymes (Tanksley *et al.*, 1982; Edwards *et al.*, 1987). The second approach, interval mapping, detects QTL by using flanking markers. This approach is more complicated analytically than the ANOVA approach and involves application of the maximum likelihood method, which requires sophisticated computer software (Lander and Botstein, 1989). Lander and Botstein (1989) have developed formulae for calculating significance levels appropriate for both methods when the genome size, number of chromosomes, number of marker intervals, and the overall false positive rate desired are given. Several statistical procedures have been developed for the application of both ANOVA and interval mapping (Soller and Brody, 1976; Edwards *et al.*, 1987; Lander and Botstein, 1989). When the same false positive rates are used, there are few reasons to suspect that the two methods would detect substantially different QTL. Stuber *et al.* (1992) compared the two methods and found that they identified basically the same QTL. Those researchers reported, however, some advantages to using the interval mapping approach. Because of the increased power associated with using flanking markers, the method gives the most likely location of the QTL under the assumption of a single QTL in the interval, and the interval mapping approach allows ambiguous or missing data. Once QTL are detected, the next step is to estimate the genotypic effect of the QTL and to localize the QTL to a precise genomic region. The interval mapping approach seems superior to the ANOVA approach for both estimation of effects and localization of the QTL (Stuber *et al.*, 1992). The success of both methods depends on the linkage between marker(s) and QTL, the number and type of progeny evaluated the heritability of the trait, and the magnitude of the effects at QTL that one desires to detect. Several methods and genetic designs have been suggested for detecting, estimating effects, and localizing QTL (Cowen, 1988; Burr, 2001).

2.6 QTL and gene action

Concerning the nature of loci controlling the variation in quantitative traits, QTL can be defined in four different ways (Mackay *et al.*, 1992). QTL are (1) “major” genes having pleiotropic effects on other traits (Barton, 1990) (2) are different from major genes in that alleles at QTL are constrained to having only small effects on the character (Mather and Jinks, 1971) (3) are modifiers of the expression of major loci (Mukai and Cockerham, 1977), and (4) have alleles with a range of effects: alleles with large effects cause recognition of the locus as a gene with major effects, and segregation of alleles with small effects gives rise to quantitative variation (Robertson, 1985).

Gene action type (additive, dominant, overdominant (Falconer, 1981) and gene effects have been studied extensively in many crop species like maize, sorghum, etc (Sprague, 1996; Hallauer and Miranda, 1988). The type of gene action controlling a trait is

very important in decisions regarding breeding method, cultivar type (inbred, hybrid, and population, etc.) and interpretation of data from quantitative genetics experiments.

2.7 QTL × Environment interactions

Leon *et al.* (2001) reported that QTLs for days to flowering in sunflower interacted with the photoperiod of the environment. A major QTL on linkage group B was not significantly associated with days to flower in a short photoperiod environment, but was significantly associated with days to flower at those environments where the photoperiod was longer. This shows that the effect of QTLs depends on the environmental conditions. Selection for the QTL on linkage group B would be expected to increase days to flower in environments with long photoperiod, but would not be effective in environments with a short photoperiod. From this experiment it was shown that not only are marker-QTL associations dependent on the population evaluated, they are also dependent on the environment in which selection is conducted. Subudhi *et al.* (2000) reported that four major QTLs for the stay-green trait in sorghum were consistently expressed across seven environments. All the environments were manipulated with irrigation treatments so that drought stress occurred after flowering and not before flowering. The stay-green trait is known to reduce drought stress after flowering, but is not effective in reducing drought stress before flowering. Subudhi *et al.* (2000) results demonstrated an absence of QTL × environment interaction, which is different from the results of Leon *et al.* (2001).

Zhu *et al.* (1999) reported that “the classification of QTL alleles as ‘favourable’ or ‘unfavourable’ may be misleading. The effect of an allele may be positive, neutral or negative depending on interactions with other loci and with environments. Therefore, for traits such as grain yield, QTL mapping and selection experiments should place more emphasis on identifying the best multi-locus allelic combinations instead of pyramiding individual favourable QTL alleles.” Their results show that using MAS will not eliminate genotype × environment interactions. Epistatic effects between QTL were also found to be important, suggesting that combinations of QTLs need to be selected for, rather than selection of individual QTLs. Due to the complications of genotype × environment interaction and epistatic effects, they suggested that phenotypic selection may be as effective as MAS for the germplasm and environments that they evaluated.

Environmental factors could have significant interactions with genetic effects on productivity and quality traits as indicated by Arora *et al.* (1975) for sorghum genotypes. Large genotype × environment (G×E) interactions would require more extensive testing and limit average gains. However, Lodhi, (1993) observed a considerable amount of genetic variability and heterosis for forage quality and yield characters, indicating that substantial gains from genetic improvement are possible.

2.8 QTL mapping in sorghum

The development of molecular marker technologies and the use of these markers in detecting and mapping quantitative trait loci has become a powerful approach for the studying the genetic and phenotypic basis of complex traits (Edwards *et al.*, 1987; Paterson *et al.*, 1988; Williams *et al.*, 1992). If individual genetic components associated with a complex trait can be identified, then research can focus on the function of each locus independently without the confounding effects of other segregating loci (Dorweiler *et al.*, 1993).

Molecular markers have been used to identify and characterize QTL associated with several different traits in sorghum including plant height and maturity (Pereira and Lee, 1995), characters concerned with plant domestication (Patterson *et al.*, 1995), disease resistance (Gowda *et al.*, 1995), and drought tolerance (Tuinstra *et al.*, 1998). In sorghum, many efforts have been made for QTL detection and mapping. Inheritance of resistance to charcoal rot disease and drought are polygenic in nature and simple phenotypic selection for such traits is difficult. Selection based on markers would assist in evaluating breeding lines for the presence of genes conditioning drought and charcoal rot tolerance and other quantitative traits in sorghum (Tuinstra *et al.*, 1997). The majority of traits related to fecundity and

adaptation in plants and animals are governed by multiple genes that interact in varying degrees to changes in the environment that produce a continuous phenotypic response.

The complex expression of plant towards biotic/abiotic stress makes the trait being associated with these stress difficult to study using traditional genetic and physiological methods. Use of molecular markers and QTL analysis of abiotic tolerance in lines grown in replicated and carefully induced drought-stressed environments has lead to a better understanding of the inheritance of this trait in sorghum (Tuinistra *et al.*, 1998; Crasta *et al.*, 1999; Tao *et al.*, 2000; Xu *et al.*, 2000; Subudhi *et al.*, 2000; Kebede *et al.*, 2001; Haussmann *et al.*, 2002).

Sorghum hybrids with the stay-green trait had a significant yield advantage even when water was limited during the grain-fill period as the rate of leaf senescence was negatively correlated ($r^2 = -0.55$) with grain dry mass (Borrell *et al.*, 1998). In sorghum QTLs for stay green in the cross B35 $\times$ Tx430 were positively associated with grain yield under stress and with yield under full irrigation (Rosenow, 1992). Molecular markers have been identified for some pre-flowering drought stress parameters (Tuinistra *et al.*, 1996) and for post-flowering stress resistance trait and stay-green trait (Nguyen and Rosenow, 1993).

Extensive studies have been carried out to map QTLs that control the stay green and chlorophyll content in sorghum, by using RFLP marker map developed from a RIL population. Xu *et al.* (2000) identified 4 staygreen 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. *Stg1* and *Stg2*, accounted for 13-20% and 20-30% of the phenotypic variability, respectively, were consistently identified in all trials at different locations in two years. In a similar study, Sanchez *et al.* 2002 identified four QTLs for stay-green trait in RILs from the cross B35 $\times$ Tx7000 with RFLP markers. Two QTLs appeared to be the most important. These stay-green QTLs were consistently identified in all field trials and accounted for 53.5% of the phenotypic variance (Sanchez *et al.* 2002). As a part of comparative mapping study between sugarcane and sorghum, a sugarcane cDNA clone with homology to maize *Rp1-D* (rust resistance) was mapped in sorghum (McIntyre *et al.*, 2004).

Molecular markers for resistance of sorghum to hemiparasitic weed, *Striga hermonthica* were mapped in two recombinant inbred populations (RIP 1 and RIP 2, $F_{(3:5)}$ lines) developed from the crosses IS 9830 $\times$ E 36-1 (RIP 1) and N 13 $\times$ E 36-1 (RIP 2). 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 set 1 and 2 of RIP 1 explaining 77.6 and 80 per cent of genetic variance, respectively (Haussmann *et al.*, 2004). Many studies of QTL mapping have been conducted in a single environment but the variation of gene expression is observed in different environments. Paterson *et al.* (1991) mapped 29 putative QTLs affecting mass per fruit, soluble solid concentration affecting mass per fruit soluble solid concentration and fruit pH. Of these 29 QTLs only 4 markers (14%) were identified in all three environments, 10 markers (34%) in two environments and 15 markers (52%) in a single environment. Zhang *et al.* (2004) reported in a study on an F_2 and two equivalent F_3 populations that only 17 of the total 44 QTLs were detected in more than one trial indicating that individual QTL seems to be sensitive to the environment. Similarly, QTLs associated with charcoal rot resistance traits (Number of internode crossed, length of infection and Lodging percent) in two different environment were mapped. A total of five QTLs were stable in expression over two environment for charcoal rot resistance traits with phenotypic variance of 14.87 to 31.1 percent (Reddy *et al.*, 2008).

QTLs for resistance to sorghum downy mildew (*Peronosclerospora sorghi*), Rajasthan downy mildew (*P. heteropogoni*) have been mapped (Nair *et al.*, 2005), midge and green bug have been identified (Tao *et al.*, 2003; Agrama *et al.*, 2002). Comparative mapping between sorghum and sugarcane suggested that the identification of markers for stalk number and suckering in sugarcane would be facilitated by focusing on sorghum QTLs associated with related traits (Jordan *et al.*, 2004). Similarly, resistance gene analysis in sugarcane and sorghum were studied and their association with QTLs for rust resistance was estimated (McIntyre *et al.*, 2005).

QTL influencing biochemical pathways such as those leading to sugar and organic acid metabolites have been reported in tomato (Fulton *et al.*, 2004). A total of 222 QTL influencing sugars, organic acid and related traits for flavour in tomato and concluded the possible effectiveness of using these QTL to produce changes in the metabolic pathway of tomato, thus leading to improved flavour and compositional quality fact been rarely identified thus far, peach (Dirlewanger *et al.*, 1999) and sweet corn (Azanza and Tadmor, 1996) being exceptions. These characteristics are very important not only for nutritional and sensory properties. Similarly QTL for biochemical trait were reported in peach (Dirlewanger *et al.*, 1999) and sweet corn (Azanza *et al.*, 1996). A range of 144 recombinant inbred lines of tomato derived from a cross between a cherry tomato line and a large-fruited line was evaluated by descriptive sensory profiling. Molecular markers were used to map quantitative trait loci (QTLs) for each sensory attribute. Causse *et al.* (2004) and Lecomte *et al.* (2004) identified the biochemical chemical components [soluble solid content (SSC), reducing sugar content (SUC), titratable acidity (TA), pH] controlling fruit quality trait and also used these QTL for introgression. Tomasz Hura, (2009) identified the high level of total phenolics in Saka genotype of triticale wheat which was associated with drought resistance and concluded that using the combination of morphological and biochemical traits one can find the candidates for identifying genes (QTL) controlling the resistance of winter triticale to drought during the tillering phase. QTLs for Invertase have been studied in tomato (Fridman *et al.*, 2005) and potato (Li *et al.*, 2005) where genetic colocation or heterologous complementation indicated that Inv polymorphisms are responsible for sugar content_composition. Sergeeva *et al.* (2006) carried out the biochemical analysis of the sucrose-splitting enzymes sucrose synthase and invertase in elongating roots and hypocotyls of *Arabidopsis*. They proved the possible role by using a combination of histochemical methods and quantitative trait locus (QTL) analysis. Lengths of roots and hypocotyls correlated better with invertase activities than with sucrose synthase activities. The highest correlations were observed with activities in the elongating zones of roots. The genetic basis of these correlations was studied by using QTL analysis. Several loci affecting invertase activity collocated with loci that had an effect on root or hypocotyl length.

2.9 Marker assisted selection

Molecular marker aided selection involves scoring segregating individuals for the presence or absence of a desired plant phenotype indirectly based on DNA banding pattern of linked markers on a gel or on autoradiogram depending on marker system. The rationale is that banding patterns reveal parental origin of the bands in segregants at a given marker locus and hence indicate presence or absence of specific chromosomal segments that carry the desired alleles. This increases the screening efficiency in breeding programmes. Previously, it was generally assumed that markers could be directly used in MAS. However, there are many factors that influence the accuracy of QTL mapping such as population size and type, level of replication of phenotypic data, environmental effects and genotyping errors. These factors are particularly important for more complex quantitative traits with many QTLs each with relatively small effects (e.g. drought tolerance, yield, biotic stress)

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

The opportunity to manipulate complex traits via MAS/backcrossing is now available. This was not previously possible without time consuming progeny testing cycles in a conventional backcrossing system using phenotypic selection. The other issue important to QTL transfer is accuracy of selection. Two separate studies by Lande and Thompson (1990) and by Zhang and Smith (1992) showed that MAS significantly improved the accuracy of selection for traits of low heritability. MAS has been advocated as a useful tool

for rapid genetic advance in case of QTLs (Lande and Thompson, 1990; Knapp, 1998). Gimelfarb and Lande (1995), presented a detailed analysis of the relationship between genetic markers and QTLs in the process of MAS. MAS could be used to pyramid major genes including disease and insect resistance genes, with the ultimate goal of producing the crop cultivars with more desirable traits (Mohan *et al.*, 1997; Witcombe and Hash, 2000).

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

2.9.1 Efficiency of MAS

Hospital and Charcosset (1997), used computer simulations to study the efficiency of MAS based on an index combining the phenotypic value and molecular score of individuals. They observed that in the first generation of selection the ratio of relative efficiency (RE) of expected efficiency of MAS over the expected efficiency of purely phenotypic selection generally increases with 1) larger population size, 2) lower heritability values of the trait, and 3) higher type-I error risk of the regression.

In studies over successive generations of selection, higher efficiency of MAS for QTLs with large effects in early generations was balanced by a higher rate of fixation of unfavorable alleles at QTLs with small effects in later generations. This suggests that MAS may become less efficient than phenotypic selection in the long-term. MAS efficiency therefore depends on the genetic determination of the target trait.

With conventional backcrossing, it takes a minimum of five to six generations to recover the recurrent parent. Data from simulation studies suggests that at least two but possibly three or even four backcross generations can be saved by using markers (Mebrouk *et al.* 2005).

The efficiency of MAS was generally reduced with increasing distance between the markers flanking the target QTL. The optimal distance recommended between two flanking markers is about 5–10 cM (Hospital *et al.*, 1992). The contribution of donor parent is reduced by half with each generation of backcrossing assuming no linkages. Molecular markers can increase the efficiency of the process in several ways. Flanking markers can be used to identify the backcross lines that are heterozygous for target genome regions. Advancing only these selected lines will also have the effect of reducing Linkage drag. (Young and Tanksley, 1989; Tanksley and Nelson, 1996). Single copy or low-copy markers with defined map locations such as RFLPS and Simple sequence repeats (SSRs) are ideal for this step. Molecular markers could also increase the efficiency of backcrossing by allowing selection of genotypes with the maximum percentage of recurrent parent genome. Markers with higher information content per reactions, such as Amplified AFLPs are ideal for this step (Waugh *et al.*, 1997).

Estimates of the probability of selecting one or more superior genotypes by MAS to predict its cost efficiency relative to phenotypic selection was presented by Knapp (1998). The frequency of superior genotypes among the selected progeny increases as the selection intensity increases. Van Berloo and Stam (1998), assessed effectiveness of MAS compared to phenotypic selection, showing that MAS appears partially promising when dominant alleles are present at QTLs and linked in coupling phase. Uncertainty in the estimated map position(s) of the QTL(s) targeted for foreground selection reduces the benefits of MAS.

Despite innovations like better marker systems and improved genetic mapping strategies, most marker associations are not sufficiently robust for successful MAS (Young, 1999) and accuracy of QTL location determination greatly affects selection efficiency of MAS (Charmet *et al.*, 1999). The number of marker data points (MDP) required in background selection and the size of the segregating population required to recover

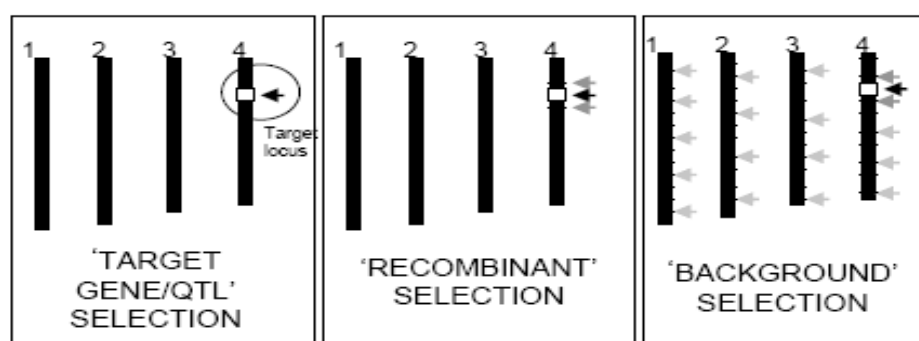
desirable individuals, comparing a two-stage selection procedure (one background selection stage and one foreground selection step) with alternative selection procedures (one foreground and two or three background selection steps) was suggested by Frisch *et al.* (1999). They observed that as the number of selection steps increases, the total number of MDP required (and hence the operational cost to generate these) decreases. The relative cost effectiveness of conventional breeding methods as compared to MAS for QPM (quality protein maize) lines

Dreher *et al.* (2003) at CIMMYT came to some preliminary conclusions on, when phenotypic screening is simple (in other words, when it is relatively easy to determine whether a given plant variety possesses a given trait, such as a certain grain color), conventional breeding is, will continue to be extremely cost-effective. Conversely, when phenotypic screening is expensive, technically difficult, or even impossible, MAS will often be advantageous. MAS offers an alternative that is simple, direct, and very reliable. Often effective selection for resistance to diseases like maize streak virus, which are strictly quarantined, can be carried out using molecular markers even at locations where the disease is not present (but with the breeding program targeting regions where the disease is currently present or is considered a significant future threat). Marker-assisted selection often allows breeders to cut down on the number of seasons needed to produce a desired product. Even a high-end MAS scheme that might run a few thousand dollars more than a conventional scheme is to the additional benefit to farmers when a variety becomes available sooner. Podlich *et al.* (2005), developed an effective approach for marker assisted selection of complex traits, i.e. Mapping As You Go (MAYG) approach, which continually revises estimates of QTL allele effects by remapping new elite germplasm generated over cycles of selection, thus ensuring that QTL estimates remain relevant to the current set of germplasm in the breeding program. They used simulation to investigate the effectiveness of the MAYG approach applied to complex traits.

2.9.2 Marker-assisted introgression in backcross breeding programs

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

Three levels of selection during marker-assisted backcrossing



With conventional backcrossing, it takes a minimum of five to six generations to recover the recurrent parent. Data from simulation studies suggests that at least two but possibly three or even four backcross generations can be saved by using markers (Mebruk *et al.*, 2005). Introgression of desirable alleles using markers may have several advantages over introgression using phenotypic information only. For the allele to be introgressed, marked chromosome segments ensure that the correct segment of donor genome is incorporated into the recipient line. For non additive acting alleles, using markers may be the only way to ensure a successful introgression program. For the background genotype, using markers gives a direct estimate of the proportion of the donor genome that is still present in

each backcross generation This may be preferred over phenotypic selection, in particular for traits with low heritabilities that are difficult to measure eg., age and sex-limited traits In several studies it has been shown that genetic markers can be used to introgress genes from one line to another (Smith *et al.*, 1987; Hillel *et al.*, 1992; Groen and Timmerman, 1992; Groen and Smith, 1995). Markers were efficient in introgression backcross programs for simultaneously introgressing an allele and selecting for the desired genomic background. Vischer (1996), investigated the efficiency of marker-assisted introgression in backcross populations of maize inbred lines by simulation studies. In marker-assisted backcross programs, usually high selection intensity is employed and only one or few individuals of a backcross population are used as non recurrent parents for the next backcross generation.

The population size of 350 individuals was used to perform both efficient control of the QTLs and efficient background selection (Thabuis *et al.*, 2004) and similar study was carried by Bouchez *et al.* (2002), where they screened 175 individuals in BC₂ and BC₃, on the basis of the computations of Hospital and Charcosset (1997), with this population size, the risk of not obtaining at least one individual carrying donor alleles at all foreground selection markers controlling the QTL segments was below 1% (minimum population size at 1%: 115 individuals), within each BC₂ and BC₃ population, eight progenies were found heterozygous for all markers on controlled segments, which was consistent with expectations. several plants could be selected carrying the donor alleles along the transferred intervals. By contrast, Lawson *et al* (1997), aimed at transferring in cultivated tomato alleles at five QTLs originating from a wild accession were unsuccessful in screening a single plant with the five favourable alleles because they used too small population size (50 to 273 plants). Marker-assisted selection appeared as a promising tool for breeding quantitative resistance. Regarding the genetic distance between perennial and bell pepper accessions (Lefebvre *et al.*, 2001), the marker-assisted backcross strategy appeared as the most suitable to transfer a limited number of QTLs. Two problems are inherent in backcross breeding, one being the large number of generations required to recover the genome of the inbred parent and the other being linkage drag, i.e. the presence of the inbred portions of the donor's genome (which can be linked to non-favorable alleles) surrounding the introgressed allele. As previously described by Tanksley *et al.* (1989), the main advantage of using DNA markers as opposed to conventional selection is to accelerate the fixation of recipient alleles in non target regions and to identify the genotypes containing crossovers close to target genes (Ribaut and Hoisington, 1998)

Young and Tanksley (1989) emphasised the size of donor chromosome segments around introgressed loci and reduction of linkage drag in Marker-Assisted Backcross (MAB) programmes. After a few backcross generations with no background selection on markers, most of the donor genes still segregating in the population are found on the chromosome carrying the introgressed gene (carrier chromosome) and more precisely on intact chromosomal segment of donor origin ("Donor segment") dragged along around this gene (Hanson, 1959; Stam and Zeven, 1981; Naveira and Barbadilla, 1992). Hence, an important objective of background selection should be the reduction of the length of this donor segment. Obviously, background selection on non carrier chromosomes is also important, though this section is more efficient in late backcross generations (Hospital *et al.*, 1992).

Drastic reduction of length of the donor segment surrounding the introgressed gene is important if undesirable genes are located close to the introgressed gene, as might be the case if the donor strain is wild genetic resources. If introgression takes place between two commercial strains, than drastic reduction of length of donor segment is not always important. In cases where the reduction is important one would like the donor segment remaining in the backcross progenies at the end of the programme to be as short as possible. This experiment involves background selection against the donor segment on the QTL carrier chromosome. Hospital *et al.* (1997) studied the minimal population size required that are necessary to obtain a selection objective, as a function of markers position and number of backcross generations performed. Selection was applied on two flanking markers, one on each side of the introgressed gene, and the selection objective was to obtain a backcross progeny that carries the donor allele at the locus of interest and recipient alleles at both flanking markers (such an individual genotype was called "double recombinant"). They derived a full theoretical treatment of the problem and provide comprehensive numerical application, which can be

used for optimisation of backcross schemes in plant breeding or any backcross scheme in which it is possible to retain a single progeny for reproduction at each generation.

According to Visscher *et al.* (1996) for practical situations, a marker distance of 10-20 cM seems appropriate. If the location of the QTL is estimated very poorly, it may be better to select on a marker haplotype based on more than two markers. In that case the width of the chromosome segment should take care of the uncertainty of the QTL position, while the markers should be spaced to give a low probability of there being undetected double recombinants selection in backcross programmes.

Examples of marker-assisted introgression

Published results of marker-assisted introgression have begun to accumulate at an increasing rate. Toojinda *et al.* (1998) successfully introgressed two QTL for stripe rust resistance in barley into a genetic background different from the one used to map the QTL. Chee *et al.* (2001) also reported the successful transfer of a QTL for grain protein concentration in wheat into a different genetic background. Ahmadi *et al.* (2001) successfully introgressed two QTL for resistance to yellow mottle virus in rice. Yousef and Juvik (2002) successfully selected on three markers linked to QTL that enhanced seedling emergence in sweet corn. The rate of success starts to decrease for introgression of larger numbers of target QTL. Stuber (1994) succeeded in increasing grain yield in maize lines by introgression of six favourable chromosome segments. Starting with the introgression lines of Eshed and Zamir (1996) and Lawson *et al.* (1997) introgressed four target chromosomal regions containing five QTL for pest resistance (acylsugar accumulation) from wild tomato into cultivated tomato.

The introgression of the four regions was successful at the genomic level. However, the level of acylsugar accumulation in the progeny introgressed for the five QTL was lower than expected, and, in particular, lower than that of the interspecific F_1 hybrid. Sebolt *et al.* (2000) performed marker-assisted backcrossing of two QTLs for seed protein concentration in soybean. Hen *et al.* (2001) manipulated four QTLs for drought resistance (root depth) in rice, a trait that is very difficult to manage phenotypically. Starting from double haploid lines, they produced a number of BC_3F_3 lines, each introgressed for one or two QTL at most. Among the four QTL, one exhibited the expected effect in the progeny, one was finally revealed as a false positive, one segment was shown to contain two QTL in repulsion phase (C/K) that reduced its expression and one segment did not exhibit the expected effect. Ribaut *et al.* (2002) introgressed five target regions containing QTL for drought tolerance (reduction of anthesis-silking interval) in maize. The results depended on the condition of the phenotypic assay of the progeny, the introgressed progeny exhibited a reduced ASI under stress conditions (drought) but the introgression had no visible effect in the absence of stress. Bouchez *et al.* (2002) performed the introgression of favourable alleles at three QTL for two traits (earliness and yield) between maize elite lines with marker assisted backcrossing. Foreground selection on markers to control the three target regions without the help of phenotypic assay was also efficient. However, results of the phenotypic evaluation of introgressed progeny, as well as the redetection of QTL among those progeny, depended upon the complexity of the trait under control. For the simple trait (earliness), QTL effects in the progeny were in general accordance with those expected from the original detection in the parental lines.

Lecomte *et al.* (2004) introgressed five QTLs strongly involved in tomato fruit quality into three different recipient lines through MAS. The breeding efficiency varied strongly with the recipient parent and significant interactions between QTL and genetic backgrounds were shown for all the studied traits. About 50% of the QTL were confirmed in each new background and new QTL were detected. The QTL with the largest effects were the most stable. Thabius *et al.* (2004) transferred resistance to *Phytophthora capsici* alleles at four QTL from a small fruited pepper into a bell pepper recipient by three cycles of marker-assisted backcrossing. Introgression was successful but a decrease of the effect from the moderate-effect QTL and of the epistatic interaction between QTL was observed. Neeraja *et al.* (2007) were successful in transferring a major QTL (*sub1*) with 70% phenotypic variance for submergence tolerance in popular mega variety of paddy. Finally, in some cases none of

the introgressed QTL had any effect, for example, three QTL for grain yield in barley (Kandemir *et al.*, 2000) and three QTL for high yield in soybean (Reyna and Sneller, 2001).

2.10 Transcription factor

A Transcription Factor (TF), also called a trans activation factor, is a DNA-binding protein that specifically interacts with a specific *cis*-acting element in the promoter region of an inducible gene of eukaryote. Transcription factors activate or inhibit the transcription of target genes by the interaction between themselves or with other proteins. In recent years, an increasing number of transcription factors that regulate the transcription of genes involved in responses to drought, high salt, cold, hormone, and pathogenesis or cell development have been identified in higher plants. According to the analysis of protein structure, a typical transcription factor is composed of four functional domains which are DNA-binding domain, transcription regulation domain, dimerization site and nuclear localization domain. With these domains, transcription factors recognize and bind separately to specific *cis*-acting elements and/or interact with the functional domains of other transcription factors, and regulate the transcription of inducible genes (Qiang *et al.*, 2001).

2.10.1 Host resistance and defence signalling pathway

Like animals, plants possess inducible defence systems to withstand pathogen and herbivore attack and other stress conditions. Defence responses are initiated upon recognition of stress signals, herbivore or pathogen-derived molecules called elicitors or PAMPs, which trigger the biosynthesis of one or a combination of the hormones like salicylic acid (SA), ethylene, and jasmonic acid (JA). The major target of these secondary signals is the cell nucleus, where the signal transduction pathways lead to the transcriptional activation of numerous genes, and consequently to the synthesis of a variety of defense proteins and protective secondary metabolites. Environmental stress, pathogens and herbivores limit plant productivity and distribution. On the other hand, stress triggers production of secondary metabolites, many of which are valuable plant compounds (Hammond-Kosack and Jones, 1996; Somssich and Hahlbrock, 1998).

Plants are capable of recognizing phytopathogens through the perception of pathogen-derived molecules or plant cell-wall degradation products due to the activities of pathogen-secreted enzymes. Such elicitor recognition events trigger an array of inducible defence responses involving signal transduction networks and massive transcriptional re-programming. The outcome of a pathogen infection relies on the balance between different signalling pathways, which are integrated by regulatory proteins.

In general, from the initial stage of recognition by the plant to the successful confinement or death of the pathogen, the distinct signalling pathways mediated by the small, signaling molecules, such as SA, JA, ethylene (ET), hydrogen peroxide (H₂O₂), nitric oxide (NO), and abscisic acid (ABA), constitute the complex signal transduction network controlling plant defence and thereby endowing the plant with a more sophisticated capacity for the highly complex, multifaceted defense response (Apel and Hirt, 2004; Gfeller and Farmer, 2004; Durrant and Dong, 2004; Mittler *et al.*, 2004; Lorenzo and Solano, 2005; Torres and Dangl, 2005; Van Loon *et al.*, 2006). The relative contribution of such signalling molecules to an inducible defence depends on the particular intruder (Reymond and Farmer, 1998; Glazebrook, 2005). Furthermore, a growing body of evidence regarding cellular signalling transduction and the regulation of expression of defence-related genes suggests that the defence signalling pathways do not function in a linear, independent fashion. Instead, each pathway can influence other pathways through positive or negative regulatory interactions (Kunkel and Brooks, 2002; Rojo *et al.*, 2003; Bostock, 2005).

Cultivar resistance is restricted to a particular pathogen species and is often referred to as a host resistance, which is tightly associated with the gene-for-gene recognition and accompanied by the HR (Dangl and Jones, 2001; Nimchuk *et al.*, 2003; Mysore and Ryu, 2004). When a plant is resistant, the interaction is then called incompatible, and when a plant is susceptible, the interaction is called compatible. This host resistance consists of the local resistance at the site of infection and the systemic acquired resistance (SAR) in the distal, non inoculated parts of plants following an activation of a local resistance (Ryals *et al.*, 1996).

Local resistance has been associated with a number of biochemical and physiological features. These include the rapid induction of the ion fluxes of H^+ , K^+ , Cl^- , and Ca^{2+} across the plasma membrane, protein phosphorylation or dephosphorylation, oxidative burst, deposition of callose and lignin, biosynthesis of proteins involved in the production of signal molecules such as ET, JA and SA as well as the accumulation of PR proteins and protective secondary metabolites (Heo *et al.*, 1999; Asai *et al.*, 2002).

2.10.2 Transcription factor and disease resistance

Plants are continually exposed to a vast array of pathogens, and generally succeed in defending themselves. An important part of this process is the induction of defence gene products that hinder pathogen growth and delay or prevent disease development. The way in which plants do this is multi-factorial, and includes regulatory proteins known as transcription factors (TFs) which directly regulate the expression of defence-related genes.

A paramount feature of the inducible defence responses upon pathogenic infection is the transcriptional regulation of a large number of plant defence related genes (Eulgem, 2005). Activation of defence responses towards pathogen infection is associated with fine-tuned transcriptional regulation of transcription factor, which can globally alter gene expression patterns (Eulgem and Somssich, 2007). The detection of a pathogen triggers several signalling cascades that are integrated in the nucleus by a diverse set of transcription factors, leading to massive transcriptional reprogramming. These transcription factors are the indispensable components of individual signalling cascades. In addition, transcription factors have been implicated in modulating the balance or cross talk between different defence signalling pathways (Anderson *et al.*, 2004; Boter *et al.*, 2004; Lorenzo *et al.*, 2004). Transcriptional reprogramming that is involved in both basal disease resistance and gene-for-gene resistance is mediated by several classes of TFs, including ERF, WRKY, and basic region/leucine zipper (bZIP) family members (Chen *et al.* 2002; Kalde *et al.*, 2003). This comprehensive transcriptional reprogramming is thought to function under a transcriptional regulatory network and eventually result in the accumulation of antimicrobial compounds and in the reinforcement of cell wall composition to prevent the multiplication and spread of pathogens in intercellular spaces or to ward off potential pathogens (Eulgem, 2005). Transcription factors regulate the temporal or spatial expression patterns of genes (Wyrick and Young, 2002). The activation or repression of these transcription factors is one critical step within the cellular regulatory circuits controlling the various biological processes (Brivanlou and Darnell, 2002). Plant defence against pathogens depends on the action of several endogenously produced hormones, including jasmonic acid (JA) and ethylene. In certain defence responses, JA and ethylene signalling pathways synergize to activate a specific set of defense genes. Martial *et al.* (2008) described the role of the Arabidopsis (*Arabidopsis thaliana*) APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) domain transcription factor ORA59 in JA and ethylene signalling and in defence. JA- and ethylene-responsive expression of several defense genes, including *PLANT DEFENSIN1.2* (*PDF1.2*), depended on ORA59. As a result, over expression of *ORA59* caused increased resistance against the fungus *Botrytis cinerea*, whereas *ORA59*-silenced plants were more susceptible. Several AP2/ERF domain transcription factors have been suggested to be positive regulators of *PDF1.2* gene expression based on over expression in stably transformed plants.

McGrath *et al.* (2005) identified 134 TF genes that showed a significant change in expression during Jasmonic acid application compared to mock-treated plants, and included many genes of previously unknown function belonging to APETALA2/Ethylene Response Factor (AP2/ERF), MYB, WRKY, and NAC TF gene families. Twenty-four of these TF genes were shown to be repressed or induced by MeJA, as well as repressed or induced by *A. brassicicola* (i.e. co-regulated). A high proportion of these co-regulated genes were members of either the AP2/ERF or WRKY TF gene families. Selected TF genes that showed significant differential regulation following MeJA exposure or *A. brassicicola* inoculation were profiled in a four-point time course over a 24 h period. It was found that the AP2/ERF TF genes identified in the primary screen were the most consistently and reproducibly regulated family of genes. Analysis of these plants revealed that this TF is a positive regulator of a subset of MeJA-responsive defence genes encoding the antimicrobial proteins PDF1.2 and CHIB. These results suggest that plants co-ordinately express multiple repressor and activator-type AP2/ERFs during pathogen challenge to modulate defence gene expression family.

A well-characterized plant defence response associated with pathogen attack is the expression of pathogenesis-related (*PR*) genes. *PR* genes are activated in both resistant and susceptible plants in response to pathogen attack. However, they often are expressed more rapidly and to a greater extent in incompatible interactions in which a resistant plant is challenged with an avirulent pathogen (Voisey and Slusarenko, 1989; van Kan *et al.*, 1992; Jia and Martin, 1999). This implies that enhanced *PR* gene expression is mediated by a recognition event involving a disease resistance (*R*) gene and its cognate avirulence (*avr*) gene. The roles of *PR* genes in plant defence responses against pathogens have been widely investigated (Cutt and Klessig, 1992). The expression of many defence-related genes is regulated by signalling molecules such as salicylic acid (SA) and ethylene. Increases in SA and its conjugates have been associated with the activation of defence genes in many plant species (Dempsey *et al.*, 1999). An important approach to understand *PR* gene regulation is to identify defence-responsive promoter regions and isolate their cognate DNA binding proteins. Several defense related transcription factors have been identified by using this method, including the WRKY transcription factors (Rushton *et al.*, 1996), a basic leucine zipper transcription factor G/HBF-1 (Dröge-Laser *et al.*, 1998), and the ethylene responsive element binding proteins (EREBPs, Ohme-Takagi and Shinshi, 1995). Isolation of these factors has provided new insights into *PR* gene regulation but also has raised the additional question of how these transcription factors themselves are regulated. In particular, because many *PR* genes are expressed rapidly during gene-for-gene incompatible interactions, it is important to understand the role of *R* genes in the regulation of defence-related transcription factors. APETALA2/EREBP-type transcription factors in plants has been proposed to play various roles in disease resistance, plant growth, development, and responses to different environmental stimuli (Okamuro *et al.*, 1997).

It has been well documented that the over expression of some *ERF* genes changes the expression of some *PR* genes and results in the increased resistance to bacterial, fungal, virus pathogens and abiotic stress (Gu *et al.*, 2002; Chakravarthy *et al.*, 2003). Four rice genes encoding ethylene-responsive transcriptional factors were cloned and their expressions were found to be responsive to biotic and abiotic stress (Cao *et al.*, 2005). The *ERF* transcription factors are shown to be involved in regulating plant pathogen-related (*PR*) genes expression via the GCC-box and non-GCC *cis*-elements (Reichman and Meyerowitz, 1998; Chakravarthy *et al.*, 2003). The over expression of *ERF* genes resulted in enhanced tolerance to pathogen attack in transgenic plants. As an example, over expression of the *Arabidopsis ERF1* enhanced resistance of transgenic plants to *Botrytis cinerea*, but the resistance to *P. syringae* infection was decreased (Berrocal-Lobo *et al.*, 2002). This shows that defined members of the *ERF* family seem to play specific roles in pathogen defence and plant response to environmental stress.

All plant transcription factors, with several exceptions, share common structural features including a DNA-binding region, an oligomerization site, a transcription-regulation domain, and a nuclear localization signal (Liu *et al.*, 1999). Generally, transcription factors are classified into distinct families according to their DNA-binding domains. In *Arabidopsis*, more than 1,500 transcription factors were identified and clustered into 34 families (Reichmann *et al.*, 2000). A large number of these transcription factors from different families have been implicated in the *Arabidopsis* defence responses because their expression is either induced or repressed following pathogen infection (Chen *et al.*, 2002; McGrath *et al.*, 2005). Of particular interest is an important role of the WRKY family in defence responses, which is defined by a DNA-binding motif containing the strictly conserved amino acid sequence WRKYGQK (Eulgem *et al.*, 2000). The first cDNA encoding a putative WRKY protein, the Sweet Potato Factor1 (SPF1), was cloned from sweet potato (Ishiguro and Nakamura 1994). Subsequently, a large number of *WRKY* genes were cloned and identified from the various plant species including *Arabidopsis*, barley, bittersweet nightshade, chamomile, legume, orchardgrass, parsley, potato, rice, sugarcane, tobacco, wheat, and wild oats (Eulgem *et al.*, 2000; Ülker and Somssich, 2004; Zhang and Wang, 2005).

WRKY transcription factors play a key role in modulating the plant defence transcriptome (Shahid *et al.*, 2008). The majority of *Arabidopsis WRKY* genes exhibit increased transcript levels after pathogen infections or after treatments with pathogen-derived elicitors and SA (Chen *et al.*, 2002; Dong *et al.*, 2003; Kalde *et al.*, 2003). The over-expression of individual *WRKY* factors such as *Arabidopsis WRKY18* and *WRKY29* reduced

the susceptibility to both bacterial and fungal pathogens (Asai *et al.*, 2002; Chen and Chen, 2002), whereas enhanced resistance to virulent *P.syringae* was also observed in *wrky7* plants (Kim *et al.*, 2006). These transgenic studies suggest that strictly regulated *WRKY* transcript levels are important for efficient pathogen defence. Some of the *WRKY* factors have been implicated in the establishment of SAR (controlling expression of *NPR1* (Yu *et al.*, 2001) and *PR-1* regulon (Maleck *et al.*, 2000).

2.10.3 Classification of transcription factors

Classification of transcription factors depends on their structural features, families sometimes subdivided according to the number and spacing of conserved residues in the most similar domain (Guilfoyle *et al.*, 1998; Riechmann and Meyerowitz, 1998).

Basic leucine zipper (bZIP)

bZIP class of DNA binding protein bind specifically to a core ABRE sequence (T/G/C)ACGTG (Guiltinan *et al.*, 1990; Izawa *et al.*, 1993; Oeda *et al.*, 1991). Sequence comparisons and functional analysis of the promoters of LEA and LEA related genes such as the wheat *Em*, rice *Osem* and *Rab16A-D* and barley *HVA22* led to identification of Absciscic Acid Responsive Elements (ABRE), required for the induction of transcription by ABA (Guiltinan *et al.*, 1990; Mundy *et al.*, 1990). Hattori *et al.* (1995) showed that the *Osem* promoter has 3 ACGTG-containing sequences, motif A, motif B and motif A', all of which contribute to a greater or lesser extent to the responsiveness of the promoter to ABA. In addition to these motifs, a sequence designated as region1 (CE3-coupling element 3) appears to function together with motif A as an ABA-response complex.

Nakagawa *et al.* (1996) isolated a bZIP protein, *OSBZ8* from rice embryo cDNA library, *OSBZ8* had structural features typical of the GBF (G-box Binding Factor)-type bZIP proteins and binds to G-box and G-box like sequences that include ABREs, which are identified in the promoters of the ABA-inducible genes, *viz.*, *Em*, *Osem*, and *RAB16*.

Homeodomain-leucine zipper (HD-ZIP) proteins

Homeodomain-leucine zipper (HD-ZIP) proteins are transcription factors as yet found only in plants. Soderman *et al.* (1996) characterized *ATHB-7* in *arabidopsis*. Induction of *ATHB7* was shown to be mediated strictly *via* ABA, since no induction was detected in the ABA deficient mutants, *aba-3* subjected to drought treatment. Induction levels in AB insensitive mutants, *abi2* and *abi3* were similar to the wild type response. These results indicate that *ATHB-7* is transcriptionally regulated in an ABA-dependent manner and may act in a signal transduction pathway which mediates a drought response.

Basic Helix turn Helix (bHTH)-MYB/Basic helix loop helix (bHLH)-MYC

MYB is an acronym derived from "myeloblastosis", an old name for a type of leukemia. MYB factors represent a family of proteins which includes a conserved domain, the MYB DNA-binding domain, found in both plants and animals. The first MYB gene identified was the 'oncogene' *v-Myb* from the avian Myeloblastosis virus (AMV). This acutely transforming retrovirus causes rapidly fatal monoclastic leukemia in chickens. Characteristics of a MYB repeat are three regularly spaced tryptophan residues which form a tryptophan cluster I, the three dimensional helix-turn-helix structures. The three repeats are referred to as R1, R2 and R3 (Stracke *et al.*, 2001). Plants *myb*-related genes constitute a large family and play a wide variety of roles in the regulation of gene expression. *Myb* genes are involved in the ABA responsive expression of drought-inducible genes in the vegetative tissues of higher plants. The Myb domain binds to a specific DNA sequence (C/TAACG/TG, for most organisms) that is closely related to the E-box hexanucleotide. Once bound, *Myb* proteins function to facilitate transcription activation (Biedenkapp *et al.*, 1988).

WRKY

WRKY proteins which are unique to plants contain either one or two WRKY domains, a 60-amino acid region highly conserved among the family members. They play a key role in

regulating the pathogen induced defense responses (Wu *et al.*, 2005), abiotic stress responses (Fowler and Thomashow, 2002; Seki *et al.*, 2002; Mare *et al.*, 2004) and are involved in various physiological processes including senescence, trichome development and biosynthesis of secondary metabolites (Eulgem *et al.*, 2000).

AP2/EREBP

AP2 (APETALA2) and EREBPs (ethylene-responsive element binding proteins) are the prototypic members of a family of transcription factors unique to plants, whose distinguishing characteristic is AP2 DNA-binding domain. AP2/EREBP genes form a large multigene family and they play a variety of roles throughout the plant life cycle being key regulators of several developmental processes, like floral organ identity, determination or control of leaf epiderma cell identity, part of the mechanisms used by plants to respond to various types of biotic and environmental stress (Riechmann and Meyerowitz, 1998). These proteins contain either one (EREBP subfamily) or two (AP2 subfamily) copies of an approximately 70-amino acid domain termed the AP2 repeat because of its initial description in the floral homeotic protein *APETALA2* (AP2).

DNA-binding specificity has been determined for three types of AP2/EREBP proteins that contain an AP2 repeat. The first class contains proteins, which bind to ethylene response elements (ERE) or GCC boxes (tobacco EREBPs, *Arabidopsis* AtEBP and AtERF 1-5, and tomato *Pti4-6*) found in the promoters of ethylene- inducible pathogenesis related genes. The second class includes proteins that bind to the C-repeat or dehydration response element (DRE) in the promoter of genes that are turned on in response to low temperatures and/or water deficit (CBF1, CBF2, CBF3/DREB1A and DREB2A). The C-repeat/DREs contain the core sequence CCGAC. The third class of AP2/EREBP-like proteins that have been shown to bind DNA consists of *Arabidopsis* RAV1 and RAV2. RAV1 and RAV2 are highly related proteins that contain two DNA binding motifs: a single AP2 repeat and a B3-like domain (Sakuma *et al.*, 2002; Liu *et al.*, 1998; Stockinger *et al.*, 1997).

ARF

Auxin-response factors (ARFs) bind with specificity to TGTCTC auxin-response elements (AuxREs), which are found in promoters of primary/early auxin-response genes. The ARF1 protein contains an amino-terminal DNA-binding domain (DBD), which has some sequence similarity to a carboxyl-terminal B3 domain found in the maize transcription factor *VIVIPAROUS1* (VP1) and its relatives. ARF1 contains a carboxyl-terminal domain (CTD) related to motifs III and IV found in the CTDs of Aux/IAA proteins, which are short-lived nuclear proteins encoded by a family of primary/early auxin-response genes. The CTDs in ARF1 and Aux/IAA proteins facilitate dimerization among the members of both ARF and Aux/IAA protein families (Ulmasov *et al.*, 1999).

bHLH

The basic/helix-loop-helix (bHLH) proteins are a super-family of transcription factors that have been well characterized in non-plant eukaryotes (Atchley and Fitch, 1997; Littlewood and Evan, 1998; Ledent and Vervoort, 2001). The reports indicate that bHLH proteins are important regulatory components in transcriptional networks in mammalian systems, controlling a diversity of processes from cell proliferation to cell lineage establishment (Grandori *et al.*, 2000; Massari and Murre, 2000).

This family is defined by the bHLH signature domain, which consists of 60-amino acids with two functionally distinct regions. The basic region, located at the N-terminal end of the domain, is involved in DNA binding and consists of 15 amino acids with a high number of basic residues. The HLH region at the C-terminal end functions as a dimerization domain and is constituted mainly of hydrophobic residues that form two amphipathic-helices separated by a loop region of variable sequence and length. Outside of the conserved bHLH domain, these proteins exhibit considerable sequence divergence. Structural analysis has shown that the interaction between the HLH regions of two separate polypeptides leads to the formation of homodimers and/or heterodimers and that the basic region of each partner binds to half of the DNA recognition sequence. The core DNA sequence motif recognized by the bHLH proteins

is a consensus hexanucleotide sequence known as the E-box (5'-CANNTG-3'). There are different types of E-boxes, depending on the identity of the two central bases. One of the most common is the palindromic G-box (5'-CACGTG-3'). Certain conserved amino acids within the basic region of the protein provide recognition of the core consensus site, whereas other residues in the domain dictate specificity for a given type of E-box. In addition, flanking nucleotides outside of the hexanucleotide core have been shown to play a role in binding specificity, and there is evidence that a loop residue in the protein plays a role in DNA binding through elements that lie outside of the core recognition sequence (Toledo-ortiz *et al.*, 2003).

YABBY

Members of the YABBY gene family are characterized by two conserved domains, a C2C2 zinc finger-like domain towards the amino terminus and a helix-loop-helix, which called as YABBY domain, sequence similarity of the first two helices of the HMG box towards the carboxyl end of the protein. In addition to the conserved YABBY and zinc finger domains, YAB1, YAB2, YAB3 and YAB5 display some sequence similarity on the carboxyl side of the YABBY domain. The region between the YABBY and zinc finger domains is variable amongst family members.

Database searches suggest that this family may be plant-specific, as genes with a similar arrangement of zinc finger and YABBY domains do not occur in either *Caenorhabditis elegans* or *Saccharomyces cerevisiae*. The YABBY genes are expressed in a polar manner in all lateral organs produced by apical and flower meristems. Their transcripts are detectable only in the abaxial domains of lateral organs when their primordia emerge and begin to differentiate. Loss of polar expression of these genes results in loss of polar differentiation of cell types in lateral organs, and gain-of-function alleles result in abaxialization of lateral organs. The expression of these genes is precisely correlated with abaxial cell fate in mutants in which abaxial cell fates are found ectopically, reduced or eliminated. The members of this gene family act redundantly to specify abaxial identity in lateral organs produced by the apical and flower meristems in *Arabidopsis* (Siegfried *et al.*, 1999).

NAC

The NAC protein family comprises a variety of plant proteins that are identified by the presence of a highly conserved N-terminal NAC domain, accompanied by diverse C-terminal domains. NAC is an acronym derived from the names of the three genes first described as containing the domain, namely NAM (no apical meristem), ATAF1, 2 and CUC2 (cup-shaped cotyledon) (Souer *et al.*, 1996; Aida *et al.*, 1997). NAC proteins have been implicated in transcriptional control in a variety of plant processes. Functions include involvement in the development of the shoot apical meristem and floral organs, and in the formation of lateral roots (Souer *et al.*, 1996; Aida *et al.*, 1997; Xie *et al.*, 2000).

Furthermore, NAC proteins have been implicated in responses to stress and viral infections (Xie *et al.*, 1999; Ren *et al.*, 2000; Collinge and Boller, 2001). Several NAC proteins have been shown to function as transcription activators (Ren *et al.*, 2000; Xie *et al.*, 2000; Duval *et al.*, 2002). The DNA-binding ability of two of these proteins, NAC1 and ANAC has been localized to the NAC domain, whereas the C-terminal regions constitute transcriptional activation domains (Xie *et al.*, 2000; Duval *et al.*, 2002). Several NAC genes are hormone inducible (Hoth *et al.*, 2002; Xie *et al.*, 2002; Greve *et al.*, 2003). NAC domains have also been implicated in interactions with other proteins such as viral proteins (Xie *et al.*, 1999; Ren *et al.*, 2000) and RING finger proteins (Xie *et al.*, 2002; Greve *et al.*, 2003). For example, interactions between the auxin-inducible NAC1 protein, involved in lateral root formation, and the RING domain protein SINAT5 attenuate the auxin signal through ubiquitination and degradation of NAC1 (Xie *et al.*, 2002). Recently, a NAC protein from *Arabidopsis* (ANAC) was identified as an interaction partner of another RING protein (Greve *et al.*, 2003). An examination of interactions between different RING domains and ANAC suggested that RING and NAC interactions may also regulate pathways controlled by the plant stress hormone abscisic acid.

HAP2 and HAP5

Proteins that bind to the CCAAT motif were first characterized in the yeast *Saccharomyces cerevisiae* through analysis of mutants with reduced levels of expression of the *CYC1* gene (encoding iso-1-Cyt c). The *CYC1* promoter comprises two UAS, one of which (UAS2) contains an inverted CCAAT motif that is required for UAS2-directed transcription. Activation of transcription from UAS2 requires HAP2, HAP3 and HAP5, which form a heterotrimeric CCAAT-box-binding complex. The HAP complex appears to control expression of genes important for mitochondrial biogenesis (Edwards *et al.*, 1998). CCAAT-box-related motifs have also been identified in the promoters of a variety of vertebrate genes. A range of transcription factors has been shown to bind to different CCAAT boxes, with varying levels of specificity and each is thought to play a distinct role in gene expression or DNA replication. Direct homologs of the yeast HAP complex (called NF-Y, CP1, or CBF) have been identified in vertebrates (Romier *et al.*, 2003).

Plant NF-Y function also appears to be important for responses to drought stress. Although a specific mechanism remains unclear, over expression of NF-YB1 and its ortholog in maize *ZmNF-YB2*, leads to enhanced drought resistance (Nelson *et al.*, 2007). Although the ability of NF-YB1 to promote drought resistance is clear, no loss-of-function data were reported to demonstrate an actual biological role in *Arabidopsis*. In contrast, a recent publication shows both over-expression and loss-of-function data for NFYA5 (Li *et al.*, 2008). Over-expression of NF-YA5 reduced drought susceptibility, anthocyanin production, and stomatal aperture, while *nf-ya5* mutants had the expected opposite phenotype in each instance.

High mobility group (HMG)

High mobility group (HMG) proteins are families of relatively low molecular weight non-histone components in chromatin. The animal HMG proteins have been classified in three families: HMG-1/2, HMG-14/17 and HMG-I/Y proteins. Members of the HMG-1/2 family contain a conserved protein motif known as the HMG box which binds to a variety of non-B forms of DNA, such as cruciform DNA. The HMG-14/17 proteins form complexes with nucleosomes on actively transcribed genes where their presence is associated with sensitivity to DNaseI. The HMG-I/Y proteins were originally detected in rapidly dividing and undifferentiated cells of human and rodents and were shown to bind to the minor groove of A/T-rich DNA with a conserved DNA-binding motif called the AT-hook. HMG1/2 are proteins of about 200 amino acid residues with a highly acidic C-terminal section which is composed of an uninterrupted stretch of from 20 to 30 aspartic and glutamic acid residues, the rest of the protein sequence is basic (Gupta *et al.*, 1997).

MADS

The best studied plant MADS-box transcription factors are those involved in floral organ identity determination. Analysis of homeotic floral mutants resulted in the formulation of a genetic model, named the ABC model, which explains how the combined functions of three classes of genes (A, B and C) determine the identity of the four flower organs. *Arabidopsis* B and C genes, which control petal, stamen, and carpel development are functionally dependent on three highly similar MADS-box genes, *SEP1*, *SEP2* and *SEP3* (Nam *et al.*, 2003). Interestingly, when mutant knockout alleles of the three *SEP* genes were combined in a triple *sep1*, *sep2*, *sep3* mutant was revealed loss of petal, stamen, and carpel resulting in a flower composed of only sepals. The *SHP* genes provide another example of MADS-box gene redundancy. *shp1* and *shp2* single mutants do not exhibit any phenotypic effect, whereas in the double mutant, development of the dehiscence zone is disturbed in the fruit, resulting in a failure to release seeds (Parenicova *et al.*, 2003).

It has been proposed that there are at least 2 lineages (type I and type II) of MADS-box genes in plants, animals, and fungi. Most of the well-studied plant genes are type II genes and have three more domains than type I genes from the N to the C terminus of the protein: intervening (I) domain (~30 codons), keratin-like coiled-coil (K) domain (~70 codons), and C-terminal (C) domain (variable length). These genes are called the MIKC-type and are specific to plants. The MADS-box is a DNA binding domain of 58 amino acids that binds DNA

at consensus recognition sequences known as CArG boxes [CC(A/T)6GG] (Santelli and Richmond, 2000).

MBF1

Multi-protein bridging factor 1 (MBF1) is known to be a transcriptional co-activator that mediates transcriptional activation by bridging between an activator and a TATA-box binding protein (TBP). Tsuda *et al.* (2004) demonstrated that expression of three MBF1 from *Arabidopsis* partially rescues the yeast *mbf1* mutant phenotype, indicating that all of them function as co-activators for GCN4-dependent transcriptional activation. Each of the subtypes showed distinct tissue-specific expression patterns and responses to phyto-hormones. These observations suggest that even though they share a similar biochemical function, each MBF1 has distinct roles in various tissues and conditions.

PHD

The Plant Homeo Domain (PHD finger, a Cys4"CHis"CCys3 zinc finger), is found in many regulatory proteins from plants or animals which are frequently associated with chromatin-mediated transcriptional regulation. The PHD finger was originally identified by comparison of the maize homeodomain (HD) protein *ZmHOX1a* to its *Arabidopsis* relative HAT3.1 and named plant homeodomain (PHD) finger due to its association with the DNA-binding HD in both genes. This motif often occurs in various regulatory genes, such as members of the trithorax (TRX-G) or polycomb (PC-G) groups and leukaemia-associated proteins (LAP finger) (Halbach *et al.*, 2000).

Trihelix

GT-2 is a plant transcriptional activator that contains two separate, but similar, trihelix DNA-binding domains. GT-1 is similar to GT-2, although it contains only one of such domains. cDNAs that encode GT-2 were isolated from rice (OS-GT2) and *Arabidopsis* (AT-GT2). Evidence is presented for the existence of an *Arabidopsis* gene family that is structurally related to AT-GT2. Two members of this GT2-like family, AT-GTL1 and AT-GTL2, have been isolated and characterized. Their sequences suggest that they evolved by a recent gene duplication event. Both AT-GT2 and AT-GTL genes contain an intron in the amino-terminal trihelix motif, indicating that this DNA-binding domain resulted from exon shuffling. RNA gel blot analysis using AT-GTL1 as a probe revealed four transcripts in the aerial part of the plant. All mRNA levels were significantly higher in siliques, suggesting that this gene family may function in fruit and/or seed development. To date, DNA-binding proteins characterized by the trihelix motif have been described only in plants, and may therefore be involved in plant-specific processes (Smalle *et al.*, 1998).

Wirly

Elicitor-induced activation of the potato pathogenesis-related gene PR-10a requires a 30-bp promoter sequence termed the ERE (elicitor response element) that is bound by the nuclear factor PBF-2 (PR-10a binding factor 2). Evidence demonstrates that inactive PBF-2 is stored in the nucleus of fresh tuber and become available for binding to the ERE upon elicitation. A protein with an apparent molecular mass of 24 kD (p24) is a DNA binding component of PBF-2. A cDNA encoding p24 has been cloned and encodes a novel protein with a potential transcriptional activation domain that could also act as a single-stranded DNA binding domain. Both PBF-2 and the cDNA-encoded protein bind with high affinity to the single-stranded form of the ERE in a sequence-specific manner. The inverted repeat sequence of the ERE, TGACAnnnnTGTCA, is critical for binding of this factor *in vitro* and for PR-10a expression *in vivo*, supporting the role of PBF-2 as a transcriptional regulator (Desveaux *et al.*, 2004).

2.11 Quantitative real time reverse transcription PCR (qRT-PCR)

A more accurate and faster method for determining of gene expression requiring minimal amount of RNA is quantitative real time PCR (qRT-PCR), a fluorescence based reverse transcription polymerase chain reaction (Bustin, 2000). However, qRT-PCR is

restricted to examining only a few genes (of Known sequence) at a time. This method involves the reverse transcription of mRNA into first stranded cDNA, which is then amplified by PCR. The amplification of the product is monitored using either a fluorescent dye (SYBR green), which binds to double stranded DNA, or gene specific probes (Taqman, molecular beacons etc). Two strategies can be performed in qRT PCR to quantify gene expression. an absolute or relative quantification of gene expression .The easier and more common method of relative gene expression requires an internal reference standard whose transcription level should remain unaffected under the experimental conditions being studied (Pfaffi, 2003; Radonic *et al.*, 2004).The expression level of gene of interest is adjusted according to the internal reference standard to account for difference in the amount and quality of starting material, and difference in RNA preparation and cDNA synthesis efficiency (Pfaffi 2003; Radonic *et al.*, 2004). Quantitative RT-PCR is becoming one of the most common methods to confirm the expression level of genes detected in microarray.

2.11.1 One step and two step qRT-PCR

When quantifying mRNA, real-time PCR can be performed as either a one step reaction, where the entire reaction from cDNA synthesis to PCR amplification is performed in a single tube. Two step reaction, where reverse transcription and PCR amplification occur in separate tubes. There are several advantages and disadvantages associated with each method. One-step real-time PCR is thought to minimize experimental variation because both enzymatic reactions occur in a single tube. However, this method uses an RNA starting template, which is prone to rapid degradation if not handled properly. Therefore, a one-step reaction may not be suitable in situations where the same sample is assayed on several occasions over a period of time. One step protocols are also reportedly less sensitive than two-step protocols (Wong and Medrano, 2005).

Two-step real-time PCR separates the reverse transcription reaction from the real-time PCR assays, allowing several different real-time PCR assays on dilutions of a single cDNA. Because the process of reverse transcription is notorious for its highly variable reaction efficiency (Mannhalter *et al.*, 2000), using dilutions from the same cDNA template ensures that reactions from subsequent assays have the same amount of template as those assayed earlier. Data from two-step real-time PCR is quite reproducible with Pearson correlation coefficients ranging from 0.974 to 0.988 (Vandesompele *et al.*, 2002). A two-step protocol may be preferred when using a DNA binding dye (such as SYBR green) because it is easier to eliminate primer-dimers through the manipulation of melting temperatures (T_m) (Vandesompele *et al.*, 2002).

2.11.2 Methods for gene quantification

Absolute quantification

Absolute quantitation uses serially diluted standards of known concentrations to generate a standard curve. The standard curve produces a linear relationship between C_t and initial amounts of total RNA or cDNA, allowing the determination of the concentration of unknowns based on their C_t values (Heid *et al.*, 1996). This method assumes all standards and samples have approximately equal amplification efficiencies (Souaze *et al.*, 1996). In addition, the concentration of serial dilutions should encompass the levels in the experimental samples and stay within the range of accurately quantifiable and detectable levels specific for both the real-time PCR machine and assay. The PCR standard is a fragment of double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), or cRNA bearing the target sequence. A simple protocol for constructing a cRNA standard for one-step PCR was reported by Fronhoffs *et al.* (2002), while a DNA standard for two-step real-time PCR can be synthesized by cloning the target sequence into a plasmid (Gerard *et al.*, 1998), purifying a conventional PCR product (Liss, 2002), or directly synthesizing the target nucleic acid. The standard used must be a pure species. DNA standards have been shown to have a larger quantification range and greater sensitivity, reproducibility, and stability than RNA standards (Pfaffl *et al.*, 2004). However, a DNA standard cannot be used for a one-step real-time RT-PCR due to the absence of a control for the reverse transcription efficiency (Giulietti *et al.*, 2001).

Relative quantification determines the changes in steady-state mRNA levels of a gene across multiple samples and expresses it relative to the levels of an internal control RNA. Relative quantification does not require standards with known concentrations and the reference can be any transcript, as long as its sequence is known. Ideally the reference gene should not be regulated or influenced by the experimental procedure. The accurate quantification of a true reference gene allows the normalization of differences in the amount of amplifiable RNA or cDNA in individual samples generated due to the following reasons *i.e.* different amounts of starting material, the quality of the starting material, differences in RNA preparation and cDNA synthesis. Since, the reference gene is exposed to the same preparation steps as the gene of interest. It is crucial to choose the appropriate reference gene for an exact comparison of mRNA transcription in different samples or tissues (Radonic *et al.*, 2004; Silver *et al.*, 2006; Dheda *et al.*, 2004). To calculate the expression of a target gene in relation to an adequate reference gene various mathematical models are established. Two types of relative quantification models are available:

- (1) Without efficiency correction

$$\text{Ratio} = 2^{-(\Delta\text{Ct sample} - \Delta\text{Ct control})}$$

- (2) with kinetic pcr correction efficiency:

$$\text{Ratio} = \frac{(\text{E target})^{\Delta\text{Ct (control - sample)}}}{(\text{E ref})^{\Delta\text{Ct (control - sample)}}} \div \frac{(\text{E ref})^{\text{Ct control}}}{(\text{E target})^{\text{Ct control}}}$$

Amplification efficiency of the reaction is an important consideration when performing relative quantification. Earlier methods of calculating gene expression have assumed the amplification efficiency of the reaction as ideal or one, means the PCR product concentration doubles during every cycle within the exponential phase of the reaction (Gibson *et al.*, 1996). However, many PCR reactions do not have ideal amplification efficiencies, and calculations without an appropriate correction factor may overestimate starting concentration (Liu and Saint, 2002). When used appropriately, relative quantification can generate useful and biologically relevant information (Pfaffl, 2004).

2.11.3 Normalisation strategies

Quantitative real-time PCR (qRT-PCR) is today the prime technique to measure gene expression (Kubista *et al.*, 2006). When properly used it offers unprecedented sensitivity, accuracy and reproducibility. But there are caveats. The target is mRNA, which must be extracted and converted to cDNA in a reverse transcription process that can proceed at highly variable yield depending on protocol. (Stahlberg *et al.*, 2004a, 2004b) RNA is further rapidly degraded by nucleases abundant in biological samples (Fleige and Pfaffl, 2006). To account for processing variation the expression of marker genes is normalised with appropriate endogenous control genes and technical repeats are performed to reduce confounding variance. Several strategies have been proposed for normalising real-time RT-PCR data. These range from ensuring that a similar sample size is chosen to using an internal housekeeping or reference gene. These approaches are not mutually exclusive and can be incorporated into a protocol at many stages

Among all the different method for normalisation, normalising to a reference gene is a simple and popular method for internally controlling error in real-time RT-PCR. This strategy targets RNAs encoded by genes, which have been collectively called housekeeping genes and benefits from the fact that all the steps required to obtain the final PCR measurement are controlled for. The procedure is simplified as both the gene of interest and the reference gene are measured using real-time RT-PCR. Reference genes can also control for different input RNA amounts used in the reverse transcription step however, because this can vary with reverse transcriptase type so it must be validated.

3. MATERIAL AND METHODS

The present investigations were carried out at the Institute of Agri-Biotechnology (IABT), Dharwad, Main Agricultural Research Station (MARS), Dharwad, Regional Agricultural Research Station (RARS), Bijapur and National Research Center on Sorghum (NRCS), Solapur, Maharashtra. The details of material used and the methodologies adopted in the present investigation are as follows.

3.1 Phenotyping of Recombinant Inbred Lines (RILs)

Experimental material

One of the major difficulties in working with charcoal rot disease caused by the fungus *Macrophomina phaseolina* Tassi (Goid) in the sorghum has been the difficulty in accurately scoring disease reaction. Plant maturity, amount of inoculum and terminal drought stress influence the disease to the maximum in susceptible genotypes. As a consequence, multiple evaluations made in different locations are required for accurate assessment of genetic contributions of component trait loci towards disease resistance. For phenotypic evaluation, the mapping population was obtained from the International Crops Research Institute for Semi Arid Tropics (ICRISAT), Patancheru, Hyderabad, India. The two parents were IS 22380, a Sudanese dwarf line (susceptible to charcoal rot) and E 36-1, a stay green source adapted to tropics (resistant to charcoal rot), and 93 Recombinant Inbred lines (RILs) derived from the cross IS 22380 x E 36-1. The pedigree information of mapping population is given in Appendix I.

Location, season and experimental design

The entries were evaluated in a sick plot at MARS, Dharwad; RARS, Bijapur and NRCS, Solapur during *rabi* 2004, 2005, 2006 and 2007 for the charcoal rot incidence and other yield related component traits. A set of 100 entries comprising of 93 RILs, parents and check (M-35-1) were taken up for phenotyping. Phenotyping was done in a sick plot for charcoal rot, which is being marked over decades. Simple lattice design was adopted on an area of 25×21 m² with four replications. Each replication consisted of 10 blocks, each with 10 entries planted randomly. Each entry was planted in a single row with 15 plants. The trial was laid out with a spacing of 45×15 cm and other recommended package of practices were followed. In order to eliminate the border effects, three rows of M-35-1 was sown on all sides of the field. Plots were thinned at 10 days after seedling emergence (DAE) to a spacing of 15 cm between plants within rows when the seedlings were at 4-leaf stage. Field evaluation was undertaken during 2007-08 to explore the contribution of two biochemical traits towards charcoal rot resistance traits at Dharwad and Bijapur location.

3.1.1 Field observations

Evaluation for charcoal rot incidence was done under natural sick plot conditions along with artificial inoculation of the fungus at 45 days after sowing (DAS). The stem tape inoculation technique developed by Mayee and Garud (1978) was followed. The methodology involves multiplication of the causal organism on sorghum grain and placing the sclerotia inoculum loaded grains directly on non-injured second internode from ground. A thin needle is pierced followed by an insertion of inoculum and covering with a cellophane tape. Three parameters *viz.*, per cent lodging, number of internodes crossed by the fungus and length of infection were used for assessing the reaction of individual genotype to the disease. Disease incidence was recorded 20 days after complete grain filling during 2004-05, 2005-06, 2006-07 and 2007-08 at all the three location.

During 2007-08 *rabi*, the field evaluation at MARS, Dharwad and RARS, Bijapur was taken up to investigate the relevance of two biochemical traits *viz.*, total phenols and lignin content in 2nd internode. This assessment was done at 75 and 95 DAS.

Charcoal rot component traits

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

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

(Sorghum descriptor, ICRISAT)

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

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

Total phenol at 75 and 95 DAS : The samples were taken from basal 2nd internode region of the sorghum stalk at 75 and 95 DAS from parents and individual RILs and the total phenol was estimated as follows.

Total phenol was estimated using Folin-Ciocalteu Reagent (FCR) method while for lignin estimation Acid Detergent Fiber (ADF) method (Sadashivam and Manikyam, 1992) was employed. First the parents E36-1 and IS 22380 were checked for significant difference for total phenol and lignin content, these were found to be significantly differing. This difference was sufficient for estimation of lignin and total phenol across all the 93 RILs. The detailed procedure for biochemical estimation is as follows.

1. Weigh exactly 1.0 g of the sample and grind it with pestle and mortar in 10 ml volume of 80% ethanol.
2. Centrifuge the homogenate at 10,000 rpm for 20 min save the supernatant, re-extract the residue with 5 ml volume of 80% ethanol, centrifuge and pool the supernatant.
3. Evaporate the supernatant to dryness.
4. Dissolve the residue in known volume of distilled water (5 ml).
5. Pipette out different aliquots (0.2 to 2 ml) into test tubes.
6. Make up the volume in each tube to 3 ml with distilled water.
7. Add 0.5 ml of Folin-Ciocalteu (FCR) reagent
8. After 3 min, add 2 ml of 20% Na₂CO₃ solution in each test tube.
9. Mix thoroughly, place the tubes in a boiling water for exactly 1 min, cool to room temperature and measure the absorbance at 650 nm against a blank reagent.
10. Prepare a standard curve using different concentration of catechol and find out the concentration of phenol in test sample and express as mg phenols / g tissue.

Lignin at 75 and 95 DAS : The samples were taken from basal 2nd internode region of the sorghum stalk at 75 and 95 DAS from parents and individual RILs and the lignin was estimated as follows.

A. Acid Detergent Fibre (ADF)

1. Weigh by difference approximately 1g of sample into a beaker suitable for refluxing.
2. Add 100 ml cold (room temperature) ADF solution and 2 ml decaline. Heat to boiling for 5 to 10 min. Reduce heat to avoid foaming as boiling begins. Reflux 60 min from onset of boiling, adjusting boiling to slow or even level.
3. Filter on previously weighed crucible, which is set on filtering apparatus, using light suction break of the filtered material with a rod and wash twice with hot water (90-100 °C), rinse the side of the crucible in the same manner.
4. Repeat washing with acetone until it removes no more color, break up all lumps so that the solvents come in contact with all particles of fibre.
5. Dry at 105 °C for 8 hr (or) overnight, cool in a dessicator to room temperature and weigh.
6. Add 72 % H₂SO₄ to this oven dried sample and determine the ash and lignin.

Acid detergent lignin

The first step is to prepare ADF as mentioned above.

1. Place the crucible in glass tray. Have one end of the tray 2 cm higher so that acid will drain away from the crucible.
2. Cover the contents of the crucible with cooled (5 °C) 72% H₂SO₄ and stir with glass rod to a smooth paste breaking all clumps. Fill crucible halfway with acid and stir. Let glass rod remain in crucible. Refill with 72% H₂SO₄ and stir at hourly interval till acid drains away.
3. Keep crucible at 20 – 23 °C.
4. After 3 hrs filter off as much acid as possible with vacuum. Rinse the residue once with 72 % acid and filter it off.
5. Wash contents with hot water (85 -95 °C) until free from acid, Rinse and remove stirring rod.
6. Dry crucible overnight at 100 °C and take the weight.
7. Ignite crucible in a muffle furnace at 500 °C for 3 hr. Cool to 250 °C and transfer to a desiccators, cool to room temperature and weigh.

$$\text{Lignin (\% dry matter basis)} = \frac{\text{Weight of crucible and ash} - \text{Weight of empty crucible}}{\text{Weight of sample on dry matter basis}} \times 100$$

Yield and related traits

Observations on early seedling vigor, plant height, ear head length, threshability, 1000-seed weight, days to 50 per cent flowering, stem thickness and seed yield, were also recorded as follows

Early seedling vigour : Early seedling vigour was recorded at 15 days after emergence of seedlings on an ordinal scale as follows

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 ear head tip of plant.

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

Threshability : The effectiveness of removing the grains by threshing was recorded as follows:

Score	Phenotype
1	Freely threshable (1-10 % unthreshed kernels)
2	Almost freely threshable (2-10 % unthreshed kernels)
3	Partly threshable (11-25 % unthreshed kernels)
4	Almost difficult to thresh (26-50 % unthreshed kernels)
5	Difficult to thresh (more than 50 % unthreshed kernels)

(Sorghum descriptor, ICRISAT)

1000-grain weight : One thousand seeds collected at random from threshed grain of each ear was counted in groups of 100, weighed and expressed in grams.

Days to 50 per cent flowering : Time interval (days) from sowing to anthesis of at least 50 per cent of the plants was recorded as days to 50 % flowering.

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

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

3.2 Genetic linkage map construction and QTL mapping

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

3.2.1 Parental polymorphism for genic SSR markers

Initial screening of parents was carried out for polymorphism before genotyping all the RILs. For this, parental DNA from IS 22380 (P_1) and E 36-1 (P_2) was subjected to PCR amplification with a total of 530 genic SSR markers to screen the parents for the polymorphism if any. The sequence information of both forward and reverse primer of genic

SSRs markers used is given in Appendix III. The experimental protocols used were as follows.

Genomic DNA extraction from sorghum leaves

DNA was extracted from a bulk of 6-7 individual plants from each entry. Three to four week old seedlings were taken from field and frozen in liquid nitrogen. These tissues were stored at -80°C until use. Genomic DNA was prepared following Krishna and Jawali (1997) method with a few minor modifications as follows

1. Frozen tissue sample (2 g) was ground into fine powder in liquid nitrogen, using autoclaved mortar and pestle.
2. The ground tissue was transferred to 2.0 ml eppendorf tube containing 900 μl of extraction buffer, then 90 μl of 20 per cent sodium dodecyl sulphate (SDS) was 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 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. DNA was redissolved by tapping the pellet suspended in RNAase and incubated at room temperature (37°C) for 30 min.
13. The DNA was precipitated by adding $1/10^{\text{th}}$ volume of 3 M 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 the tubes were put upside down on paper towel to get rid of excess ethanol.
18. Pellet was dissolved in 100 μl of T_{10}E_1 .

Purification of extracted genomic DNA

1. Equal volume (100 μl) of phenol: chloroform: isoamylalcohol (25:24:1) mixture was added to each tube and the contents were mixed by inverting.
2. The contents were spun at 2500 rpm for 10 min and supernatant was transferred to fresh tubes.

- Steps 1 and 2 were repeated.
- To the above supernatant, 10 μ l of 3 M sodium acetate (1/10 volume of aqueous layer) and 2.5 volumes of chilled absolute ethanol was added, mixed gently and incubated at -20°C for 15-20 min.
- The DNA was spooled in 1.5 ml eppendorf tubes using a glass hook, washed with 70 % alcohol and dried.
- DNA was dissolved in 100 μ l $T_{10}E_1$ and kept at -20°C till its further use.

Determination of quantity and quality of isolated DNA

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

- Initialization of the instrument was done with nanopure water.
- The instrument was set blank with help of 2 μ l $T_{10}E_1$
- The quantity of DNA was measured by loading 2 μ l DNA sample on Nanodrop spectrophotometer pedestal.
- The machine gives the DNA quantity in ng/ μ l and OD value.

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

$$M_1V_1 = M_2V_2$$

$$(100 \text{ ng}/\mu\text{l}) \quad V_1 = (5 \text{ ng}/\mu\text{l}) (100 \mu\text{l})$$

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

$$V_1 = 5 \mu\text{l}$$

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

PCR conditions for amplifying of genic SSR alleles

EST-SSR developed at IABT, UAS, Dharwad were used in the present study and were synthesized from Sigma-Aldrich, Pvt. Ltd, USA. Primer dilutions were done by giving a brief spin to collect the amorphous primer stock at bottom of tubes supplied by the company and then distilled water was added to prepare stock solution of 100 pM and the tubes were incubated at 37°C for 30 mins. Then the working solution of 5 pM concentration was done using the formula mentioned in DNA dilution.

PCR optimization

Three concentrations of each of primer pair (0.2, 0.3, 0.5 pM), Mg^{2+} (1.0, 2.0, 5.0 mM), and *Taq* polymerase enzyme (In-house), and two concentrations each of DNA (5, 10 ng) and dNTP (0.1, 0.2 mM, Eppendorf, USA) were varied in different combinations and the combination that gave good amplification was selected. Amplifications were performed in a final volume of 25 μ l with 1x assay buffer. Three different programs of "Touchdown" PCR (Don *et al.*, 1991) with base annealing temperature ranges of 55°C – 50°C , 60°C – 55°C , and

65 °C–60 °C were used for standardization of annealing temperatures and primer pairs were classified into three groups based on annealing temperature range required by them to produce sharp bands without much of spurious products. In the initial annealing steps, the annealing temperature was decreased by 1°C after two subsequent cycles for first 10 cycles. Products were thereafter amplified for 30 cycles at the appropriate optimum annealing temperature with a final extension of 20 min.

Amplification reaction mixture for genic SSRs was prepared in 96 well PCR plate tubes (Tarson Pvt. Ltd, India) containing following components in a total volume of 25 µl.

Components	Quantity (µl/reaction)
Genomic DNA (5 ng/ml)	2.0
dNTPs mix (2.5 mM each)	1.0
Forward Primer (5 pM/µl)	1.0
Reverse primer (5 pM/µl)	1.0
MgCl ₂ (25 mM)	1.0
10x assay buffer	2.5
<i>Taq DNA polymerase</i> (in-house)	0.34
Sterile water	16.16

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

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

3.2.2 Separation of genic SSR PCR products

Separation and visualization of PCR products was done on both agarose (3 %) as well as polyacrylamide gels (6%). Agarose gels were used to check amplification and polymorphism between the two parents, if any could be detected than we carried out the gnotyping for all the 93 RILs using agarose. The genic SSRs which did not show any

detectable polymorphism between parents on 3.5 % agarose gels with ethidium bromide staining were carried forward to silver staining pipeline.

Agarose gel electrophoresis

Agarose was casted in 3.5 % gels in TAE buffer (1×). Slabs were casted in a horizontal gel frame (Hoefer HE99X 18 x 30 cm Amersham Bioscience. Pvt. Ltd. USA) and products were visualized by incorporating 1 µl (10 mg/ml) ethidium bromide per 10 ml of gel solution and viewed in a gel documentation system (Syngene. Pvt. Ltd. USA). The procedure of gel electrophoresis is as follows

1. The perspex tray and comb were thoroughly was cleaned with 70% alcohol with tissue paper.
2. The ends of perspex tray were sealed with spacers and comb was inserted.
3. Agarose gel (3.5 %) was prepared by adding 3.5 g agarose to 100 ml of TAE (1×) buffer (EDTA 0.5 M at pH 8).
4. The solution was boiled by putting the flask in microwave oven and cooled to 60°C.
5. Ethidium bromide (5 µl of conc. 10 mg/ml) was added to the gel and mixed gently.
6. The gel was poured into the tray and air bubbles were removed by using pipette. After the gel was completely set, tape was removed and the gel was placed into the electrophoresis tank.
7. Approximately 500 ml of TAE (1×) buffer was poured into the electrophoresis tank enough to cover the gel to a depth of 5 mm.
8. Comb was removed carefully.
9. About 1/10th volume of loading buffer (6×) bromophenol blue dye was added to DNA samples and mixed by gentle tapping and spinning for 2-3 sec in a microcentrifuge.
10. DNA samples were loaded onto the wells and the power supply of about 80 V was provided to run the gel.
11. The power supply was switched off when was about 2 cm from positive end, and the gel was removed from the gel apparatus.
12. The gel was viewed and photographed by using gel documentation system (UVI Tech England).

Polyacrylamide gel electrophoresis

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

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

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

For casting each gel, 80 ml of 6 per cent acrylamide gel was prepared. Just prior to pouring, 60 µl of N, N, N'N'-tetramethylethylenediamine (TEMED) and 600 µl of 10 per cent ammonium per sulphate (APS) was added to initiate polymerization. The contents were mixed gently by swirling and the bubbles were avoided. Before pouring the assembly was kept on the bench top so that it made 45-degree angle with the bench top. Assembly was tilted such that one of the bottom corners is raised above the other (off the bench top) and then carefully the solution was poured into the space between the glass plates starting at the lower corner. As the acrylamide solution filled the space, gel assembly was lowered 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 the pouring, they were dislodged by tapping. Gel was left for 20 to 40 min for complete polymerization.

Electrophoresis of polyacrylamide

1. After the polymerization process, 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.
2. Tris free base, boric acid and 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).
3. Comb was removed and then excess polyacrylamide gel was removed with razor blade. Tissue paper was used to clean the glass plates with buffer.
4. Air bubbles and unpolymerised acrylamide on the top of gel were removed by squirting with 5x TBE.
5. 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.
6. SSR loading dye (3x STR dye) was added to PCR products to a final of 1x and samples were denatured by heating to 95 °C for 4 min and immediately cooled on ice.
7. After the pre-run, flushed the urea from the well area 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.
8. Six µl of each samples were loaded into the wells for facilitating the sizing of the various alleles. DNA ladder of 100 bp size was loaded into the first or last well after denaturing.
9. Gel was run using the same conditions as in pre-run. It was electrophoresed till the dye reached bottom of the gel.

Visualization of genic SSR alleles

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

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

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 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.

Genotyping RILs with genic SSRs

Mapping population consisting of 93 RILs and parents were screened with polymorphic genic SSR markers.

Scoring of genic SSRs alleles and genotyping

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

1. Homozygote for the allele from parental strain P_1 (IS 22380) at the locus as $A=1$
2. Homozygote for the allele for parental strain P_2 (E 36-1) at the locus as $B=3$
3. Heterozygote carrying the alleles from both P_1 and P_2 strains $H=2$
4. Off types showing banding pattern different from the parents and missing data for the individual at the locus - = 0

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

3.2.3 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 2.0 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.

The linkage map with 85 marker published by Reddy *et al.* (2008) was used as reference map for assigning genic SSR markers to defined linkage groups. A total of 63 genic SSR markers were assigned to linkage groups using MAPMAKER/EXP version 3.0 b, as

described above. Together, a total of 148 markers were available to construct the linkage map.

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

3.2.4 QTL analysis

QTL analysis was carried out following multiple algorithms to ensure robustness, cross validation of the data

MAPMAKER/EXP performs full multipoint linkage analysis for dominant and co-dominant markers in a mapping population. The output of MAPMAKER/EXP contain information on linkage groups and linkage distances between the markers on each linkage group are included as input file for QTL Cartographer (2.5b) (Basten *et al.* 1997). QTL Cartographer is a suite of programs for mapping quantitative trait loci (QTLs) onto a genetic linkage map. The QTLs identified for components of resistance were assigned to the linkage groups based on linkage positions of markers on respective linkage maps. Threshold values with 300 times permutation and significance at 0.05 per cent was set as the cutoff for QTL detection.

3.3 Marker assisted introgression of charcoal rot resistance QTLs

Charcoal rot/stalk rot is a devastating disease of sorghum worldwide. High yielding lines with partial levels of resistance to charcoal rot resistance have been identified through traditional breeding approaches. Breeding for resistance to charcoal rot disease has been difficult due to its quantitatively inheritance with low to moderate heritability. Identification of genomic regions harbouring the gene cascades responsible for such genetically complex traits is made possible through QTL mapping approach. Identification for a marker pairs conditioning such QTLs can facilitate specific transfer of QTLs to desired background through marker assisted backcrossing method. Three stable QTLs, with major effect and coming up at all 3 environments were identified from the result of present investigations and were chosen for marker-assisted introgression one QTL each for lodging per cent (*Cr1*, LG-I), number of internode crossed by fungus (*Cr2*, LG-B) and length of infection (*Cr3*, LG-B) accounted for 43% of phenotypic variation of the available variation for charcoal rot resistance. These stable and major QTLs were introgressed in to the background of M35-1 and SPV86; both varieties are agronomically superior while highly susceptible for charcoal rot disease.

3.3.1 Plant material

The source of QTLs, E 36-1 was used as donor parent, M 35-1 and SPV 86 were two recurrent parent in the introgression programme.

Brief Description of parental line used in the introgression programme.

Donor parent used

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

Recurrent parent used

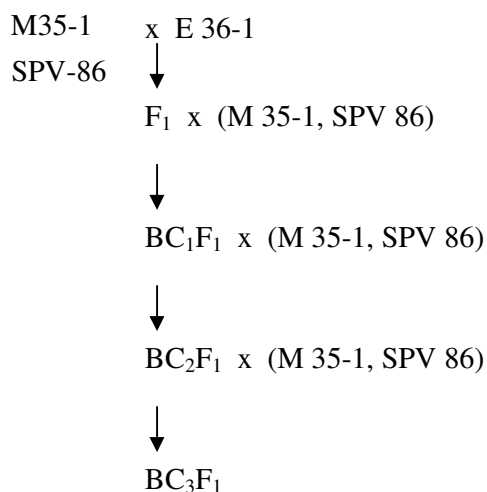
SPV 86: Is highly susceptible to charcoal rot disease. It is high yielding variety popular in North Karnataka and Maharashtra.

M 35-1: Widely cultivated popular genotype of Karnataka region for *rabi* growing situation. It is known for its agronomic eliteness and good grain traits (lusturous and bold seed grain. Though this variety is agronomically very good but is not tolerant to charcoal rot disease. It has good plasticity under field condition.

Hybridisation and segregating generations

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

Marker assisted backcross scheme



DNA isolation

The F₁s were heterozygous for all the target QTLs and so no MAS was employed. The F₁s were backcrossed to recipient line M35-1 and SPV 86 to produce BC₁F₁ population. For isolation of DNA Six-inch long leaf strips from two-week-old seedlings were collected from individual progeny of the BC₁F₁ and BC₂F₁ populations derived from the crosses SPV 86 × E 36-1 and M 35-1 × E 36-1.

3.3.2 Selection of the marker for marker assisted selection

SSR markers linked to QTLs for charcoal rot resistance component traits mapped to n two linkage groups LG-B and LG-I were used for selection of the individuals presumably having the donor alleles (foreground selection). At the same time to avoid the other genome region from donor parent from linkage group B and I, limited background selection was exercised to avoid the linkage drag around target QTL position. Two flanking markers outside the QTL region were used for identifying the backcross progenies having the recurrent parent allele at both the markers termed as double homozygotes at that particular loci.

SSR markers that were flanked to target QTL regions and were polymorphic between two parental lines were used for MAS. Backcross progenies with desired allele combination (heterozygous state in BC₁F₁ and BC₂F₁ at target QTL region) depending upon the resolution of marker for polymorphism, either 3.5 % agarose gel (ethidium bromide staining) or 6 % polyacrylamide gels were used for screening of backcross progenies

The list of markers used for foreground and limited background selection

Foreground marker	LG	Limited background marker
<i>xtxp176</i>	I	<i>xtxp 96</i>
<i>xtxp 312</i>	I	<i>xtxp274</i>
<i>xtxp297</i>	B	<i>xtxp48</i>
<i>xiabt 73</i>	B	<i>xiabt82</i>
<i>xiabt275</i>	B	<i>xiabt60</i>
<i>xiabt241</i>	B	<i>xiabt87</i>

The genotype E36-1 was used as donor parent for all the three stable QTL (*cr1*, *cr2*, *cr3*). The widely cultivated genotype M 35-1 and charcoal rot susceptible genotype SPV 86 was used as recurrent parent in Backcross programme. The F_1 was emasculated and pollinated with pollen of recurrent parent M35-1 and SPV 86 to produce BC_1F_1 seeds. The selected BC_1F_1 progeny heterozygous for all the three target QTL region was used for emasculation and simultaneously backcrossing with recurrent parent M35-1 and SPV 86 to produce BC_2F_1 progeny seed. The progeny of BC_2F_1 were screened for foreground and limited background selection and selected plant were backcrossed with recurrent parent to get the $BC_3 F_1$ progeny seed.

Scoring nuclear SSRs and genic SSRs alleles in the Backcross progenies

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

1. "A"-Homozygote at marker locus as that of donor parent (E 6-1)
2. "B"- Homozygote at marker locus as that of recurrent parent (M35-1 / SPV86).
3. "H"-Heterozygote at marker locus as that of E36-1 and M35-1/ SPV86.
4. "O" - Off types showing banding pattern different from the parents and
5. " _ " missing data for the individual at the locus

3.4 Real-time functional analyses of transcription factor genes

Plants are continually exposed to a vast array of pathogens, and generally succeed in defending themselves. An important part of this process is the induction of defence gene products that hinder pathogen growth and delay or prevent disease development. The way in which plants do this is multi-factorial, and includes regulatory proteins known as transcription factors (TFs) which form the early responsive genes directly regulating the expression of defense related genes. The expression and activity of these TFs can be differentially regulated by pathogen challenge. To identify TFs potentially involved in plant defence signaling, the expression of 185 TF genes of 18 different families were selected to screen through Quantitative real-time PCR (qRT-PCR).

3.4.1 Selection of transcription factors

The sorghum transcription factor database from centre for bioinformatics, Peking University (<http://plantfdb.cbi.pku.edu.cn/web/index>) having 54 TFs families and 397 Transcription factors. Analysis of *Sorghum* transcription factors was thoroughly analysed. Analysis of *Sorghum* TF database shows different members in a family of transcription factors. They used the assembled transcripts data from PlantGDB to predict TFs and extensive annotations of the TFs, including similarity searches against major databases (Uniprot, Refseq, EMBL, and Transfac *et al*), InterPro domain information and EST expression. A set of 18 important families having 185 transcription factor gene member were selected for their expression analyses.

3.4.2 Disease manifestation and tissue collection

The resistant variety E36-1 and susceptible variety SPV 86 were chosen to study the differential gene expression of transcription factor genes through qRT-PCR. These two genotypes were sourced from MARS, Dharwad, All India co-ordinated sorghum improvement project, Dharwad.

Fungus inoculum used in this experiment was obtained from the working collection maintained at IABT. The D4 isolate which was previously identified in our lab (Rajkumar, 2004) as a virulent strain among the strains prevailing in the northern Karnataka sorghum growing situations was used. The stem tape inoculation technique developed by Mayee and Garud (1978) was followed. The mock inoculation to serve as control was carried out with SPV 86 and E36-1 without inoculum loaded grains. Both of these genotypes with mock inoculated and inoculated condition were grown in pots with two biological replicates. Moisture stress was induced at terminal growth stage for proper manifestation of charcoal rot disease.

Sample collection

Second internode region was carefully excised from randomly tagged plants of E36-1 and SPV 86 at 75 and 90 days after sowing, The tissue were immediately frozen in liquid nitrogen and later stored at -80 °C until RNA was isolated.

3.4.3 Preparation of cDNA.

Isolation of total RNA

Total RNA was isolated from 2nd internode region of inoculated and mock inoculated samples of E36-1 & SPV 86 using Trizol reagent (Sigma-Aldrich Pvt. Ltd. USA).

Approximately 0.5-1g of tissue was grounded into a very fine powder in a pre-chilled mortar and pestle. The ground tissue was transferred into a pre-chilled 50 ml conical tube and 5-10 ml of TRIzol reagent (1 ml of TRIzol/100 mg of tissues) was added. Contents were mixed well with vortex and 1 ml of the mixture was transferred into a labeled, RNase-free 1.5 ml tubes to incubate for 5 min at room temperature. After incubation, 200µl of chloroform per 1 ml of TRIzol was added and vortexed for 20 sec. This was incubated for 10 min at room temperature and centrifuged at 13,200 rpm for 10 min at 4 °C. The aqueous phase was carefully transferred into a new RNase-free 1.5 ml tube without disturbing the other phases. The tube was placed on the ice as soon as transferred. Equal volume of isopropanol was added and mixed gently by inverting (2-3 times). This was incubated on ice for 30 min and centrifuged at 13,200 rpm for 20 min at 4 °C. A very small pellet was visible at the bottom of the tube which was the total RNA. The supernatant was decanted and allowed to stand upside down on kimwipes for 5 min. The pellet was washed with 500µl of 75 % ethanol. The liquid was decanted and the inside of the tube was wiped dry with a clean kimwipe, without touching the pellet. The pellet was resuspended in 500 µl of RNase-free water and was incubated on ice for at least an hour and pipetted occasionally for dissolving the pellet. This was centrifuged at 13,200 rpm for 20 min at 4 °C and the supernatant was transferred into a new RNase-free 1.5 ml tube. It was precipitated with 10 % 3M sodium acetate and equal volume of isopropanol. Contents were incubated on ice for 1 hour or overnight at -80 °C and

Table 3: Details of transcription factor families and members selected for qRT-PCR quantification in sorghum

Sl. No.	TF family	Known members	Selected members	Annotated function
1	<i>Whirly</i>	2	2	Salicylic acid responsive and critical for plant disease resistance responses
2	<i>WRKY</i>	7	7	defence-response, Senescence, biosynthesis of secondary metabolites and
3	<i>MADS</i>	16	14	Signal-transduction pathway (pathogen induced and drought stress), floral organ identity determination
4.	<u><i>bHLH</i></u>	24	23	ABA responsive , modulating plant defence transcriptome
5	<u><i>AP2-EREBP</i></u>	19	19	Developmental processes, biotic and abiotic stress
6.	<i>bZIP</i>	25	22	Different environmental stress, ABA-responsive
7	<i>PHD</i>	15	15	Chromatin-mediated transcription regulation
8	<i>HB</i>	8	8	ABA-responsive
9	<i>MYB</i>	9	7	Plant stress response due to pathogen, wounding, and anaerobic stress.
10	<i>NAC</i>	19	19	Development of shoot apical meristem, floral organ, and formation of lateral root.
11	<u><i>ARF</i></u>	8	8	Auxin responsive,
12	<i>Trihelix</i>	1	1	ABA responsive, Fruit and seed development
13	<i>HMG</i>	14	14	Low temperature and ABA responsive, mostly present in rapidly dividing and undifferentiated cells.
14	<i>MBF1</i>	1	1	Phytohormone response
15	<i>CCAAT-HAP2</i>	3	3	Mitochondrial biogenesis
16	<i>CCAAT-HAP5</i>	7	7	Mitochondrial biogenesis
17.	<i>C2C2-YABBY</i>	3	3	Apical and floral meristem,
18.	<i>MYB-related</i>	25	12	control the cell proliferation and cell differentiation
Total			185	

next day, centrifuged at 13,200 rpm for 20 min at 4 °C. The final pellet was resuspended into 50 µl of RNase-free water and was stored at -80 °C.

Purification and quality check

The total RNA was treated to remove DNA using Turbo DNA-free™ kit (cat#AM1907 Ambion, USA) as per the manufacturer's instruction. In order to eliminate the genomic DNA, 20 µg of total RNA was digested with RNase-free DNaseI and finally the total RNA was precipitated into desired volume of water.

The quantity and quality of total RNA was checked using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA). The total-RNA was subjected to Nanodrop ND-1000 using RNase-free water as blank: absorbance was recorded at 260/280 and 260/230. Further, the sample was fractionated over a formaldehyde agarose gel. Absence of genomic contamination was subsequently confirmed by PCR with total-RNA as template (Caldana *et al.*, 2007).

Preparation of cDNA

Single stranded cDNA was prepared by using High Capacity cDNA Reverse Transcription Kit (cat#4374966, Ambion, USA) as per the manufacturer's instruction. All reagents were thawed and stored on ice in RNase and DNase free work environment. All individual reagents were mixed thoroughly and spun down.

Composition of components used for the cDNA synthesis

Sl. No.	Components	Volume/reaction (µl)
1.	10X RT buffer	2.00
2.	25X dNTP (100 mM)	0.80
3.	10X RT random primers	2.00
4.	MultiScribe™ Reverse Transcriptase	1.00
5.	RNase inhibitor	1.00
6.	Nuclease free water	3.20
7.	Total RNA (2 µg)	10.00
	Total volume	20.00

Components of cDNA reaction were pipetted and 2µg of total-RNA in a single 20µl reaction was quantitatively converted to single-stranded cDNA

Thermal condition for single-stranded cDNA conversion

	Step 1	Step 2	Step3	Step 4
Temperature	25 °C	37 °C	85 °C	4 °C
Time	10 min	120 min	5 sec	hold

3.4.4 Standardisation of Real-Time PCR condition

Primer design

Each of 185 TF member sequences from sorghum were carefully analyzed by comparing among members of same family for similarity and distinction; the distinct portion of the sequence of each member to differentiate from others was identified and targeted to design the PCR primer pairs. All Transcription factor families have a conserved region which common for all members. Non conserved region specific to each members was scanned for primer designing. The primer pair specific to all 185 TFs were designed using primer3plus software (http://frodo.wi.mit.edu/cgi-bin/primer3plus/primer3plus_www.cgi) and the primers were synthesized at Sigma-Aldrich, Pvt. Ltd, USA. The following parameters, predicted melting temperature (T_m) of $60 \pm 2^\circ\text{C}$, primer lengths of 20-24 nucleotides, guanine-cytosine (GC) contents of 45-55 % and PCR amplicon length of 100-200 base pairs (bp) were used for designing the primer pair for all 185 TFs. Again, primer pair specificity was confirmed using BLAST analysis in Sorghum Transcription Factor Database.

Primer concentration

In real-time quantitation of any gene expression, primer concentration is a one of the key factors. Primer concentrations ranging from 150 nM, 200 nM, 250 nM, 300 nM, 350 nM, and 400 nM were used to optimize the amplification. Primer at 200 nM concentration gave single melting curve, low Ct value, high fluorescence value and no primer dimer when loaded on gel. Hence, this concentration of primer was used for all the experimental study.

Annealing temperature

It is essential to determine optimal annealing temperature for each primer pair before its actual use. All 185 TFs were titered for optimal annealing temperature and divided into three group ranges; “ $59-60^\circ\text{C}$, 60°C and $61-63^\circ\text{C}$ ”.

Rreaction mixture

The master mix of different components of real-time PCR was prepared fresh to avoid handling errors. The reaction mixture of 10 μl containing 1.0 ng cDNA, 200 nM of each gene-specific primer and 5 μl of 2x SYBR green reagents (Ambion, USA) were used. Individual components of reaction mixture were standardized for 10 μl reaction volume.

Setting baseline and threshold level

The point of measurement (baseline and threshold level) should be accurately determined to reflect the quantity of a particular target within reaction. During PCR, changing the reaction conditions and environment can influence fluorescence. In general, the level of fluorescence in any well corresponds to the amount of target present in it. Fluorescence levels may fluctuate due to changes in the reaction medium creating a background signal. The background signal is most evident during the initial cycles of PCR prior to significant accumulation of the target amplicon. During these early PCR cycles, the background signal in all wells is used to determine the “*baseline fluorescence*” across the entire reaction plate. For data analysis, more accurate measurements of fluorescence are needed which are attained when the target amplification is sufficiently above the background signal.

The Eppendorf realplex detection software sets the default baseline from 3 - 15 cycles. To determine if the baseline needs to be adjusted, it was important to determine which reaction in the assay emerges earliest above the baseline. The highly abundant gene 18S rRNA and few other TFs gene started to amplify very early in 20th PCR cycle. Therefore baseline was not changed and set default between 3-15 cycles.

The threshold value is a numerical value assigned for each run, which reflects a statistically significant point above the calculated baseline. Once the baseline has been set correctly, the software automatically sets the threshold at 10 standard deviations above the mean baseline fluorescence. It was ensured that threshold line should be placed in the

exponential phase to increase the precision and quality of the experimental data and same baseline and threshold default setting was used for all PCR reactions.

Relative gene quantification analysis

The two most commonly used methods to analyze data from real-time PCR experiments are absolute quantification and relative quantification. Absolute quantification determines the input copy number, usually by relating the PCR signal to a standard curve and the relative quantification relates the PCR signal of the target transcript in a treatment group to that of other samples such as an untreated control. In this experiment, the relative quantification approach was used to measure the expression level of 185 transcription factor genes in charcoal rot disease infestation in resistant and susceptible cultivar at 75 and 90 DAS. The mathematical model $\Delta\Delta C_t$ method (livak 1997 and 2001; livak and schmittgen 2001) was used to determine relative expression ratio (fold change).

In real-time PCR, fluorescence was recorded at each cycle to monitor the generation of amplified product. For proper calculation of initial target levels, differences in efficiency of amplification (E) must be taken into consideration. Even small differences in amplification efficiencies (E) will get added up making large apparent differences in mRNA levels. The absolute quantification requires a set up of standard curves from which PCR efficiency will be deduced. But the disadvantages of standard curves are (i) the extra efforts and cost needed to set up additional samples (ii) Non matching E due to presence of inhibitors and serial dilutions.

The relative quantification with PCR efficiency correction was adopted to calculate the fold change expression. PCR efficiency of all the TF genes was obtained from the exponential phase of each individual amplification plot using the equation $(1+E) = 10^{\text{slope}}$ (Ramakers *et al.*, 2003). The LinRegPCR software based on the above equation proposed a linear regression on the log fluorescence per cycle number data as an assumption-free method was used to calculate starting concentrations of mRNA and PCR efficiencies for each sample. The log-linear part of the PCR data was determined for each sample by selecting a lower and an upper limit of a “window of linearity”. Linear regression analyses was used to calculate the intercept and the slope, log (No) and log (eff) respectively, from the straight line that fits best to the included data points. The individual PCR efficiency follows from the slope of the linear regression line ($\text{Eff} = 10^{\text{slope}}$) and used as a quality check to exclude possible contained samples.

To ensure unambiguous selection of data point within the “window of linearity”, the lines consisting of at least 4 and not more than 6 data points with the highest R^2 value (0.99) and slope close to the maximum slope were selected.

Processing the raw fluorescence data

Pre-requisite for LinRegPCR to achieve maximum PCR efficiency is background corrected fluorescence data points of each well. Raw fluorescence data was obtained from the eppendorf realplex using data extractor software (Eppendorf Pvt. Ltd, India).and this background was due to residual fluorescence of the dye, differences in tube transparency, dust, noise of the electronics etc. In the majority of cases, a variable background makes a near-linear contribution to the curves generated by the amplifier and it should be subtracted from the raw fluorescence without distorting the data considerably.

For background correction, the baseline fluorescence data was collected from 3-15 cycles. The fluorescence increments (raw fluorescence – Y_0) were normalized to reaction fluorescence background (Y_0) for each sample reaction (Yu, *et al.*, 2006), which mentions below:

Normalized fluorescence = raw fluorescence – Y_0 / Y_0

The proposed method minimized the influence of the initial vertical background shift of reaction. The background corrected or normalized fluorescence data was used to calculate

PCR efficiency by LinRegPCR software. The calculated PCR efficiency was used to derive fold expression of TFs gene using the following method:

$$Ratio = \frac{E(target)^{-\Delta Ct}}{E(control)^{-\Delta Ct}}$$

E_{target} = PCR efficiency of target gene in sample.

$E_{control}$ = PCR efficiency of target gene in control.

ΔCt = (Ct of target gene Ct of reference gene)

Real-time PCR condition and analysis of data

An eppendorf realplex instrument (Eppendorf Pvt. Ltd., India) was used for all real-time PCR amplifications. The data was analyzed by using delta-delta Ct method (Livak and Schmittgen, 2001; Czechowski *et al.*, 2004). To generate a baseline-subtracted plot of the logarithmic increase in fluorescence signal (ΔRn) versus cycle number, baseline data were collected between cycles 3 and 15. All amplification analyzed with threshold automatically set by the instrument. In order to compare data from different PCR runs or cDNA samples, C_t values for all the TF genes were normalized to the C_t value of 18S and Actin, which was the most stable and having lowest gene expression stability (M). Gene expression stability (M) of three genes Actin, B-tubulin and 18S in various tissue samples under different conditions was measured by Normfinder (Molecular Diagnostic Laboratory, Dept. of Clinical Biochemistry, Aarhus University Hospital, Denmark.). Different methods are available for estimating PCR efficiency; the classical method uses C_t values obtained from a series of template dilutions. An alternate method utilizes absolute fluorescence data capture during the exponential phase of amplification of each real-time PCR (Ramakers *et al.*, 2003). Comparison of the two methods yields very similar amplification efficiencies. Hence, PCR efficiency (E) of each primer pair of the TFs was estimated using LinRegPCR software from data obtained from the exponential phase of each individual amplification plot and the equation $(1+E) = 10^{\text{slope}}$ (Ramakers *et al.*, 2003). RT-PCR product was resolved on 3% agarose gel to check the specific amplification.

Real-time PCR amplification conditions

Stage	Step	Temperature ($^{\circ}\text{C}$)	Duration	No. of cycles
1.	Initial denaturation	95	10 min.	1
2.	Denaturation	95	15 sec.	40
3.	Annealing (vary with primers)	59.5, 60, 61.5	20 sec.	
4.	Extension	68	20 sec.	
5.	Melting curve	95	10 min.	1

3.5 Homology based functional annotation

BLAST similarity comparison was used to reveal possible functional relationship of each EST containing microsatellite which were mapped on to different linkage group. BLAST search was performed by using BLASTx program against the non redundant (nr) database. An E-value cut off was used as criteria to assign tentative identity to any sequence. BLASTx search results were visually inspected to ensure that the sequence similarity was contiguous before conclusion was made. The comparison was also done against cured, highly annotated, proteins in SWISS-PROT database, which contains proteins of demonstrated function. Upon close scrutiny, the EST sequences with function were classified based on their role in any organism.

3.6 Statistical analysis

3.6.1 Phenotypic characters

Statistical analysis was carried out by pooling of the phenotypic data for all the four years within the location rather than pooling of data across location for different season as per the calculation using Spearman's rank correlation (Spearman, 1906).

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

1. Rank both sets of data from the highest to the lowest. Make sure to check for tied ranks.
2. Subtract the two sets of ranks to get the difference 'd'.
3. Square the values of 'd'.
4. Add the squared values of d to get $\sum d^2$.
5. Use the formula $R_s = 1 - (6 \sum d^2 / n^3 - n)$ where n is the number of ranks.

If the R_s value is -1, there is a perfect negative correlation.

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

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

If the R_s value is 0, there is no correlation

If the R_s value falls between 0 and 0.5, there is a weak positive correlation.

If the R_s value falls between 0.5 and 1, there is a strong positive correlation

Analysis of variance (ANOVA)

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

Phenotypic and genotypic coefficients of variation

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

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

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

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 Sivasubramanian and Menon (1973), as follows.

0-10% : Low

10-20% : Moderate

20% and above : High

Phenotypic correlation

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

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

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 for (n-2) degrees of freedom for test of significance.

Heritability

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

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

Where,

H% = Heritability

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)

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

H = Heritability

P = Phenotypic standard deviation

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

Genetic advance as percent mean

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

Where,

GA = Genetic advance

$\bar{X}$ = General mean

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

0-10% : Low

10-20% : Moderate

Above 20% : High

3.6.2 Marker segregation

Using chi-square test the goodness of fit for segregation of marker loci was checked. The calculated χ^2 values were compared with table values at one degree of freedom for each marker locus. The marker status of any RIL or parents for a locus was scored either as P₁ or P₂ allele.

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

Where,

'O'-Observed frequency of allele

'E'-Expected frequency of the allele

3.6.3 Statistics used in qRT-PCR study

Use of statistical analysis on real-time PCR data is a new subject in gene quantitation and it is gaining importance. The Statistical data analysis in gene quantitation is performed on the basis of classical standard parametric test such as correlation coefficient, t-tests, standard deviation and standard error. The technical precision or reproducibility of real-time PCR measurement was assessed by performing replicate measurements in separate PCR runs (technical and Biological replicate). Correlation coefficient was calculated between two technical replicate and two biological replicate (technical replicate: same pool of cDNA; biological replicate: two different pool of cDNA obtained independently from different batch of total-RNA) to determine the reproducibility of the experiment. The standard deviation of ΔCt was calculated from the standard deviation of the target and reference values using the formula $S = (S_1^2 + S_2^2)^{1/2}$ (S₁= std. deviation of target gene and S₂ = std. deviation of reference gene).

A Student *t*-test was used to compare statistically the gene expression between two different treatments. The expression level ratio between two conditions with *P* values <0.05 (95% confidence) are taken into consideration to state that they were significantly up regulated or down regulated.

4. EXPERIMENTAL RESULTS

The results of various experiments of the investigation on “Genome wide molecular mapping, introgression of stable QTLs and expressional quantitation of transcription factor genes in charcoal rot manifestation in *Sorghum bicolor* (L.) Moench.” are presented under the following headings

- 4.1 Field evaluation of RILs for component traits of charcoal rot and yield
- 4.2 Construction of genetic linkage maps
- 4.3 QTL analyses and mapping
- 4.4 Introgression of stable QTLs into recurrent parents
- 4.5 Functional analyses of transcription factor genes

4.1 Field evaluation of RILs for component traits of charcoal rot and yield

One of the major difficulties in working with charcoal rot disease in sorghum has been in the accurate scoring the disease reaction. Plant maturity and the amount of inoculum influence the severity of the disease in a susceptible genotype (Plate 1). As a consequence, multiple evaluations made in different locations over years are essential to get an accurate account of the reaction of the genetic material and to compute the genetic contributions towards resistance. Results of the field experiments carried out in three locations over four seasons are as follows for charcoal rot and yield component traits.

The phenotypic data sets were compared for their consistency both over seasons and locations through a ranked correlation approach to determine the data pooling strategy. It was found that the data sets were more consistent within each location over season as compared to within a season over locations (Table 4). Hence, pooling of four years (2004, 2005, 2006 and 2007) data was done for statistical analysis separately for each of the three location viz., Dharwad, Bijapur and Solapur.

The data of all the parameters distributed normally at all the three locations of field evaluation and hence it was used directly for analysis of statistical and QTL mapping without transformation. During *rabi* 2007, the field evaluation of RIP was done to explore two biochemical traits viz., total phenols and lignin at 75 DAS and 95 DAS along with other traits associated with charcoal rot resistance and yield.

4.1.1 Estimates of variability and character association

Pooled analysis for variance was carried out for 11 characters. The appropriate degree of freedom (DF) and mean sum of squares (MSS) for all the three different location for each along with other genetic parameters viz., range, mean, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability estimates and predicted genetic advance as per cent of mean for all the characters were worked out. The analysis of variance revealed highly significant difference among RILs for all the 11 traits at all the three locations of Dharwad, Bijapur and Solapur. Data on biochemical parameters together with all other traits from Dharwad and Bijapur locations were separately analysed for the year 2007.

4.1.1.1 Dharwad location

a. Component traits of charcoal rot resistance

The estimate of variability parameters of charcoal rot resistance and component traits are presented in Table 5a to 5d. The frequency distribution patterns for all the traits are presented in Fig. 1 and graphical presentation of PCV and GCV are shown in Fig. 2.



Plate.1. Charcoal rot manifestation : pith discolouration, at 2nd internode in the field

Table 4: Pearson ranked correlations for component traits of charocoal rot resistance across locations and seasons of field experimentation

a) Lodging per cent

Season/Location	Dharwad	Bijapur	Solapur
2005 on 2006	0.95	0.94	0.94
2005 on 2007	0.95	0.94	0.95
2006 on 2007	0.94	0.94	0.96
	2005	2006	2007
Dharwad on Bijapur	0.93	0.92	0.92
Dharwad on Solapur	0.92	0.92	0.91
Bijapur on Solapur	0.93	0.89	0.93

b) Length of infection

	Dharwad	Bijapur	Solapur
2005 on 2006	0.95	0.94	0.96
2005 on 2007	0.93	0.92	0.95
2006 on 2007	0.95	0.94	0.95
	2005	2006	2007
Dharwad on Bijapur	0.92	0.9	0.91
Dharwad on Solapur	0.91	0.91	0.9
Bijapur on Solapur	0.92	0.92	0.92

c) Internodes crossed by the fungus

	Dharwad	Bijapur	Solapur
2005 on 2006	0.91	0.95	0.94
2005 on 2007	0.94	0.93	0.96
2006 on 2007	0.95	0.93	0.96
	2005	2006	2007
Dharwad on Bijapur	0.88	0.9	0.89
Dharwad on Solapur	0.9	0.91	0.91
Bijapur on Solapur	0.91	0.92	0.92

Number of internodes crossed by the pathogen

There was a significant difference among RILs and significant G x E interaction over seasons of field evaluation. The mean average was 2.91 cm with a range of 2.88 - 3.0 cm. The phenotypic and genotypic coefficients of variation for this trait were 1.45 and 1.05, respectively with a heritability of 52.7 per cent.

Length of infection

There was significant G x E interaction with significant difference between the RILs. Wide range of values was recorded for length of spread of infection with range of values between 10.15 and 47.05cm. There was normal distribution for this trait. High PCV (29.46) and GCV (29.24) with high heritability of 98.5 per cent and an moderate expected genetic advance of 16.62 per cent was noticed.

Lodging per cent

The mean average value for lodging per cent was 48.1cm with maximum and minimum value of 84.38 and 24.38 cm, respectively. The RILs differed significantly in there values with significant G x E interaction. High PCV (26.94) and GCV (23.75) with high heritability of 77.7 per cent with an associated genetic advance of 20.83 per cent over the mean were recorded for this trait.

Lignin at 75 DAS

The RILs used for phenotyping showed significant difference among themselves and showed a normal and continuous distribution, indicating the quantitative nature of the trait. The mean average of 4.06 with a range 3.15 - 5.06 was recorded. A PCV and GCV of 11.97 and 10.86, respectively with high heritability of 98 per cent was recorded for this trait.

Lignin at 95 DAS

Significant difference was observed between RILs for lignin content at 95 DAS, with mean average value of 5.33 with range between 4.28 to 5.98. The PCV of 9.78 and GCV of 9.66 with high heritability (97 per cent) and low genetic gene advance was recorded.

Total phenol at 75 DAS

Significant difference between the genotype of RILs were recorded. The variation of 3.59 -7.32 with mean average value of 5.00 was noticed. The PCV and GCV values were moderate with the values of 15.70 and 15.39, respectively with high heritability estimate of 94.96 per cent.

Total phenol at 95 DAS

All the RILs significantly differed in the values for total phenol content at 95 DAS. Mean average value of 4.74 with range of 3.26 - 6.08 was recorded. Moderate PCV and GCV of 15.59 and 15.53, respectively were recorded with high heritability estimate of 99.3 per cent was noticed.

b. Yield and other related traits

Early seedling vigour

The Mean sum of squares due to seasons was significant with high G x E interaction for early seedling vigour scored on an ordinal scale. The analysis of variance revealed significant differences between the RILs of IS 22380 and E36-1 derived RIP. A pooled mean value of 2.29 with a range of 1.25 - 3.56 was recorded. The PCV and GCV values of 26.59 and 16.00 respectively were recorded with a moderate heritability of 36 per cent for this trait.

Table 5a: Pooled analysis of variance of eleven traits in the recombinant inbred lines derived from the cross IS 22380 x E 36-1 evaluated during 2004, 2005, 2006 and 2007 at Dharwad location

Source of variation	DF	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁
Environment	3	0.0026**	11.71**	94.68**	311.42**	1892.31**	4.53**	119.51**	4.62**	0.15	0.56*	579.12**
Replication	3	0.001	0.125	9.67	4.92	7.91	0.25	1.33	1.12	0.097	1.15	3.43
Genotypes	92	0.0027**	1254.11**	2595.51**	75.76**	1411.3**	1.99**	131.51**	159.11**	3.67**	2.56**	846.32**
G × E	276	0.0027**	197.05**	491.22**	18.75**	277.81**	0.57**	19.63**	27.23**	0.41**	0.31**	191.31**
Error	828	0.0009	0.99	37.74	0.36	5.01	0.02	0.53	1.22	0.23	0.23	2.45
S.Em.±		0.0106	0.35	2.18	0.25	0.8	0.06	0.25	0.39	0.2	0.17	0.67
CD at 5 %		0.02	0.94	5.84	0.69	2.14	0.16	0.67	1.11	0.57	0.49	1.92
CD at 1 %		0.03	1.29	8.02	3.68	2.94	0.22	0.92	1.50	2.23	1.92	2.61
CV (%)		7.80	13.51	12.73	12.93	5.58	5.51	12.09	13.91	20.94	21.24	11.52

* Significance at 5 % probability level

** Significance at 1% probability level

X₁ : Number of internode crossed

X₂ : Length of infection (cm)

X₃ : Lodging per cent

X₄ : Panicle length (cm)

X₅ : Seed yield /plant (g)

X₆ : Stem thickness (cm)

X₇ : Days to 50% flowering

X₈ : 1000 seed weight (g)

X₉ : Threshability

X₁₀ : Early seedling vigour

X₁₁ : Plant height (cm)

Table 5b: Magnitude of genetic variability parameters of eleven traits in the recombinant inbred lines derived from the cross IS 22380 x E 36-1 evaluated during 2004, 2005, 2006 and 2007 at Dharwad location

Trait	Range		Grand mean	PCV (%)	GCV (%)	Heritability (%)	GA as % of mean
	Minimum	Maximum					
X ₁	2.88	3.10	2.91	1.45	1.05	52.70	0.05
X ₂	10.15	47.05	27.81	29.46	29.24	98.50	16.62
X ₃	24.38	84.38	48.10	26.94	23.75	77.70	20.83
X ₄	13.8	25.82	19.51	9.98	9.54	91.40	3.72
X ₅	20.82	62.06	40.45	21.53	20.81	93.40	16.76
X ₆	2.41	4.45	3.63	10.97	9.48	74.70	0.53
X ₇	57.34	74.56	67.64	4.11	3.96	92.90	5.25
X ₈	18.16	37.67	27.92	11.02	10.28	87.00	5.52
X ₉	1.00	3.31	1.63	38.61	27.76	51.10	0.66
X ₁₀	1.25	3.56	2.29	26.59	16.00	36.20	0.46
X ₁₁	107.51	148.90	133.10	4.95	4.81	94.30	12.80

* Significance at 5 % probability level

** Significance at 1% probability level

X₁ : Number of internode crossed

X₂ : Length of infection (cm)

X₃ : Lodging per cent

X₄ : Panicle length (cm)

X₅ : Seed yield /plant (g)

X₆ : Stem thickness (cm)

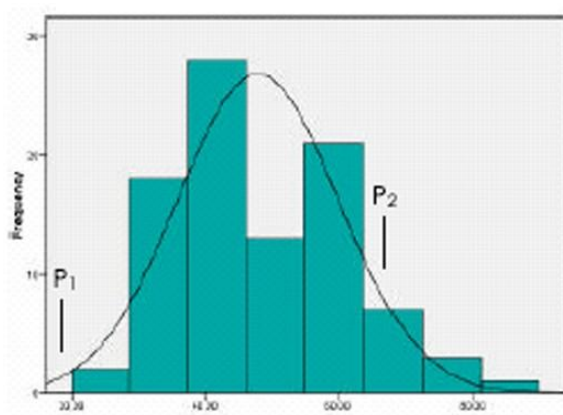
X₇ : Days to 50% flowering

X₈ : 1000 seed weight (g)

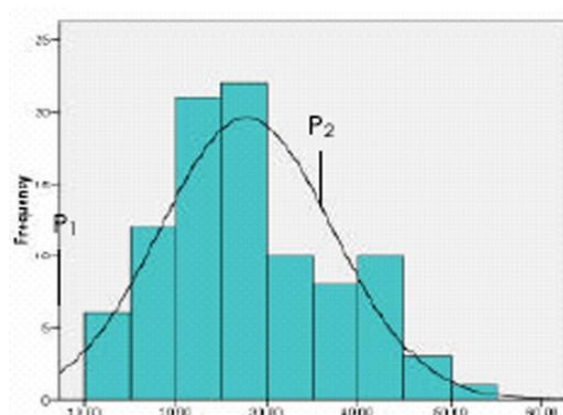
X₉ : Threshability

X₁₀ : Early seedling vigour

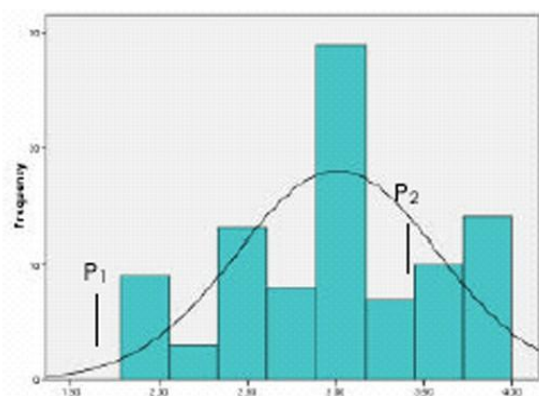
X₁₁ : Plant height (cm)



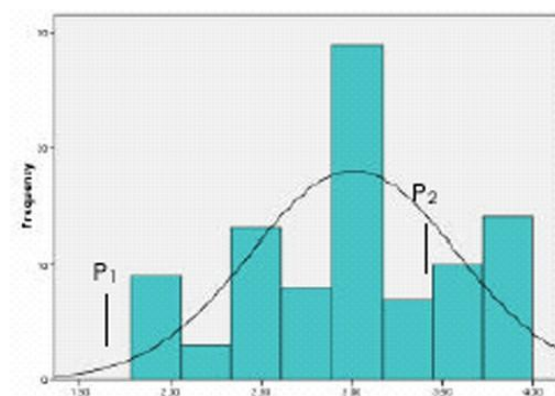
A. Lodging percent



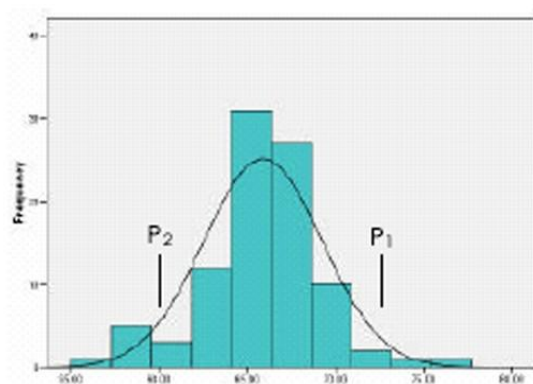
B. Length of infection



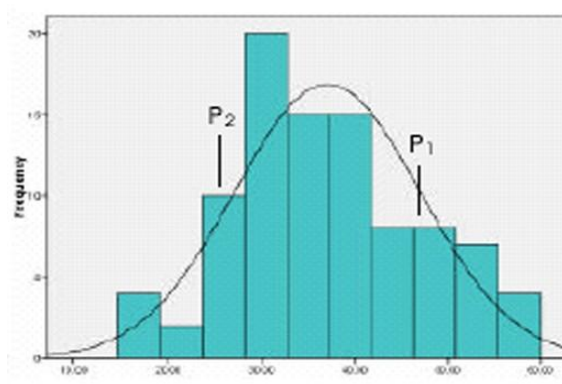
C. Number of internode crossed by fungus



C. Number of internode crossed by fungus



E. Days to 50% flowering

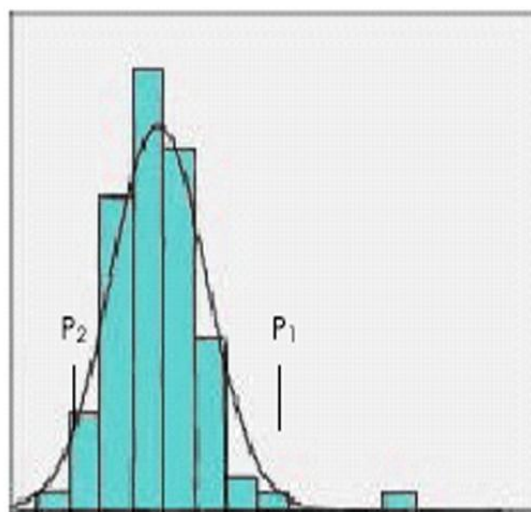


F. Grain yield

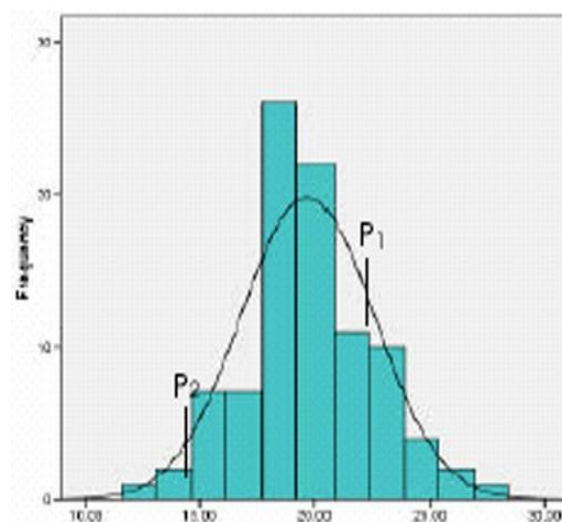
Legend : P₁ – E36-1 P₂ – IS 22380

Fig.1. Frequency distribution of charcoal rot resistance component and other yield traits at Dharwad location

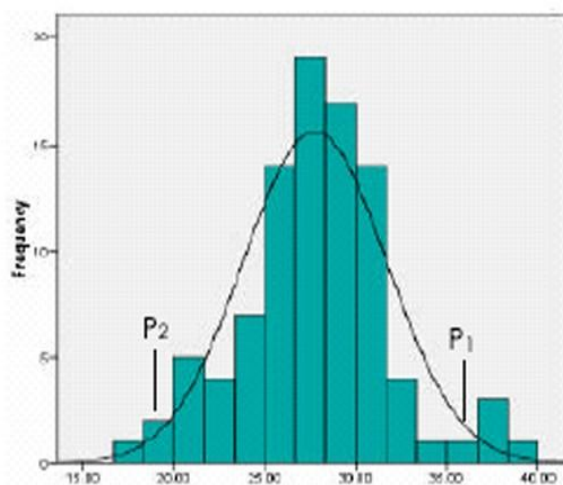
Fig.1. Contd....



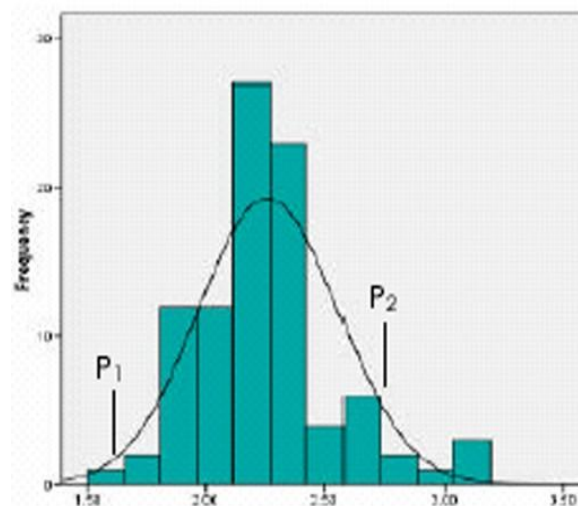
G. Stem thickness



H. Panicle length



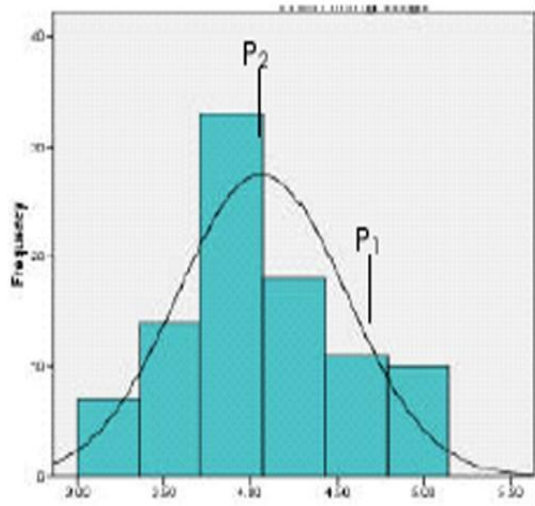
I. Seed weight



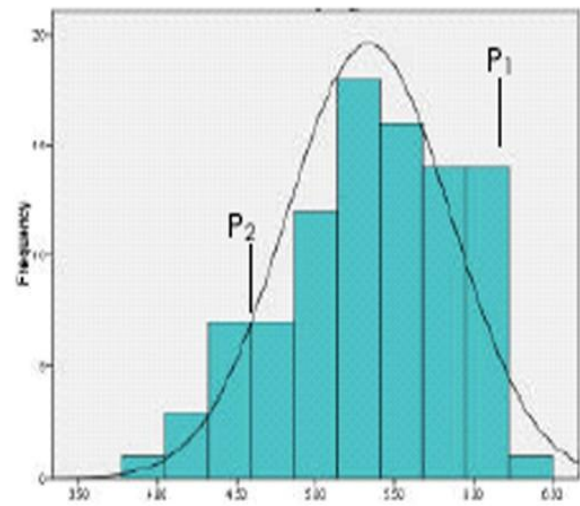
J. Early seedling vigour

Legend : P₁ – E36-1 P₂ – IS 22380

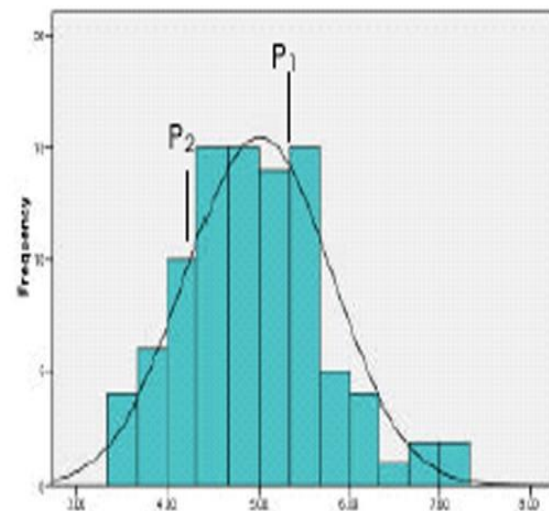
Fig.1. Contd....



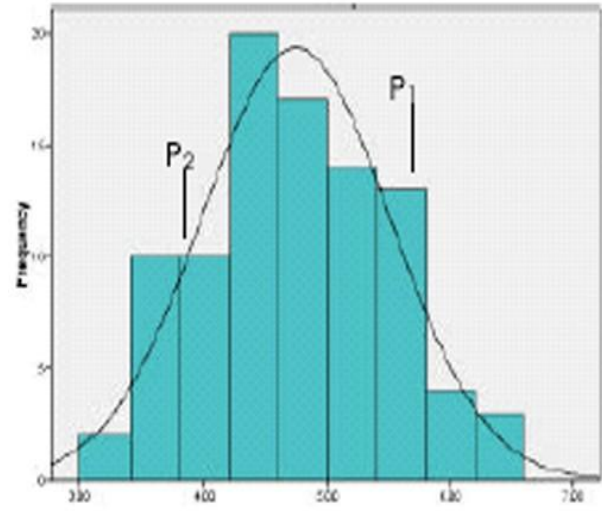
A. Lignin content at 75 DAS



B. Lignin content at 95 DAS



C. Total phenols at 75 DAS



D. Total phenol at 95 DAS

Legend: P₁—E36-1 P₂—IS22380

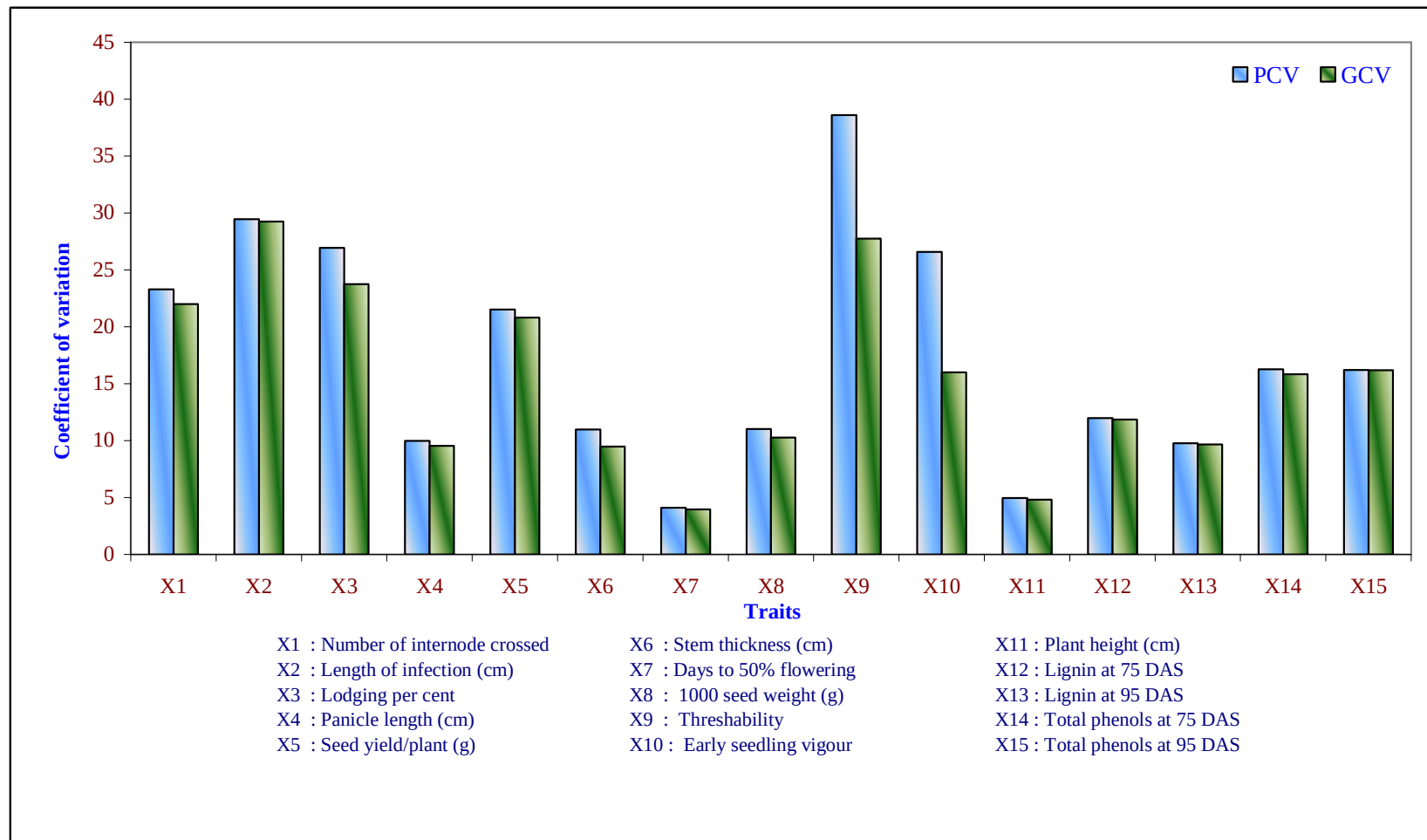


Fig.2. PCV and GCV of pooled data for 15 traits at Dharwad location

Table 5c: Analysis of variance for morphological and biochemical traits in the recombinant inbred lines derived from the cross IS 22380 × E 36-1 evaluated during 2007 at Dharwad location

Source of variation	DF	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅
Replication	3	0.25	0.69	44.81	15.53	38.95	1.15	4.87	0.34	0.08	0.82	5.66	0.02	0.015	0.04	0.006
Genotypes	92	1.31 **	359.71 **	585.01 **	23.73 **	400.61 **	0.69 **	44.85 **	56.10 **	1.09 **	0.94 **	282.15 **	0.93 **	1.06	2.54	2.35
Error	276	0.19	0.91	34.65	0.85	1.57	0.09	0.39	2.66	0.21	0.23	2.70	0.004	0.006	0.03	0.008
S.Em.±		0.311	0.67	4.17	0.64	0.89	0.21	0.44	1.15	0.32	0.34	1.16	0.04	0.05	0.13	0.02
CD at 5 %		0.87	1.86	11.61	1.80	2.42	0.55	1.22	3.24	0.89	0.94	3.24	0.11	0.14	0.36	0.05
CD at 1 %		0.91	2.41	15.34	2.35	3.34	0.74	1.62	4.23	1.17	1.28	4.38	0.15	0.19	0.5	0.08
CV (%)		14.71	3.61	12.25	14.99	3.41	10.31	10.95	5.91	20.26	22.6	1.24	1.7	1.54	3.89	2.67

*Significant at 5% probability level

**Significant at 1% probability level

X₁ : Number of internode crossed

X₆ : Stem thickness (cm)

X₁₁: Plant height (cm)

X₂ : Length of infection (cm)

X₇ : Days to 50% flowering

X₁₂ : Lignin at 75 Days after sowing

X₃ : Lodging per cent

X₈ : 1000 seed weight (g)

X₁₃: Lignin at 95 Days after sowing

X₄ : Panicle length (cm)

X₉ : Threshability

X₁₄ : Total phenols at 75 Days after sowing

X₅ : Seed yield/plant (g)

X₁₀ : Early seedling vigour

X₁₅: Total phenols at 95 Days after sowing

Table 5d: Magnitude of genetic variability parameters for morphological and biochemical traits in the recombinant inbred lines derived from the cross IS 22380 × E 36-1 evaluated during 2007 at Dharwad location

Trait	Range		Grand mean	PCV (%)	GCV (%)	Heritability (%)	GA as % of mean
	Minimum	Maximum					
X ₁	2.00	4.00	2.99	22.96	17.64	59.11	0.84
X ₂	12.93	50.85	27.80	34.28	34.11	99.02	19.41
X ₃	25.00	87.00	48.01	27.34	24.43	79.91	21.60
X ₄	12.20	25.30	18.41	13.93	13.00	87.21	4.60
X ₅	16.70	58.80	37.07	27.13	26.92	98.45	20.40
X ₆	2.12	4.20	2.97	16.67	13.07	61.56	0.63
X ₇	57.00	77.60	65.90	5.15	5.06	96.65	6.75
X ₈	19.10	38.80	27.86	14.37	13.12	83.34	6.87
X ₉	1.00	3.25	1.62	40.59	29.00	51.05	0.69
X ₁₀	1.00	3.75	2.27	27.87	17.97	41.61	0.54
X ₁₁	103.80	150.80	132.20	6.44	6.32	96.36	16.91
X ₁₂	3.15	5.06	4.06	11.97	11.86	98.11	0.98
X ₁₃	4.28	5.98	5.33	9.78	9.66	97.53	1.05
X ₁₄	3.59	7.32	5.00	16.28	15.83	94.61	1.59
X ₁₅	3.26	6.08	4.74	16.20	16.19	99.91	1.58

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

X₁ : Number of internode crossed

X₆ : Stem thickness (cm)

X₁₁: Plant height (cm)

X₂ : Length of infection (cm)

X₇ : Days to 50% flowering

X₁₂ : Lignin at 75 Days after sowing

X₃ : Lodging per cent

X₈ : 1000 seed weight (g)

X₁₃: Lignin at 95 Days after sowing

X₄ : Panicle length (cm)

X₉ : Threshability

X₁₄ : Total phenols at 75 Days after sowing

X₅ : Seed yield/plant (g)

X₁₀ : Early seedling vigour

X₁₅: Total phenols at 95 Days after sowing

Plant height

The RILs differed significantly for plant height with a pooled mean plant height of 133.1cm and with wide ranging values between 107.5 cm and 148.9 cm. The PCV and GCV of 4.95 and 4.81 respectively were recorded for this trait at Dharwad location with a high heritability value of 94.3 per cent together with an expected genetic advance of 16.74 per cent over mean.

Days to 50 per cent flowering

There was a significant difference in the mean sum of squares across the RILs of the mapping population. The mean number of days to 50 per cent flowering was 67.60 with a range of 57.34 to 74.56 days. The phenotypic and genotypic coefficients of variability were 4.11 and 3.96, respectively. The heritability of this trait was very high at 92.90 per cent with an expected genetic advance of 5.29 per cent.

Stem thickness

There was significant difference among RILs for this trait in the RIP evaluated at Dharwad location over four consecutive years. A significant G x E interaction was recorded in the pooled results. The mean value was 3.6 cm with a range of 2.41 to 4.45 cm among RILs. The PCV and GCV for this trait were 10.97 and 9.48, respectively with a high heritability of 74.1 per cent was recorded.

Ear head length

The analysis of variance showed significant difference among RILs both for mean performance and G x E interactions as revealed by the ANOVA of ear head length. The range of variation recorded was between 13.80 cm and 25.82 cm with a grand mean of 19.5 cm. The PCV and GCV were 9.98 and 9.54 per cent respectively. The heritability estimate was high with 91.4 per cent while a less genetic advance of 3.72 per cent over mean for this trait was recorded.

Threshability

The mean average grain mean of 1.63 with a range of 1.30 - 3.00 was recorded where the G x E interaction for this trait was found to be highly significant. A high PCV (38.61), GCV (27.6) and heritability (51 per cent) was recorded for this trait Dharwad field evaluation.

1000 seed weight

Significant differences between RILs were recorded for mean 1000 seed weight and G x E interactions. The mean 1000 seed weight among the RILs was 27.92 g. The RIP recorded a range of 18.16 - 37.67 g. The data distributed normally for this trait with moderate value of PCV (11.02) and GCV (10.28) with high heritability estimates of 87.1 per cent.

Grain yield per plant

Both mean performance and G x E interactions among RILs were significantly differed among RILs at Dharwad field evaluation. A mean grain yield 40.45g per plant with a wide range of 20.82 - 62.06g was recorded. High phenotypic and genotypic coefficients of variability of 21.53 and 20.81, respectively were recorded with high heritability (93.4 per cent).

c. Character association studies

The genotypic and phenotypic correlations among all the 11 traits were computed to understand the nature of association among and possible interactions leading to the manifestation of phenotypes (Table 6a to 6d).

Table 6a: Genotypic correlation coefficients (pooled) among eleven traits in the recombinant inbred lines derived from the cross IS 22380 x E 36-1 evaluated during 2004, 2005, 2006 and 2007 at Dharwad location

Traits	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁
X ₁	1	0.410**	0.027	0.047	-0.08	-0.023	0.128	0.024	0.036	0.094	-0.011
X ₂		1	0.045	0.194	-0.169	-0.055	-0.191	-0.047	-0.246**	-0.061	-0.073
X ₃			1	-0.025	-0.51	-0.031	0.069	-0.455**	0.189	-0.046	0.292**
X ₄				1	-0.087	-0.299**	0.299**	0.009	-0.061	0.052	-0.031
X ₅					1	-0.079	-0.202	0.461	-0.095	0.055	0.067
X ₆						1	0.017	-0.043	-0.15	-0.292**	0.034
X ₇							1	0.16	-0.187	0.362**	0.290**
X ₈								1	0.132	-0.151	0.235*
X ₉									1	-0.006	0.120
X ₁₀										1	-0.448**
X ₁₁											1

* Significance at 5 % probability level

** Significance at 1% probability level

X₁: Number of internode crossed
X₂: Length of infection (cm)
X₃: Lodging per cent
X₄: Panicle length (cm)
X₅: Seed yield /plant (g)
X₆: Stem thickness (cm)

X₇: Days to 50% flowering
X₈: 1000 seed weight (g)
X₉: Threshability
X₁₀: Early seedling vigour
X₁₁: Plant height (cm)

Table 6b: Phenotypic correlation coefficients (pooled) among eleven traits in the recombinant inbred lines derived from the cross IS 22380 x E 36-1 evaluated during 2004, 2005, 2006 and 2007 at Dharwad location

Traits	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁
X ₁	1	0.330**	0.033	0.033	-0.057	-0.017	0.097	0.015	0.037	0.075	-0.013
X ₂		1	0.025	0.187	-0.175	-0.046	-0.190	-0.032	-0.166	-0.033	0.07
X ₃			1	-0.024	-0.486**	-0.012	0.076	-0.408**	0.114	-0.037	0.269**
X ₄				1	-0.074	-0.266**	0.278**	0.008	-0.043	0.041	-0.035
X ₅					1	-0.074	-0.203*	0.44**	-0.069	0.037	0.081
X ₆						1	0.011	-0.04	-0.101	-0.161	0.009
X ₇							1	0.147	-0.131	0.215*	0.200*
X ₈								1	0.082	-0.081	0.216*
X ₉									1	0.001	0.083
X ₁₀										1	-0.256*
X ₁₁											1

* Significance at 5 % probability level

** Significance at 1% probability level

X₁ : Number of internode crossed

X₂ : Length of infection (cm)

X₃ : Lodging per cent

X₄ : Panicle length (cm)

X₅ : Seed yield /plant (g)

X₆ : Stem thickness (cm)

X₇ : Days to 50% flowering

X₈ : 1000 seed weight (g)

X₉ : Threshability

X₁₀ : Early seedling vigour

X₁₁ : Plant height (cm)

Number of internodes crossed

There was a positive association of number of internodes crossed by fungus with length of infection, lodging per cent, 1000 seed weight, days to flowering, threshability, panicle length and the trait was found to be negatively associated with seed yield and stem thickness both at genotypic and phenotypic level.

Length of infection

Length of infection was found to be positively correlated with number of internodes crossed by fungus at both genotypic (0.41) and phenotypic (0.33) level while negatively correlated with seed yield, threshability, early seedling vigour and plant height at Dharwad location.

Lodging per cent

This trait was positively correlated with number of internodes crossed, plant height, days to 50 per cent flowering, threshability and length of infection both at genotypic and phenotypic level. However, it recorded negative correlation with 1000 seed weight and early seedling vigor.

Lignin at 75 DAS

There was a positive and significant correlation at both genotypic and phenotypic level with lignin at 95 DAS, total phenol at 75 DAS. However, there was a negative correlation with length of spread of infection and lodging per cent at both phenotypic and genotypic level among the RILs.

Total phenol at 75 DAS

Total phenols at 75 DAS recorded a negative correlation with all the three charcoal rot traits viz., length of spread of infection, number of internodes crossed by the fungus and lodging per cent. There was a negative correlation with stem thickness, panicle length, plant height and days to 50 per cent flowering as well. However, this trait recorded a positive correlation with total phenol at 95 DAS, lignin at 75 DAS and 95 DAS both at genotypic and phenotypic level.

Total phenol at 95 DAS

This trait recorded a negative and significant correlation with length of spread of infection and number of internodes crossed by the fungus, lodging per cent and panicle length both at genotypic and phenotypic levels, while a positive and significant correlation with total phenol at 75 DAS, lignin at 75 and 95 DAS, both at genotypic and phenotypic level were noticed.

Early seedling vigour

Early seedling vigour showed significant positive correlation with days to 50 per cent flowering both genotypic (0.36) and phenotypic (0.21) level. Significant negative correlation (0.29) was recorded at genotypic level with stem thickness.

Stem thickness

Stem thickness showed significant negative correlation both at genotypic (0.30) and phenotypic (0.26) level with panicle length.

Days to 50 per cent flowering

Significant positive correlation with plant height was recorded both at genotypic (0.36) and phenotypic level (0.27) and also with early seedling vigour at both genotypic (0.23) and phenotypic (0.20) was observed at both genotypic and phenotypic level.

Table 6c: Genotypic correlation coefficient among the morphological and biochemical traits in the recombinant inbred lines derived from the cross IS 22380 × E 36-1 evaluated during 2007 at Dharwad location

Traits	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅
X ₁	1	-0.29*	0.194	0.005	-0.185	-0.017	0.099	0.032	0.008	0.116	-0.22	0.185	0.043	-0.097	-0.119
X ₂		1	0.072	0.11	-0.119	-0.023	-0.109	-0.007	-0.169	-0.085	0.076	-0.013	0.118	-0.067	-0.056
X ₃			1	-0.035	-0.327**	-0.015	0.053	-0.302**	0.063	-0.216*	0.206*	-0.136	-0.02	-0.048	-0.037
X ₄				1	0.015	-0.254*	0.27*	0.003	-0.011	-0.074	-0.042	-0.205*	0.011	-0.029	-0.009
X ₅					1	-0.014	-0.118	0.354**	-0.001	0.091	-0.013	-0.085	-0.027	0.093	0.144
X ₆						1	-0.161	-0.027	-0.007	-0.141	0.031	-0.139	-0.13	-0.091	-0.129
X ₇							1	0.028	-0.263**	0.379**	0.227*	0.061	0.032	-0.113	-0.189
X ₈								1	0.106	-0.117	0.055	0.004	-0.062	0.094	0.169
X ₉									1	-0.077	0.097	-0.029	-0.123	0.007	0.023
X ₁₀										1	-0.532*	0.148	0.185	-0.022	-0.111
X ₁₁											1	-0.191	0.097	0.028	0.062
X ₁₂												1	0.319	0.224*	0.17
X ₁₃													1	0.208	0.162
X ₁₄														1	0.952**
X ₁₅															1

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

X₁ : Number of internode crossed
X₂ : Length of infection (cm)
X₃ : Lodging per cent
X₄ : Panicle length (cm)
X₅ : Seed yield/plant (g)

X₆ : Stem thickness (cm)
X₇ : Days to 50% flowering
X₈ : 1000 seed weight (g)
X₉ : Threshability
X₁₀ : Early seedling vigour

X₁₁ : Plant height (cm)
X₁₂ : Lignin at 75 Days after sowing
X₁₃ : Lignin at 95 Days after sowing
X₁₄ : Total phenols at 75 Days after sowing
X₁₅ : Total phenols at 95 Days after sowing

Table 6d: Phenotypic correlation coefficient among the morphological and biochemical traits in the recombinant inbred lines derived from the cross IS 22380 × E 36-1 evaluated during 2007 at Dharwad location

Traits	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅
X ₁	1	0.25	0.137	-0.017	-0.143	-0.029	0.068	0.027	-0.016	0.077	-0.159	0.137	0.025	-0.086	-0.091
X ₂		1	0.065	0.105	-0.117	-0.017	-0.105	-0.009	-0.116	-0.053	-0.075	-0.013	0.116	-0.063	-0.056
X ₃			1	-0.033	-0.29**	-0.004	0.044	-0.242*	0.033	-0.097	0.19	-0.115	-0.026	-0.045	-0.034
X ₄				1	0.015	-0.217*	0.157	0.019	-0.005	-0.023	-0.044	-0.188	0.01	-0.028	-0.008
X ₅					1	-0.016	-0.115	0.324**	-0.021	0.06	-0.013	-0.083	-0.026	0.092	0.142
X ₆						1	-0.128	-0.027	0.002	-0.064	0.021	-0.103	-0.088	-0.056	-0.101
X ₇							1	0.026	-0.197	0.231	0.200*	0.059	0.029	-0.107	-0.186
X ₈								1	0.057	-0.063	0.044	0.005	-0.05	0.082	0.155
X ₉									1	-0.019	0.064	-0.034	-0.092	0.006	0.017
X ₁₀										1	-0.332**	0.103	0.122	-0.035	-0.069
X ₁₁											1	-0.185	0.092	0.03	0.061
X ₁₂												1	0.316**	0.215*	0.168
X ₁₃													1	0.21*	0.16
X ₁₄														1	0.925**
X ₁₅															1

*Significant at 5% probability level

**Significant at 1% probability level

X₁ : Number of internode crossed

X₆ : Stem thickness (cm)

X₁₁: Plant height (cm)

X₂ : Length of infection (cm)

X₇ : Days to 50% flowering

X₁₂ : Lignin at 75 Days after sowing

X₃ : Lodging per cent

X₈ : 1000 seed weight (g)

X₁₃: Lignin at 95 Days after sowing

X₄ : Panicle length (cm)

X₉ : Threshability

X₁₄ : Total phenols at 75 Days after sowing

X₅ : Seed yield/plant (g)

X₁₀ : Early seedling vigour

X₁₅: Total phenols at 95 Days after sowing

Panicle length

Panicle length showed significant positive correlation with days to 50 per cent flowering at genotypic (0.30) and phenotypic (0.26) level and negative correlation with stem thickness at genotypic (0.29) and phenotypic (0.26) level.

1000 seed weight

Positive correlation at both genotypic(0.46) and phenotypic (0.43) level with seed yield per plant was observed while negative correlation at both genotypic and phenotypic level with correlation coefficient values of 0.45 and 0.40, respectively with lodging per cent was observed.

Threshability

Threshability of the grains was negatively correlated with length of infection at genotypic level with a correlation coefficient value of 0.25.

Plant height

There was positive correlation at both genotypic and phenotypic level with lodging per cent (0.30), days to 50 per cent flowering (0.29) and 1000 seed weight (0.23) while negative correlation was noticed with early seedling vigour at both genotypic and phenotypic level with coefficient of correlation 0.48 and 0.26 .

4.1.1.2 Bijapur

a. Component parameters of charcoal rot disease resistance

The estimate of variability parameters of charcoal rot resistance and component traits are presented in Table 7a to 7d. The frequency distribution patterns for all the traits are presented in Fig. 3 and graphical presentation of PCV and GCV are shown in Fig. 4.

Number of internodes crossed by the pathogen

Significant difference among RILs with a significant G x E interaction was observed. The values recorded were in the range of 2.86 to 3.0 with a mean of 2.91. The RILs showed continuous normal distribution. The phenotypic and genotypic coefficients of variation for this trait were 21.85 and 16.4, respectively with moderate heritability of 56.33 per cent.

Length of infection

All the RILs differed significantly with respect to spread of charcoal rot infection with significant G X E interactions. A wider range of between 13.07 and 41.60 cm, with a grand mean of 27.8 was recorded. Moderate values of PCV (25.53) and GCV (25.53) were recorded. High heritability of 97.6 per cent with moderate genetic advance of 13.23 per cent over mean was noticed.

Lodging per cent

There was significant difference among the RILs. A wide range of 28.12 to 77.5 per cent with a grand mean of 47.5 per cent lodging was recorded. The PCV of 25.45, GCV of 23.14 per cent and a high heritability (82.7) with an associated genetic advance of 20.56 per cent over mean was observed

Lignin at 75 DAS

The RILs showed significant difference among themselves and showed a normal and continuous distribution indicating quantitative nature of this trait .The mean average value of 4.07 with range of 3.16 - 5.18 was recorded. Moderate PCV (11.29) and GCV (11.22) with high heritability (98 per cent) values were observed for this trait.

Table 7a: Pooled analysis of variance of eleven traits in the recombinant inbred lines derived from the cross IS 22380 x E 36-1 evaluated during 2004, 2005, 2006 and 2007 at Bijapur location

Source of variation	DF	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁
Environment	3	1.09**	10382.00**	312.53**	8905.14**	1641.01**	10.67**	602.51**	113.51**	61.13**	41.05**	1025.32**
Replication	3	0.13	4.06	7.51	1.36	4.95	0.53	301.23	3.20	0.91	0.37	8.66
Genotypes	92	4.41**	782.2**	2047.01**	148.19**	1723.98**	2.47**	536.19**	233.19**	2.35**	1.54**	1188.17**
G × E	276	0.44*	106.6**	119.9**	86.30**	35.61**	0.53**	338.59**	3.98**	0.70**	0.94**	47.22**
Error	828	0.19	1.09	25.19	0.49	2.57	0.07	328.11	1.76	0.15	0.16	5.77
CD at 5 %		0.42	1.00	5.21	0.67	1.56	0.25	17.28	1.34	0.36	0.39	2.34
CD at 1 %		0.55	1.02	6.96	0.89	2.10	0.92	23.25	1.76	0.47	0.52	3.12
CV (%)		14.40	3.90	10.58	13.20	3.92	8.28	26.83	4.90	20.53	18.73	11.80

* Significance at 5 % probability level

** Significance at 1% probability level

X₁ : Number of internode crossed

X₂ : Length of infection (cm)

X₃ : Lodging per cent

X₄ : Panicle length (cm)

X₅ : Seed yield /plant (g)

X₆ : Stem thickness (cm)

X₇ : Days to 50% flowering

X₈ : 1000 seed weight (g)

X₉ : Threshability

X₁₀ : Early seedling vigour

X₁₁ : Plant height (cm)

Table 7b: Magnitude of genetic variability parameters of eleven traits in the recombinant inbred lines derived from the cross IS 22380 x E 36-1 evaluated during 2004, 2005, 2006 and 2007 at Bijapur location

Trait	Range		Grand mean	PCV (%)	GCV (%)	Heritability (%)	GA as % of mean
	Minimum	Maximum					
X ₁	2.00	4.31	3.02	21.85	16.40	56.38	0.77
X ₂	13.07	41.60	27.80	25.53	25.23	97.62	13.23
X ₃	28.12	77.50	47.50	25.45	23.14	82.70	20.56
X ₄	15.31	29.02	21.36	9.77	9.20	88.61	3.81
X ₅	23.22	64.42	40.60	25.61	25.31	97.71	20.91
X ₆	2.38	4.56	3.14	13.87	11.12	64.30	0.58
X ₇	58.56	82.60	67.60	27.32	5.20	36.92	1.38
X ₈	18.24	38.32	27.10	14.81	13.97	89.01	7.35
X ₉	1.12	3.56	1.86	28.72	20.08	48.98	0.54
X ₁₀	1.31	3.12	2.13	20.83	9.08	19.02	0.17
X ₁₁	103.00	154.70	134.20	6.55	6.30	92.51	16.74

* Significance at 5 % probability level

** Significance at 1% probability level

X₁ : Number of internode crossed

X₂ : Length of infection (cm)

X₃ : Lodging per cent

X₄ : Panicle length (cm)

X₅ : Seed yield /plant (g)

X₆ : Stem thickness (cm)

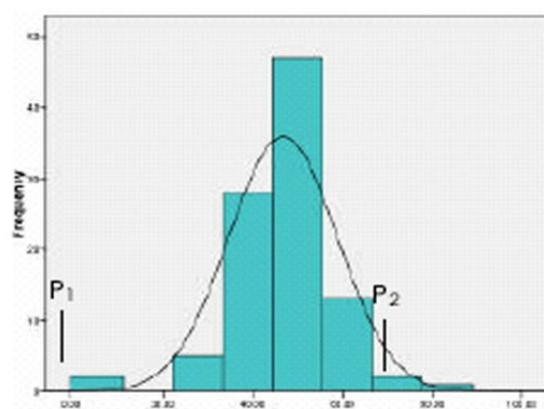
X₇ : Days to 50% flowering

X₈ : 1000 seed weight (g)

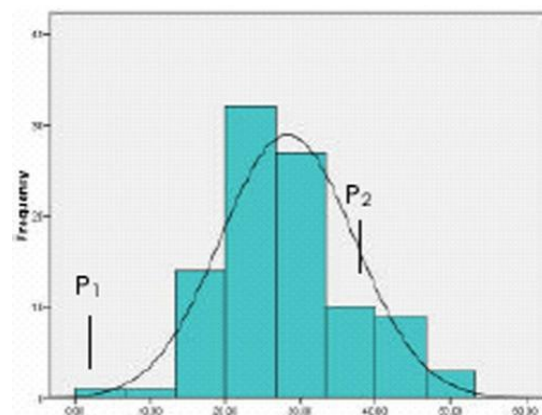
X₉ : Threshability

X₁₀ : Early seedling vigour

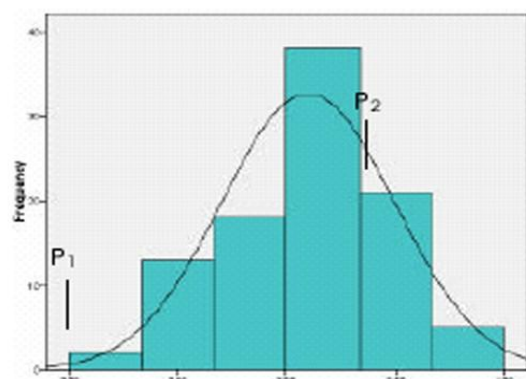
X₁₁ : Plant height (cm)



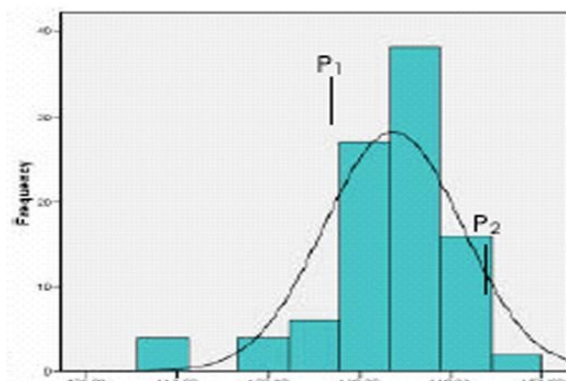
A. Lodging Percent



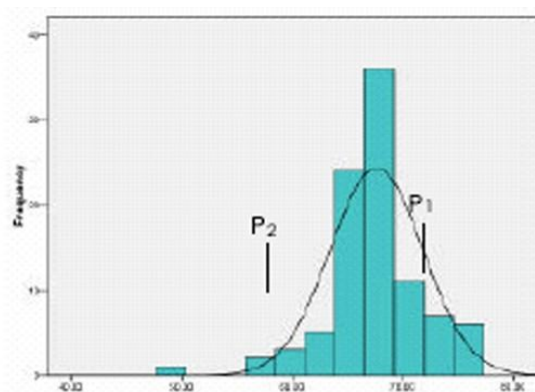
B. Length of infection



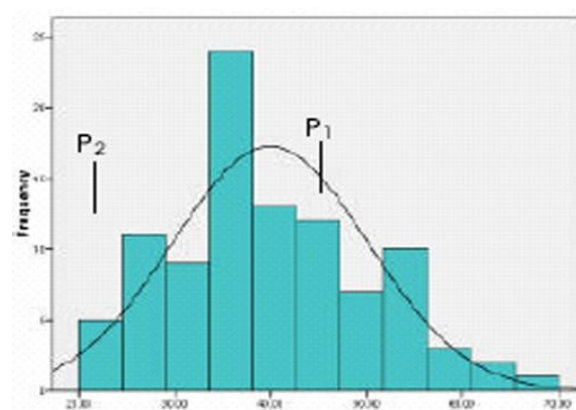
C. Number of internode crossed



D. Plant height



E. Days to 50 % flowering

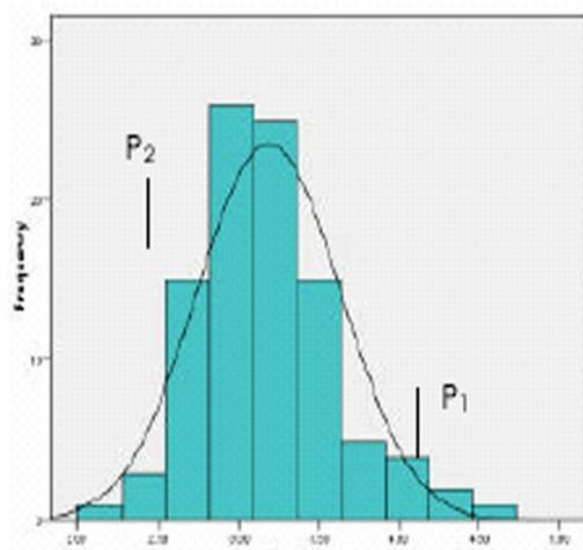


F. Grain yield

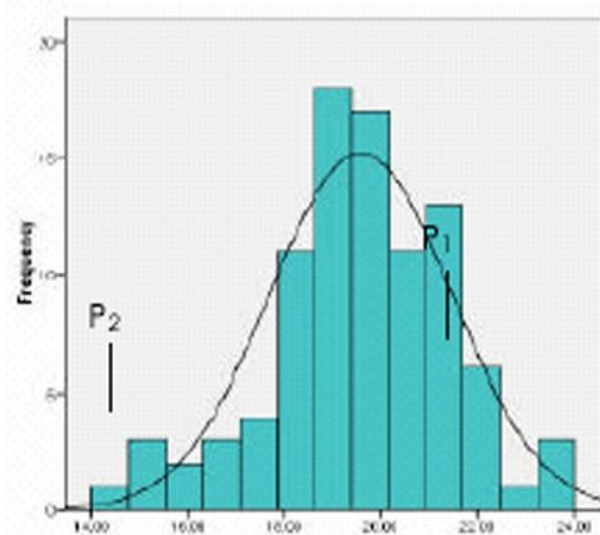
Legend : P₁ – E36-1 P₂ – IS 22380

Fig.3. Frequency distribution of charcoal rot resistance component and other yield traits at Bijapur location

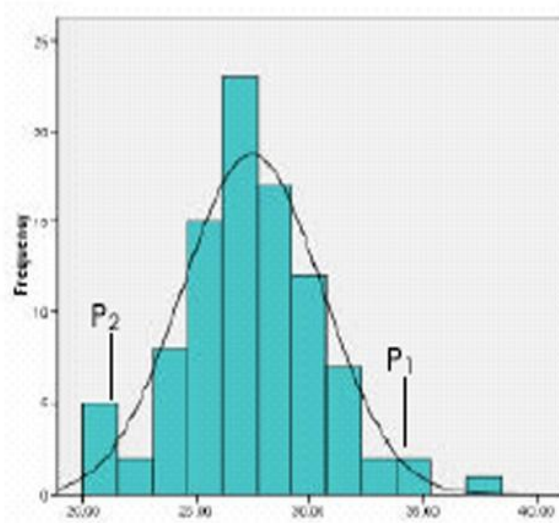
Fig.3. Contd....



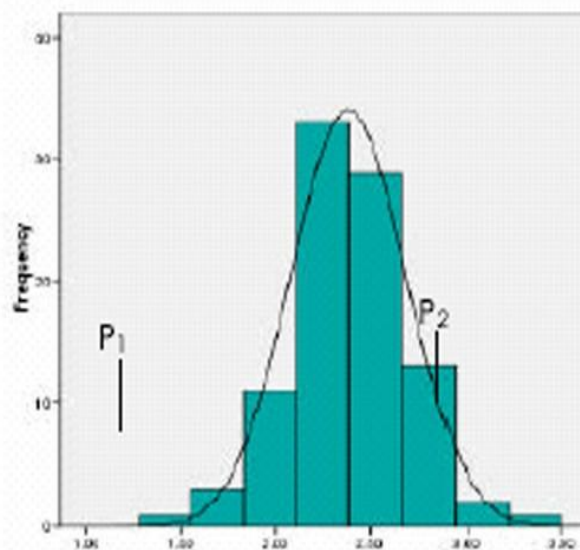
G. Stem thickness



H. Panicle Length



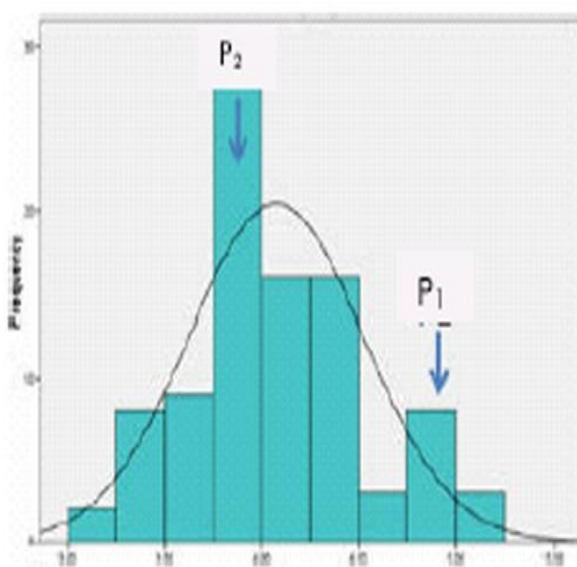
I. Seed weight



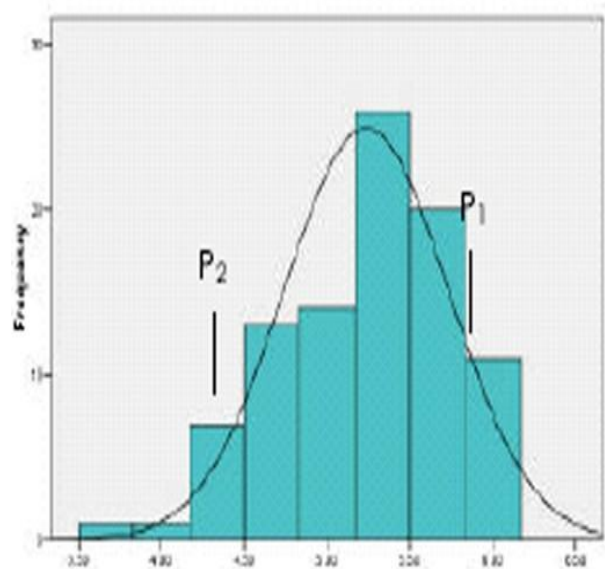
J. Threshability

Legend : P₁ – E36-1 P₂ – IS 22380

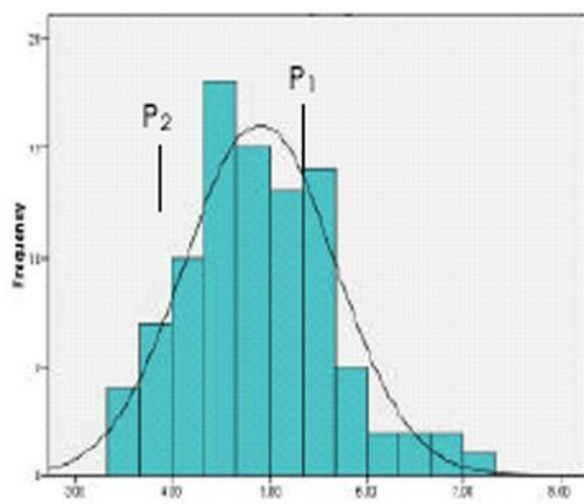
Fig.3. Contd.....



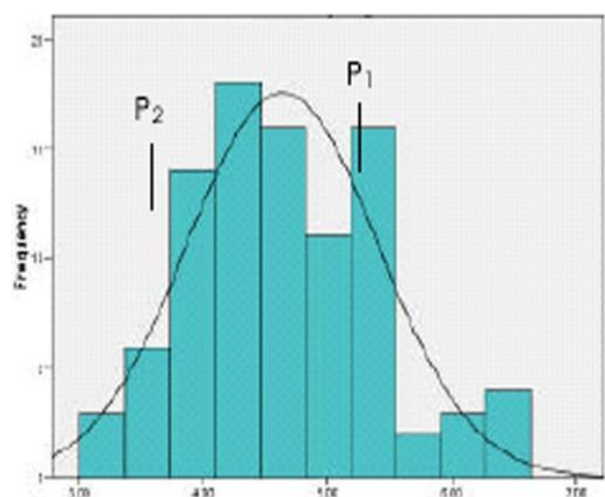
A. Lignin content at 75 DAS



B. Lignin content at 95 DAS



C. Total phenol at 75 DAS



D. Total phenols at 95 DAS

Legend : P₁ – E36-1 P₂ – IS 22380

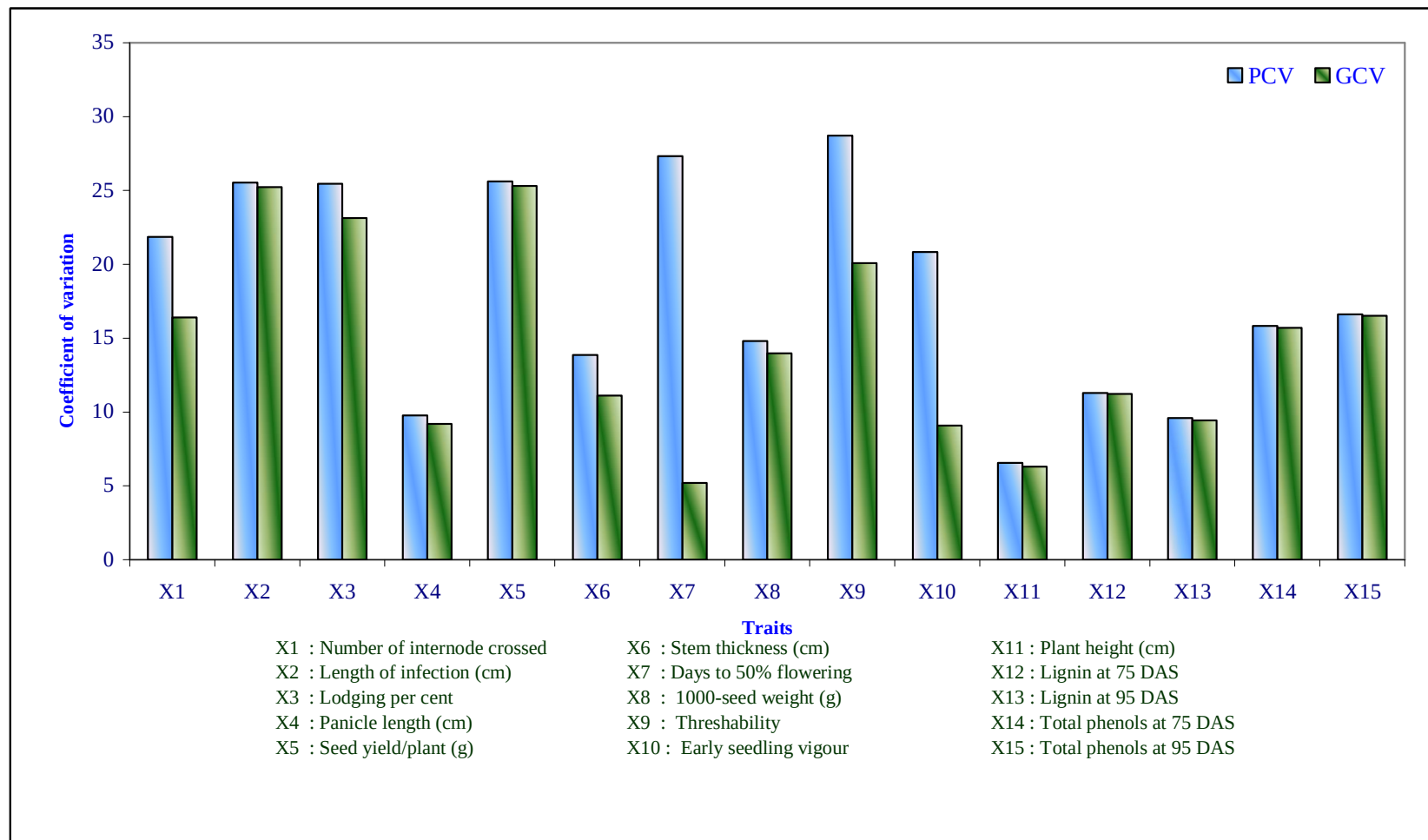


Fig.4. PCV and GCV of pooled data 15 traits at Bijapur location

Lignin at 95 DAS

Significant difference was observed between RILs for lignin content at 95, with mean average value of 5.23 with range of 3.76 - 6.06. Low values of PCV (9.78) and GCV (9.66) with high heritability (97 per cent) and low genetic gene advance was recorded.

Total phenol at 75 DAS

Significant difference was observed among the RILs for total phenol at 75 DAS with range of values between 3.64 -7.24. The PCV (15.83) and GCV (15.70) values were moderate with high heritability estimate (98.3 per cent) and low genetic gain.

Total phenol at 95 DAS

Significant difference was observed among RILs for total phenol content at 95 DAS. Mean value of 4.63 with range of values between 3.33 and 6.45 were recorded. Moderate PCV (16.2) and GCV (16.52) with heritability estimate of 99.3 per cent were noticed.

b. Other yield traits

Early seedling vigour

The analysis of variance revealed significant difference among the RILs .The environment effects was also significant for this trait with significant G x E interaction. 1.31 to 3.12 was the observed range with mean value of 1.86. The PCV and GCV were 20.83 and 9.08, respectively. Moderate heritability of 19.0 per cent and genetic gain of 17.0 were recorded.

Plant height

A large significant variation was observed for plant height with the values among RILs ranging from 103.0 to 154.7cm with an overall mean of 134.2 cm. The phenotypic and genotypic coefficients of variability were 6.55 and 6.3, respectively. The estimate of heritability was high with per cent heritability value of 92.5 per cent and an expected genetic advance of 16.74 per cent over mean.

Day to 50 per cent flowering

The mean number of days required to 50 per cent flowering was 67.6 with a range of 58.56 - 82.1. The phenotypic and genotypic coefficients of variability were 27.32 and 15.2, respectively. The heritability estimate of 36.0 per cent with an expected genetic advance of 13.8 was observed for this trait.

Stem thickness

There was significant difference for this trait among RILs. The mean value for this trait was 3.14 cm with range of 2.38 to 4.86 cm. The PCV and GCV for this trait were 82.6 and 67.6, respectively. Heritability of 14.81 per cent and GA of 13.97 were recorded.

Ear head length

The analysis of variance showed significant GxE interaction in RILs. The range of variation recorded was between 15.31 and 29.02 cm with an overall mean of 21.36 cm. The PCV and GCV were 9.77 and 9.20 per cent, respectively. The heritability estimate was high with 88.6 per cent while a less genetic advance of 3.81 per cent over mean was recorded.

Threshability

Mean value for threshability was 1.63 with a range between 1 and 3.31. Significant differences between RILs for threshability were recorded. PCV and GCV were 38.61 and 27.6 respectively with moderate heritability of 51 per cent.

Table 7c: Analysis of variance for morphological and biochemical traits in the recombinant inbred lines derived from the cross IS 22380 × E 36-1 evaluated during 2007 at Bijapur location

Source of variation	DF	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅
Replication	3	0.19	0.64	37.62	0.29	0.13	0.69	1286.2	0.72	0.59	0.50	9.51	0.001	0.012	0.013	0.02
Genotypes	92	1.08**	293.19**	422.53**	22.99**	422.42**	0.81**	1398.16**	57.86**	1.39**	0.92**	334.11**	0.83**	0.98**	2.39**	2.35
Error	276	0.29	2.11	32.73	0.61	2.86	0.07	1312.2	0.47	0.08	0.19	5.43	0.002	0.008	0.01	0.007
S.Em.±		0.42	1.03	4.04	0.55	1.20	0.19	25.61	0.48	0.20	0.30	1.67	0.04	0.06	0.07	0.06
CD at 5 %		1.16	2.86	1.23	1.53	3.36	0.53	71.13	1.33	0.57	0.83	4.41	0.11	0.17	0.95	0.17
CD at 1 %		1.54	3.81	14.82	2.02	4.41	0.71	92.31	1.77	0.75	1.13	6.32	0.15	0.22	0.26	0.22
CV (%)		17.14	5.07	12.05	4.31	4.22	8.67	13.6	12.60	17.35	17.15	1.73	1.24	1.75	2.06	1.83

*Significant at 5% probability level

**Significant at 1% probability level

X₁ : Number of internode crossed

X₆ : Stem thickness (cm)

X₁₁: Plant height (cm)

X₂ : Length of infection (cm)

X₇ : Days to 50% flowering

X₁₂ : Lignin at 75 Days after sowing

X₃ : Lodging per cent

X₈ : 1000 seed weight (g)

X₁₃: Lignin at 95 Days after sowing

X₄ : Panicle length (cm)

X₉ : Threshability

X₁₄ : Total phenols at 75 Days after sowing

X₅ : Seed yield/plant (g)

X₁₀ : Early seedling vigour

X₁₅: Total phenols at 95 Days after sowing

Table 7d: Magnitude of genetic variability parameters for morphological and biochemical traits in the recombinant inbred lines derived from the cross IS 22380 × E 36-1 evaluated during 2007 at Bijapur location

Trait	Range		Grand mean	PCV (%)	GCV (%)	Heritability (%)	GA as % of mean
	Minimum	Maximum					
X ₁	2.00	4.25	3.09	22.42	14.45	41.51	0.59
X ₂	13.62	50.73	28.65	30.24	29.81	97.23	17.35
X ₃	30.00	72.50	47.47	24.04	20.79	74.94	17.59
X ₄	10.00	23.08	17.89	13.92	13.22	90.12	4.62
X ₅	20.40	62.20	40.25	25.79	25.44	97.31	20.81
X ₆	2.20	4.57	3.14	16.17	13.66	71.35	0.75
X ₇	59.00	78.80	69.17	52.80	6.72	75.91	1.22
X ₈	18.65	37.83	26.68	14.42	14.19	96.85	7.68
X ₉	1.00	4.00	1.70	37.70	33.48	78.91	1.05
X ₁₀	1.00	3.75	2.47	24.69	17.37	49.55	0.62
X ₁₁	101.00	153.10	133.67	7.00	6.78	93.81	18.09
X ₁₂	3.16	5.18	4.07	11.29	11.22	98.83	0.94
X ₁₃	3.76	6.06	5.23	9.60	9.44	96.72	1.00
X ₁₄	3.64	7.24	4.90	15.83	15.70	98.32	1.57
X ₁₅	3.33	6.45	4.63	16.62	16.52	98.81	1.57

* Significant at 5% probability level

** Significant at 1% probability level

X₁ : Number of internode crossed

X₆ : Stem thickness (cm)

X₁₁: Plant height (cm)

X₂ : Length of infection (cm)

X₇ : Days to 50% flowering

X₁₂ : Lignin at 75 Days after sowing

X₃ : Lodging per cent

X₈ : 1000 seed weight (g)

X₁₃: Lignin at 95 Days after sowing

X₄ : Panicle length (cm)

X₉ : Threshability

X₁₄ : Total phenols at 75 Days after sowing

X₅ : Seed yield/plant (g)

X₁₀ : Early seedling vigour

X₁₅: Total phenols at 95 Days after sowing

1000 seed weight

There was significant variation among RILs for 1000 seed weight. The range of variation recorded was between 18.16 to 37.67g with an overall mean of 27.92g. The per cent PCV and GCV were 11.02 and 10.28, respectively. High heritability estimates of 87 per cent were observed.

Grain yield per plant

Significant difference was recorded between RILs for this trait. Grain yield per plant varied from 20.82 to 62.06g across RILs with a mean of 40.45g. The per cent phenotypic and genotypic coefficients of variability for this trait were 21.53 and 20.81, respectively. High heritability of 93.4 per cent with moderate genetic gain of 16.76 was recorded.

c. Character association studies

The genotypic and phenotypic correlations among all traits were computed to understand the nature of association among the characters and are presented in Table 8a to 8d.

Number of internodes crossed

The number of internodes crossed by *M. phaseolina* had positive correlation at genotypic and phenotypic level with lodging per cent and length of infection while with all other eight traits including plant height, seed yield, 1000 seed weight, stem thickness, ear head length, early seedling vigour showed negative association with this trait at both phenotypic and genotypic levels.

Length of infection

A highly significant and positive association at both genotypic (0.80) and phenotypic (0.68) level was recorded between length of infection, and ear head length. In contrast, length of infection was negatively correlated with early seedling vigour and days to 50 per cent flowering, seed yield, threshability, plant height and 1000 seed weight at phenotypic and genotypic level. Length of infection was positively correlated with other two charcoal rot traits viz., lodging per cent and number of internodes crossed at both phenotypic and genotypic level.

Lodging per cent

There was a positive correlation with length of infection and number of internodes crossed at phenotypic level but negatively correlated with length of infection and positively correlated with number of internodes crossed at genotypic level. For other yield traits lodging per cent was positively related with days to flowering, threshability and plant height while this trait had negative association with seed yield, 1000 seed weight, stem thickness and panicle length. at both genotypic and phenotypic level.

Lignin at 75 DAS

Negative correlation with two charcoal rot traits (length of infection and per cent lodging) was observed. However, there was significant positive correlation at genotypic and phenotypic level with lignin at 95 DAS, total phenol at 75 DAS and total phenol at 95 DAS.

Total phenol at 75 DAS

Negative correlation with all the three charcoal rot traits viz., Length of spread of infection, number of internodes crossed and lodging per cent was noticed with this trait. However, there was a significant and positive correlation with panicle length.

Table 8a: Genotypic correlation coefficients (pooled) among eleven traits in the recombinant inbred lines derived from the cross IS 22380 x E 36-1 evaluated during 2004, 2005, 2006 and 2007 at Bijapur location

Traits	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁
X ₁	1	0.232*	0.133	0.075	-0.08	-0.014	-0.073	-0.019	-0.151	-0.11	-0.107
X ₂		1	0.009	0.805**	-0.099	-0.013	-0.241*	-0.065	-0.205	-0.329**	0.011
X ₃			1	0.071	-0.446**	-0.011	0.111	-0.378**	0.081	-0.18	0.215*
X ₄				1	-0.176	-0.280**	0.441**	-0.185	-0.22	-0.277**	0.083
X ₅					1	-0.018	-0.008	0.409**	-0.003	0.187	-0.032
X ₆						1	0.027	-0.167	-0.157	-0.083	-0.051
X ₇							1	0.133	0.157	0.328**	0.220*
X ₈								1	0.134	-0.038	0.064
X ₉									1	0.045	0.038
X ₁₀										1	-0.592**
X ₁₁											1

* Significance at 5 % probability level

** Significance at 1% probability level

X₁ : Number of internode crossed

X₂ : Length of infection (cm)

X₃ : Lodging per cent

X₄ : Panicle length (cm)

X₅ : Seed yield /plant (g)

X₆ : Stem thickness (cm)

X₇ : Days to 50% flowering

X₈ : 1000 seed weight (g)

X₉ : Threshability

X₁₀ : Early seedling vigour

X₁₁ : Plant height (cm)

Table 8b: Phenotypic correlation coefficients (pooled) among eleven traits in the recombinant inbred lines derived from the cross IS 22380 x E 36-1 evaluated during 2004, 2005, 2006 and 2007 at Bijapur location

Traits	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁
X ₁	1	0.169	0.085	0.042	-0.06	-0.015	-0.038	-0.029	-0.078	-0.052	-0.076
X ₂		1	0.01	0.681**	-0.097	-0.013	-0.038	-0.061	-0.143	-0.14	0.014
X ₃			1	0.057	0.401**	-0.01	0.023	-0.321**	0.054	-0.064	0.193
X ₄				1	-0.163	-0.234*	0.075	-0.169	-0.149	-0.126	0.072
X ₅					1	-0.014	-0.001	0.381**	-0.003	0.071	-0.031
X ₆						1	-0.019	-0.117	-0.066	-0.017	-0.038
X ₇							1	0.021	0.033	0.042	0.16
X ₈								1	0.087	-0.02	0.056
X ₉									1	0.041	0.022
X ₁₀										1	-0.25*
X ₁₁											1

* Significance at 5 % probability level

** Significance at 1% probability level

X₁ : Number of internode crossed

X₂ : Length of infection (cm)

X₃ : Lodging per cent

X₄ : Panicle length (cm)

X₅ : Seed yield /plant (g)

X₆ : Stem thickness (cm)

X₇ : Days to 50% flowering

X₈ : 1000 seed weight (g)

X₉ : Threshability

X₁₀ : Early seedling vigour

X₁₁ : Plant height (cm)

Total phenol at 95 DAS

There was negative correlation with length of spread of infection and number of internodes crossed by the fungus and lodging per cent both at genotypic and phenotypic level. A positive and significant correlation with total phenols at 75 DAS, lignin at 75 and 95 DAS both at genotypic and phenotypic level was noticed.

Panicle length

There was positive correlation with days to 50 per cent flowering at both genotypic (0.28) and phenotypic (0.23) level, while negative correlation was observed for stem thickness and early seedling vigour

Seed yield

Significant positive correlation was observed with 1000 seed weight at both genotypic and phenotypic level with correlation coefficient of 0.40 and 0.38, respectively.

Days to flowering

Significant positive correlation was observed at genotypic level with early seedling vigour and plant height with correlation coefficient values of 0.33 and 0.22, respectively.

1000 seed weight

Significant positive correlation was observed with seed yield at genotypic and phenotypic level with correlation coefficient of 0.41 and 0.38, respectively. Negative correlation with lodging per cent was observed at both genotypic (0.37) and phenotypic (0.32) level.

Early seedling vigour

Early seedling vigour was negatively correlated with length of spread of infection (0.33) and panicle length (0.28) while positively correlated with days to 50 per cent flowering with genotypic correlation of coefficient value of 0.32.

Plant height

Plant height was positively correlated with early seedling vigour (0.21) and lodging per cent (0.22) at genotypic level. Negative correlation with early seedling vigour was observed with genotypic correlation of coefficient of 0.25.

4.1.1.3 Solapur

a. Component parameters of charcoal rot disease resistance

The estimate of variability parameters of charcoal rot resistance and component traits are presented in Table 9a and 9b. Frequency distribution patterns of all the traits are presented in Fig. 5 and graphical presentation of PCV and GCV is shown in Fig. 6.

Number of internodes crossed by the pathogen

There was significant difference between RILs used for phenotyping. The range of variation was between 2.0 and 4.5 with a mean value of 2.9. The phenotypic and genotypic coefficients of variation for this trait were 26.51 and 21.87 respectively with heritability of 68.1 per cent. Positive correlation was recorded with other two charcoal rot component traits viz., lodging per cent and length of infection crossed by the fungus. A negative correlation was recorded for seed yield per plant and days to flowering.

Table 8c: Genotypic correlation coefficient among the morphological and biochemical traits in the recombinant inbred lines derived from the cross IS 22380 × E 36-1 at Bijapur location during 2007

Traits	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅
X ₁	1	0.258*	0.104	-0.03	-0.023	-0.084	0.477**	-0.049	-0.125	0.051	0.089	0.067	0.08	-0.049	-0.056
X ₂		1	0.045	0.061	-0.08	-0.031	0.502**	-0.045	-0.117	-0.12	0.054	-0.052	0.098	-0.008	-0.126
X ₃			1	-0.127	-0.426**	-0.074	0.238	-0.38**	0.118	-0.305**	0.19**	-0.046	-0.026	-0.182	-0.156
X ₄				1	-0.051	-0.354**	0.146	-0.127	-0.095	-0.016	0.106	-0.241**	-0.065	0.42**	0.037
X ₅					1	-0.022	0.164	0.387**	-0.011	0.156	-0.012	-0.031	-0.029	0.122	0.082
X ₆						1	-0.102	-0.169	-0.203	0.115	0.011	-0.091	-0.013	-0.039	-0.007
X ₇							1	0.255	0.712**	0.267**	0.141	0.016	-0.683**	-0.389	-0.254
X ₈								1	0.138	-0.059	0.094	-0.057	-0.074	0.131	0.128
X ₉									1	-0.119	0.164	-0.158	-0.062	0.022	-0.004
X ₁₀										1	-0.454**	0.188	0.24*	0.01	-0.116
X ₁₁											1	-0.145	-0.01	0.041	0.148
X ₁₂												1	0.269**	0.254*	0.296**
X ₁₃													1	0.2	0.165
X ₁₄														1	0.934**
X ₁₅															1

*Significant at 5% probability level

**Significant at 1% probability level

X₁ : Number of internode crossed

X₆ : Stem thickness (cm)

X₁₁: Plant height (cm)

X₂ : Length of infection (cm)

X₇ : Days to 50% flowering

X₁₂ : Lignin at 75 Days after sowing

X₃ : Lodging per cent

X₈ : 1000 seed weight (g)

X₁₃: Lignin at 95 Days after sowing

X₄ : Panicle length (cm)

X₉ : Threshability

X₁₄ : Total phenols at 75 Days after sowing

X₅ : Seed yield/plant (g)

X₁₀ : Early seedling vigour

X₁₅: Total phenols at 95 Days after sowing

Table 8d: Phenotypic correlation coefficient among the morphological and biochemical traits in the recombinant inbred lines derived from the cross IS 22380 × E 36-1 evaluated during 2007 at Bijapur location

Traits	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅
X ₁	1	0.155	0.058	-0.037	0.009	-0.040	-0.012	-0.044	-0.092	0.006	0.054	0.055	0.048	-0.028	-0.038
X ₂		1	0.039	0.062	-0.078	-0.032	-0.052	-0.046	-0.102	-0.083	0.049	-0.051	0.101	-0.009	-0.123
X ₃			1	0.107	-0.371**	0.075	0.032	-0.313**	0.077	-0.158	0.267**	-0.033	-0.026	-0.157	-0.131
X ₄				1	-0.048	0.235*	0.011	-0.121	-0.061	-0.036	0.100	-0.227**	-0.066	0.001	0.034
X ₅					1	-0.011	0.02	0.376**	-0.011	0.09	-0.01	-0.031	-0.028	0.122	0.079
X ₆						1	-0.051	-0.13	-0.119	0.05	0.016	-0.076	-0.015	-0.033	-0.013
X ₇							1	-0.027	0.1	0.048	0.017	0.005	-0.082	-0.048	-0.033
X ₈								1	0.118	-0.049	0.092	-0.056	-0.074	0.128	0.125
X ₉									1	-0.065	0.147	-0.148	-0.046	0.012	-0.005
X ₁₀										1	-0.315**	0.131	0.173	0.003	-0.078
X ₁₁											1	-0.137	-0.002	0.04	0.144
X ₁₂												1	0.263**	0.251*	0.292**
X ₁₃													1	0.195	0.162
X ₁₄														1	0.921**
X ₁₅															1

*Significant at 5% probability level

**Significant at 1% probability level

X₁ : Number of internode crossed

X₆ : Stem thickness (cm)

X₁₁: Plant height (cm)

X₂ : Length of infection (cm)

X₇ : Days to 50% flowering

X₁₂ : Lignin at 75 Days after sowing

X₃ : Lodging per cent

X₈ : 1000 seed weight (g)

X₁₃: Lignin at 95 Days after sowing

X₄ : Panicle length (cm)

X₉ : Threshability

X₁₄ : Total phenols at 75 Days after sowing

X₅ : Seed yield/plant (g)

X₁₀ : Early seedling vigour

X₁₅: Total phenols at 95 Days after sowing

Table 9a: Pooled analysis of variance of six traits in the recombinant inbred lines derived from the cross IS 22380 x E 36-1 evaluated during 2005, 2006 and 2007 at Solapur location

Source of variation	DF	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆
Replication	3	1.09	0.12	18.62	0.29	0.014	0.25
Environment	2	1.74*	145.53**	53.87**	182.31**	5.02**	197.8*
Genotypes	92	3.23*	1007.92*	2434.42*	1252.57*	2.016*	19.26*
G × E	184	0.11	0.004	0.03	0.054	0.003	0.31
Error	644	14.98	0.31	23.81	0.28	0.004	0.27
S.Em.±		2.909	0.281	2.44	0.268	0.034	0.25
CD at 5%		8.09	0.78	6.78	0.75	0.09	0.71
CD at 1%		10.70	1.06	9.22	0.99	0.13	0.93
CV (%)		10.20	12.02	10.01	11.29	12.16	10.77

** Significant at 1% probability * Significant at 5% probability

X₁ : Number of internodes crossed

X₂ : Length of infection (cm)

X₃ : Lodging per cent

X₄ : Seed yield /plant (g)

X₅ : Stem thickness (cm)

X₆ : Days to 50% flowering

Table 9b: Magnitude of genetic variability parameters of six traits in the recombinant inbred lines derived from the cross IS 22380 x E 36-1 evaluated during 2005, 2006 and 2007 at Solpaur location

Traits	Mean	Range		GCV (%)	PCV (%)	Heritability (%)	GA as % of mean
		Minimum	Maximum				
X ₁	2.90	2.07	4.25	21.87	26.51	68.10	2.08
X ₂	7.91	10.2	15.19	40.32	42.00	99.70	15.50
X ₃	48.75	20.51	89.27	35.81	37.19	92.70	22.50
X ₄	41.62	20.26	55.05	30.71	32.10	99.00	18.65
X ₅	3.23	2.20	6.20	15.64	15.71	98.00	1.58
X ₆	67.01	56.25	75.51	5.26	5.31	97.90	5.75

* Significant at 5% probability ** Significant at 1% probability

X₁ : Number of internodes crossed

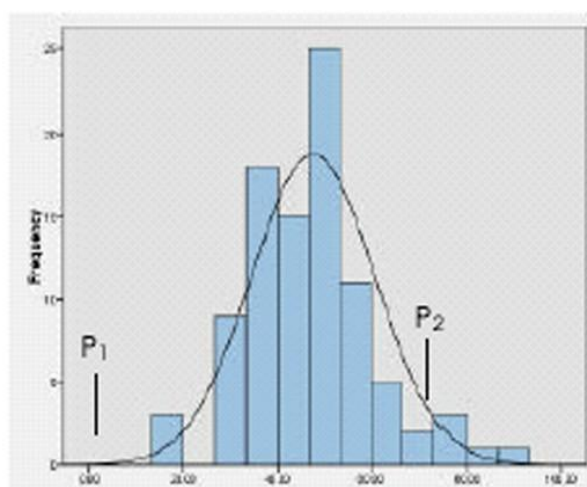
X₂ : Length of infection (cm)

X₃ : Lodging per cent

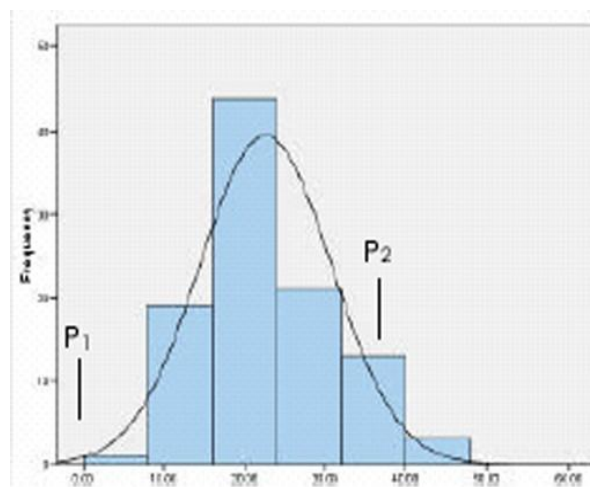
X₄ : Seed yield /plant (g)

X₅ : Stem thickness (cm)

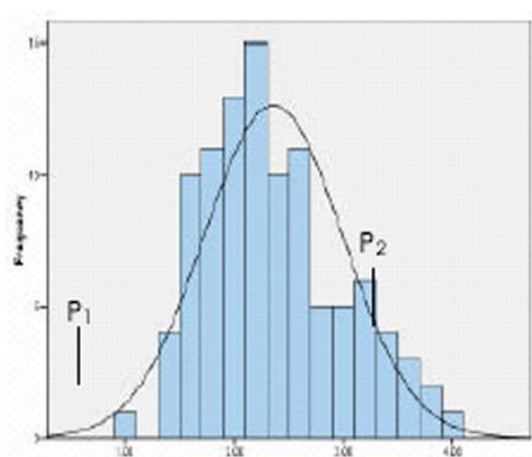
X₆ : Days to 50% flowering



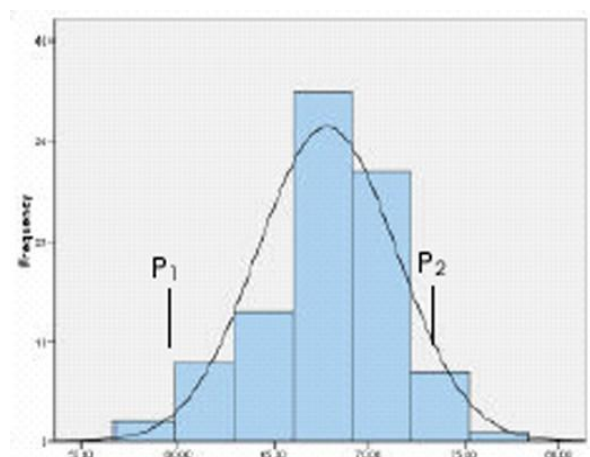
A. Lodging Percent



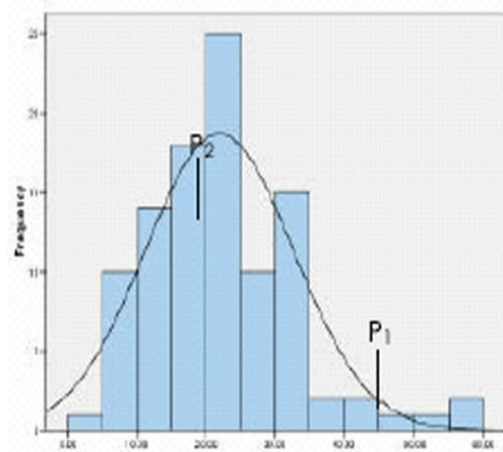
B. Length of infection



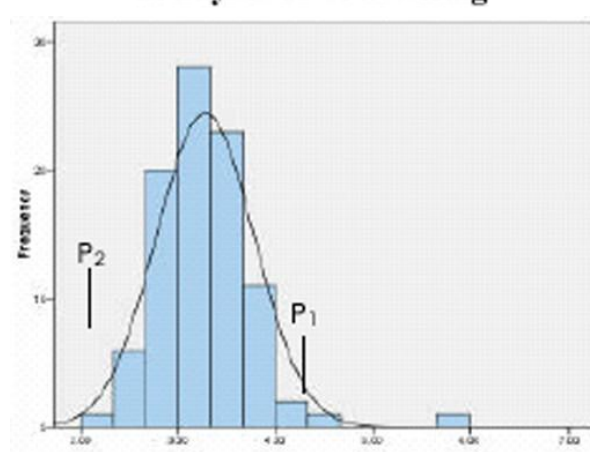
C. Number of internodes crossed by fungus



D. Days to 50 % flowering



E. Grain yield



F. Stem thickness

Fig.5. Frequency distribution of charcoal rot resistance component and other yield traits at Solapur location

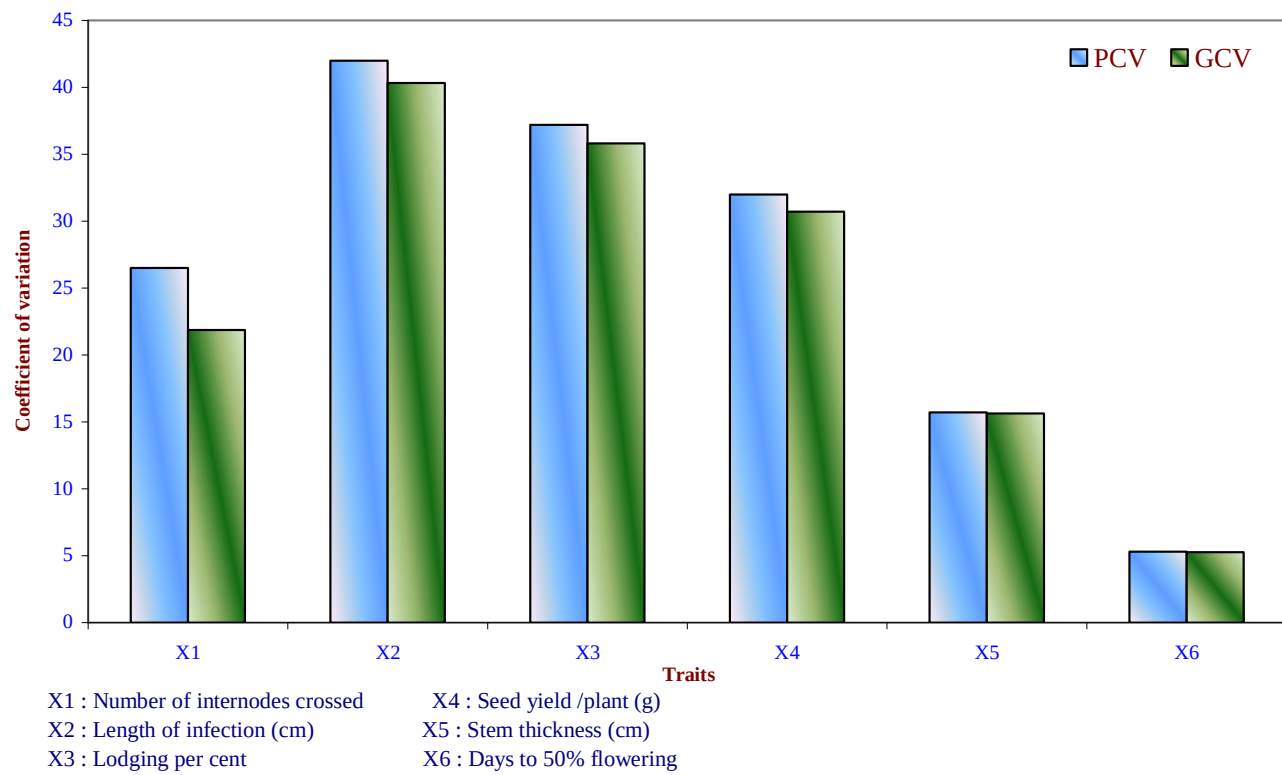


Fig.6. PCV and GCV of pooled data for 15 traits at Solapur location

Length of infection

All the RILs were differing with respect to spread of charcoal rot infection with mean average value of 7.9 cm and range of 10.2 to 15.9 cm. The PCV and GCV were 40.3 and 40.2, respectively. High heritability of 99.7 per cent was noticed.

Lodging per cent

For lodging per cent the RILs differed significantly in their values. There existed a wide range of variability ranging from 20.5 to 89.5 per cent with a grand mean of 48.7 per cent. The PCV of 37.19 and GCV of 35.81 per cent with a heritability of 92.7 were recorded. Positive correlation with length of infection and number of internodes crossed by fungus and negative correlation for seed yield and stem thickness was observed.

b. Other yield traits

Day to 50 per cent flowering

Significant difference between the genotypes for days to 50 per cent flowering was recorded with mean of 67 days required for flowering and range of 56 – 75 days. The GCV and PCV values were 15.64 and 15.71, respectively. The heritability value of 98 per cent was recorded for this trait. Positive correlation with length of infection and lodging per cent was recorded while there was negative correlation with number of internodes crossed; stem thickness and seed yield both at genotypic and phenotypic level.

Stem thickness

There was significant difference for this trait among the RILs. The mean value for this trait was 3.2 cm with a range of 2.2 to 6.2 cm. The PCV and GCV for this trait were 15.71 and 15.64, respectively. High Heritability of 98 per cent was recorded for stem thickness. There was positive correlation with seed yield while negative correlation was observed with respect to lodging per cent and length of infection caused by fungus.

Grain yield per plant

Grain yield per plant varied from 20.2 to 55.05g across RILs with a mean of 41.6 g. The phenotypic and genotypic coefficients of variability for this trait were 30.1 and 30.7, respectively. High heritability of 99 per cent was recorded. There was negative correlation with all the three charcoal rot component trait viz., number of internodes crossed, lodging per cent and length of infection caused by fungus.

c. Character association studies

The genotypic and phenotypic correlations among all traits were computed to understand the nature of association among the characters and are presented in Table 9c and 9d.

Number of internodes crossed

Positive correlation was recorded with lodging per cent with genotypic and phenotypic correlation coefficient values of 0.37 and 0.35, respectively. While negative correlation was recorded for seed yield with genotypic and phenotypic correlation coefficient values of 0.42 and 0.40, respectively.

Day to 50 per cent flowering

Positive correlation with length of infection and lodging per cent was recorded while there was negative correlation with number of internode crossed, stem thickness and seed yield both at genotypic and phenotypic level.

Table 9c: Genotypic correlation coefficients (pooled) among six traits in the recombinant inbred lines derived from the cross IS 22380 x E 36-1 evaluated during 2005, 2006 and 2007 at Solapur location

Traits	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆
X ₁	1	0.087	0.37**	-0.421**	-0.036	0.06
X ₂		1	0.153	-0.106	-0.037	-0.058
X ₃			1	-0.089	-0.035	0.009
X ₄				1	0.001	-0.09
X ₅					1	-0.016
X ₆						1

** Significant at 1% probability

* Significant at 5% probability

X₁ : Number of internodes crossed

X₂ : Lodging per cent

X₃ : Length of infection (cm)

X₄ : Seed yield /plant (g)

X₅ : Stem thickness (cm)

X₆ : Days to 50% flowering

Table 9d : Phenotypic correlation coefficients (pooled) among six traits in the recombinant inbred lines derived from the cross IS 22380 x E 36-1 evaluated during 2005, 2006 and 2007 at Solapur location

Traits	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆
X ₁	1	0.053	0.35**	-0.405**	-0.037	0.055
X ₂		1	0.126	-0.089	0.03	-0.049
X ₃			1	-0.089	-0.035	0.01
X ₄				1	0.001	-0.008
X ₅					1	-0.015
X ₆						1

** Significant at 1% probability * Significant at 5% probability

X₁ : Number of internodes crossed

X₂ : Lodging per cent

X₃ : Length of infection (cm)

X₄ : Seed yield /plant (g)

X₅ : Stem thickness (cm)

X₆ : Days to 50% flowering

Stem thickness

There was positive correlation with seed yield while negative correlation was observed with respect to lodging per cent and length of infection.

Grain yield per plant

There was negative correlation with all the three charcoal rot component trait viz., number of internodes crossed, lodging per cent and length of infection crossed by fungus.

4.1.5 Estimation of phenol and lignin in resistance and susceptible genotypes

The estimation of total phenol and lignin in resistant and susceptible genotypes was worked out to see if there would be patterns among previously known resistant and susceptible genotypes of sorghum (Table 10a and 10b). It was found that there was significant difference between resistance and susceptible genotypic groups for total phenol and lignin content indicating the role of these compounds in disease resistance. Of all resistant genotypes tested for the total phenol content, high total phenol was observed in A-1 (5.334 mg) followed by DSH 4 (5.268 mg), DSV 2 (5.223 mg), DSV 3 (5.201 mg), DSV 4 (5.136 mg) and DSV 5 (5.143 mg). Among susceptible genotypes lowest value was recorded by CSH 8R (4.075) followed by SPV 82 (4.387), SPH 24 (4.505), SPH 29 (4.578), and SB 1066 (4.567).

4.2 Construction of genetic linkage map

4.2.1. Parental polymorphism

In the present investigation a set of 530 genic SSR markers were used to screen the parents of the RIP, IS22380 and E36-1 to identify the polymorphic ones for further genotyping the RILs. Screening was done on a two tier pipeline; first the fractionation of PCR products was done on a 3.5 per cent agarose followed by 6 per cent PAGE gels where silver staining recipe was adopted. The markers found monomorphic on agarose were taken through the PAGE format to check if they could exhibit polymorphism between parents. Taken together, out of 530 genic SSRs, 473 (89.2 %) marker showed scorable amplification, of which 63 (13.3%) genic SSRs detected polymorphism between the parents (Table 11). All the polymorphic markers were used to genotype individual RILs of mapping population and the gels were scored into excel format suitable for mapping pipeline (Plate 2).

4.2.1.1 Marker segregation and segregation distortion

The segregation pattern of all the 63 marker loci in RILs was compared and checked for their fitness to expected 1:1 ratio. The calculated χ^2 values were compared with table value at 1 and 5 per cent confidence level for each marker locus. All marker loci were segregated in 1:1 ratio (Table 12). The list of all polymorphic markers which include previously identified nuclear SSRs and RAPDs together accounting for 141 markers is presented in Appendix IV along with their calculated χ^2 fitness values. The banding pattern obtained during genotyping of RILs were scored as follows, homozygote for the allele from parental strain P₁ (E 36-1) at the locus as A=1, heterozygote carrying the alleles from both P₁ and P₂ strains H=2, homozygote for the allele for parental strain P₂ (IS 22380) at the locus as B=3 and 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 format suitable for linkage analysis by MAPMAKER/EXP (3.0b).

4.2.2 Construction of linkage map

The size of mapping population used for construction of linkage map was 93 RILs derived from a cross IS 22380 x E36-1. The genotypic data of 148 markers was used for assigning the linkage position and construction of linkage map. The marker data of 148 markers was used to construct a linkage map using MAPMAKER/EXP (3.0b) software

Table 10a: Total phenol content in resistant and susceptible genotypes of sorghum at 75 DAS

Resistant	Total phenol (mg/g)	Susceptible	Total phenol (mg/g)	t test
A-1	5.334	SPH 24	4.505	0.000008
DSV2	5.2233	SPV-86	4.5356	
DSV 3	5.2013	CSH 8R	4.075	
DSV 4	5.1366	SPV 81	4.3878	
DSV 5	5.1438	SB 1066	4.5675	
DSH 4	5.2688	SPH 29	4.5788	

Table 10b: Lignin content in resistant and susceptible genotypes of sorghum at 75 DAS

Resistant	Lignin (%)	Susceptible	Lignin (%)	P value
A-1	4.7632	SPH 24	4.0999	0.000001
DSV2	4.8213	SPV-86	4.1245	
DSV 3	4.7234	CSH 8R	4.0231	
DSV 4	4.6998	SPV 81	4.1067	
DSV 5	4.6865	SB 1066	4.0977	
DSH 4	4.7688	SPH 29	4.1024	

Table 11: Summary statistics of EST-SSR markers used to develop a genetic linkage map of RIP derived from the cross IS 22380 $\times$ E 36-1

Sl. No.	Particulars	Values
1.	Total number of EST-SSR marker screened	530
2.	Number of EST SSR marker amplified	473
3.	Per cent amplification	89.2%
4.	Number of polymorphic markers for IS 22380 $\times$ E 36-1	63
5.	Per cent polymorphism	13.3%
6.	Number of markers mapped on to the linkage groups	63

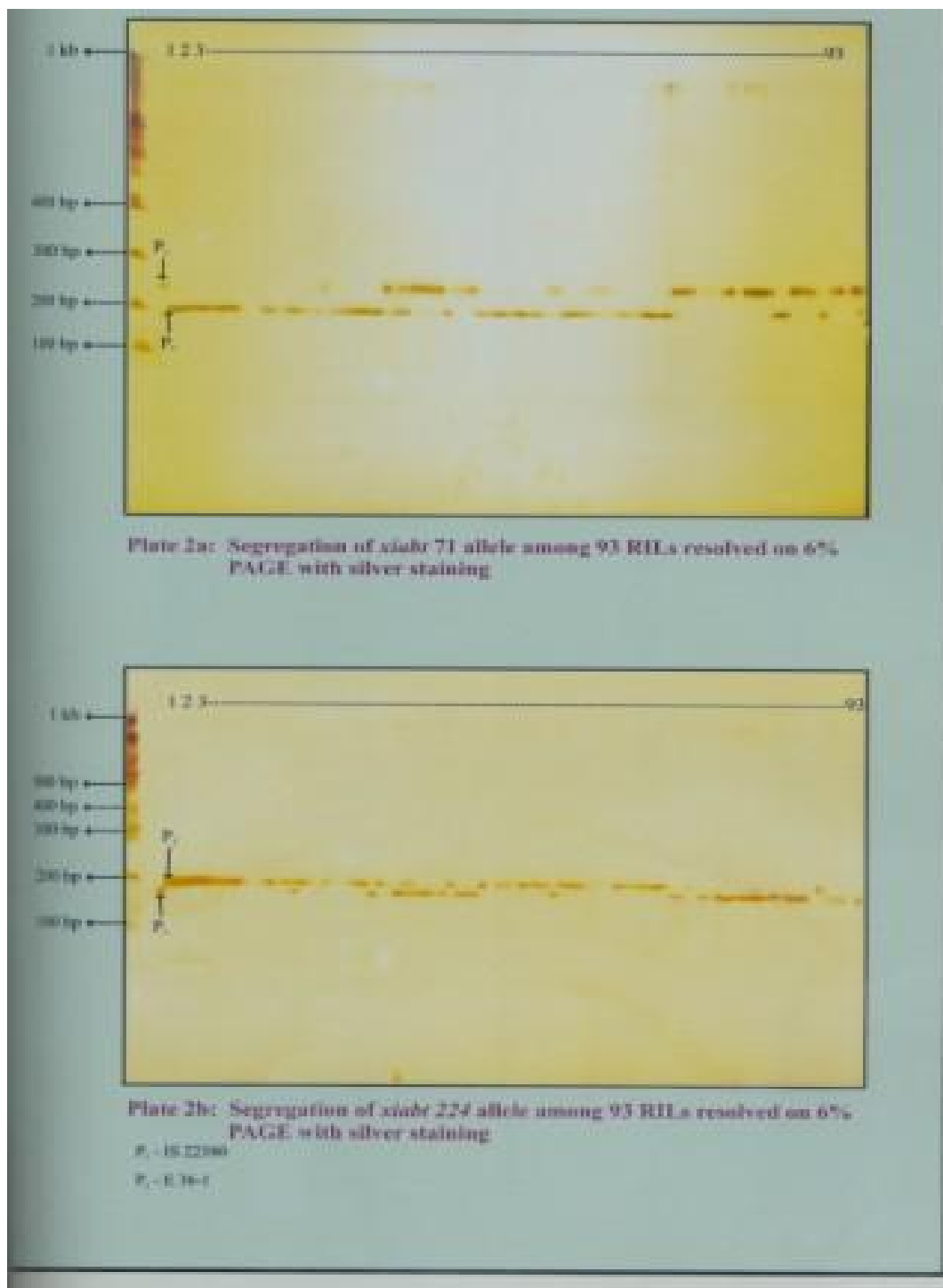


Plate.2a. Segregation of *xiaht* 71 allele among 93 RILs resolved 6% PAGE with silver staining

Plate.2b. segregation of *xiaht* 224 allele among 93 RILs resolved on 6% PAGE with silver staining

Table 12: Test of significance for segregation of polymorphic genic SSR markers in RILs derived from the cross IS 22380 x E 36-1

Sl. No.	Marker	P ₁ allele	P ₂ allele	χ^2
1	<i>xiabt2</i>	37	51	2.227**
2	<i>xiabt9</i>	43	49	0.391**
3	<i>xiabt20</i>	39	54	2.419**
4	<i>xiabt1</i>	47	46	0.011**
5	<i>xiabt46</i>	48	40	0.01**
6	<i>xiabt29</i>	40	49	0.19**
7	<i>xiabt05</i>	38	39	0.01**
8	<i>xiabt32</i>	44	40	0.19**
9	<i>xiabt13</i>	38	54	3.4**
10	<i>xiabt49</i>	42	43	0.01**
11	<i>xiabt01</i>	41	51	1.08**
12	<i>xiabt22</i>	44	49	0.26**
13	<i>xiabt82</i>	35	53	3.68**
14	<i>xiabt83</i>	45	48	0.09**
15	<i>xiabt45</i>	37	56	3.80**
16	<i>xiabt47</i>	45	48	0.09**
17	<i>xiabt85</i>	43	42	0.01**
18	<i>xiabt96</i>	38	49	1.39**
19	<i>xiabt72</i>	47	46	0.1**
20	<i>xiabt27</i>	44	47	0.09**
21	<i>xiabt94</i>	44	47	0.09**
22	<i>xiabt21</i>	50	43	0.52**
23	<i>xiabt22</i>	43	48	0.27**
24	<i>xiabt51</i>	38	54	3.4**
25	<i>xiabt224</i>	42	49	0.53**
26	<i>xiabt434</i>	40	51	1.32**
27	<i>xiabt380</i>	45	48	0.09**
28	<i>xiabt378</i>	40	50	0.53**
29	<i>xiabt243</i>	39	55	2.72**
30	<i>xiabt275</i>	37	54	0.87**
31	<i>xiabt241</i>	39	55	2.72**
32	<i>xiabt420</i>	42	51	0.72**
33	<i>xiabt389</i>	55	39	1.6**
34	<i>xiabt374</i>	40	48	3.1**
35	<i>xiabt30</i>	40	51	3**
36	<i>xiabt60</i>	38	55	0.72**
37	<i>xiabt80</i>	55	38	0.63**
38	<i>xiabt07</i>	40	48	0.05**
39	<i>xiabt57</i>	42	35	1.16**
40	<i>xiabt92</i>	41	39	0.68**

Contd.....

Sl. No.	Marker	P ₁ allele	P ₂ allele	χ^2
41	<i>xiabt97</i>	48	38	2.36**
42	<i>xiabt37</i>	43	50	3.8**
43	<i>xiabt31</i>	40	53	0.72**
44	<i>xiabt53</i>	37	55	0.91**
45	<i>xiabt61</i>	45	46	0.011**
46	<i>xiabt63</i>	40	53	1.816**
47	<i>xiabt62</i>	37	54	0.87**
48	<i>xiabt65</i>	39	55	2.72**
49	<i>xiabt66</i>	38	55	0.72**
50	<i>xiabt67</i>	40	55	2.041**
51	<i>xiabt69</i>	42	49	0.53**
52	<i>xiabt68</i>	37	56	3.80**
53	<i>xiabt64</i>	47	44	0.099**
54	<i>xiabt71</i>	46	49	0.094**
55	<i>xiabt73</i>	35	53	3.68**
56	<i>xiabt74</i>	42	47	0.103**
57	<i>xiabt75</i>	42	49	0.53**
58	<i>xiabt76</i>	37	54	0.87**
59	<i>xiabt77</i>	45	46	0.011**
60	<i>xiabt78</i>	41	54	0.085**
61	<i>xiabt79</i>	40	53	1.8**
62	<i>xiabt81</i>	45	48	0.093**
63	<i>xiabt86</i>	43	50	3.8**
P<0.01>		: 3.81		
Expected ratio		: 1:1		

Table 13: Distance (cM) between pair of markers mapped to different linkage groups of IS 22380 × E 36-1 derived recombinant inbred lines

Sl. No	LG	Pairs of markers	Distance (cM)	Sl. No	LG	Pairs of markers	Distance (cM)
1	A	SbAGB02 - xtxp318	0.55	7	C	xiabt 96-xiabt 20	48.5
2		txtp 318 - Corn 61	9.88	8		xiabt20 – xiabt53	58.7
3		Corn 61 – xtxp267	7.26	9		xiabt 53-xiabt47	58.7
4		txtp 267 – xiabt71	17.33	10		xiabt 47-xiabt51	86.75
5		xiabt71-xiabt434	33.53	1	D	txtp343-xtxp12	4.35
6		xiabt434-xtxp250	3.67	2		txtp12-xiabt79	0.04
7		txtp250-AC13	23.74	3		xiabt79-xiabt77	4.2
8		AC13-xiabt224	18.82	4		xiabt77-xiabt374	13.7
9		xiabt224-AT5	23.24	5	E	xiabt374-xtxp9	14.3
10		AT5-H4	7.20	6		txtp9-xiabt389	21.5
11		H4 – xiabt75	19.48	7		xiabt389-xiabt01	53.1
12		xiabt75 – xtxp327	10.13	8		xiabt01-Xcup9	53.6
13		txtp327 – xiabt2	4.46	9	F	Xcup9-M9	9
14		xiabt2 – xiabt37	19.48	10		M9-xiabt22	23.1
15		xiabt 37- xiabt97	34.49	11		xiabt22-xtxp213	25.5
16		xiabt97-xtxp302	18.50	12		txtp213-xiabt1	16.5
17		txtp302-xiabt38	7.50	13	G	xiabt1-xiabt61	24.6
18		xiabt38-xtxp350	8.03	14		xiabt61-xiabt63	4.4
19		txtp350-xtxp183	2.30	1		txtp295- SbAgE03	24.9
20		txtp183-xiabt21	32.80	2		SbAgE03 – xtxp358	9.86
21		xiabt21-xiabt57	51.52	3	H	txtp358-xtxp204	8.46
22		xiabt57-xiabt92	18.30	4		txtp204-Corn73	2.82
23		xiabt92-xiabt67	27.51	5		Corn73-xtxp92	7.94
24		xiabt67-xiabt69	19.19	6		txtp92-xiabt13	41.02
1	B	txtp63-xtxp8	3.8	7	I	xiabt13- xiabt27	37.07
2		txtp8-xtxp211	6.5	8		xiabt 27-xiabt49	28.26
3		txtp211-xtxp216	15.7	1		txtp67-xtxp230	8.58
4		txtp216-xtxp287	6.8	2		txtp230-xtxp258	4.81
5		txtp287-Corn74	3.2	3	J	txtp258-SbAG04	8.03
6		Corn74-Corn59	5.9	4		SbAG04-xtxp284	15.14
7		Corn59-xtxp303	8.8	5		txtp284-AC20	19.91
8		txtp303-xtxp201	7.15	6		AC20-AK10	13.72
9		txtp201-xiabt378	8.4	7	K	AK10-BH13	2.92
10		xiabt378-xtxp348	12.5	8		BH13-xiabt78	0.5
11		txtp348-xiabt31	52.9	9		xiabt78-xiabt83	30.6
12		xiabt31-SvpepCAA	30.1	10		xiabt83-xiabt30	53.52
13		SvpepCAA-G16	0.5	11	L	xiabt30-G19	48.2
14		G16-L18	10.68	1		PepC-xtxp45	17.8
15		L18-xiabt243	14.83	2		txtp45-xiabt81	1.1
16		xiabt243-AC12	22.9	3		xiabt81-xiabt9	13.75
17		AC12-xiabt80	34.65	4	M	xiabt9-xtxp159	6.4
18		xiabt80-M10	49.92	5		txtp159-xiabt46	27.1
19		M10-xtxp48	17.2	6		txtp217-xtxp329	11.5
20		txtp48-xtxp297	10.09	7		txtp329-AC19	4.2
21		txtp297-xiabt73	18.23	8	N	AC19-xtxp254	12.2
22		xiabt73-xiabt82	0.56	9		txtp254-SbagA01	11.1
23		xiabt82-xiabt74	17.64	10		SbagA01-xtxp273	9.9
24		xiabt74-xiabt07	13.78	11		txtp273-xiabt72	45.4
25	C	xiabt07-xiabt76	18.26	1	O	txtp248-xiabt86	19.9
26		xiabt76-xiabt60	32.33	2		txtp86-xtxp96	3.4
27		xiabt60-xiabt275	34.65	3		txtp96-xtxp176	6.7
28		xiabt275-xiabt241	17.23	4		txtp176-xtxp312	8.1
29		xiabt241-xiabt87	18.53	5	P	txtp312-xtxp274	3.7
30		xiabt87-xiabt62	0.54	6		txtp274-xiabt29	76.5
31		xiabt62-xiabt65	17.23	7		xiabt29-xiabt05	69
32		xiabt65-xiabt66	3.72	8		xiabt05-xiabt94	90.5
1		Corn24-xtxp210	11.83	1	Q	txtp325-xtxp324	3.08
2		txtp210-xtxp353	5.9	2		txtp324-xtxp338	18.9
3		txtp353-xtxp71	1.79	3		txtp338-xiabt420	21.3
4		txtp71-xtxp160	12.9	4		xiabt420-xiabt45	138.7
5		txtp160-xiabt85	25.62	5	R	xiabt45-xiabt58	0.04
6		xiabt85-xiabt96	51.2				

(Lander *et al.*, 1987). Linkage distances in terms of centimorgan (cM) values were calculated using Kosambi mapping function. A total of 140 markers were found associated among themselves with varied distances on ten linkage groups. The details of linkage groups to which these 140 markers belonged and linkage distance in cM are given in Table 13. It was not possible to establish relationship for five RAPD (AB4, AT9, G17, M17 and H3) and two SSR markers (*xtxp290* and *xtxp354*). The anchor marker loci were selected from the previously published sorghum linkage maps of Bhatramakki *et al.* (2000), Qaramerie *et al.* (1997), Haussmann *et al.* (2004) and Reddy *et al.* (2008). The linked markers in the present study were assigned to ten linkage groups viz., LG A, LG B.....B, C, D, E, F, G, H, I and J. The number of markers mapped per linkage group ranged from six in LG-G and LG-J to 33 in LG-B. The linkage map thus constructed had a total length of 2905 cM spanned across all the 10 linkage group (Fig. 7).

Further, functional assignment for all 63 genic SSRs found mapped on to the linkage map were annotated for their function using the available information. Computational tools are now regularly used to infer function based upon significant sequence similarity to experimentally verified proteins or putative proteins. The EST sequences were compared against known databases using these tools. Protein homology searches were performed in order to identify the putative function of the ESTs. BLAST similarity comparison was used to reveal possible functional relationship of each EST containing microsatellite. BLAST search was performed by using BLASTx (<http://www.ncbi.nih.gov/blastx>) program against the non redundant (nr) database. An E-value cut off was used as criteria to assign tentative identity to any sequence. BLASTx search results were visually inspected to ensure that the sequence similarity was contiguous before conclusion was made. The comparison was also done against curated, highly annotated, proteins in SWISS-PROT database, which contains proteins of demonstrated function. Out of 63 EST-SSR mapped on to different linkage group the functional annotation was available only for 27 (42.1%) EST-SSR while for remaining 38 (60.3%) EST-SSR no putative function could be assigned. The possible reasons for 'no matches' for majority of sequences may be due to truncated open reading frames, which is characteristic of EST sequences or that they represent long untranslated regions, structural RNAs or bonafide proteins which are unique to sorghum at this level of comparison.

On linkage group A only two markers showed similarity with the known proteins, Ferredoxin-NADP reductase (*xiabt* 37) and catalytic coenzyme binding protein (*xiabt*224). In linkage group B, seven markers were assigned with putative function with the possible role in disease resistance, transcription initiation, fatty acid metabolism and photosynthesis. In linkage group C, three markers showed putative function viz., *xiabt*20 (RPT-2 like protein), *xiabt* 47 (NADP-dependent malic enzyme) and *xiabt* 85 (glucan endo-1, 3-beta-glucosidase 7 precursor). In linkage group D, three markers *xiabt* 389 (NADPH toxin reductase), *xiabt*1 (Inositolphosphorylceramide-B-hydroxylase) and *xiabt* 61(receptor protein kinase) showed functional similarity with known protein. The marker on linkage group E (*xiabt* 49) showed similarity with AATL2 like protein while the markers on linkage group F showed similarity with xyloglucan endotransglucosylase (*xiabt* 30) and ring H2 finger protein (*xiabt*78). Eukaryotic translation initiation factor protein showed similarity with the marker (*xiabt* 83) on linkage group F. None of the markers mapped on linkage group G and LG-H showed putative functional role with known protein while on linkage group J two markers viz., *xiabt* 45 (para-hydroxybenzoate-polyprenyltransferase) and *xiabt* 420 (phytosulfokines 5 precursor) showed putative function. The functional annotation for all 63 genic SSRs mapped on to the linkage map are presented in the Table 14.

4.3 QTL analyses and mapping

The linkage map constructed using 93 RILs derived from the cross IS22380 x E36-1 was used for QTL localization. The software QTL Cartographer (2.5b) was used to analyse the data by Composite Interval Mapping (CIM), implemented with a LOD score of 2.0 as threshold value for QTL significance (Fig. 8). The additive effect and the estimates of the portion of phenotypic variation explained by individual QTL were computed. In this mapping population, the female parent (the marker allele homozygote scored as A) was IS 22380 (susceptible to charcoal rot disease) and male parent (the marker allele homozygote scored as B) was E36-1 (resistant to charcoal rot disease). For QTL analysis the individual season data (2004, 2005, 2006 and 2007) separately from each location (Dharwad, Bijapur and

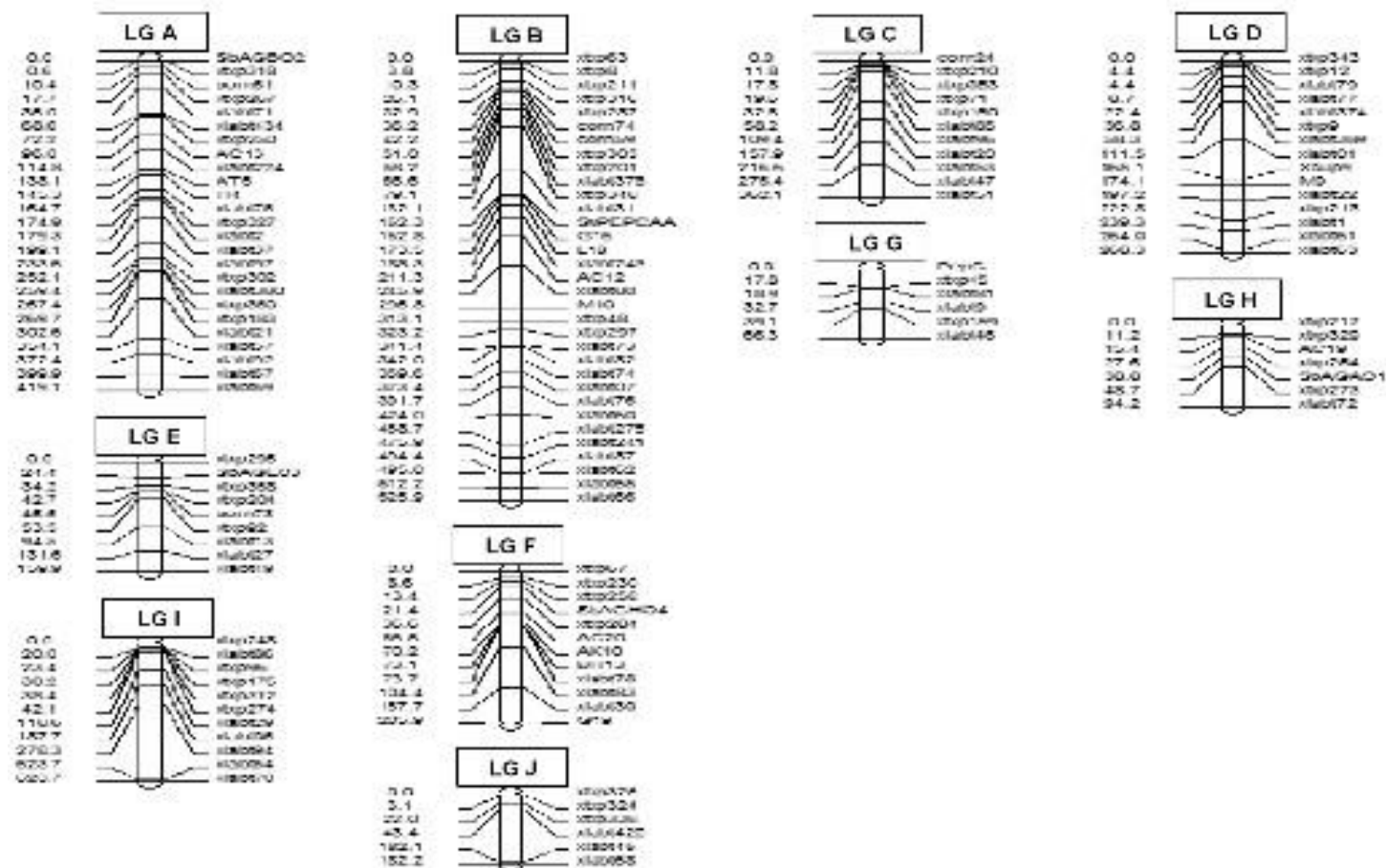


Fig.7. Genetic linkage map constructed using SSR, RAPD and EST-SSR markers in RILs derived from the cross IS 22380XE36-1

Solapur) was used and the QTLs were computed to compare further to realize the consistency leading to robustness. All the QTLs associated with 11 different traits during different season were worked separately for Dharwad, Bijapur and Solapur. Finally, of common and stable QTLs found consistent over four seasons and three locations were noted down. The QTL analysis for lignin and total phenol was carried out on the data obtained during 2007 at Dharwad and Bijapur locations..

4.3.1 Dharwad location

All the QTLs associated with 11 different traits during different season were worked separately for Dharwad location (Table 15a to 15f). Graphical representation of chromosomal positions of common QTLs found at all the four seasons with flanking markers is presented in Fig. 9.

Number of internodes crossed by fungus

Four QTLs conferring to length of infection were identified on linkage group A (*txp* 302 – *xiabt* 380), B (*txp* 297 – *xiabt* 73 and *xiabt* 92 – *xiabt* 62) and D (*txp* 213 – *xiabt* 1) of which two QTLs were detected on same linkage group (LG-B). Out of four QTLs detected one QTL on LG-B was consistently detected for all the four seasons which was flanked by marker *txp* 297 and *xiabt* 81 at position 18.01 - 19.5 cM with additive effect ranging from 4.1-6.8 and phenotypic variance of 10.9-13.5.

Length of infection

Total of three QTLs were detected of which, two QTLs, one each on linkage group A and LG-B were found consistent for all the four season. On linkage group A the QTL were flanked by the marker AC13 and *xiabt* 224 at position 96.1-100.7cM with additive effect ranging from 2.1-4.6 and phenotypic variance of 8.9-10.7. On linkage group B the QTL was flanked with *xiabt* 275 and *xiabt* 241 with phenotypic variance of 8.0 to 16.1 with additive effect of 3.1 to 4.9.

Per cent Lodging

Four QTLs were detected on three different linkage groups D (*xiabt* 343 – *txp* 12), LG-I (*txp* 176 – *xiabt* 312 and *txp* 274 – *xiabt* 29) and LG-J (*txp* 338 – *xiabt* 420) found common for all the season. Three QTLs (*txp* 343 – *txp* 12, *txp* 176 – *xiabt* 312 and *txp* 274 – *xiabt* 29) showed additive effect contributing from E36-1 and one QTL on linkage group J flanked by the marker *txp* 338 and *xiabt* 420 showed additive effect contributing from IS 22380 parent. The phenotypic variance and LOD score were in the range of 8.7 to 11.17 and 2.1 to 3.8, respectively.

Total phenol at 75 DAS

Three QTLs were detected one each on linkage group H (AC 19–*txp* 254), LG I (*txp* 274-*xiabt* 29) and LG-J (*xiabt* 420-*xiabt* 45) with peak LOD score of 3.0, 2.4 and 2.9, respectively. Two QTL on linkage group H and LG-J were showing the phenotypic variance of 29.11 and 10 respectively with additive effect of 1.54 and 3.0. The QTL on linkage group showed the phenotypic variances of 12.15 with additive effect of 1.43. For all these QTL the resistant parent (E 36-1) was contributing the allele for this loci.

Lignin at 75 DAS

A total of three QTLs were detected on three different linkage group B (*txp* 303 – *txp* 301), LG-C (*xiabt* 47 – *xiabt* 51), LG-D (*xiabt* 374 – *txp* 9) with phenotypic variance of 10.11, 11.2, 22.01 and 14 respectively with peak LOD value of 2.8, 2.9, 4.02 and 3.62. For the QTL on linkage group B (*txp* 303-*txp*301) the additive effect of 1.25 was recorded and the contribution was from susceptible parent (IS 22380) while for other three QTLs on linkage group C, LG-D and LG-H the additive effect value of 6.3, 0.29 and 2.41 was recorded with the major contribution from resistant parent (E36-1).

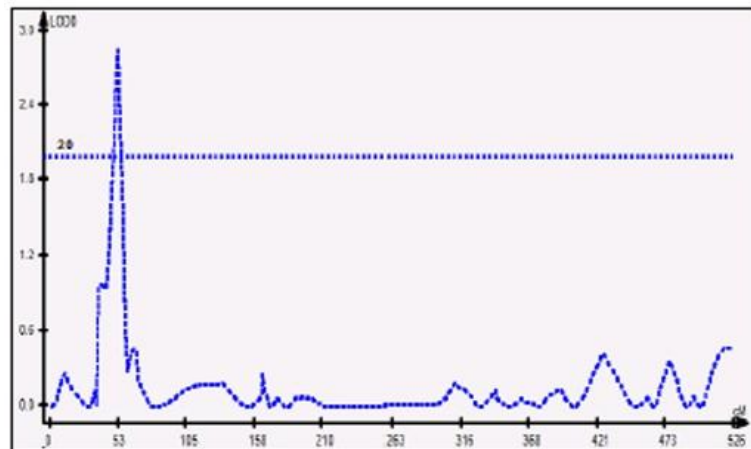
Table 14: Functional annotation (BLASTx) of ESTs harbouring microsatellites mapped on to different linkage groups of IS 22380 × E 36-1 derived from recombinant inbred lines

Sl. No.	Gene ID	Primer	LG	Functional annotation	E- value
1	31332334	<i>xiabt</i> 2	A	No significant similarity found	
2	45949294	<i>xiabt</i> 21	A	Unknown protein (<i>Zea mays</i>)	4.00E-56
3	30973845	<i>xiabt</i> 380	A	No significant similarity found	
4	31334597	<i>xiabt</i> 37	A	Ferredoxin--NADP reductase, root isozyme	8.00E-56
5	17886525	<i>xiabt</i> 57	A	Hypothetical protein Osl_12585 (<i>Oryza sativa</i>)	3.2
6	30978605	<i>xiabt</i> 67	A	No significant similarity found	
7	9850502	<i>xiabt</i> 224	A	Binding/ catalytic/ coenzyme binding (<i>Arabidopsis</i>)	1.00E-08
8	30942962	<i>xiabt</i> 75	A	Hypothetical protein OsJ_03583	3.00E-25
9	9305677	<i>xiabt</i> 69	A	Unknown protein (<i>Zea mays</i>)	8.00E-31
10	13390776	<i>xiabt</i> 71	A	Hypothetical protein Osl_01023 (<i>Oryza sativa</i>)	2.00E-11
11	5042862	<i>xiabt</i> 434	A	Unknown protein (<i>Zea mays</i>)	1.00E-05
12	30932238	<i>xiabt</i> 92	A	Hypothetical protein Osl_21838 (<i>Oryza sativa</i>)	9.00E-09
13	18060131	<i>xiabt</i> 97	A	Hypothetical protein LOC565026	3.7
14	45961441	<i>xiabt</i> 7	B	No significant similarity found	
15	18052712	<i>xiabt</i> 31	B	ZIM motif family protein (<i>Zea mays</i>)	1.00E-07
16	30974381	<i>xiabt</i> 378	B	myb-related protein Hv33 (<i>Zea mays</i>)	3.00E-19
17	31347844	<i>xiabt</i> 60	B	mRNA turnover protein 4 (<i>Zea mays</i>)	3.00E-85
18	30949145	<i>xiabt</i> 87	B	Hypothetical protein <i>Oryza sativa</i>	5.00E-76
19	45965503	<i>xiabt</i> 62	B	No significant similarity found	
20	45992006	<i>xiabt</i> 243	B	Cytochrome P450 [<i>Triticum aestivum</i>]	9.00E-29
21	45994116	<i>xiabt</i> 65	B	Omega-3 fatty acid desaturase (<i>Zea mays</i>)	2.00E-44
22	34442563	<i>xiabt</i> 66	B	Transcription regulator (<i>Zea mays</i>)	1.00E-40
23	12775306	<i>xiabt</i> 73	B	Hypothetical protein OsJ_03583 (<i>Oryza sativa</i>)	3.00E-25
24	30948481	<i>xiabt</i> 74	B	No significant similarity found	
25	45996483	<i>xiabt</i> 275	B	Unknown protein (<i>Zea mays</i>)	2.00E-56
26	13784591	<i>xiabt</i> 76	B	Unknown protein (<i>Zea mays</i>)	3.00E-11
27	12775835	<i>xiabt</i> 80	B	No significant similarity found	
28	14569915	<i>xiabt</i> 82	B	Unknown protein (<i>Zea mays</i>)	3.00E-25
29	45986595	<i>xiabt</i> 241	B	Cytochrome P450	1.00E-79
30	31334672	<i>xiabt</i> 20	C	RPT2-like protein (<i>Sorghum bicolor</i>)	4.00E-52
31	33109119	<i>xiabt</i> 47	C	NADP-dependent malic enzyme (<i>Sorghum bicolor</i>)	2.00E-15
32	34509336	<i>xiabt</i> 51	C	No significant similarity found	
33	9852958	<i>xiabt</i> 53	C	No significant similarity found	
34	31346189	<i>xiabt</i> 85	C	Glucan endo-1,3-beta-glucosidase 7 precursor	2.00E-29
35	12618782	<i>xiabt</i> 96	C	Expressed protein (<i>Oryza sativa</i>)	9.00E-10

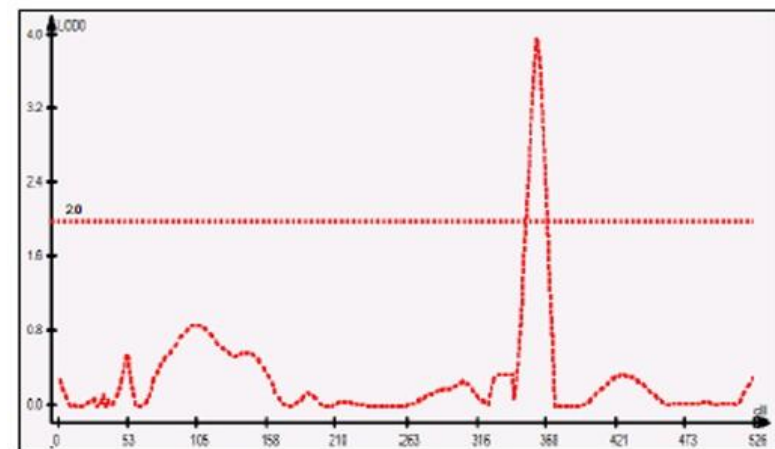
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Sl. No.	Gene ID	Primer	LG	Functional annotation	E- value
36	30937893	<i>xiabt</i> 389	D	NADPH HC toxin reductase-like (<i>Oryza sativa</i>)	2.00E-17
37	57815147	<i>xiabt</i> 1	D	Inositolphosphorylceramide-B C-26 hydroxylase	6.00E-08
38	30964510	<i>xiabt</i> 22	D	Hypothetical protein Osl_06053 (<i>Oryza sativa</i>)	4.00E-15
39	14328763	<i>xiabt</i> 77	D	Unknown protein (<i>Zea mays</i>)	4.00E-02
40	31384321	<i>xiabt</i> 61	D	Receptor protein kinase-like protein (<i>Zea mays</i>)	8.00E-57
41	57804841	<i>xiabt</i> 63	D	Unknown protein (<i>Zea mays</i>)	3.00E-15
42	18062597	<i>xiabt</i> 01	D	No significant similarity found	
43	30942986	<i>xiabt</i> 79	D	No significant similarity found	
44	30976670	<i>xiabt</i> 374	D	Hypothetical protein LOC100191809 (<i>Zea mays</i>)	1.00E-29
45	11679325	<i>xiabt</i> 13	E	Hypothetical protein OsJ_12011 (<i>Oryza sativa</i>)	3.00E-39
46	34441344	<i>xiabt</i> 27	E	Unknown protein (<i>Zea mays</i>)	8.00E-77
47	34509854	<i>xiabt</i> 49	E	AATL2 (<i>Zea mays</i>)	3.00E-52
48	7536147	<i>xiabt</i> 30	F	Xyloglucan endotransglucosylase/hydrolase prot.	2.00E-15
49	7218421	<i>xiabt</i> 78	F	Ring H2 finger protein ATL 2K	1.00E-18
50	30936043	<i>xiabt</i> 83	F	Eukaryotic translation initiation factor 3 sub.	5.00E-43
51	45968710	<i>xiabt</i> 9	G	Unknown protein (<i>Zea mays</i>)	6.00E-85
52	30932211	<i>xiabt</i> 46	G	Hypothetical protein Osl_04777 (<i>Oryza sativa</i>)	3.00E-09
53	9856009	<i>xiabt</i> 81	G	Hypothetical protein (<i>Zea mays</i>)	1.00E-25
54	45974008	<i>xiabt</i> 72	H	Unknown protein (<i>Zea mays</i>)	5.00E-11
55	13064958	<i>xiabt</i> 5	I	Unknown protein (<i>Zea mays</i>)	5.00E-38
56	33108946	<i>xiabt</i> 29	I	[<i>Drosophila erecta</i>] 47829	2.00E-08
57	34509330	<i>xiabt</i> 64	I	Unknown protein (<i>Zea mays</i>)	1.00E-58
58	13585195	<i>xiabt</i> 70	I	Hypothetical protein LOC100193036 (<i>Zea mays</i>)	1.00E-76
59	9301623	<i>xiabt</i> 86	I	Hypothetical protein Osl_12212 (<i>Oryza sativa</i>)	2.00E-23
60	30970132	<i>xiabt</i> 94	I	Hypothetical protein LOC100193708 (<i>Zea mays</i>)	8.00E-52
61	31332570	<i>xiabt</i> 45	J	Para-hydroxybenzoate-polyprenyltransferas.	4.5
62	57820833	<i>xiabt</i> 68	J	Unknown protein (<i>Zea mays</i>)	5.00E-33
63	11680215	<i>xiabt</i> 420	J	Phytosulfokines 5 precursor (<i>Zea mays</i>)	3.00E-07

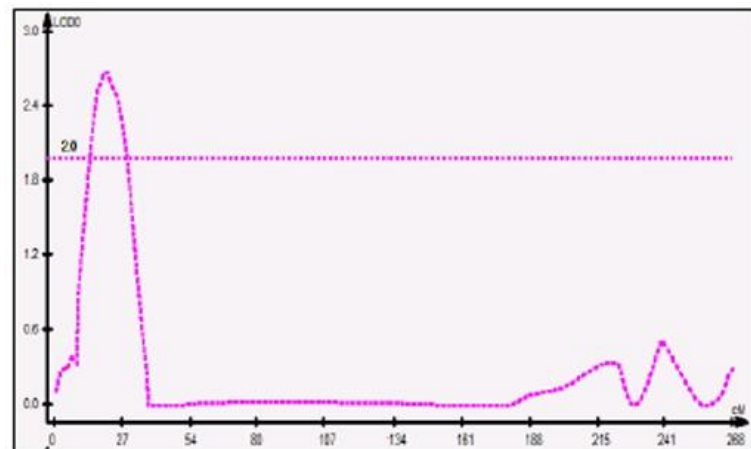
LG = Linkage group



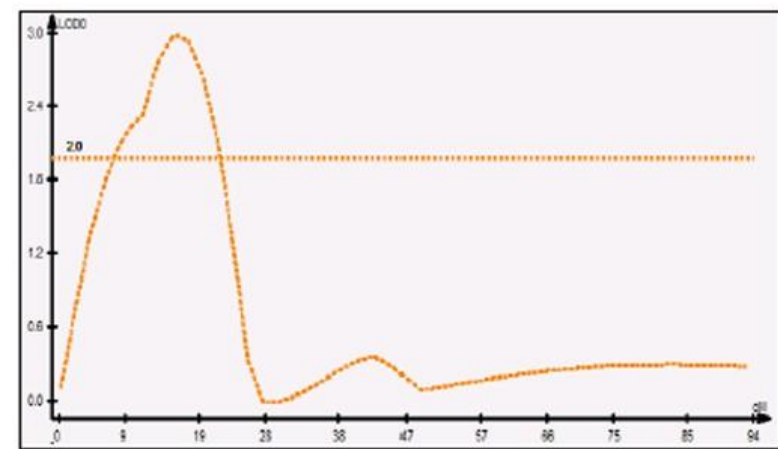
a) Length of infection on linkage group B with peak LOD² value of 2.9



b) Number of internode crossed on linkage group B with peak LOD² value of 4.0



c) Lignin content at 75 DAS on linkage group D with peak LOD² value of 2.6



d) Total phenol at 75 DAS on linkage group II with peak LOD² value of 3.0

Fig.8. Graphical output of Win Cartographer (2.5b) depicting QTLs for different traits

Table 15a: Features of the QTLs computed by composite interval mapping for charcoal rot resistance and yield component traits in the RILs derived from the cross IS 22380 × E 36-1 at Dharwad location during 2004

Traits	Marker	Linkage group	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internodes crossed	<i>txtp297-xiabt273</i>	B	331.9	4.4798	0.3934	19.29	18.23
	<i>txtp213-xiabt1</i>	D	229.8	2.7918	0.2758	12.54	16.50
Length of infection (cm)	<i>AC13-xiabt224</i>	A	100.7	2.2607	2.1356	10.76	18.82
Per cent lodging	<i>txtp343-txtp12</i>	D	1.8	3.0673	9.8628	11.01	4.35
	<i>txtp176-txtp312</i>	I	33.7	2.194	6.3081	7.89	8.10
Early seedling vigour	<i>txtp267-xiabt 71</i>	A	19.75	2.4697	-0.1926	9.86	17.33
	<i>txtp297-xiabt73</i>	B	327.9	3.8006	-0.1937	14.35	18.23
	<i>txtp12-xiabt 79</i>	D	4.36	5.1397	-0.2745	18.21	
	<i>txtp9-xiabt359</i>	D	51.6	2.5327	-0.1969	11.45	21.50
	<i>xcup9-M9</i>	D	171.7	2.3719	-0.2063	12.15	
Days 50% flowering	<i>AC12-xiabt80</i>	B	232.8	2.51	1.4861	11.97	34.65
Plant height (cm)	<i>corn24-txtp210</i>	C	10.01	2.2789	4.9936	12.83	11.83
Stem thickness (cm)	<i>AT5-H4</i>	A	139.8	3.6003	0.5276	11.14	7.2
	<i>txtp297-xiabt73</i>	B	328.5	2.3342	0.639	9.54	18.23
	<i>AK10-BH13</i>	F	70.23	3.9911	0.6199	12.47	2.92
	<i>txtp176-txtp312</i>	I	38.8	3.0152	0.711	9.85	8.10
Panicle length (cm)	<i>txtp176-txtp312</i>	I	32.7	2.0476	-1.8022	7.94	8.1
Threshability	<i>H4-xiabt75</i>	A	146.7	2.4151	0.3067	15.42	19.48
	<i>txtp348-xiabt31</i>	B	93.6	3.3335	0.3236	13.74	52.90
	<i>G16-L18</i>	B	165.8	3.0126	0.2604	11.28	10.68
	<i>txtp297-xiabt273</i>	B	318.9	2.5158	-0.2463	9.69	18.23
	<i>AC20-AK10</i>	F	60.9	2.1647	0.2163	7.93	2.93
1000-seed weight (g)	<i>corn59-txtp201</i>	B	47.5	2.1777	1.5678	8.04	15.10
	<i>txtp348-xiabt81</i>	B	91.6	2.2489	2.1385	8.65	52.90
Seed yield /plant (g)	<i>corn24-txtp210</i>	C	3.8	2.8309	5.9158	11.39	11.85
	<i>txtp45-xiabt81</i>	G	19.9	2.693	6.132	13.11	1.10

Table 15b: Features of the QTLs computed by composite interval mapping for charcoal rot resistance and yield component traits in the RILs derived from the cross IS 22380 × E 36-1 at Dharwad location during 2005

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed	<i>txtp297-xiabt73</i>	B	338.2	3.1	5.3	10.46	18.23
Length of infection (cm)	<i>AC13-xiabt224</i>	A	96.8	3.2	2.1	10.0	18.82
	<i>xiabt275-xiabt241</i>	B	465.8	2.43	3.1	8.0	17.23
Lodging per cent	<i>txtp176-xiabt312</i>	I	33.2	2.39	6.36	8.05	8.10
	<i>txtp274-xiabt29</i>	I	45.2	2.0	.6	10.0	76.50
	<i>txtp343-txtp12</i>	D	2.2	3.8	6.95	11.01	4.35
	<i>txtp338-xiabt420</i>	J	22.07	2.78	-7.62	11.17	21.30
Early seedling vigour	<i>txtp267-xiabt71</i>	A	18.9	2.97	-1.13	7.55	17.33
	<i>txtp297-xiabt 73</i>	B	326.5	2.33	-0.27	10.13	18.23
	<i>xiabt79-xiabt 77</i>	D	5.93	4.73	-1.33	16.11	4.20
	<i>txtp 9-xiabt 359</i>	D	39.5	3.22	-2.35	10.85	21.50
Days to 50% flowering	<i>AC12-xiabt 80</i>	B	219.7	2.98	2.13	9.22	34.65
	<i>xiabt 31-SvPEPCAA</i>	B	138.9	3.67	2.69	5.97	30.10
	<i>xiabt 78-xiabt83</i>	F	80.18	2.88	1.88	6.89	30.60
Plant height (cm)	<i>Corn 24-txtp210</i>	C	10.23	2.73	2.11	11.28	11.83
	<i>xiabt 81-xiabt9</i>	G	23.89	3.23	3.48	7.29	13.75
Stem thickness (cm)	<i>AT 5-H4</i>	A	142.9	2.73	0.15	7.75	7.20
	<i>txtp 297-xiabt73</i>	B	324.8	3.87	1.77	11.90	18.23
	<i>AK 10-BH13</i>	F	71.23	2.99	1.65	10.87	2.92
	<i>txtp 176-txtp312</i>	I	32.9	3.98	1.39	13.89	8.10
	<i>xiabt 13-xiabt27</i>	E	95.27	2.96	1.77	11.88	37.07
Panicle length (cm)	<i>txtp176-txtp312</i>	I	30.76	3.99	-2.88	8.99	8.10
Threshability	<i>H4-xiabt75</i>	A	149.78	2.98	1.67	10.87	19.48
	<i>txtp348-xiabt31</i>	B	85.9	2.84	0.76	8.90	52.90
	<i>G16-L18</i>	B	163.3	2.98	1.33	10.34	10.68
	<i>txtp 297-xiabt 273</i>	B	326.9	2.34	-0.93	8.98	18.23
	<i>AC20-AK10</i>	F	58.51	2.56	0.35	9.98	2.93
1000-seed weight (g)	<i>Corn 59-txtp 201</i>	B	43.19	2.55	1.3	7.01	15.10
	<i>txtp 348-xiabt 81</i>	B	80.1	2.16	2.19	9.76	52.90
	<i>xiabt27-xiabt49</i>	E	142.9	3.1	3.6	17.3	28.26
Seed yield /plant (g)	<i>Corn 24-txtp210</i>	C	6.89	2.23	3.98	6.88	11.83
	<i>txtp 45-xiabt81</i>	G	18.01	2.78	5.88	10.9	1.10
	<i>txtp 160-xiabt85</i>	C	39.8	2.19	4.33	17.9	25.62

Table 15c: Features of the QTLs computed by composite interval mapping for charcoal rot resistance and yield component traits in the RILs derived from the cross IS 22380 × E 36-1 at Dharwad location during 2006

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed by fungus	<i>txp297-xiabt73</i>	B	336.2	2.7	4.7	9.80	18.23
	<i>xiabt 92-xiabt62</i>	B	389.3	2.3	4.9	11.80	27.51
Length of infection (cm)	<i>AC13-xiabt224</i>	A	97.1	2.2	3.1	8.90	18.82
	<i>xiabt275-xiabt241</i>	B	465.8	3.5	4.9	16.1	17.23
Lodging per cent	<i>txp176-xiabt312</i>	I	32.8	3.2	3.8	10.90	8.10
	<i>txp274-xiabt29</i>	I	56.4	3.2	4.3	11.10	76.50
	<i>txp343-txp12</i>	D	3.9	2.8	4.3	9.20	4.35
	<i>txp338-xiabt420</i>	J	23.5	2.1	-3.67	8.77	21.30
Early seedling vigour	<i>txp267-xiabt71</i>	A	19.8	2.10	-2.8	5.8	17.33
	<i>txp297-xiabt 73</i>	B	329.5	2.89	-1.9	9.87	18.23
	<i>xiabt79-xiabt 77</i>	D	8.0	3.22	-2.1	10.9	4.20
	<i>txp 9-xiabt 359</i>	D	45.1	2.78	-2.9	18.9	21.50
	<i>txp213-xiabt1</i>	D	229.1	2.9	-1.9	5.98	16.50
Days to 50% flowering	<i>AC12-xiabt 80</i>	B	220.9	3.2	3.9	5.8	34.65
	<i>xiabt 31-SvPEPCAA</i>	B	134.7	2.99	2.8	7.8	30.10
	<i>xiabt 78-xiabt83</i>	F	83.9	2.6	1.88	10.9	30.60
Plant height (cm)	<i>Corn 24-txp210</i>	C	9.11	3.1	3.89	10.9	11.83
	<i>xiabt 81-xiabt9</i>	G	20.4	2.8	2.9	8.90	13.75
	<i>txp92-xiabt13</i>	E	58.9	2.3	1.98	7.99	41.02
Stem thickness (cm)	<i>AT 5-H4</i>	A	141.6	2.89	1.9	8.9	7.20
	<i>txp 297-xiabt73</i>	B	326.8	2.9	1.98	10.1	18.23
	<i>AK 10-BH13</i>	F	72.9	2.10	2.8	8.9	2.92
	<i>txp 176-txp312</i>	I	31.1	2.3	2.1	10.6	8.10
	<i>xiabt 13-xiabt27</i>	E	95.27	2.1	2.98	9.99	37.07
Panicle length (cm)	<i>txp176-txp312</i>	I	31.8	3.2	-3.2	10.9	8.10
Threshability	<i>H4-xiabt75</i>	A	150.8	2.11	2.33	11.9	19.48
	<i>txp348-xiabt31</i>	B	85.9	2.3	1.9	9.2	52.90
	<i>G16-L18</i>	B	163.2	2.1	2.38	11.09	10.68
	<i>txp 297-xiabt 73</i>	B	330.1	2.3	-2.1	11.9	18.23
	<i>AC20-AK10</i>	F	62.6	2.6	0.89	10.8	2.93
1000-seed weight (g)	<i>Corn 59-txp 303</i>	B	43.19	2.6	2.7	9.88	15.10
	<i>txp 348-xiabt 81</i>	B	80.1	2.3	3.2	11.90	52.90
	<i>xiabt27-xiabt49</i>	E	138.9	2.1	3.6	17.30	28.60
Seed yield /plant (g)	<i>Corn 24-txp210</i>	C	7.89	2.5	4.8	5.50	11.83
	<i>txp 45-xiabt81</i>	G	19.2	2.78	5.79	10.90	1.10
	<i>txp 160-xiabt85</i>	C	31.1	2.3	3.3	15.50	25.62
	<i>txp92-xiabt13</i>	E	57.8	3.2	7.8	10.10	40.02
	<i>txp230-txp258</i>	F	10.9	2.3	5.5	11.90	4.81

Table 15d: Features of the QTLs computed by composite interval mapping for charcoal rot resistance and yield component traits in the RILs derived from the cross IS 22380 × E 36-1 at Dharwad location during 2007

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed	<i>txp297-xiabt73</i>	B	340.0	3.3	7.8	5.9	18.23
	<i>xiabt 92-xiabt62</i>	B	374.9	3.3	5.5	10.9	27.51
	<i>txp302-xiabt380</i>	A	256.8	2.3	3.9	9.8	7.50
Length of infection (cm)	<i>AC13-xiabt224</i>	A	97.1	2.2	3.1	8.9	18.82
Lodging per cent	<i>txp176-xiabt312</i>	I	36.9	2.7	4.8	11.8	8.10
	<i>txp274-xiabt29</i>	I	59.8	2.2	3.2	10.1	76.50
	<i>txp343-txp12</i>	D	2.9	3.3	1.1	11.9	4.35
	<i>txp338-xiabt420</i>	J	29.8	3.2	-1.1	9.9	21.30
Early seedling vigour	<i>txp297-xiabt 73</i>	B	35.8	3.2	-3.9	10.99	17.33
	<i>xiabt79-xiabt 77</i>	D	4.9	4.7	-5.6	11.9	4.20
	<i>txp 9-xiabt 359</i>	D	48.1	3.1	-4.9	11.1	21.50
Days to 50% flowering	<i>AC12-xiabt 80</i>	B	211.3	2.8	4.8	15.9	34.65
	<i>xiabt 45-xiabt68</i>	J	181.1	2.6	4.3	10.8	0.04
	<i>xiabt 78-xiabt83</i>	F	91.7	2.8	3.4	15.8	30.60
	<i>txp 297-xiabt73</i>	B	339.7	3.7	2.8	11.9	17.33
	<i>AK 10-BH13</i>	F	71.3	4.90	3.7	15.9	2.92
	<i>xiabt 13-xiabt27</i>	E	111.8	3.1	2.6	11.9	37.07
Plant height (cm)	<i>Corn 24-txp210</i>	C	9.22	4.8	4.3	11.8	11.83
	<i>xiabt 81-xiabt9</i>	G	29.9	2.8	5.5	18.9	13.75
	<i>txp92-xiabt13</i>	E	67.8	2.0	9.8	10.9	41.02
Panicle length (cm)	<i>txp176-txp312</i>	I	33.7	2.8	-5.6	13.8	8.10
	<i>txp338-xiabt420</i>	J	28.9	3.7	-1.9	10.6	21.30
Threshability	<i>txp348-xiabt31</i>	B	89.9	2.6	2.4	10.8	52.90
	<i>txp 297-xiabt 73</i>	B	329.9	3.2	-5.7	6.7	18.23
	<i>AC20-AK10</i>	F	59.8	2.7	1.88	11.9	2.93
1000-seed weight (g)	<i>Corn 59-txp 303</i>	B	46.19	2.8	2.9	18.9	15.10
	<i>txp 348-xiabt 81</i>	B	89.1	2.9	3.2	16.8	52.90
	<i>xiabt27-xiabt49</i>	E	142.9	3.1	3.6	17.3	28.26
	<i>xiabt 86-txp96</i>	I	22.8	4.3	3.8	7.9	3.40
Seed yield /plant (g)	<i>Corn 24-txp210</i>	C	6.89	2.23	3.98	6.88	11.83
	<i>txp 45-xiabt81</i>	G	18.01	2.78	5.88	10.9	1.10
	<i>txp 160-xiabt85</i>	C	39.8	2.19	4.33	17.9	25.62
	<i>txp92-xiabt13</i>	E	90.2	3.9	8.7	11.9	41.02
	<i>txp230-txp258</i>	F	11.88	4.3	2.8	8.6	4.81
Total phenol at 75 DAS	<i>Ac19-txp254</i>	H	15.41	3.0	1.4	12	12.20
	<i>txp274-xiabt29</i>	I	50.11	2.4	1.54	29	76.50
	<i>xiabt 420 -xiabt 45</i>	J	80.0	2.9	3.0	10.0	138.70
Lignin content at 75 DAS	<i>txp303-txp301</i>	B	51.05	2.8	-0.25	10	7.15
	<i>xiabt 47-xiabt 51</i>	C	280.2	2.9	6.3	11.2	14.30
	<i>xiabt374-txp9</i>	D	28.46	4.02	0.29	22	4.20
Total phenol at 95 DAS	<i>txp329-Ac19</i>	H	13.17	3.62	2.52	14	76.50
	<i>txp274-xiabt29</i>	I	42.11	2.7	3.42	19	27.51
Lignin content at 95 DAS	<i>xiabt 92-xiabt 67</i>	A	372.4	6.41	2.2	23	2.82
	<i>txp 204-Corn 73</i>	E	43.0	2.9	7.2	10	3.52

Table 15e: Features of the QTLs that were specifically detected for charcoal rot and yield related component traits in RILs derived from the cross IS 22380 x E 36-1 at Dharwad location

Traits	Marker	Linkage group	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internodes crossed	<i>xtxp213-xiabt1</i>	D	229.80	2.79	0.27	12.54	18.00
Early seedling vigour	<i>xtxp267-xiabt71</i>	A	19.75	2.46	-0.19	9.86	17.33
Stem thickness (cm)	A75-H4	A	139.80	3.60	0.52	11.14	7.20
	<i>xtxp176-xtxp312</i>	I	38.80	3.01	0.71	9.85	8.10
Threshability	G16-L18 I	B	165.80	3.01	0.26	11.28	10.68

Table 15f: Features of the QTLs that were commonly detected across four seasons for charcoal rot and yield related component traits in RILs derived from the cross IS 22380 x E 36-1 at Dharwad location

Traits	Marker	Linkage group	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internodes crossed	<i>txtp297-xiabt273</i>	B	331.9-340.	2.7-4.4	0.39-7.8	5.9-19.29	18.23
Length of infection (cm)	<i>AC13-xiabt224</i>	A	96.1-100.7	2.2-3.2	2.1-4.6	8.9-10.7	18.82
	<i>xiabt275-xiabt241</i>	B	465.8-466.8	2.5-3.5	3.1-4.9	8-16.1	17.23
Per cent lodging	<i>txtp343-txtp12</i>	D	1.8-3.9	2.8-3.8	1.1-9.8	9.2-11.01	4.35
	<i>txtp176-txtp312</i>	I	32.8-36.9	3.1-3.2	3.8-6.3	7.8-11.8	8.10
	<i>txtp274-xiabt29</i>	I	45.2-59.8	2.0-3.2	3.2-4.3	10-11.1	76.50
	<i>txtp338-xiabt420</i>	J	22.07-29.8	2.1-3.2	-1.1-7.62	8.7-11.17	21.30
Early seedling vigour	<i>txtp267-xiabt 71</i>	A	18.9-19.8	2.1-2.9	-0.19-2.8	5.8-9.8	17.33
	<i>txtp297-xiabt73</i>	B	325.9-329.5	2.33-3.8	-0.19-3.9	9.87-14.35	18.23
	<i>txtp9-xiabt359</i>	D	39.5-51.6	2.53-3.22	-0.19-4.9	10.85-18.9	21.50
	<i>xiabt79-xiabt 77</i>	D	4.9-8.0	3.22-4.9	-1.3—5.6	10.9-16.11	4.20
Days 50% flowering	<i>AC12-xiabt80</i>	B	211-232.8	2.13-2.8	1.4-4.8	5.8-15.9	34.65
	<i>xiabt 78-xiabt83 d</i>	F	80.18-91.7	2.6-2.8	1.8-3.4	6.89-15.8	30.60
Plant height (cm)	<i>corn24-txtp210</i>	C	9.11-10.23	2.3-4.8	2.11-4.9	10.9-12.8	11.83
	<i>xiabt 81-xiabt9</i>	G	20.4-29.9	2.8-3.23	2.9-5.5	7.29-18.9	13.75
Stem thickness (cm)	<i>AT5-H4</i>	A	139.8-142.9	2.7-3.6	0.5-1.9	7.75-11.14	7.20
	<i>txtp297-xiabt73</i>	B	324.8-328.5	2.33-3.88	0.63-1.98	9.54-11.9	18.23
	<i>AK10-BH13</i>	F	70.23-72.9	2.10-3.99	0.61-2.8	8.9-12.4	2.92
	<i>txtp176-txtp312</i>	I	30.76-32.7	2.1-3.98	0.7-1.39	9.85-13.89	8.10
Panicle length (cm)	<i>txtp176-txtp312</i>	I	30.76-33.7	2.0-3.99	-1.8—5.6	7.94-13.8	8.10
Threshability	<i>H4-xiabt75 I</i>	A	146.7-150.8	2.1-2.9	0.3-2.33	10.87-15.42	19.48
	<i>txtp348-xiabt31</i>	B	85.9-95.6	2.3-3.3	0.3-2.4	8.9-13.74	52.90
	<i>G16-L18</i>	B	163.2-165.8	2.1-3.0	0.26-2.38	10.34 -11.28	10.68
	<i>txtp297-xiabt273</i>	B	318.9-329.9	2.3-3.2	-0.24-5.7	6.7-11.9	18.23
	<i>AC20-AK10</i>	F	58.51-62.6	2.1-2.7	0.2-1.88	7.93-11.9	2.93
1000-seed weight (g)	<i>corn59-txtp201</i>	B	43.19-47.5	2.1-2.8	1.3-2.9	7.01-18.9	15.10
	<i>txtp348-xiabt81</i>	B	80.1-91.6	2.16-3.2	2.1-3.2	8.65-16.8	52.90
	<i>xiabt27-xiabt49</i>	E	138.9-143.9	2.1-4.7	2.3-3.6	11.9-18.3	28.26
Seed yield /plant (g)	<i>corn24-txtp210</i>	C	3.8-7.89	2.1-3.1	2.56-5.9	4.4-11.6	11.83
	<i>txtp45-xiabt81</i>	G	18.01-19.5	2.6-3.3	4.1-6.8	10.9-13.5	1.10

Total phenol at 95 DAS

One QTL was detected for this trait on linkage group I (*xtxp* 274 - *xiabt* 29) with phenotypic variance 19 and LOD score of 2.7. The additive effect of 3.42 for the QTL on linkage group I showed that the parent E 36-1 was contributing to this locus.

Lignin content at 95 DAS

Two QTLs were detected for this trait one each on linkage group A (*xiabt* 92 – *xiabt* 67) and LG-E (*xtxp* 204 – *Corn* 43) with phenotypic variance of 23 and 10 respectively with peak LOD score of 6.41 and 2.9, respectively. The additive effect of 2.2 and 7.2 were on linkage group A and E, respectively

Early seedling vigour

A total of five QTLs were detected for early seedling vigour at Dharwad location of which four QTLs were found to be consistent during all the four seasons, while one QTL (*xtxp* 213 – *xiabt* 1) was detected separately during 2006 season. Four QTL (*xtxp* 267 – *xiabt* 71, *xtxp* 297 – *xiabt* 73, *xtxp* 9 – *xiabt* 359, *xiabt* 79 – *xiabt* 77) were found on the LG-D while one QTL was mapped on LG-A (*xtxp* 213 – *xiabt* 1) All the QTL showed additive effect with favorable allele contributing from E36-1 parent. The Phenotypic variance and peak LOD score value were in the range of 5.8 to 16.11 and 2.1 to 4.9, respectively. One QTL flanked by marker *xtxp* 9 and *xiabt* 359 on LG D with show a relatively high LOD score (3.22) and phenotypic variance (18.9).

Plant height

Three QTLs were detected for plant height one each on linkage group C (*Corn* 24 – *xiabt* 80), LG-G (*xiabt* 81 – *xiabt* 9), and LG-E (*xtxp* 92 – *xiabt* 13). Two QTL one each on LG-C and LG-G were consistent at all the four season while one QTL on LG E was detected for only two seasons. One QTL on linkage group G was flanked by genic SSR (*xiabt* 81 and *xiabt* 9), which showed LOD score of 2.8 - 3.23 with phenotypic variance of 7.29 - 18.9.

Days to 50 per cent flowering

Seven QTLs bracketed by the pairs of markers, AC 12 – *xiabt* 80, *xiabt* 45 – *xiabt* 68, *xiabt* 78 – *xiabt* 83, *xtxp* 297 – *xiabt* 73, AK 10 – BH 13, *xiabt* 13 – *xiabt* 27 and AC 12 – *xiabt* 80 were detected for this trait, out of which two QTLs conditioned by the marker pairs AC 12 – *xiabt* 80 and *xiabt* 78 – *xiabt* 83 were consistent for all the season while four QTLs (*xiabt* 45 – *xiabt* 68, *xiabt* 78 – *xiabt* 83, *xtxp* 297 – *xiabt* 73, AK 10 – BH 13, *xiabt* 13 – *xiabt* 27) were detected only during 2007 and one QTL flanked by the marker *xiabt* 31 and SvPEPCAA on LG-B was detected during 2005 and 2006. One QTL was flanked by genic SSR which was consistent for all the season was on linkage group F with additive effect of 1.8 - 3.4 and phenotypic variance of 6.89 - 15.8.

Stem thickness

Five QTLs conditioned by the marker pairs AT 5 – H 4, *xtxp* 297 – *xiabt* 73, AK 10 – BH 13, *xtxp* 176 – *xtxp* 312 and *xiabt* 13 - *xiabt* 27, were detected for stem thickness. All the five QTLs were detected on different linkage group such as LG-A, LG-B, LG-F, LG-I and LG-E. Four QTLs were consistent for all the season out of which one QTL flanked by the marker *xtxp* 176- *xtxp* 312 on linkage group I showed overlap with QTL for ear head length. The QTL on linkage group E flanked by the marker *xiabt* 13 and *xiabt* 27 was common for two season with LOD score of 2.1 - 2.9 with phenotypic variance of 9.99 - 11.8.

Ear head length

Two QTLs were detected on two different linkage group viz., LG-I (*xtxp* 176 - *xtxp* 312, and LG-J (*xtxp* 338 – *xiabt* 420) out of which only one QTL on linkage group I flanked by marker *xtxp* 176 and *xtxp* 312 was consistent for all the four season with peak

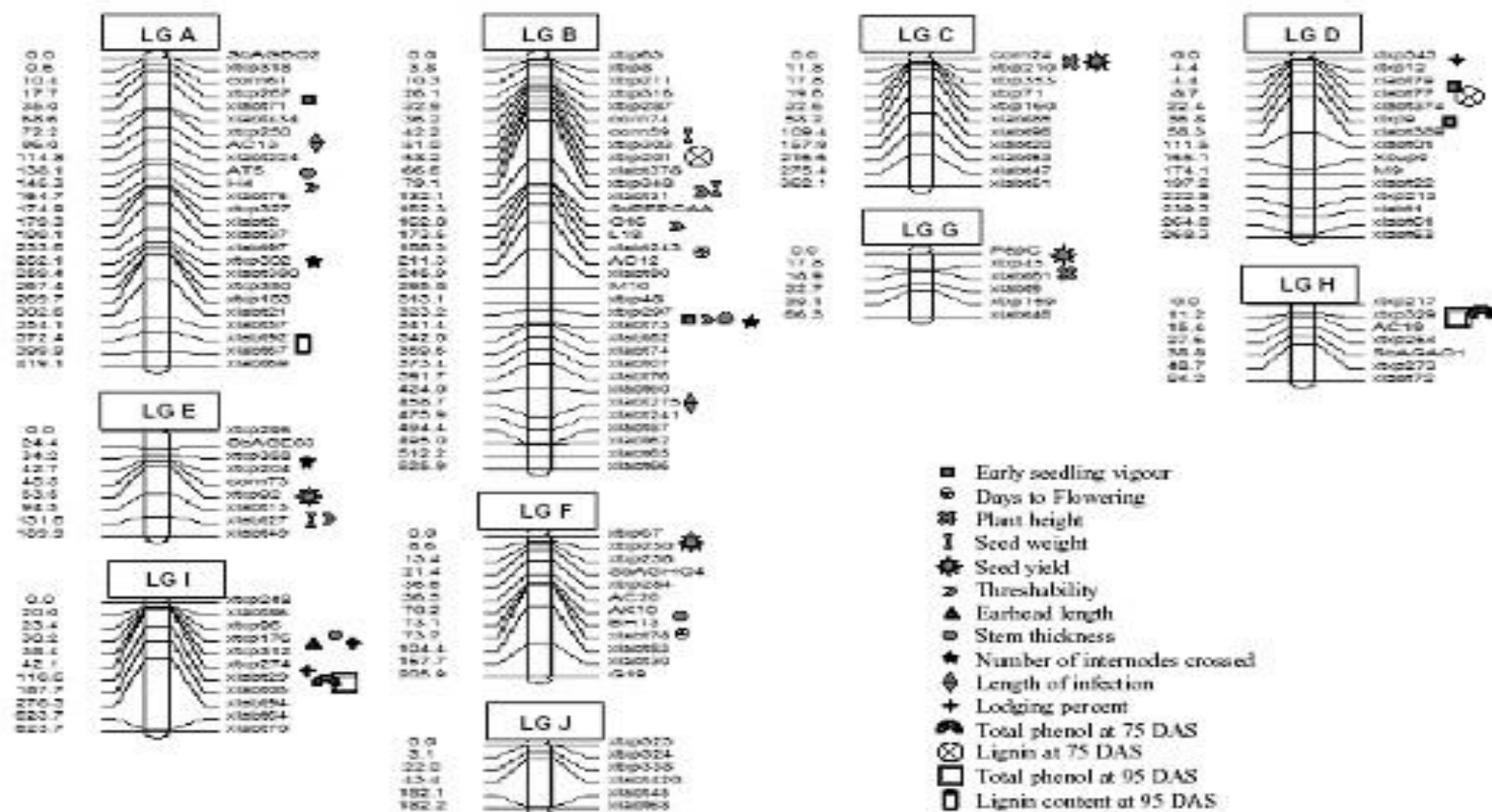


Fig.9. Map localization of common and stable QTLs for morphological and biochemical trait over four season at Dharwad location

LOD score of 3.99 with phenotypic variance of 13.8. The phenotypic variance and LOD score were in range of 10.6 - 13.8 and 2.8 – 3.99, respectively.

Threshability

Total of five QTLs were detected consistently for all the four season. Out of Five QTL three on linkage group B (G 16 - L 18, *txp* 297 – *xiabt* 273 and *txp* 348 – *xiabt* 31) and one each on linkage group A (H4 – *xiabt* 75) and LG-F (AC 20 – AK 10) were located. The phenotypic variance and LOD score were in range of 7.93- 15.42 and 2.4 – 3.3, respectively. The QTL on linkage group A flanked by the marker H4 and *xiabt* 275 showed the high LOD value of 3.3 with phenotypic variance of 15.42.

1000 seed weight

Three QTLs were detected of which two QTL were consistent and were found on same linkage group B (*Corn* 59 – *txp* 202 and *txp* 348 – *xiabt* 81). The phenotypic variance and LOD score were in range of 7.01 – 18.3 and 2.1 – 4.7, respectively. One QTL which was found on linkage group E was flanked by *xiabt* 27 and *xiabt* 49 with LOD score of 4.7 and phenotypic variance of 18.3.

Seed yield

Out of five QTLs detected on linkage group C (*Corn* 24 – *txp* 210, *txp* 160 – *xiabt* 85, and, LG-G (*txp* 45 – *xiabt* 81), LG-E (*txp* 92 – *xiabt* 13) and LG-F (*txp* 230 – *txp* 258), only one QTL on linkage group C was consistent for all the season. The QTL on linkage group C was flanked by *Corn* 24 – *txp*210 at position 7.89 cM this QTL showed Phenotypic variance of 11.6 with LOD value of 3.8 and additive effect of 5.9.

4.3.2 Bijapur location

All the QTLs associated with 11 different traits during different season were worked separately for Bijapur location (Table 16a to 16f). Graphical representation of chromosomal positions of common QTLs found at all the four seasons with flanking markers is presented in Fig. 10.

Number of internodes crossed by fungus

Four QTLs were detected consistently on linkage group A (*txp* 302 – *xiabt* 380), LG-B (*txp* 297 – *xiabt* 73 and *xiabt* 92 – *xiabt* 62), and LG-E (*txp*358 – *txp* 204) for all the season for number of internodes crossed by the fungus. On linkage group B two QTLs were detected for this trait at position 338cM (*txp* 297-*xiabt* 73) and 380.0 cM (*xiabt* 92-*xiabt* 62) with high phenotypic variance of 15.5 and 16.9, respectively. The QTL on linkage group B with flanking marker *txp* 297 – *xiabt* 73 was co-localized with QTL for stem thickness and early seedling vigour.

Length of infection

Total of three QTLs were detected for this trait at Dharwad of which two QTL showed consistent expression for all the seasons. Two QTLs were found on the same linkage group B at distance 60.3cM (*txp*201-*xiabt*378) and 465.8 cM (*xiab* 275-*xiabt* 241) with phenotypic variance of 17.7 and 16.1, respectively. The additive effect for these two QTLs showed that the allele from E36-1 is contributing positively for this trait.

Lodging per cent

Five QTLs were detected for this trait one each on linkage group D (*txp* 343 – *txp* 12), LG-H (AC 19 – *txp* 254), LG-I (*txp* 176 – *xiabt* 312 and *txp* 274 – *xiabt* 312), and LG-J (*txp* 338 – *xiabt* 420). Three QTLs were common for all the four season which were located on linkage group I (*txp* 176 – *xiabt* 312 and *txp* 274 – *xiabt* 29) and linkage group D (*txp* 343 – *txp* 12). The QTLs on linkage group I located at position 36.9 cM (*txp* 176 - *xiabt* 312) and 59.8cM (*txp*274-*xiabt* 29) showed high phenotypic variance of 11.8 and 10.1, respectively.

Table 16a: Features of the QTLs computed by composite interval mapping for charcoal rot resistance and yield component traits in the RILs derived from the cross IS 22380 × E 36-1 at Bijapur location during 2004

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed	<i>txp297-xiabt73</i>	B	338.0	3.5	6.5	15.5	18.23
	<i>xiabt 92-xiabt62</i>	B	380.5	2.5	4.6	16.9	27.51
	<i>txp302-xiabt38</i>	A	256.8	2.6	1.7	10.5	7.50
	<i>txp358-txp204</i>	E	37.8	2.3	3.6	9.9	8.46
Length of infection (cm)	<i>xiabt275-xiabt241</i>	B	465.8	3.5	4.9	16.1	17.23
	<i>txp201-xiabt378</i>	B	60.3	2.3	7.8	17.7	8.40
Lodging per cent	<i>txp176-xiabt312</i>	I	36.9	2.7	4.8	11.8	8.10
	<i>txp274-xiabt29</i>	I	59.8	2.2	3.2	10.1	76.50
	<i>txp343-txp12</i>	D	2.9	3.3	1.1	11.9	4.35
Early seedling vigour	<i>txp273-xiabt72</i>	H	51.9	2.2	-2.9	8.9	17.33
	<i>txp297-xiabt 73</i>	B	326.9	2.7	-2.7	13.3	18.23
	<i>xiabt79-xiabt 77</i>	D	8.2	2.1	-2.2	10.8	4.20
	<i>txp 9-xiabt 359</i>	D	42.1	2.6	-3.1	9.8	21.50
	<i>xiabt96-xiabt20</i>	C	123.3	2.3	-2.6	10.9	48.50
Days to 50% flowering	<i>AC12-xiabt 80</i>	B	230.9	2.3	3.8	11.9	34.65
	<i>xiabt 45-xiabt68</i>	J	182.1	2.8	6.8	8.9	0.05
	<i>xiabt 78-xiabt83</i>	F	101.8	3.8	7.9	18.9	30.60
Plant height (cm)	<i>Corn 24-txp210</i>	C	10.3	2.9	3.3	9.8	11.83
	<i>xiabt 81-xiabt9</i>	G	233.7	3.3	4.9	10.2	13.75
	<i>txp92-xiabt13</i>	E	90.3	3.2	7.6	9.1	41.02
	<i>BH13-xiabt 78</i>	F	71.9	2.7	5.5	11.9	0.50
	<i>txp338-xiabt420</i>	J	26.9	3.9	4.4	17.7	21.30
Stem thickness (cm)	<i>txp318-corn61</i>	A	3.3	2.2	6.8	10.0	9.88
	<i>txp 297-xiabt73</i>	B	339.7	3.7	2.8	11.9	18.23
	<i>AK 10-BH13</i>	F	71.3	3.8	3.9	11.8	2.93
	<i>BH13-xiabt78</i>	F	73.5	2.2	8.9	10.9	0.50
	<i>xiabt 13-xiabt27</i>	E	113.7	2.5	7.9	10.8	37.07
Panicle length (cm)	<i>txp176-txp312</i>	I	37.9	3.8	-3.9	10.7	8.10
	<i>txp338-xiabt420</i>	J	42.2	2.2	-2.8	9.1	21.30
	<i>Corn 73-txp92</i>	E	49.8	2.8	-4.5	19.9	7.93
Threshability	<i>H4-xiabt75</i>	A	162.2	2.9	3.33	15.1	19.48
	<i>txp348-xiabt31</i>	B	90.8	3.2	3.4	13.3	52.90
	<i>xiabt 27-xiabt49</i>	E	140.7	2.2	4.28	10.1	28.26
	<i>txp 297-xiabt 73</i>	B	330.9	2.9	-2.7	15.6	18.23
	<i>AC20-AK10</i>	F	70.1	2.1	3.3	18.3	2.93
1000-seed weight (g)	<i>Corn 59-txp 303</i>	B	49.9	2.5	5.5	16.1	15.10
	<i>txp 348-xiabt 81</i>	B	128.9	2.6	4.4	10.7	52.90
	<i>xiabt27-xiabt49</i>	E	139.8	3.6	4.4	10.5	28.26
	<i>xiabt 86-txp96</i>	I	22.8	4.3	3.8	7.9	3.40
Seed yield /plant (g)	<i>Corn 24-txp210</i>	C	8.89	2.2	3.3	15.1	11.83
	<i>txp 45-xiabt81</i>	G	18.01	2.1	4.6	9.6	1.10
	<i>txp 160-xiabt85</i>	C	43.8	2.6	3.3	15.6	25.62
	<i>txp92-xiabt13</i>	E	91.7	2.5	6.7	10.8	41.02
	<i>txp230-txp258</i>	F	14.7	3.5	6.6	11.9	4.81

Table 16b: Features of the QTLs computed by composite interval mapping for charcoal rot resistance and yield component traits in the RILs derived from the cross IS 22380 × E 36-1 at Bijapur location during 2005

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed	<i>txp297-xiabt73</i>	B	325.0	2.2	3.6	11.7	18.23
	<i>xiabt 92-xiabt62</i>	B	391.8	3.3	8.8	9.7	27.51
	<i>txp302-xiabt380</i>	A	258.7	2.1	3.7	16.7	7.50
	<i>txp358-txp204</i>	E	40.9	2.8	3.8	19.8	8.46
Length of infection (cm)	<i>AC13-xiabt224</i>	A	100.7	3.8	8.9	17.8	18.82
	<i>xiabt275-xiabt241</i>	B	471.8	2.1	3.7	13.8	17.23
	<i>txp201-xiabt37</i>	B	60.3	3.1	5.6	13.6	8.40
Lodging per cent	<i>txp176-xiabt312</i>	I	37.1	2.2	3.5	11.0	8.10
	<i>txp274-xiabt29</i>	I	62.8	3.9	5.5	11.8	76.50
	<i>txp343-txp12</i>	D	3.1	2.7	3.9	13.7	4.35
	<i>txp338-xiabt420</i>	J	31.9	3.8	-2.7	11.9	21.30
	<i>AC19-txp254</i>	H	18.6	3.1	-3.1	4.1	12.20
Early seedling vigour	<i>txp273-xiabt72j</i>	H	56.9	3.6	-2.9	18.9	17.33
	<i>txp297-xiabt 73</i>	B	329.6	3.8	-1.9	10.8	18.23
	<i>xiabt79-xiabt 77</i>	D	5.3	2.5	-2.8	11.8	4.20
	<i>txp 9-xiabt 359</i>	D	39.7	2.7	-2.9	10.8	21.50
Days to 50% flowering	<i>AC12-xiabt 80</i>	B	243.0	2.1	6.7	8.8	34.65
	<i>xiabt 78-xiabt83</i>	F	82.1	2.9	5.9	10.8	30.60
Plant height (cm)	<i>Corn 24-txp210</i>	C	10.3	4.3	6.7	11.9	11.83
	<i>xiabt 81-xiabt9</i>	G	231.8	2.2	5.5	8.9	13.75
	<i>BH13-xiabt 78</i>	F	72.8	2.1	6.7	18.0	0.50
	<i>txp338-xiabt420</i>	J	39.2	2.7	5.5	15.7	21.30
Stem thickness (cm)	<i>txp318-corn 61</i>	A	7.9	3.6	7.7	9.1	9.88
	<i>txp 297-xiabt73</i>	B	338.1	2.9	6.6	20.4	18.23
	<i>AK 10-BH13</i>	F	71.9	2.5	6.9	16.5	2.93
	<i>BH13-xiabt78</i>	F	72.5	2.7	7.9	5.1	0.50
	<i>xiabt 13-xiabt27</i>	E	126.9	2.6	6.1	9.9	37.07
Panicle length (cm)	<i>txp176-txp312</i>	I	33.3	4.4	-2.9	18.1	8.10
	<i>txp338-xiabt420</i>	J	27.1	3.3	-3.3	17.1	21.30
	<i>Corn 73-txp92</i>	E	47.5	3.3	-5.6	10.8	7.93
Threshability	<i>H4-xiabt75</i>	A	160.1	2.1	4.7	11.9	19.48
	<i>txp348-xiabt31</i>	B	101.8	3.9	7.8	6.7	52.90
	<i>xiabt 27-xiabt49</i>	E	150.1	3.8	1.1	8.8	28.26
	<i>txp 297-xiabt 73</i>	B	335.9	3.8	-2.7	10.1	18.23
	<i>AC20-AK10</i>	F	60.1	2.9	1.1	8.9	2.93
1000-seed weight (g)	<i>Corn 59-txp 303</i>	B	50.9	2.0	3.9	10.9	15.10
	<i>txp 348-xiabt 81</i>	B	123.3	3.8	5.5	18.9	52.90
	<i>xiabt27-xiabt49</i>	E	140.7	2.8	2.1	10.9	28.26
	<i>xiabt 86-txp96</i>	I	23.3	2.1	8.8	19.9	3.40
Seed yield /plant (g)	<i>Corn 24-txp210</i>	C	9.1	3.2	4.5	10.3	11.83
	<i>txp 45-xiabt81</i>	G	16.5	2.8	3.6	17.9	1.10
	<i>txp 160-xiabt85</i>	C	45.6	2.2	2.6	13.3	25.62
	<i>txp92-xiabt13</i>	E	87.6	2.8	3.3	7.8	41.02
	<i>txp230-txp258</i>	F	9.8	2.9	1.1	7.9	4.81
	<i>txp325-txp324</i>	J	7.3	2.5	7.8	10.9	3.08

Table 16c: Features of the QTLs computed by composite interval mapping for charcoal rot resistance and yield component traits in the RILs derived from the cross IS 22380 × E 36-1 at Bijapur location during 2006

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed	<i>txp297-xiabt73</i>	B	329.1	2.61	3.7	10.9	18.23
	<i>xiabt 92-xiabt62</i>	B	392.8	2.1	7.9	11.8	27.51
	<i>txp302-xiabt380</i>	A	256.9	2.3	1.8	10.9	7.50
	<i>txp358-txp204</i>	E	36.8	2.78	2.7	13.7	8.46
Length of infection (cm)	<i>AC13-xiabt224</i>	A	102.7	2.8	9.0	13.3	18.82
	<i>xiabt275-xiabt241</i>	B	460.3	2.3	4.3	11.8	17.23
	<i>txp201-xiabt378</i>	B	62.5	2.6	3.8	11.9	8.40
Lodging per cent	<i>txp176-xiabt312</i>	I	37.1	2.2	3.4	10.7	8.10
	<i>txp274-xiabt29</i>	I	62.8	3.3	2.7	11.8	76.50
	<i>xiabt83-xiabt30</i>	F	130.9	2.3	3.8	10.9	53.52
Early seedling vigour	<i>txp273-xiabt72</i>	H	72.8	2.8	-3.8	10.9	17.33
	<i>txp297-xiabt 73</i>	B	329.3	2.7	-1.8	11.6	18.23
	<i>txp267-xiabt71</i>	A	28.9	2.3	-2.3	10.3	17.33
	<i>xiabt79-xiabt 77</i>	D	6.3	3.4	-4.5	10.9	4.20
	<i>txp 9-xiabt 359</i>	D	40.9	2.1	-1.8	8.7	21.50
Days to 50% flowering	<i>AC12-xiabt 80</i>	B	216.8	2.7	4.8	7.9	34.65
	<i>txp284-Ac20</i>	F	38.9	2.1	3.8	11.9	19.91
	<i>xiabt 78-xiabt83</i>	F	82.7	2.6	3.6	11.8	30.60
Plant height (cm)	<i>Corn 24-txp210</i>	C	7.9	2.6	5.4	10.8	11.83
	<i>xiabt 81-xiabt9</i>	G	229.8	2.8	1.8	18.9	13.75
	<i>txp159-xiabt46</i>	G	40.9	2.3	2.7	10.3	27.10
	<i>BH13-xiabt 78</i>	F	71.8	2.8	5.3	17.6	0.50
	<i>txp338-xiabt420</i>	J	42.8	3.8	4.7	18.9	21.30
Stem thickness (cm)	<i>txp318-corn 61</i>	A	8.8	2.5	3.8	11.9	9.88
	<i>txp 297-xiabt73</i>	B	339.3	2.3	5.4	18.7	18.23
	<i>AK 10-BH13</i>	F	71.9	2.3	7.1	11.8	2.93
	<i>BH13-xiabt78</i>	F	73.2	2.1	2.9	15.6	0.50
	<i>xiabt 13-xiabt27</i>	E	129.8	2.5	3.5	11.7	37.07
Panicle length (cm)	<i>txp176-txp312</i>	I	34.5	3.5	-3.7	14.5	8.10
	<i>Corn 73-txp92</i>	E	46.9	2.3	-3.3	11.9	7.93
Threshability	<i>H4-xiabt75</i>	A	161.3	2.7	3.9	10.9	19.48
	<i>xiabt 27-xiabt49</i>	E	151.9	3.5	2.3	17.6	28.26
	<i>txp 297-xiabt 73</i>	B	339.1	2.3	-2.6	7.8	18.30
	<i>AC20-AK10</i>	F	61.6	3.2	2.1	17.8	2.93
1000-seed weight (g)	<i>Corn 59-txp 303</i>	B	49.6	2.7	2.7	11.8	15.10
	<i>txp 348-xiabt 81</i>	B	125.7	2.7	4.3	16.3	52.90
	<i>xiabt27-xiabt49</i>	E	157.8	2.1	3.2	7.8	28.26
	<i>xiabt 86-txp96</i>	I	20.0	2.6	6.6	16.5	3.40
Seed yield /plant (g)	<i>Corn 24-txp210</i>	C	7.6	2.5	3.7	17.8	11.83
	<i>txp 45-xiabt81</i>	G	18.1	2.1	3.3	15.7	1.10
	<i>txp 160-xiabt85</i>	C	51.6	2.3	3.2	17.6	25.62
	<i>txp92-xiabt13</i>	E	88.9	2.1	3.9	15.8	41.02

Table 16d: Features of the QTLs computed by composite interval mapping for charcoal rot resistance and yield component traits in the RILs derived from the cross IS 22380 × E 36-1 at Bijapur location during 2007

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed	<i>txp297-xiabt73</i>	B	327.8	3.3	4.4	19.9	18.23
	<i>xiabt 92-xiabt62</i>	B	393.4	2.7	6.5	18.9	27.51
	<i>txp302-xiabt380</i>	A	254.3	2.8	10.9	18.9	7.50
	<i>txp358-txp204</i>	E	42.1	5.7	2.1	16.9	8.46
Length of infection (cm)	<i>xiabt275-xiabt241</i>	B	473.8	2.8	3.9	11.9	17.23
	<i>txp201-xiabt378</i>	B	62.7	3.9	6.7	14.8	8.40
Lodging per cent	<i>txp176-xiabt312</i>	I	36.8	2.8	3.8	11.9	8.10
	<i>txp338-xiabt420</i>	J	40.9	3.1	-3.8	10.9	21.30
	<i>AC19-txp254</i>	H	23.9	2.8	-6.7	18.9	12.20
Early seedling vigour	<i>txp273-xiabt72</i>	H	91.5	2.8	-3.9	11.2	17.33
	<i>txp297-xiabt 73</i>	B	333.8	2.9	-1.8	11.9	18.23
	<i>xiabt79-xiabt 77</i>	D	8.1	3.2	-1.6	11.8	4.20
Days to 50% flowering	<i>AC12-xiabt 80</i>	B	243.8	3.2	7.0	7.3	34.65
	<i>txp284-Ac20</i>	F	47.9	3.2	1.9	18.9	19.91
	<i>xiabt 78-xiabt83</i>	F	85.7	3.3	4.4	11.0	30.60
Plant height (cm)	<i>Corn 24-txp210</i>	C	11.1	4.3	5.5	15.6	11.83
	<i>xiabt 81-xiabt9</i>	G	231.8	2.9	5.8	13.8	1.75
	<i>BH13-xiabt 78</i>	F	74.3	3.2	3.6	16.5	0.50
	<i>txp 45-xiabt81</i>	G	18.1	2.3	4.5	13.7	1.10
Stem thickness (cm)	<i>txp 297-xiabt73</i>	B	335.9	2.5	7.9	17.6	18.30
	<i>BH13-xiabt78</i>	F	73.6	2.3	5.5	13.7	0.50
Panicle length (cm)	<i>txp176-txp312</i>	I	37.8	4.2	-3.3	11.9	8.10
	<i>txp338-xiabt420</i>	J	39.3	4.3	-2.7	15.6	21.30
	<i>Corn 73-txp92</i>	E	48.7	3.6	-8.9	12.9	7.93
Threshability	<i>H4-xiabt75</i>	A	165.1	3.5	3.9	10.8	19.48
	<i>txp348-xiabt31</i>	B	105.8	2.8	5.8	11.8	52.90
	<i>xiabt78-xiabt83</i>	F	89.9	2.7	5.8	15.8	30.60
1000-seed weight (g)	<i>txp 348-xiabt 81</i>	B	130.9	2.7	3.8	14.5	52.90
	<i>xiabt27-xiabt49</i>	E	144.4	2.2	3.8	11.0	28.26
	<i>xiabt 86-txp96</i>	I	22.8	3.7	10.9	17.9	3.40
Seed yield /plant (g)	<i>txp 45-xiabt81</i>	G	18.1	2.1	3.7	16.8	1.10
	<i>txp 160-xiabt85</i>	C	44.8	2.9	3.7	11.8	25.62
	<i>txp92-xiabt13</i>	E	84.3	3.8	4.3	16.8	41.02
	<i>txp230-txp258</i>	F	13.3	2.1	2.9	10.9	4.81
Total phenol at 75 DAS	<i>Ac19-txp254</i>	H	16.61	2.9	2.39	11.13	12.20
	<i>txp274-xiabt29</i>	I	49.2	2.3	1.45	25.10	76.50
Lignin content at 75 DAS	<i>txp303-txp301</i>	B	51.5	2.6	2.2	12.70	7.15
	<i>xiabt374-txp9</i>	D	26.4	3.90	3.25	23.25	14.30
Total phenol at 95 DAS	<i>txp329-Ac19</i>	H	12.14	3.50	2.3	15.60	4.20
	<i>txp274-xiabt29</i>	I	43.2	2.5	1.5	17.10	76.50
Lignin content at 95 DAS	<i>xiabt 92-xiabt 67</i>	A	372.11	6.41	3.1	19.23	27.51

Table 16e: Features of the QTLs that were specifically detected for charcoal rot and yield related component traits in RILs derived from the cross IS 22380 x E 36-1 at Bijapur location

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed	<i>txp302-xiabt380</i>	A	256.8	2.6	1.7	10.5	7.50
	<i>txp358-txp204</i>	E	37.8	2.3	3.6	9.9	8.46
Lodging per cent	<i>AC19-txp254</i>	H	18.6	3.1	-3.1	4.1	12.20
	<i>xiabt83-xiabt30</i>	F	130.9	2.3	3.8	10.9	53.52
Early seedling vigour	<i>txp273-xiabt72</i>	H	51.9	2.2	-2.9	8.9	17.33
	<i>xiabt96-xiabt20</i>	C	123.3	2.3	-2.6	10.9	48.50
	<i>txp92-xiabt1304</i>	E	90.3	3.2	7.6	9.1	41.02
	<i>BH13-xiabt 78</i>	F	71.9	2.7	5.5	11.9	0.50
	<i>txp338-xiabt420</i>	J	26.9	3.9	4.4	17.7	27.30
	<i>txp 45-xiabt81</i>	G	18.1	2.3	4.5	13.7	1.10
Days to 50% flowering	<i>xiabt 45-xiabt68</i>	J	182.1	2.8	6.8	8.9	0.05
	<i>txp284-Ac20</i>	F	47.9	3.2	1.9	18.9	19.91
Stem thickness (cm)	<i>txp318-corn</i>	A	3.3	2.2	6.8	10.0	9.88
	<i>BH13-xiabt78</i>	F	73.5	2.2	8.9	10.9	0.50
	<i>xiabt 13-xiabt27</i>	E	113.7	2.5	7.9	10.8	37.07
Panicle length (cm)	<i>txp338-xiabt420</i>	J	42.2	2.2	-2.8	9.1	21.30
	<i>Corn 73-txp92</i>	E	49.8	2.8	-4.5	19.9	7.93
Threshability	<i>xiabt 27-xiabt49</i>	E	140.7	2.2	4.28	10.1	28.26
	<i>xiabt78-xiabt83</i>	F	89.9	2.7	5.8	15.8	30.60
1000-seed weight (g)	<i>xiabt 86-txp96</i>	I	22.8	4.3	3.8	7.9	3.40
Seed yield /plant (g)	<i>txp92-xiabt13</i>	E	91.7	2.5	6.7	10.8	41.02
	<i>txp230-txp258</i>	F	14.7	3.5	6.6	11.9	4.81
	<i>txp325-txp324</i>	J	7.3	2.5	7.8	10.9	3.08

Table 16f: Features of the QTLs that were commonly detected across four seasons for charcoal rot and yield related component traits in RILs derived from the cross IS 22380 × E 36-1 at Bijapur location

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed	<i>txtp297-xiabt73</i>	B	325.0-338.0	2.2-3.5	2.6-6.5	10.9-19.9	18.23
	<i>xiabt 92-xiabt62</i>	B	380.5-393.4	2.1-3.3	4.6-8.8	9.7-18.9	27.51
	<i>txtp302-xiabt380</i>	A	254.3-258.7	2.1-2.8	1.7-10.9	10.5-18.9	7.50
	<i>txtp358-txtp204</i>	E	36.8-42.1	2.3-5.7	2.1-3.8	9.9-19.8	8.46
Length of infection (cm)	<i>xiabt275-xiabt241</i>	B	460.3-473.8	2.1-3.5	3.7-4.9	11.8-16.1	17.23
	<i>txtp201-xiabt378</i>	B	60.3-62.7	2.3-3.9	3.8-7.8	11.9-17.7	8.40
	<i>AC13-xiabt224</i>	A	100.7-102.7	2.8-3.8	8.9-9.0	13.3-17.8	18.82
Lodging per cent	<i>txtp176-xiabt312</i>	I	36.8-37.1	2.2-3.8	3.4-4.8	10.7-11.9	8.10
	<i>txtp274-xiabt29</i>	I	59.8-62.8	2.2-3.9	2.7-5.5	10.1-11.8	76.50
	<i>txtp343-txtp12</i>	D	2.9-3.1	2.7-3.3	1.1-3.9	11.9-13.7	4.35
	<i>txtp338-xiabt420</i>	J	31.9-40.9	3.1-3.8	-2.7-3.8	10.9-11.9	21.30
	<i>AC19-txtp254</i>	H	18.6-23.9	3.1-2.8	-3.1-6.7	4.1-18.9	12.20
Early seedling vigour	<i>txtp273-xiabt72</i>	H	51.9-91.5	2.2-3.6	-2.9-3.9	8.9-18.9	17.33
	<i>txtp297-xiabt 73</i>	B	326.9-333.8	2.7-3.8	-1.8-2.7	10.8-13.3	18.23
	<i>xiabt79-xiabt 77</i>	D	5.3-8.2	2.1-3.4	-1.6-4.5	10.8-11.8	4.20
	<i>txtp 9-xiabt 359</i>	D	39.7-42.1	2.1-2.7	-1.8-3.1	9.8-10.9	21.50
Days to 50% flowering	<i>AC12-xiabt 80 all</i>	B	216.8-243.0	2.1-3.2	3.8-7.0	7.3-11.9	34.65
	<i>xiabt 78-xiabt83all</i>	F	82.1-101.8	2.6-3.8	3.6-7.9	10.8-18.9	30.60
Plant height (cm)	<i>Corn 24-txtp210</i>	C	7.9-11.1	2.9-4.3	3.3-6.7	9.8-15.6	11.83
	<i>xiabt 81-xiabt9</i>	G	29.8-33.7	2.8-3.3	1.8-5.8	8.9-18.9	13.75
	<i>BH13-xiabt 78</i>	F	71.8-74.3	2.1-3.2	3.6-6.7	11.9-18.0	0.50
	<i>txtp338-xiabt420</i>	J	26.9-42.8	2.7-3.9	4.4-4.7	15.7-18.9	21.30
Stem thickness (cm)	<i>txtp318-corn 61</i>	A	3.3-8.8	2.2-3.6	3.8-7.7	9.1-11.9	9.88
	<i>txtp 297-xiabt73</i>	B	335.9-339.7	2.3-3.7	2.8-7.9	11.9-20.4	18.30
	<i>AK 10-BH13</i>	F	71.3-71.9	2.1-3.8	3.9-7.1	11.8-16.5	2.93
	<i>BH13-xiabt78</i>	F	72.5-73.6	2.1-2.7	2.3-8.9	5.1-15.6	0.50
	<i>xiabt 13-xiabt27</i>	E	113.7-129.7	2.5-2.7	3.3-7.9	5.1-11.7	37.07
Panicle length (cm)	<i>txtp176-txtp312</i>	I	33.3-37.9	3.5-4.4	-2.9-3.9	10.7-18.1	8.10
	<i>txtp338-xiabt420</i>	J	27.1-42.2	2.2-4.3	-2.8-3.3	9.1-17.1	21.30
	<i>Corn 73-txtp92</i>	E	46.9-49.8	2.3-3.6	-3.3-8.9	10.8-19.9	7.93
Threshability	<i>H4-xiabt75</i>	A	160.1-165.1	2.1-3.5	3.33-4.7	10.8-15.1	19.48
	<i>txtp348-xiabt31</i>	B	90.8-105.8	2.8-3.9	3.4-7.8	6.7-13.3	52.90
	<i>xiabt 27-xiabt49</i>	E	140.7-151.9	2.2-3.8	1.1-4.28	8.8-17.6	28.26
	<i>txtp 297-xiabt 73</i>	B	330.9-339.1	2.3-3.8	-2.6-2.7	7.8-15.6	18.30
	<i>AC20-AK10</i>	F	60.1-70.1	2.1-3.2	1.1-3.3	8.9-18.3	13.72
1000-seed weight (g)	<i>Corn 59-txtp 303</i>	B	49.9-50.9	2.0-2.7	2.7-5.5	10.9-16.1	8.80
	<i>txtp 348-xiabt 81l</i>	B	123.3-130.9	2.6-3.8	3.8-5.5	10.7-18.9	52.90
	<i>xiabt27-xiabt49</i>	E	139.8-157.8	2.1-3.6	2.1-4.4	7.8-11.0	28.26
	<i>xiabt 86-txtp96</i>	I	20.0-23.3	2.7-4.3	3.8-10.9	7.9-19.9	3.40
Seed yield /plant (g)	<i>Corn 24-txtp210</i>	C	7.6-8.89	2.2-3.2	3.3-4.5	10.3-17.8	11.83
	<i>txtp 45-xiabt81</i>	G	16.5-18.1	2.1-2.8	3.3-4.6	9.6-17.9	1.10
	<i>txtp 160-xiabt85</i>	C	43.8-51.6	2.3-2.9	2.6-3.9	13.3-17.6	25.62
	<i>txtp92-xiabt13</i>	E	84.3-91.7	2.1-3.8	3.3-6.7	7.8-16.8	41.02
	<i>txtp230-txtp258</i>	F	9.8-14.7	2.9-3.5	1.1-6.6	7.9-11.9	4.81

Lignin at 75 DAS

A total of two QTLs were detected on two different linkage group viz., LG-B (*txp* 303 – *txp* 301) and LG-D (*xiabt* 374 – *txp* 9) with phenotypic variance of 12.7 and 23.01, respectively with peak LOD score of 2.6 and 3.9. For the QTL on linkage group B (*txp* 303-*txp*301) the additive effect of -2.2 was recorded and the contribution was from susceptible parent (IS 22380) while for other QTL on linkage group D the additive effect value of 3.25 was recorded with the major contribution from resistant parent (E36-1)

Total phenol at 95 DAS

One QTL was detected for this trait on linkage group I (*txp* 274 - *xiabt* 29) with phenotypic variance of 17 and LOD score of 2.5. The additive effect of 1.5 for the QTL on linkage group I showed that the parent E 36-1 was contributing to this locus.

Lignin content at 95 DAS

One QTL was detected for this trait on linkage group A (*xiabt* 92 - *xiabt*67) with phenotypic variance of 19 and with peak LOD score of 4.3 with additive effect of 3.1.

Early seedling vigour

Five QTLs were detected on linkage group A, LG-B, LG-D and LG-H for early seedling vigour, out of which four QTLs were consistent for all the four season. Two QTLs were detected on linkage group D (*xiabt* 79 – *xiabt* 77 and *txp* 9 – *xiabt* 359) and one each on linkage group H (*txp* 273- *xiabt* 72) and B (*txp* 297 – *xiabt* 73). The QTL on linkage group D flanked by the marker *xiabt* 79-*xiabt* 77 at position 8.2cM showed high phenotypic variance and LOD score of 11.8 and 3.4, respectively.

Plant height

Total of Six QTLs were detected across five linkage group C (*Corn*24 – *xiabt* 80), LG-E (*txp* 92 – *xiabt* 13), LG-F (BH 13 – *xiabt* 78), LG-G (*xiabt* 81 – *xiabt* 9 and *txp* 45 – *xiabt* 81) and LG-J (*txp* 338 – *xiabt* 20). Four QTL were consistently detected for all the season which were four one each on linkage group C (*Corn*24 – *xiabt* 80), LG-F (BH 13 – *xiabt* 78), LG-G (*xiabt* 81 – *xiabt* 9) and LG-J (*txp* 338 – *xiabt* 20) at position 10.3, 71.9, 28.7 and 26.9 cM, respectively. One QTL which was common on linkage group G flanked by maker *xiabt* 81 and *xiabt* 9 showed high phenotypic variance of 10.22 with LOD score of 3.3 and additive effect of 4.9.

Days to 50 per cent flowering

Four QTLs were detected for this trait at Bijapur location on linkage group B(Ac12-*xiabt* 80), LG-F(*xiabt* 78- *xiabt*83 and *txp* 284 – Ac 20) and LG-J (*xiabt* 45 – *xiabt* 83).The phenotypic variance and LOD score were in the range of 7.3 – 18.9 and 2.1 – 3.8, respectively .Two QTL were consistent in expression towards this trait for all the four season which were located on linkage group B (Ac12- *xiabt* 80) and LG-F (*xiabt* 78- *xiabt*83) at distance 230.9 and 101.8cM, respectively.

Stem thickness

Five QTLs were detected on linkage group A (*txp* 318 - *Corn* 61), LG-B (*txp* 297 – *xiabt* 73), LG-F (AK 10 – BH 13 and BH 13 – *xiabt* 78) and LG-E (*xiabt* 13 – *xiabt* 27) which were consistently detected for all the season. The phenotypic variance and LOD score were in the range of 9.1 – 20.4 and 2.1 – 3.8, respectively. Two QTLs were found on same linkage group F at position 71.3 - 73.5cM flanked by marker AK 10 and *xiabt* 78. High phenotypic variance (20.4) and LOD score (3.8) was observed on linkage group B (*txp* 297 – *xiabt* 73).

Ear head length

Three QTLs were detected for the entire season one each on linkage group E (*Corn* 73 – *txp* 92), LG-J (*txp* 338 – *xiabt* 420) and LG-I (*txp* 176 – *txp* 312) at distance 49.8,

42.2 and 37.9 cM respectively. The phenotypic variance and LOD score were observed with range of 9.1 – 19.9 and 2.2 – 4.4, respectively. The QTL on linkage group E flanked by the marker *Corn* 73 and *txp* 92 expressed high phenotypic variance of 19.9 with LOD score of 2.8 and additive allele effect of 4.5.

Threshability

Six QTLs were detected on linkage group A (*H4* – *xiabt* 75), LG-B (*txp* 297 – *xiabt* 273 and *txp* 348 – *xiabt* 31), LG-E (*xiabt* 29 – *xiabt* 47) and LG-F (AC 20 – AK 10) for threshability of which five QTL were consistently expressing for all the season. The phenotypic variance and LOD score were observed with range of 6.7 – 18.3 and 2.1 – 4.3. The QTL flanked by marker *txp* 297-*xiabt* 73 showed co-localization with QTL for stem thickness on linkage group B with phenotypic variance of 15.6 and LOD score of 2.9.

1000 seed weight

Four QTLs were consistently expressed and found on three different linkage group viz., LG-B (*Corn* 59 – *txp* 202 and *txp* 348 – *xiabt* 81) LG-E (*xiabt* 29 – *xiabt* 49) and LG-I (*xiabt* 86- *txp* 96). Two QTLs were found on the same linkage group B at distance 49.9cM (*Corn* 59-*txp*303) and 128.9 cM (*txp*348-*xiabt*49) showed phenotypic variance of 16.1 and 10.7, respectively. QTL on linkage group E was flanked by marker *xiabt* 27 –*xiabt* 49 with phenotypic variance of 10.5.

Seed yield

Six QTLs were detected on linkage group C (*Corn* 24 – *txp* 210 and *txp* 160 – *xiabt* 85), LG-E (*txp* 92 – *xiabt* 13), LG-F (*txp* 230 – *txp* 258), LG-G (*txp* 45 – *xiabt* 81), LG-J (*txp*325 – *txp*324) and LG-I of which five QTL were expressing consistently for all the season. Two QTLs were detected on same linkage group C at position 8.89cM (*Corn*24-*txp*210) and 43.8 cM (*txp* 160 - *xiabt* 81) with high phenotypic variance of 15.1 and 15.6, respectively.

4.3.3 Sholapur location

All the QTLs associated with 6 different traits during three season were worked separately for Solapur location (Table 17a to 17e). Graphical representation of chromosomal positions of common QTLs found at all the four seasons with flanking markers is presented in Fig. 11.

Number of internodes crossed by the fungus

As a component trait for charcoal rot resistance two QTLs were consistently detected for this trait on the same linkage group B (*txp*297 – *xiabt* 73 and *xiabt* 92 – *xiabt*. The QTL at position 324.7 cM was found to be colocalized with QTL for stem thickness with phenotypic variance of 13.6 and additive effect of 3.8. The QTL at position 367.9 cM was flanked by the genic SSR marker (*xiabt* 92 and *xiabt* 62) with phenotypic variance of 11.9 and additive effect of 4.5.

Length of infection

Three QTLs were consistently expressed in all the season and were found on two linkage groups (LG-A and LG-B). Two QTLs on the same linkage group (B) were positioned at 63.3 cM (*txp* 201- *xiabt* 378) and 463.4 cM (*xiabt* 275 - *xiabt* 241) with phenotypic variance of 13.1 and 10.1, respectively with additive effect of 5.5 to 2.7. On linkage group A the QTL flanked by AC13 and *xiabt* 224 was positioned at 110.1cM with 10.9 and 5.3 as phenotypic variance and additive effect, respectively.

Lodging Per cent

All the three QTLs which were found for lodging per cent were consistent in there expression during all the seasons. One Each QTL was detected on linkage group H, LG-I and LG-J. The QTL were flanked by the marker *txp* 274 - *xiabt*378 (LG-I), *txp*338-*xiabt*420 (LG-

Table 17a: Features of the QTLs computed by composite interval mapping for charcoal rot resistance and yield component traits in the RILs derived from the cross IS 22380 × E 36-1 at Solapur location during 2005

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed	<i>txp297-xiabt73</i>	B	324.7	2.8	3.8	13.6	18.30
	<i>xiabt 92-xiabt62</i>	B	367.9	2.7	4.5	11.9	27.51
Length of infection (cm)	<i>AC13-xiabt224</i>	A	110.1	2.3	5.5	10.9	18.82
	<i>xiabt275-xiabt241</i>	B	463.4	2.51	2.7	10.1	17.23
	<i>txp201-xiabt378</i>	B	63.3	2.7	5.5	13.1	8.40
Lodging per cent	<i>txp274-xiabt29</i>	I	51.7	2.1	-5.7	10.8	76.50
	<i>txp338-xiabt420</i>	J	25.6	2.5	-5.6	17.8	21.30
	<i>AC19-txp254</i>	H	28.6	2.7	-3.3	10.1	12.20
Days to 50% flowering	<i>AC12-xiabt 80</i>	B	233.8	2.9	4.5	11.9	34.65
	<i>txp204-corn73</i>	E	43.7	2.5	3.5	13.1	2.84
	<i>xiabt 78-xiabt83</i>	F	91.8	2.1	5.5	18.9	19.91
Stem thickness (cm)	<i>xiabt86-txp96</i>	I	22.6	2.7	3.3	11.5	3.40
	<i>txp 297-xiabt73</i>	B	330.8	3.6	6.3	13.6	18.30
	<i>BH13-xiabt78</i>	F	73.3	2.5	4.8	19.8	0.50
Seed yield /plant (g)	<i>txp 45-xiabt81</i>	G	18.3	2.5	5.7	11.8	1.10
	<i>txp 160-xiabt85</i>	C	42.7	2.9	4.6	9.8	25.62
	<i>txp230-txp258</i>	F	9.8	3.5	8.9	19.5	4.81

Table 17b: Features of the QTLs computed by composite interval mapping for charcoal rot resistance and yield component traits in the RILs derived from the cross IS 22380 × E 36-1 at Solapur location during 2006

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed	<i>txp297-xiabt73</i>	B	333.7	2.5	3.5	11.9	18.10
	<i>xiabt 92-xiabt62</i>	B	378.9	2.2	4.4	9.8	27.51
Length of infection (cm)	<i>AC13-xiabt224</i>	A	100.8	2.1	3.3	9.6	18.82
	<i>xiabt275-xiabt241</i>	B	465.5	2.6	5.5	11.1	17.23
	<i>txp201-xiabt378</i>	B	60.8	2.1	4.5	9.8	8.40
Lodging per cent	<i>txp274-xiabt29</i>	I	55.8	2.8	-3.3	19.9	76.50
	<i>txp338-xiabt420</i>	J	27.8	2.1	-6.6	18.9	21.30
	<i>AC19-txp254</i>	H	23.6	2.3	-5.9	8.8	12.20
Days to 50% flowering	<i>AC12-xiabt 80</i>	B	216.8	2.3	3.3	9.7	34.05
	<i>txp204-corn73</i>	E	44.3	3.8	2.7	10.5	2.84
	<i>xiabt 78-xiabt83</i>	F	88.5	2.6	3.5	15.6	19.91
Stem thickness (cm)	<i>xiabt86-txp96</i>	I	31.9	4.5	2.9	8.9	3.40
	<i>txp 297-xiabt73</i>	B	334.7	2.7	5.4	10.9	18.30
Seed yield /plant (g)	<i>txp 45-xiabt81</i>	G	17.9	3.8	6.6	9.9	1.10
	<i>txp 160-xiabt85</i>	C	51.7	2.6	3.8	10.5	25.62
	<i>txp 284-AC20</i>	F	48.7	2.5	3.7	8.9	19.91
	<i>txp230-txp258</i>	F	10.8	2.7	5.5	10.8	4.81

Table 17c: Features of the QTLs computed by composite interval mapping for charcoal rot resistance and yield component traits in the RILs derived from the cross IS 22380 × E 36-1 at Solapur location during 2007

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed	<i>txp297-xiabt73</i>	B	325.8	2.3	4.4	11.9	18.30
	<i>xiabt 92-xiabt62</i>	B	378.9	2.1	3.5	8.8	27.51
Length of infection (cm)	<i>AC13-xiabt224</i>	A	109.8	2.3	4.3	9.8	18.82
	<i>xiabt275-xiabt241</i>	B	465.5	2.5	5.5	9.8	17.23
	<i>txp201-xiabt378</i>	B	59.8	2.5	6.6	11.9	8.40
Lodging per cent	<i>txp274-xiabt29</i>	I	52.3	5.5	-3.3	6.9	76.50
	<i>txp338-xiabt420</i>	J	27.9	2.8	-4.3	13.3	21.30
	<i>AC19-txp254</i>	H	16.7	2.1	-5.6	9.8	12.20
Days to 50% flowering	<i>AC12-xiabt 80</i>	B	233.2	2.7	3.3	10.9	34.60
	<i>txp204-corn73</i>	E	43.7	2.1	2.7	9.8	2.84
	<i>xiabt 78-xiabt83</i>	F	93.3	2.7	4.4	10.9	19.91
Stem thickness (cm)	<i>xiabt86-txp96</i>	I	22.9	2.2	2.8	9.8	3.40
	<i>txp 297-xiabt73</i>	B	328.8	2.2	4.4	10.9	18.30
	<i>BH13-xiabt78</i>	F	74.5	3.3	7.8	19.9	0.50
Seed yield /plant (g)	<i>txp 45-xiabt81</i>	G	17.9	3.7	2.7	9.8	1.10
	<i>txp 160-xiabt85</i>	C	43.1	2.3	3.4	11.1	25.62
	<i>txp230-txp258</i>	F	10.9	3.8	5.5	9.8	4.81

Table 17d: Features of the QTLs that were specifically detected for charcoal rot and yield related component traits in RILs derived from the cross IS 22380 x E 36-1 at Solapur location

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Lodging per cent	AC19- <i>xtxp</i> 254	H	28.6	2.7	-3.3	10.1	12.20
Days to 50% flowering	<i>xtxp</i> 204-corn73	E	43.7	2.5	3.5	13.1	2.84
Stem thickness (cm)	<i>xiabt</i> 86- <i>xtxp</i> 96	I	22.6	2.7	3.3	11.5	3.40
	BH13- <i>xiabt</i> 78	F	73.3	2.5	4.8	19.8	0.50
Seed yield /plant (g)	<i>xtxp</i> 230- <i>xtxp</i> 258	F	9.8	3.5	8.9	19.5	4.81
	<i>xtxp</i> 284-AC20	F	48.7	2.5	3.7	8.9	19.91

Table 17e: Features of the QTLs that were commonly detected across three seasons for charcoal rot and yield related component traits in RILs derived from the cross IS 22380 x E 36-1 at Solapur location

Trait	Flanking marker	LG	QTL position	Peak LOD ²	Additive effect	Phenotypic variance (%)	Distance (cM)
Number of internode crossed	<i>txxp297-xiabt73</i>	B	324.7-333.7	2.3-2.8	3.5-4.5	11.9-13.6	18.30
	<i>xiabt 92-xiabt62</i>	B	367.9-378.9	2.1-2.7	3.5-4.5	8.8-11.9	27.51
Length of infection (cm)	<i>AC13-xiabt224</i>	A	100.8-110.1	2.1-2.3	3.3-5.5	9.8-10.9	18.82
	<i>xiabt275-xiabt241</i>	B	463.4-465.5	2.1-2.51	2.7-5.5	9.6-10.1	17.23
	<i>txxp201-xiabt378</i>	B	59.8-63.3	2.1-2.7	4.5-6.6	9.8-13.1	8.40
Lodging per cent	<i>txxp274-xiabt29</i>	I	51.7-55.8	2.1-5.5	3.3-5.7	6.9-19.9	76.50
	<i>txxp338-xiabt420</i>	J	25.6-27.9	2.5-2.8	-4.3-5.6	13.3-17.8	21.30
	<i>AC19-txtp25</i>	H	16.7-28.6	2.1-2.7	-3.3-5.9	8.8-10.1	12.20
Days to 50% flowering	<i>AC12-xiabt 80</i>	B	216.8-233.8	2.3-2.9	3.3-4.5	9.7-11.9	34.60
	<i>txxp204-corn73</i>	E	43.7-44.3	2.1-3.8	2.7-3.5	9.8-13.1	2.84
	<i>xiabt 78-xiabt83</i>	F	88.5-93.3	2.1-2.7	3.5-5.5	10.9-18.9	19.91
Stem thickness (cm)	<i>xiabt86-txtp96</i>	I	31.9-22.6	2.2-4.5	2.8-3.3	8.9-11.5	3.40
	<i>txxp 297-xiabt73</i>	B	328.8-334.7	2.2-3.6	4.4-6.3	10.9-13.6	18.30
	<i>BH13-xiabt78</i>	F	73.3-74.5	2.5-3.3	4.8-7.8	19.8-19.9	0.50
Seed yield /plant (g)	<i>txxp 45-xiabt81</i>	G	17.9-18.3	2.5-3.8	2.7-6.6	9.8-11.8	1.10
	<i>txxp 160-xiabt85</i>	C	42.7-51.7	2.3-2.9	3.4-4.6	9.8-11.1	25.62
	<i>txxp230-txtp258</i>	F	9.8-10.9	2.7-3.8	5.5-8.9	9.8-19.5	4.81
	<i>txxp 284-AC20</i>	F	48.7	2.5	3.7	8.9	19.91

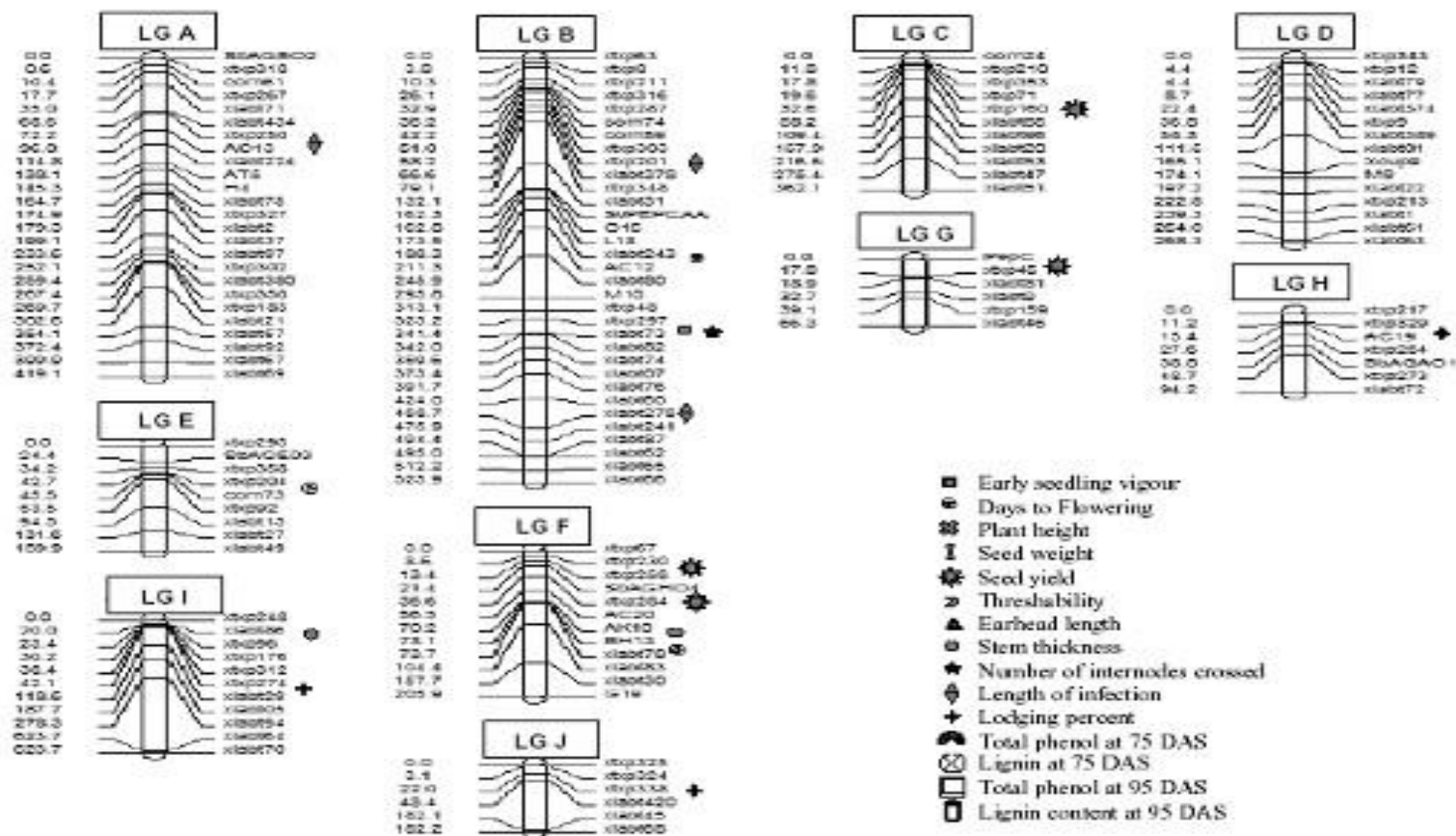


Fig.11. Map localization of common and stable QTL for morphological trait over three season at Solapur location

J) and AC19 –*xtxp* 254 (LG-H) with phenotypic variance of 10.8, 17.8 and 10.1 respectively with additive effect of -5.7, 5.6 and -3.3

Days to 50% flowering

Three QTLs were detected consistently for all the season, one each on linkage group LG-B (AC 12 – *xiabt* 80), LG-E (*xtxp* 204 – *Corn* 73) and LG-F (*xiabt* 78 – *xiabt* 83). The QTL on linkage group F was flanked by genic SSRs *xiabt* 78 and *xiabt* 83 with high phenotypic variance of 18.9 with additive effect of 5.3 and LOD score of 2.1

Stem thickness

Three QTLs were found to be associated for stem thickness one each on linkage group B (*xtxp* 297 – *xiabt* 73), LG-F (BH 13 – *xiabt* 78) and LG-I (*xiabt* 86 – *xtxp* 96). These three QTLs showed consistency over all the season. The QTL on linkage group B was found to be co-localized with QTL for number of internodes crossed by the fungus. This QTL was found at position 330.8 cM with phenotypic variance of 13.6 and additive effect of 6.3 with peak LOD score of 3.6.

Seed yield

Four QTLs were detected on linkage group C(*xtxp* 160 – *xiabt* 85), LG F (*xtxp* 230 – *xtxp* 258 and *xtxp* 284 – AC 20) and LG G (*xtxp* 45 – *xiabt* 81). The observed range of phenotypic variance and LOD score was 8.9 – 19.5 and 2.3 – 3.8, respectively. The QTL on linkage group F (*xtxp* 230 – *xtxp* 258) showed high phenotypic variance (19.8) and LOD score (3.8). The QTL on linkage group C (*xtxp* 45-*xiabt* 81) and LG-G (*xtxp* 160-*xiabt* 85) showed phenotypic variance of 9.8 and 11.8, respectively with additive effect of 4.6 and 5.7.

Common and stable QTL for charcoal rot resistance and yield traits.

Common and stable QTLs across all three location and four season were noted down to find out the consistency and stability of QTL and which can be worthwhile to use in introgression programme for improving different traits. Features of QTLs commonly detected across four seasons and three location for charcoal rot resistance and related component traits are presented in Table 18. Finally, graphical representation of chromosomal positions of common QTLs found consistent over four seasons and three locations is presented in Fig. 12.

Number of internodes crossed

Two QTLs were stably expressing at all the locations and over years which were found on same linkage group B. The QTL on linkage group B positioned at 324.0 - 340.0cM was flanked by the marker *xtxp* 297 and *xiabt* 273 with phenotypic variance of 5.9 - 19.29, this QTL was found co-localized with the QTL for stem thickness. The other QTL on linkage group B was flanked by genic SSRs (*xiabt* 92- *xiabt* 62), with phenotypic variance of 8.8 -18.9 and additive effect of 3.5 - 8.8 .The additive effect indicated that the allele contributing for these QTL were contributed from E 36-1.

Length of infection

Three QTLs were detected for this trait which were stably expressed at all the locations over the seasons. Three QTLs were located on linkage group LG-A (one QTL) and LG B (two QTLs). Two QTLs on linkage group B were flanked with the marker *xiabt* 275 - *xiabt*241 and *xtxp* 201-*xiabt* 378 with phenotypic variance of 8.0-16.1 and 9.8-17.7, respectively with Peak LOD value of 2.1-3.5 and 2.1-3.9. The QTL on linkage group A was flanked with the marker AC13-*xiabt*224 with phenotypic variance of 8.9 - 17.9. For all the three QTLs the allele for additive effect was contributed from E36-1.

Lodging Percent

Four QTLs were stably expressed for all the season and location. These were located on three different linkage groups (LG-D, LG-I and LG-J). The data from all the locations showed the normal distribution indicating quantitative nature of this trait. For the QTL on

Table 18: Features of the QTLs that were commonly detected across four years and three locations for charcoal rot and yield related component traits in RILs derived from the cross IS 22380 x E 36-1

Traits	Marker	Linkage group	QTL position	Peak LOD ²	Additive effect	Phenotypic variance	Distance (cM)
Length of infection (cm)	AC13- <i>xiabt</i> 224	A	96.1-110.1	2.1-3.8	3.3-9.0	8.9-17.8	18.82
	<i>xiabt</i> 275- <i>xiabt</i> 241	B	460.3-473.8	2.1-3.5	2.7-5.5	8.0-16.1	17.23
	txp201- <i>xiabt</i> 378	B	59.8-63.3	2.1-3.9	3.8-7.8	9.8-17.7	8.40
Number of internodes crossed	txp297- <i>xiabt</i> 73	B	324.0-340.0	2.2-4.4	0.39-7.8	5.9-19.29	18.30
	<i>xiabt</i> 92- <i>xiabt</i> 62	B	367.9-393.4	2.1-3.3	3.5-8.8	8.8-18.9	27.51
Per cent lodging	txp343-txp12	D	1.8-3.9	2.7-3.8	1.1-9.8	9.2-13.7	4.35
	txp176-txp312	I	32.8-37.1	2.2-3.8	3.4-6.3	7.89-11.9	8.10
	txp274- <i>xiabt</i> 29	I	45.2-62.8	2.1-5.5	2.7-5.7	6.9-19.9	76.50
	txp338- <i>xiabt</i> 420	J	22.0-40.9	2.1-3.8	-1.1-7.62	8.7-17.8	21.30
Early seedling vigour	txp297- <i>xiabt</i> 73	B	325.9-333.8	2.3-3.8	-0.19-3.9	5.8-13.3	18.23
	<i>xiabt</i> 79- <i>xiabt</i> 77	D	4.9-9.2	2.1-4.9	-1.3-5.6	10.8-16.11	4.20
Days to 50% flowering	AC12- <i>xiabt</i> 80	B	211.0-243.0	2.1-3.2	1.4-7.0	5.8-15.9	34.60
	<i>xiabt</i> 78- <i>xiabt</i> 83	F	80.18-101.8	2.1-3.8	1.8-7.9	6.89-15.8	30.60
Plant height	Corn24-txp210	C	10.01	2.27	4.99	12.83	11.83
Stem thickness (cm)	txp297- <i>xiabt</i> 73	B	324.8-339.7	2.2-3.8	0.63-6.3	9.54-20.4	18.30
	AK10-BH13	F	70.23-72.9	2.1-3.9	0.61-7.1	8.9-16.5	2.93
Ear head length	txp176-txp312	I	30.7-33.7	2.0-4.4	-1.8-5.6	7.9-18.1	8.10
Threshability	H4- <i>xiabt</i> 75	A	146.7-164.7	2.1-3.5	0.30-4.7	10.8-15.42	19.48
	txp348- <i>xiabt</i> 31	B	85.9-105.8	2.3-3.9	0.3-7.8	6.7-13.74	52.90
	txp297- <i>xiabt</i> 73	B	318.9-339.1	2.3-3.8	-0.24-5.7	6.7-15.6	18.30
	AC20-AK10	F	58.51-70.1	2.1-3.2	0.2-3.3	7.93-18.3	13.72
1000 seed weight	corn59-txp201	B	43.19-50.9	2.0-2.8	1.3-5.5	7.9-16.1	15.15
	txp348- <i>xiabt</i> 81	B	80.1-130.9	2.1-3.8	2.1-5.5	8.6-18.9	52.90
	<i>xiabt</i> 27- <i>xiabt</i> 49	E	138.0-157.8	2.1-4.7	2.1-4.4	7.8-18.3	28.26
Seed yield /plant (g)/plant	corn24-txp210	C	3.8-8.89	2.1-3.2	2.5-5.9	4.4-17.8	11.83
	txp45- <i>xiabt</i> 81d	G	16.5-19.5	2.1-3.8	2.7-6.6	9.6-17.9	1.10
	txp160- <i>xiabt</i> 85	C	42.7-51.7	2.3-2.9	2.6-4.6	9.8-17.6	25.62
Total phenols at 75 DAS	Ac19-txp254	H	15.41-16.61	2.9-3.0	1.43-2.39	11-12	12.20
	txp274- <i>xiabt</i> 29	I	49.2-50.1	2.3-2.4	1.45-1.54	25-29	76.50
Lignin content at 75 DAS	txp303-txp301	B	51.05-51.5	2.6-2.8	-0.25-2.2	10-12	7.15
	<i>xiabt</i> 374-txp9	D	26.4-28.46	3.90-4.02	0.29-3.25	22-23	14.30
Total phenols at 95 DAS	txp329-Ac19	H	12.14-13.17	3.50-3.62	2.3-2.41	14-15	4.20
	txp274- <i>xiabt</i> 29	I	42.11-43.2	2.5-2.7	1.5-3.2	17-19	76.50
Lignin content at 95 DAS	<i>xiabt</i> 92- <i>xiabt</i> 67	A	372.4-372.11	4.3-6.4	2.2-3.1	19-23	27.51

linkage group J (*txp* 338-*xiabt*420) the additive effect (-1.1-7.62) was contributed from the IS 22380 allele while for all the other three QTLs on linkage group D and I the allele was contributed from E36-1.

Early seedling vigour

For early seedling vigour two QTLs were common in all the locations and seasons which were located on linkage group B and D. The allele contributing to additive effect was from IS 22380 parent for both the QTLs. On linkage group B, the QTL was positioned between flanking marker *txp* 297 and *xiabt* 73 with phenotypic variance of 5.8 - 13.3 and peak LOD value of 2.3 - 3.8. The QTL on linkage group D was bracketed by genic SSRs (*xiabt* 9 – *xiabt* 77) with 10.8-16.11 phenotypic variance and LOD value of 2.1-4.9.

Plant height

For plant height two QTLs were found stable in all the locations and season. The QTL on linkage group C was flanked by marker *Corn* 24 and *txp* 210 with a phenotypic variance of 9.8 - 15.6 and peak LOD score of 2.3 - 4.8. The allele contributing to additive effect came from IS 22380 parent. The QTL on linkage group G was flanked by genic SSRs (*xiabt* 81 and *xiabt* 9) with phenotypic variance of 7.29 - 18.9 with additive effect of 1.8 - 5.8.

Days to 50% flowering

Two QTLs were detected one each on linkage group B and LG-F consistently for all the season. The allele contributing for early flowering was contributed from IS 22380. The QTL on linkage group B was flanked by AC12 and *xiabt* 80 with additive effect of 1.4 - 7.0 and phenotypic variance of 5.8 -15.9 while the QTL on linkage group F (*xiabt* 78-*xiabt* 83) showed the peak LOD score of 2.1 to 3.8 with phenotypic variance of 6.89-15.8.

Stem thickness

Out of two stable QTLs detected in all the season, one QTL on linkage group B (*txp*297 and *xiabt*73) with phenotypic variance of 9.54 - 20.4 showed co localization with QTLs for number of internodes crossed by the fungus. The QTL on linkage group F was flanked by the marker AK 10 and BH 13 with phenotypic variance of 8.9 -16.5 with wider range of additive effect (1.61-7.1). The allele contributing for stem thickness was contributed from E 36-1.

Ear head length

One QTL showed stable expression which was positioned on linkage group I. The QTL on linkage group I was bracketed by marker *txp* 176 and *xtp* 312 with phenotypic variance of 7.9 -18.1 and peak LOD value of 2.0 - 4.4. The additive effect of -1.8 -5.6 was recorded which indicates the allele contributed from E36-1 for this trait.

Threshability

Four stable QTLs were detected for this trait on three different linkage groups (LG-A, LG-B and LG-F). For three QTLs the allele was contributed from E36-1 while for the one QTL on linkage group B (*txp* 297 - *xiabt* 273) the allele was contributed from IS 22380 with phenotypic variance of 6.7 -15.6 with peak LOD value of 2.3 -3.8.

1000 seed weight

Three QTLs were stable in there expression at all the locations over the years .Two QTLs were located on linkage group B (*Corn* 59 – *txp* 209 and *txp* 348 – *xiabt* 81) while one QTL was located on linkage group E (*xiabt* 27 - *xiabt*49). For all the QTLs the additive effect indicated that the allele for this trait was contributed from E 36-1.

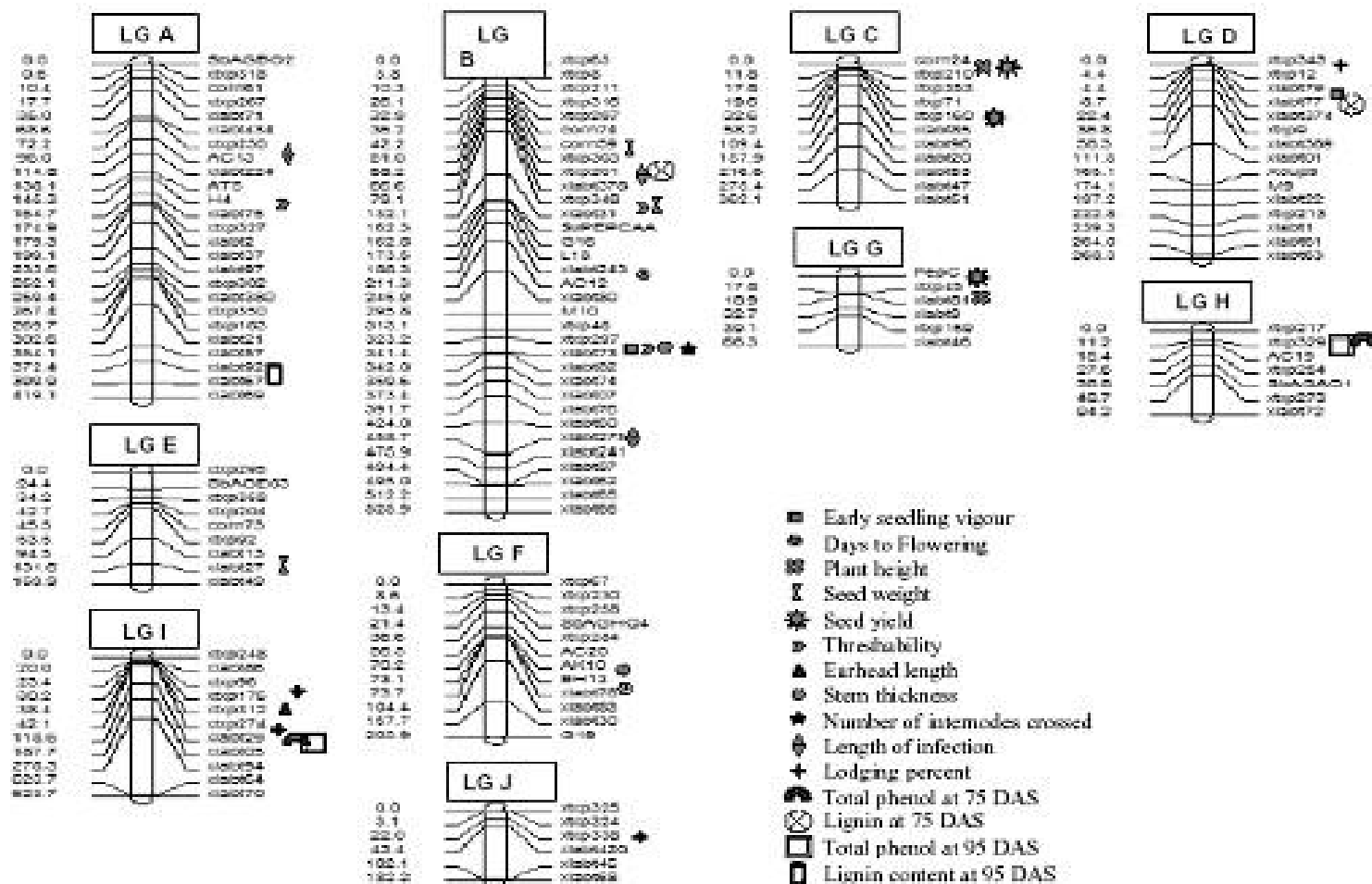


Fig.12.Map localization of common and stable QTL for morphological and biochemical trait over four season and three location

Seed yield per plant

Three QTLs were stably expressed at all the three locations over four seasons. The QTLs were located on linkage group C (two QTLs) and LG-G (one QTL). Two QTLs on linkage group C were flanked by marker *Corn24-xtxp201* and *xtxp160-xiabt 85* with phenotypic variance of 4.4 -17.8 and 9.6 -17.9, respectively with peak LOD value of 2.1 - 3.2 and 2.3 - 2.9. The additive effect for all the three QTLs indicated that the allele from E36-1 was mostly contributing for seed yield.

Total phenol and Lignin

The QTL for total phenol at 75 DAS were found on linkage group H (AC 19 - *xtxp 254*) and LG-I (*xtxp 274 – xiabt 29*) with LOD score ranging from 2.3 - 3.0 with phenotypic variance of 12 - 29. The QTL for per cent lignin during 75 DAS were found on linkage group B and LG-D having LOD score of 2.8 to 4.02 with phenotypic variance of 10 - 23.

One QTL (*xtxp 274 – xiabt 29*) for total phenol content during 95 DAS was on same linkage group as that of QTL for phenol content during 75 DAS i.e on linkage group I with LOD score of 2.5 - 2.7 and phenotypic variance of 17-19. The other QTL was on linkage group B (LOD score of 3.62) and phenotypic variance of 15.

One QTL (*xiabt 92 – xiabt 62*) was detected for per cent lignin content during 95 DAS on LG A and with high LOD score of 6.8 and phenotypic variance of 23 per cent.

There is common genomic region (LGI) flanked by marker *xtxp274–xiabt29*, associated with QTL for lodging per cent, phenol content at 75 DAS and lignin content at 95 DAS on linkage group H, two QTLs governing total phenol at 75 DAS and 95 DAS share same genomic region.

It was found that total phenol content was reduced from 75 DAS to 95 DAS while lignin content increased during the same period. There is possible some role of total phenol towards disease resistance as evident from decrease in phenol content in resistant (E36-1) and susceptible parent (IS 22380) during 75 DAS to 95 DAS with increase in virulence during corresponding period with symptoms of typical discoloration and disintegration of pith region while, lignin has less role to play in disease control but helps in strengthening of tissue cells to withstand the lodging

4.4 Introgression of stable QTLs

Three stable QTLs, one each for lodging per cent (*xtxp 176 – xtxp 312, (cr1)*), number of internodes crossed by fungus (*xtxp 297 – xiabt 73, (cr2)*) and length of infection (*xiabt 275 – xiabt 241, (cr3)*). These three QTLs contributed 18.05, 10.46 and 15.01, respectively to a collective effect of 43.52 per cent of phenotypic variation for charcoal rot resistance. For all the three QTLs the favourable allele was found to be contributed by E36-1 parent. At first level the target markers conditioning the QTLs were screened among the recurrent parents (M35-1 and E36-1) and the donor parent (E36-1) to achieve the foreground selection. Selecting backcross progeny with the target QTLs and tightly-linked flanking markers in order to minimize linkage drag was done which is referred to as 'recombinant selection' or limited background selection. Further, the Marker Assisted Backcrossing (MAB) involved selecting the backcross progeny (that have already been selected for the target trait) with 'background' markers. In other words, markers can be used to select against the donor genome, which may accelerate the recovery of the recurrent parent genome (background selection).

Two recurrent parents, M35-1 and SPV 86, both highly susceptible for charcoal rot disease while being agronomically superior were backcrossed with donor parent E36-1 and subsequently advanced to BC₃ generations separately. All backcross progenies obtained for M35-1 and SPV 86 were screened with foreground markers to identify the progenies with target QTLs and then also screened with background markers for recombinant selection termed as limited background selection (Plate 3).

4.4.1 Foreground selection in M35-1 derived progenies

The target QTL from donor parent is ensured in recurrent parent background by selecting for heterozygous state for the flanking marker at all the three targeted QTL region till BC₃F₁.

A total of 400 progenies were screened with six polymorphic foreground markers (*txp* 176, *xiabt* 312, *txp* 297, *xiabt* 73, *xiabt* 75, *xiabt* 241) flanking *cr1*, *cr2* and *cr3* QTLs on LG-B and LG-I. A total of 2285 scorable marker data point was generated and a part of it is presented in Table 19. The bands in the gels were scored as A, B, H, O and “—” based on their pattern compared with those of the parents. “A” was defined as the presence of allele from the donor parent (E36-1), “B” was defined as the presence of allele from recurrent parent M35-1, “H” was defined as the heterozygous (presence of both recurrent and donor parent alleles), “O” was defined as an allele from neither from donor parent nor from the recurrent parent allele and “—” was a missing sample. At BC₁F₁ generation only the progenies with marker status “H” for all the target marker pairs were scored and selected (Table 20a and 20b). A total of six progeny were found to be heterozygous (“H”) for all three targeted QTL region. Out of which one progeny (#233) was selected for backcrossing with recurrent parent M35-1 (Plate 5). It was ensured that each plant produced 600-700 seed after seed set and these seed were advanced to next backcross generation (BC₂F₁).

4.4.2 Foreground and limited background selection of M35-1 derived BC₂F₁ progeny

In order to overcome the linkage drag it was ensured that a strong selection with marker outside the donor QTL region was carried on chromosome and the progenies that showed banding pattern as that of recipient type (homozygote) outside donor region on carrier chromosomes were marked. At BC₂F₁ generation two rounds of selection (foreground and limited background) was carried out. First each progeny were screened with six foreground polymorphic marker (*txp* 176, *xiabt*312, *txp*297, *xiabt*73, *xiabt* 75 and *xiabt* 241) flanking the target QTL region and thus noting down the marker status for each progeny to ensure the heterozygous nature for all the six primer (foreground selection). Thereafter, a second level of selection was performed to avoid the other genome region from donor parent from QTL carrying linkage group B and I, so as to take care of linkage drag around target QTL position. Two flanking markers outside each targeted QTL region were used for identifying the backcross progenies having the recurrent parent allele at both the markers called as double homozygote at those particular loci. The foreground and limited background selection for all the background progenies simultaneously rather than sequentially to maximize chances of getting a single progeny with all the marker status required though the genotyping effort was more/increased. Each of the 12 markers (six flanking marker targeting the QTL region and six flanking marker outside donor QTL region) were screened across all the 509 progenies. A total of 6002 marker data points were generated encompassing foreground and background selection (Table 21). After screening on gel the marker status of each progeny was recorded in excel format with proper notation and progeny number as described earlier (Table 22). A total of five plants fulfilled these requirements and of which one progeny (#217) was selected and emasculated for backcrossing with recurrent parent M35-1 to produce BC₃F₁ seeds.

4.4.3 Foreground selection of SPV 86 derived BC₁F₁ progeny

A total of 390 progeny were screened with six polymorphic foreground markers (*txp* 176, *xiabt*312, *txp*297, *xiabt*73, *xiabt* 75, *xiabt* 241) flanking the three QTL regions (*Cr1*, *Cr2*, and *Cr3*) on two linkage group (B and I). A total of 2033 scorable marker data point were generated (Table 23). The bands in the gels were scored as A, B, H, O and “—” based on their pattern compared with those of the parents. “A” was defined as the presence of allele from the donor parent (E36-1), “B” was defined as the presence of allele from recurrent parent SPV 86, “H” was defined as the heterozygous (presence of both recurrent and donor parent alleles), “O” was defined as an allele from neither from donor parent nor from the recurrent parent allele and “—” was a missing sample. The progenies with marker status “H” for all the primer screened were selected for advancement (Table 24a and 24b). A total of seven progeny were found to be heterozygous (“H”) for all the three targeted QTL region and out of



Plate.3. Advancement of backcross progenies in greenhouse condition during marker assisted backcross programme

Table 19: Summary statistics of foreground selection for M35-1 x E 36-1 derived BC₁F₁ backcross progenies

Sl. No.	Particulars	M35-1 x E 36-1
1.	Number of progeny screened BC ₁ F ₁	400
2.	Number of marker screened for foreground selection	6
3.	Scorable marker data points generated	2285
4.	Number of progenies satisfying the foreground selection for all the targeted QTL region	6
5.	Marker status of selected plants at target QTL region	Heterozygous
6.	Number of plant selected for backcrossing	1

Table 20a: Part of score sheet indicating the genotypic status for flanking markers for three charcoal resistance QTLs (*cr1*, *cr2* and *cr3*) among BC₁F₁ progenies derived from M35-1 × E 36-1 crosses (foreground selection)

Progeny	<i>cr1</i>		<i>cr2</i>		<i>cr3</i>	
	Flanking marker		Flanking marker		Flanking marker	
	<i>xtxp176</i>	<i>xiabt312</i>	<i>xtxp297</i>	<i>xiabt 73</i>	<i>xiabt 275</i>	<i>xiabt 241</i>
1	H	H	B	O	O	A
2	O	B	B	B	B	H
3	H	H	H	H	H	H
4	A	O	-	H	O	B
5	H	B	A	A	B	H
6	-	-	H	H	O	B
7	B	B	-	H	O	B
8	-	B	A	H	H	B
9	B	B	H	H	B	A
10	B	B	H	B	H	A
11	-	B	B	B	B	B
12	O	H	B	B	B	O
13	H	H	B	H	H	H
14	H	H	B	O	H	A
15	O	B	B	B	B	H
16	-	-	H	-	B	H
17	B	B	B	H	B	B
18	A	B	B	H	H	B
19	B	-	O	H	H	H
20	B	H	O	H	H	B
21	H	B	A	-	O	B
22	H	H	B	B	B	B
23	A	B	O	H	H	H
24	B	-	B	H	B	O
25	O	H	O	B	A	O
26	H	H	H	H	B	H
27	H	H	A	O	O	A
28	B	B	H	B	B	H
29	B	-	B	-	O	H
30	B	B	B	H	H	B
31	B	H	H	H	B	B
32	B	O	H	H	H	H
33	B	H	B	O	B	B
34	B	H	B	-	H	B
35	H	B	B	B	H	H
36	B	B	H	O	-	-

- 'A' - Homozygote at marker locus as that of donor parent (E 36-1)
 'B' - Homozygote at marker locus as that of recurrent parent (M 35-1)
 'H' - Heterozygote at marker locus as that of E 36-1
 'O' - Off type showing banding pattern different from parents
 '-' - Missing data for the individual at the locus

Table 20b: Part of score sheet indicating the genotypic status for flanking markers for three charcoal resistance QTLs (*cr1*, *cr2* and *cr3*) among BC₁F₁ progenies derived from M35-1 × E 36-1 crosses (foreground selection)

Progeny	<i>cr1</i>		<i>cr2</i>		<i>cr3</i>	
	Flanking marker		Flanking marker		Flanking marker	
	<i>txp176</i>	<i>xiabt312</i>	<i>txp297</i>	<i>xiabt 73</i>	<i>xiabt 275</i>	<i>xiabt 241</i>
105	O	O	H	B	B	A
106	H	H	H	H	H	H
107	H	B	H	A	H	H
108	B	H	H	H	B	H
109	A	O	A	H	-	B
110	B	-	B	O	B	H
111	-	H	B	B	H	B
112	H	A	H	A	H	B
113	H	H	H	H	H	H
114	B	A	B	H	B	B
115	B	H	A	-	B	O
116	H	H	H	H	H	B
117	B	H	-	A	H	B
118	B	H	B	H	B	B
119	A	H	A	H	-	B
120	B	-	B	O	B	H
121	-	-	H	O	-	O
122	B	H	H	H	B	H
123	H	B	B	B	A	B
124	-	-	-	-	H	O
125	H	-	B	-	O	B
126	H	B	H	B	B	H
127	-	B	O	B	A	-
128	O	B	A	H	B	B
129	B	A	B	O	B	B
130	B	B	H	O	H	H
131	O	O	B	-	B	B
132	B	H	H	A	H	B
133	B	H	B	H	B	B
134	O	B	-	H	A	B
135	H	B	-	H	B	B
136	H	A	H	B	H	B
137	H	B	B	A	B	B
138	B	B	O	-	H	A
139	-	H	H	B	A	H
140	H	H	B	H	B	B

'A' - Homozygote at marker locus as that of donor parent (E 36-1)
 'B' - Homozygote at marker locus as that of recurrent parent (M 35-1)
 'H' - Heterozygote at marker locus as that of E 36-1
 'O' - Off type showing banding pattern different from parents
 '-' - Missing data for the individual at the locus

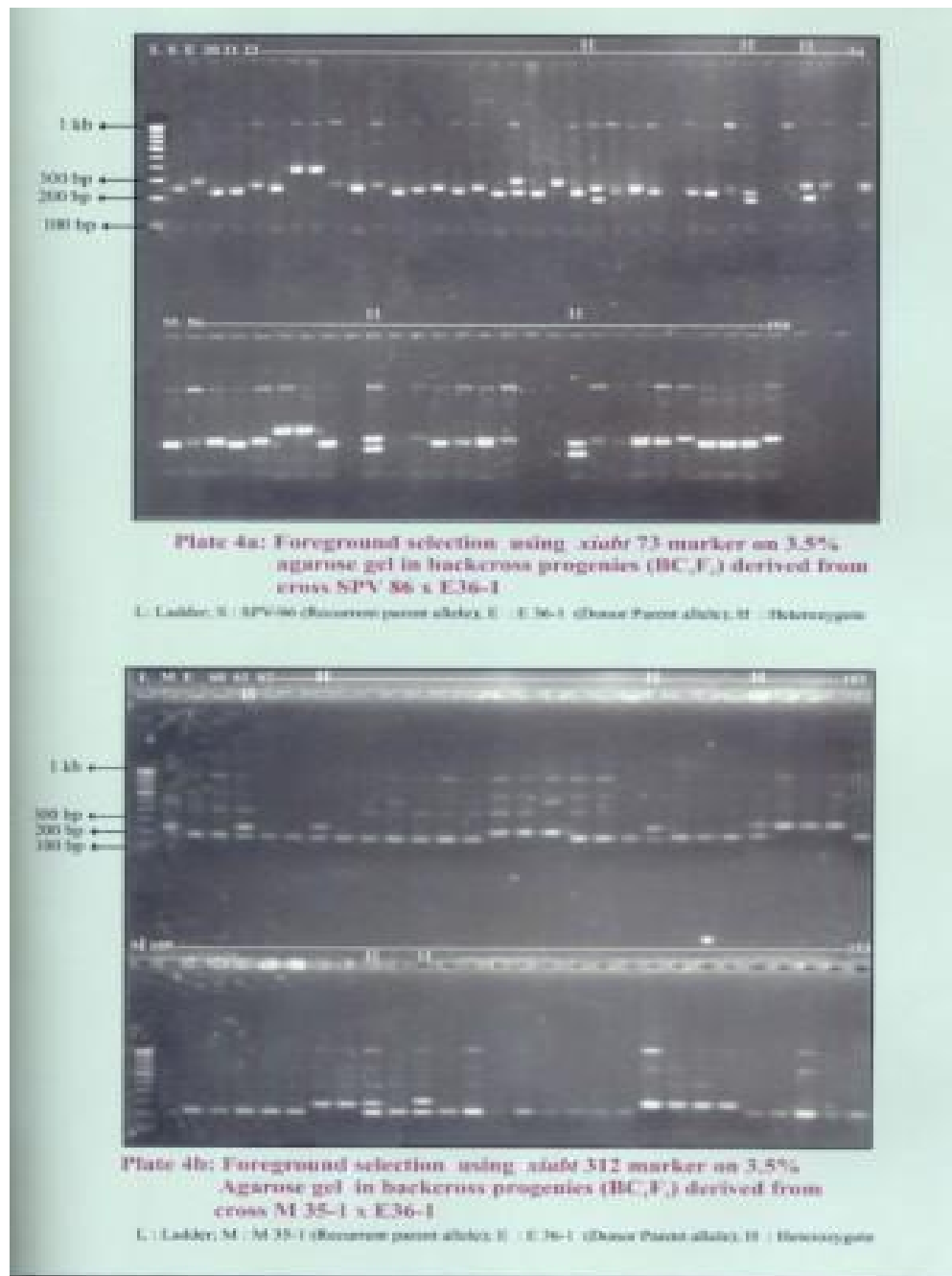


Plate.4a. Foreground selection using xiab73 marker on 3.5% agarose gel in backcross progenies (BC₁F₁) derived from cross SPV 86xE36-1

Plate.4b. Foreground selection using xiab312 marker on 3.5% Agarose gel in backcross progenies (BC₁F₁) derivd from cross M35-1 xE36-1

Table 21: Summary statistic of foreground and limited background selection for M35-1 x E 36-1 derived BC₂F₁ backcross progenies

Sl. No.	Particulars	M35-1 × E 36-1
1.	Number of progeny screened BC ₂ F ₁	509
2.	Number of marker used for foreground selection	6
3.	Number of marker used for limited background selection	6
4.	Scorable Marker data points generated	6002
5.	Number of Progeny satisfying the foreground & background selection for all the targeted QTL region	5
6.	Marker status of selected plants at target QTL region	Heterozygous for foreground marker and homozygote for background marker
7.	Number of plant selected for backcrossing	1

Table 22: Part of score sheet for BC₂F₁ population of cross the M35-1 × E 36-1 for three target QTLs genotyped with SSR and genic SSR markers

Progeny No.	<i>cr1</i>				<i>cr2</i>				<i>cr3</i>			
	Flanking marker (foreground selection)		Flanking marker (limited background selection)		Flanking marker (foreground selection)		Flanking marker (limited background selection)		Flanking marker (foreground selection)		Flanking marker (limited background selection)	
	<i>txp176</i>	<i>xiabt312</i>	<i>txp96</i>	<i>txp274</i>	<i>txp297</i>	<i>xiabt73</i>	<i>txp48</i>	<i>xiabt 82</i>	<i>xiabt275</i>	<i>xiabt241</i>	<i>xiabt 60</i>	<i>xiabt 87</i>
211	A	H	-	B	A	O	H	A	H	A	H	B
212	B	O	B	H	B	-	H	H	H	H	H	H
213	B	B	H	B	-	H	B	A	B	H	B	B
214	H	B	O	B	A	H	O	O	H	B	B	A
215	B	B	A	H	B	H	H	H	H	H	H	-
216	A	A	B	O	B	-	H	B	H	A	H	H
217	H	H	B	B	H	H	B	B	H	H	B	B
218	H	H	B	H	B	O	B	-	B	O	B	H
219	B	B	-	H	A	-	-	-	H	O	-	O
220	B	B	-	H	B	A	B	H	H	H	B	H
221	H	A	H	B	H	H	H	B	B	B	A	B
222	A	B	B	A	B	B	O	-	H	H	B	B
223	B	-	-	H	-	H	H	B	A	B	H	B
224	O	H	H	O	H	H	B	H	B	O	B	B
225	B	H	A	H	H	A	-	B	O	-	B	B
226	B	B	-	-	H	H	H	H	B	A	A	B
227	B	H	O	H	H	-	A	H	B	H	B	H
228	H	H	-	H	H	B	H	B	B	B	B	H
229	H	H	H	O	O	H	-	H	O	H	O	B
230	H	O	B	-	B	B	O	B	H	H	H	H
231	H	H	H	A	H	B	B	B	B	B	O	H
232	H	H	-	H	H	H	A	H	H	H	H	-

Contd.....

Progeny No.	cr1				cr2				cr3			
	Flanking marker (foreground selection)		Flanking marker (limited background selection)		Flanking marker (foreground selection)		Flanking marker (limited background selection)		Flanking marker (foreground selection)		Flanking marker (limited background selection)	
	<i>txtp176</i>	<i>xiabt312</i>	<i>txtp96</i>	<i>txtp274</i>	<i>txtp297</i>	<i>xiabt73</i>	<i>txtp48</i>	<i>xiabt 82</i>	<i>xiabt275</i>	<i>xiabt241</i>	<i>xiabt 60</i>	<i>xiabt 87</i>
233	B	B	-	O	H	-	B	H	B	H	B	O
234	H	B	-	O	O	-	B	-	H	-	-	O
235	H	O	O	-	-	-	O	-	O	O	H	-
236	B	H	H	H	B	B	B	A	B	B	A	A
237	A	B	B	O	-	H	H	B	B	B	B	B
238	H	-	H	H	B	A	B	H	B	O	H	B
239	O	H	H	B	H	B	O	B	B	B	B	B
240	H	H	A	-	B	O	-	B	B	B	H	B
241	-	H	H	H	H	B	A	A	B	O	B	B
242	H	H	-	A	H	B	H	B	H	H	B	B
243	A	B	O	B	-	H	B	H	O	H	-	B
244	B	H	O	H	O	B	H	H	H	H	B	B
245	H	B	H	B	O	B	-	B	B	B	-	-
246	B	-	H	A	-	-	-	H	B	-	H	-
247	B	-	H	B	A	B	H	H	H	H	H	O
248	A	H	B	H	H	H	B	B	B	H	B	O
249	B	B	A	B	B	O	-	H	A	H	B	H
250	H	H	O	B	O	B	H	-	H	H	H	O
251	O	H	A	H	B	B	B	A	B	B	B	A
252	H	H	B	O	-	H	H	B	B	B	B	B
253	H	-	H	H	B	A	B	H	B	O	H	B
254	H	O	B	B	H	B	O	B	B	B	B	H
255	B	O	B	-	B	O	-	B	B	B	A	B

'A' - Homozygote at marker locus as that of donor parent (E 36-1)

35-1)

'H' - Heterozygote at marker locus as that of E 36-1

'-' - Missing data for the individual at the locus

'B' - Homozygote at marker locus as that of recurrent parent (M

'O' - Off type showing banding pattern different from parents

which one progeny (#106) was selected for emasculation and backcrossing with recurrent parent SPV 86. The seed obtained from this cross were advanced to next generation (BC₂F₁).

4.4.4 Foreground and limited background selection of SPV 86 derived BC₂F₁ progeny

In BC₂F₁ generation two rounds of selection (foreground and limited background) was carried out. First each progeny were screened with six polymorphic foreground marker (*xtxp* 176, *xiabt*312, *xtxp*297, *xiabt*73, *xiabt* 75, *xiabt* 241) flanking the target QTL region and thus noting down the marker status for each progeny and ensuring the heterozygous nature for marker loci for all the six primer. Thereafter a second level of selection was performed to avoid the other genome region from donor parent from QTL carrying linkage group B and LG-I, i.e. limited background selection was exercised so as to take care of linkage drag around target QTL position. Two flanking markers outside each targeted QTL region were used for identifying the backcross progenies having the recurrent parent allele at both the markers termed as double homozygote at that particular locus. Foreground and limited background selection was carried out for all the background progenies simultaneously rather than sequentially to maximize our chances of getting a single progeny with all the marker status of our requirement though the genotyping effort was increased. Each of the 12 markers (six flanking marker targeting the donor QTL region and six flanking marker outside of QTL markers) were screened across all the 526 progenies. A total of 5989 marker data points were generated encompassing foreground and background selection (Table 25). After screening on gel the marker status of each progeny was recorded in excel format with proper notation and progeny number as described earlier. We selected the progeny which showed heterozygosity ("H") and double homozygote ("B") for each of the three QTL regions (Table 26). A total of three plants fulfilled these requirements and out of which one progeny (#442) was selected and emasculated for backcrossing with recurrent parent M35-1 to produce BC₃F₁ seeds

4.5 Functional analyses of transcription factor genes

Plants are continually exposed to a vast array of pathogens, and generally succeed in defending themselves. An important part of this process is the induction of defence gene products that hinder pathogen growth and delay or prevent disease development. The way in which plants do this is multi-factorial, and includes regulatory proteins known as transcription factors which directly regulate the expression of defence-related genes. The expression and activity of these TFs can be differentially regulated by pathogen challenge. Further, it is important to pin-down the specific member/s of TF families actually involved in resistance manifestation against specific pathogens.

Selection of TF families

Based on the available information about various TFs and their annotated functions in model systems such as *Arabidopsis thaliana*, *Zea mays* and *Oryza sativa* a set of 18 important TF families having 185 members were selected for their expression analysis on the basis of their direct and indirect role in biotic and abiotic stress. Since charcoal rot disease has drought stress as an important factor in its manifestation, the TFs that are involved in drought stress as well were included.

Generally in qRT-PCR, transcripts of stably expressed genes (reference genes) are used for data normalization. In *Oryza sativa* and *Arabidopsis thaliana*, previous publications have suggested 18S rRNA, EF-1 α , β -actin, β -tubulin, and ubiquitin (UBQ) as good reference genes (Caldana, 2007; Czechowski, 2004). To identify the most suitable reference genes in sorghum, we initially selected four candidate genes, viz., actin (AC1), β -tubulin, 18S-rRNA, and elongation factor-1 α which are commonly housekeeping genes in plants. The gene expression stability measure (M) was estimated through qRT-PCR in a set of different cDNA samples (pathogen infected and water stressed root and shoot tissues), using NormFinder software (model-based variance estimation approach). The difference in gene expression level of two ideal control genes should be the same in all experimental conditions compared (tissues, treatment), the lower the M value of a gene, the more stably it is expressed

Table 23: Summary statistics of foreground selection for SPV 86 x E 36-1 derived BC₁F₁ backcross progenies

Sl. No.	Particulars	SPV 86 x E 36-1
1.	Number of progeny screened BC ₁ F ₁	390
2.	Number of marker screened for foreground selection	6
3.	Scorable marker data points generated	2033
4.	Number of progenies satisfying the foreground selection for all the targeted QTL region	7
5.	Marker status of selected plants at target QTL region	Heterozygous
6.	Number of plant selected for backcrossing	1

Table 24a: Part of score sheet indicating the genotypic status for flanking markers for three charcoal resistance QTLs (*cr1*, *cr2* and *cr3*) among BC₁F₁ progenies derived from SPV 86 × E 36-1 crosses (foreground selection)

Progeny	<i>cr1</i>		<i>cr2</i>		<i>cr3</i>	
	Flanking marker		Flanking marker		Flanking marker	
	<i>xtxp176</i>	<i>xiabt312</i>	<i>xtxp297</i>	<i>xiabt 73</i>	<i>xiabt 275</i>	<i>xiabt 241</i>
1	H	B	A	A	H	H
2	-	-	H	H	H	B
3	B	B	H	B	B	B
4	A	O	-	H	B	B
5	B	B	O	-	B	B
6	H	H	O	B	H	B
7	B	B	-	H	B	H
8	-	B	A	H	H	B
9	B	B	H	H	B	B
10	H	H	H	H	H	H
11	-	B	H	B	B	H
12	O	H	H	B	A	H
13	H	H	O	H	A	H
14	H	H	H	O	O	A
15	H	B	B	B	B	A
16	O	-	B	-	O	B
17	B	B	B	H	H	H
18	O	-	H	H	B	B
19	O	-	-	H	H	H
20	H	H	H	H	H	H
21	B	B	-	-	O	H
22	H	H	B	B	B	H
23	O	-	B	B	A	-
24	A	B	H	H	-	O
25	B	B	B	H	A	B
26	H	H	B	B	-	H
27	H	B	B	A	B	B
28	H	O	A	-	H	H
29	H	B	H	B	H	H
30	B	-	H	B	O	H
31	B	B	B	B	B	B
32	H	-	-	H	-	B
33	O	-	B	B	H	B
34	B	O	B	B	H	H
35	B	H	H	H	A	O

'A' - Homozygote at marker locus as that of donor parent (E 36-1)
 'B' - Homozygote at marker locus as that of recurrent parent (M 35-1)
 'H' - Heterozygote at marker locus as that of E 36-1
 'O' - Off type showing banding pattern different from parents
 '-' - Missing data for the individual at the locus

Table 24b: Part of score sheet indicating the genotypic status for flanking markers for three charcoal resistance QTLs (*cr1*, *cr2* and *cr3*) among BC₁F₁ progenies derived from SPV86 × E 36-1 crosses (foreground selection)

Progeny	<i>cr1</i>		<i>cr2</i>		<i>cr3</i>	
	Flanking marker		Flanking marker		Flanking marker	
	<i>xtxp176</i>	<i>xiabt312</i>	<i>xtxp297</i>	<i>xiabt 73</i>	<i>xiabt 275</i>	<i>xiabt 241</i>
231	H	O	-	-	O	H
232	O	-	A	H	H	H
233	H	H	H	H	H	H
234	H	H	H	H	B	B
235	A	B	H	O	-	A
236	B	H	O	-	B	B
237	B	B	B	H	H	-
238	H	B	A	A	H	H
239	-	-	H	H	H	B
240	B	B	H	B	B	B
241	A	O	-	H	B	B
242	B	B	-	H	B	H
243	-	B	A	H	H	B
244	B	B	H	H	B	B
245	A	B	H	O	-	A
246	B	H	O	-	B	B
247	H	O	O	-	-	-
248	H	H	H	H	B	B
249	B	B	B	B	A	H
250	-	O	-	H	H	H
251	B	B	-	-	O	H
252	H	H	B	B	B	H
253	O	-	B	B	A	-
254	A	B	H	H	-	O
255	B	B	O	-	B	B
256	H	H	H	H	H	H
257	B	B	-	H	B	H
258	-	B	A	H	H	B
259	B	B	H	H	B	B
260	-	B	H	H	A	O
261	-	B	H	B	B	H
262	H	B	B	-	H	H
263	B	B	A	B	B	H
264	O	A	-	H	H	B
265	H	H	B	B	A	-
266	A	B	H	B	H	B

- 'A' - Homozygote at marker locus as that of donor parent (E 36-1)
 'B' - Homozygote at marker locus as that of recurrent parent (M 35-1)
 'H' - Heterozygote at marker locus as that of E 36-1
 'O' - Off type showing banding pattern different from parents
 '-' - Missing data for the individual at the locus

Table 25: Summary statistic of foreground and limited background selection for SPV 86 x E 36-1 derived BC₂F₁ backcross progenies

Sl. No.	Particulars	SPV 86 × E 36-1
1.	Number of progeny screened BC ₂ F ₁	526
2.	Number of marker used for foreground selection	6
3.	Number of marker used for limited background selection	6
4.	Scorable Marker data points generated	5989
5.	Number of Progeny satisfying the foreground & background selection for all the targeted QTL region	3
6.	Marker status of selected plants at target QTL region	Heterozygous for foreground marker and homozygote for background marker
7.	Number of plant selected for backcrossing	1

Table 26: Part of score sheet for BC₂F₁ population of cross the SPV86 × E 36-1 for three target QTLs genotyped with SSR and genic SSR markers

Progeny No.	<i>cr1</i>		<i>cr2</i>				<i>cr3</i>					
	Flanking marker (foreground selection)		Flanking marker (limited background selection)		Flanking marker (foreground selection)		Flanking marker (limited background selection)		Flanking marker (foreground selection)		Flanking marker (limited background selection)	
	<i>txtp176</i>	<i>xiabt312</i>	<i>txtp96</i>	<i>txtp274</i>	<i>txtp297</i>	<i>xiabt 73</i>	<i>txtp48</i>	<i>xiabt 82</i>	<i>xiabt 275</i>	<i>xiabt 241</i>	<i>xiabt 60</i>	<i>xiabt 87</i>
418	B	A	H	B	H	H		H	B	B	B	A
419	B	H	B	B	B	B	H	-	H	O	H	B
420	H	-	H	O	H	A	O	H	H	-	H	B
421	H	H	H	-	H	-	B	-	H	B	H	H
422	B	-	H	B	H	H	A	H	B	H	-	B
423	A	H	B	H	-	-	H	B	B	B	H	A
424	H	-	H	H	O	-	H	O	-	B	H	B
425	H	H	B	H	B	O	B	-	B	O	B	H
426	B	B	-	H	A	-	-	-	H	O	-	O
427	B	A	H	-	B	H	H	H	B	A	A	B
428	A	B	O	B	H	B	A	H	B	H	B	H
429	H	B	B	H	B	B	H	B	B	B	B	H
430	A	H	B	O	B	H	-	H	O	H	O	B
431	O	B	B	A	H	H	H	H	-	H	B	H
432	H	A	A	B	O	B	-	H	B	H	H	H
433	B	H	A	H	H	A	H	B	H	-	A	H
434	B	H	O	H	H	B	B	A	B	B	H	B
435	H	H	-	H	H	-	-	H	-	H	-	H
436	H	H	H	O	O	A	H	B	H	-	H	H
437	A	B	B	H	O	B	B	A	B	B	H	A
438	A	B	H	H	H	B	H	B	H	B	O	H

Contd.....

Progeny No.	cr1				cr2				cr3			
	Flanking marker (foreground selection)		Flanking marker (limited background selection)		Flanking marker (foreground selection)		Flanking marker (limited background selection)		Flanking marker (foreground selection)		Flanking marker (limited background selection)	
	xtxp176	xiabt312	xtxp96	xtxp274	xtxp297	xiabt 73	xtxp48	xiabt 82	xiabt 275	xiabt 241	xiabt 60	xiabt 87
439	H	H	B	B	B	A	B	B	O	H	H	-
440	B	O	-	H	H	B	B	H	H	O	B	B
441	H	H	B	A	B	H	B	H	H	-	B	B
442	H	H	B	B	H	H	B	B	H	H	B	B
443	A	-	B	O	-	B	B	B	B	H	B	B
444	H	A	-	-	H	O	-	B	H	H	H	B
445	B	H	A	H		H	H	H	-	H	H	B
446	B	H	H	B	B	B	O	B	H	-	O	B
447	B	H	B	A	H	H	H	B	O	O	H	B
448	B		A	B	B	H	O	O	H	H	-	B
449	A	B	O	B	B	B	O	B	H	H	H	B
450	H	-	B	H	H	B	B	B	B	B	O	B
451	A	A	A	A	H	H	A	H	H	H	H	B
452	H	H	H	B	H	-	B	H	B	H	B	-
453	H	B	H	B	O	-	B	-	H	-	-	-
454	-	O	O	H	-	-	O	-	O	O	H	O
455	O	H	A	H	B	B	B	A	B	B	A	O
456	B	A	A	-	-	H	H	B	B	B	B	H
457	B	H	H	B	B	A	B	H	B	O	H	O
458	A	H	H	H	H	B	O	B	B	B	B	A
459	-	H	O	H	B	O	-	B	B	B	H	B
460	O	B	O	B	O	B	O	B	B	B	O	B
461	H	A	H	O	H	-	B	H	H	B	B	B
462	H	-	B	O	B	A	A	A	H	H	A	H

'A' - Homozygote at marker locus as that of donor parent (E 36-1)

35-1)

'H' - Heterozygote at marker locus as that of E 36-1

'-' - Missing data for the individual at the locus

'B' - Homozygote at marker locus as that of recurrent parent (M

'O' - Off type showing banding pattern different from parents

(suggested limit of $M < 1.5$). The gene expression stability (M) values for actin (AC1), 18S rRNA, and β -tubulin, were 0.312, 0.300, and 0.315, respectively.

The genes 18S and actin were found to be the best combination of the reference genes as their expression stability value (M) is 0.235 (calculated by pair wise variation (V), which indicates the optimal number of reference genes to be analyzed in a given experiment). This also indicates that this combination is the most stable, indicating the absence of significant differences in the expression levels of reference genes in varied experimental conditions

qRT-PCR conditions and efficiency

qRT-PCR reactions were performed in an optical 96-well eppendorf plates with an Eppendorf realplex detection system, using the Power SYBR Green master mix dye to monitor cDNA amplification. The 185 TFs genes were divided into four groups on the basis of melting temperature and reactions were carried out on 96 well format.

Subsequently, the average PCR efficiencies were computed for each individual primer pair across all analyzed samples tissue in different condition (75 DAS and 90 DAS). The analyses indicated that 28 genes registered a PCR efficiency greater than 1.91, and 151 genes registered a PCR efficiencies ranging from 1.71-1.90 and six TF genes found ranging their PCR efficiencies between 1.60 and 1.70.

Accuracy and precision of gene expression quantitation

The specificity of the PCR reaction was determined by loading the PCR products on 3 % agarose gel. Approximately, all TF genes yielded specific sized amplicon as predicted (Plate 5). A set of 43 TF genes were found failed in accumulating detectable PCR product in resistant and susceptible genotypes at both the time points viz., 75 DAS and 90 DAS. Normalised Ct values of 185 TF genes in mock inoculated (control) and inoculated (treated) samples of E36-1 (resistant) and SPV 86 (susceptible) at 75 and 95 DAS are presented in Appendix V. The list of transcription factor genes used in qRT-PCR study are presented in Appendix VI.

The technical precision or reproducibility of real-time PCR was assessed by performing replicate measurements in separate PCR runs, using the same pool of cDNA (technical replicates) and two different pools of cDNA obtained independently from two different batches of total-RNA under same condition (biological replicates). Precision, as reflected by the correlation coefficient, was high in both cases, with the technical replicates ($r > 0.98$), lower than the biological replicates ($r > 0.96$) as expected (Czechowski *et al.*, 2004) for both susceptible and resistant genotypes (Fig. 13 and 13b).

Expression patterns of TF genes

Out of 185 TF genes a set of 43 genes largely belong to the families bHLH (9), PHD (10) and NAC (9) families failed to express in situation tested (Fig. 14). About 121 TF genes were up-regulated in resistant and susceptible genotypes at 75 DAS and 95 DAS (Fig. 15a and 15b). In all these experimental situation a TF gene was called significantly up regulated only if the P value < 0.05 and fold change < 0.5 or > 2 was recorded at 75 and 90 DAS in resistant and susceptible cultivar,

The expression status of the TF genes in resistant and susceptible cultivar at two different time points is as follows

TF genes expression in E36-1 at 75 DAS

The expression data of 142 TF genes during pathogen infestation (target) and mock inoculated (control) conditions in resistant (E36-1) genotypes at 75 DAS was analyzed. The threshold Ct value for target genes in target sample ranged between 22.1 and 40 while in control sample it ranged between 27.1 and 40 with a mean Ct value of 27.3 for internal reference genes (Actin and 18s). A set of 21 TF genes recorded up regulation, 50 genes

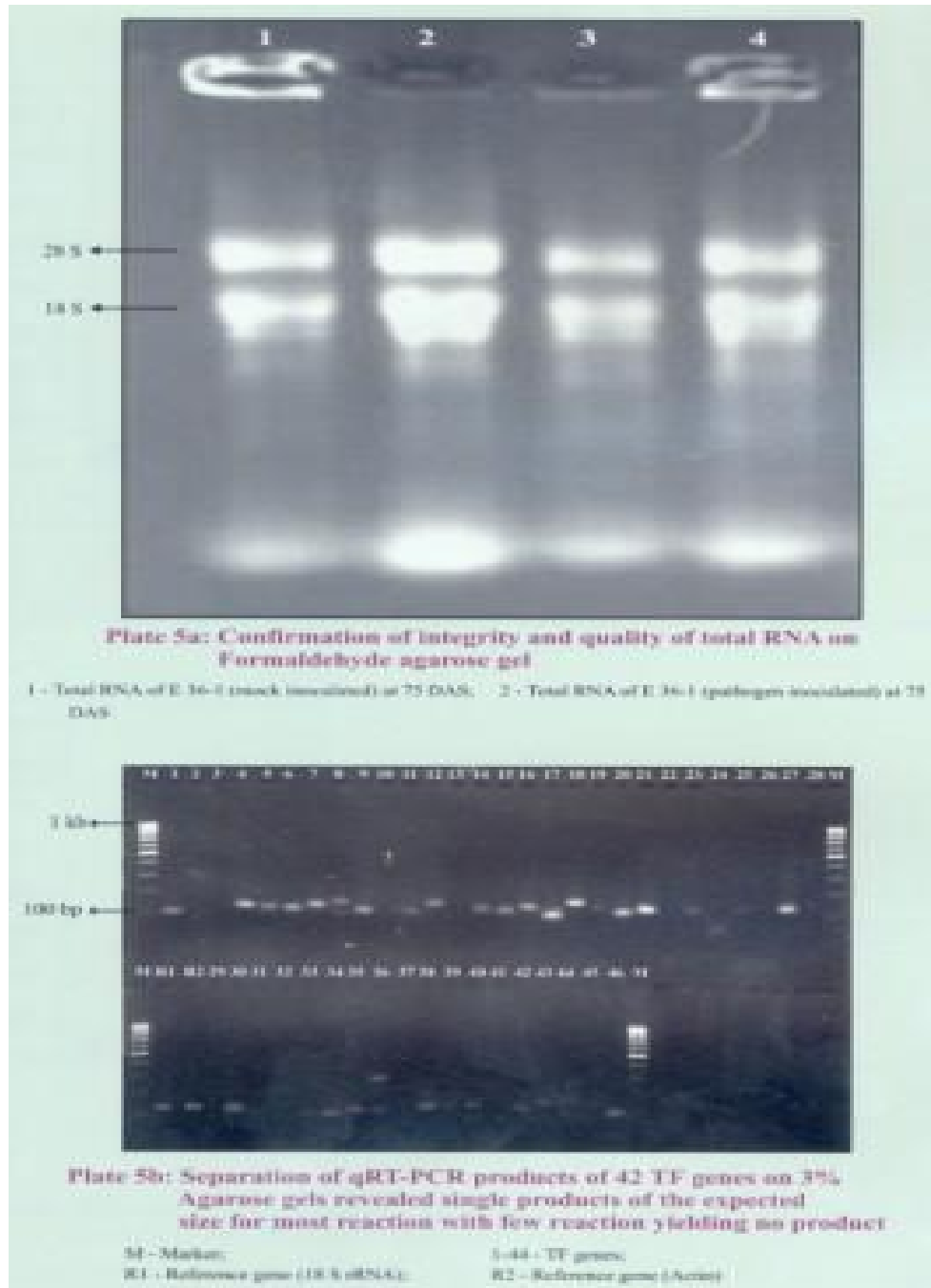


Plate.5a. Confirmation of integrity and quality of total RNA on Formaldehyde agarose gel

Plate.5b. Separation of qRT-PCR products of 42 TF genes on 3% Agrose gels reveled single products of the expected size for most reaction with few reaction yielding no product

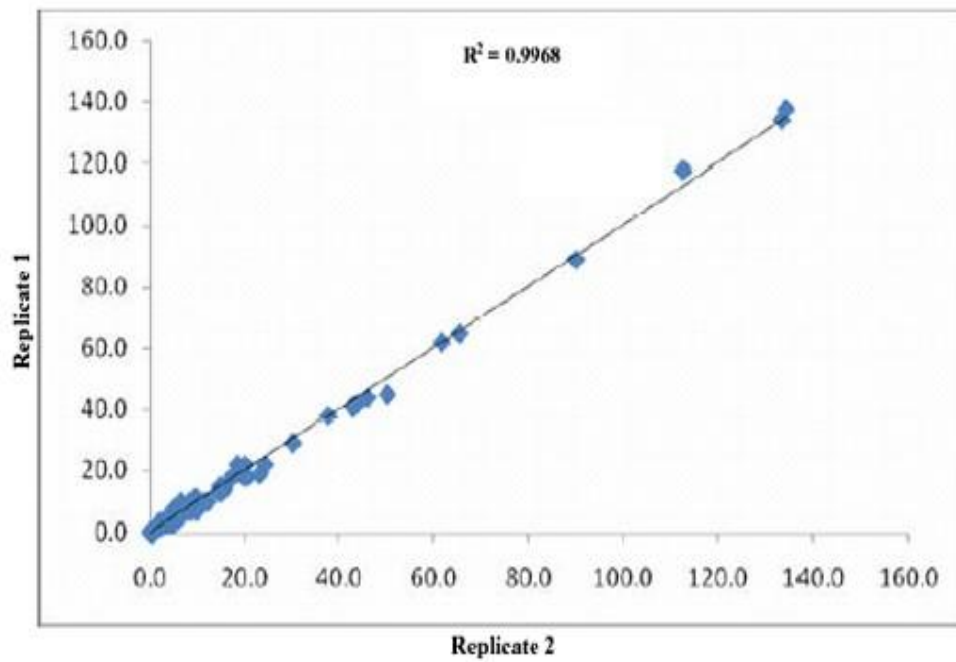


Fig.13a. Regression value between two technical replicates for pathogen inoculated sample of SPV 86 used in qRT-PCR experiment

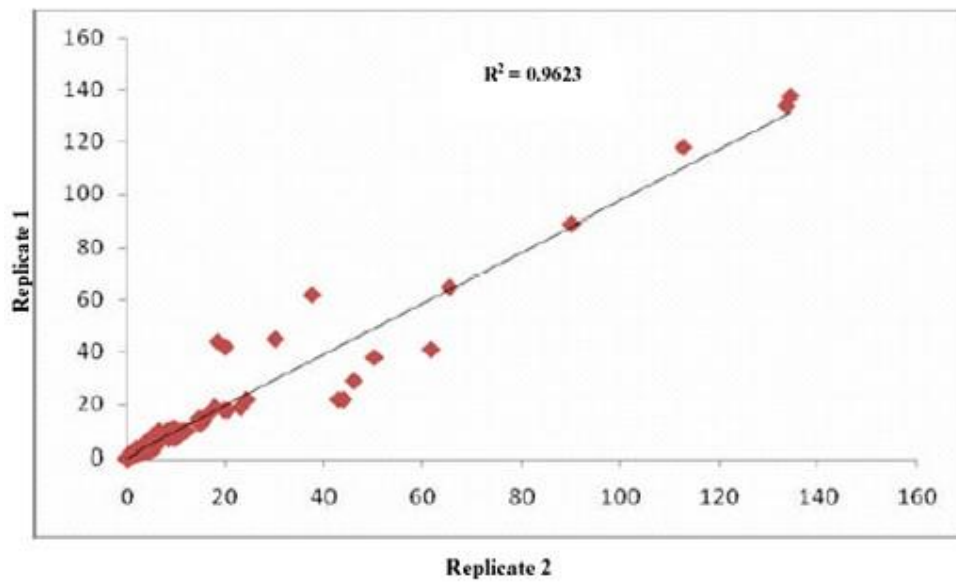


Fig.13b. Regression value between two biological replicates for pathogen inoculated sample of SPV 86 USED IN qRT-PCR experiment

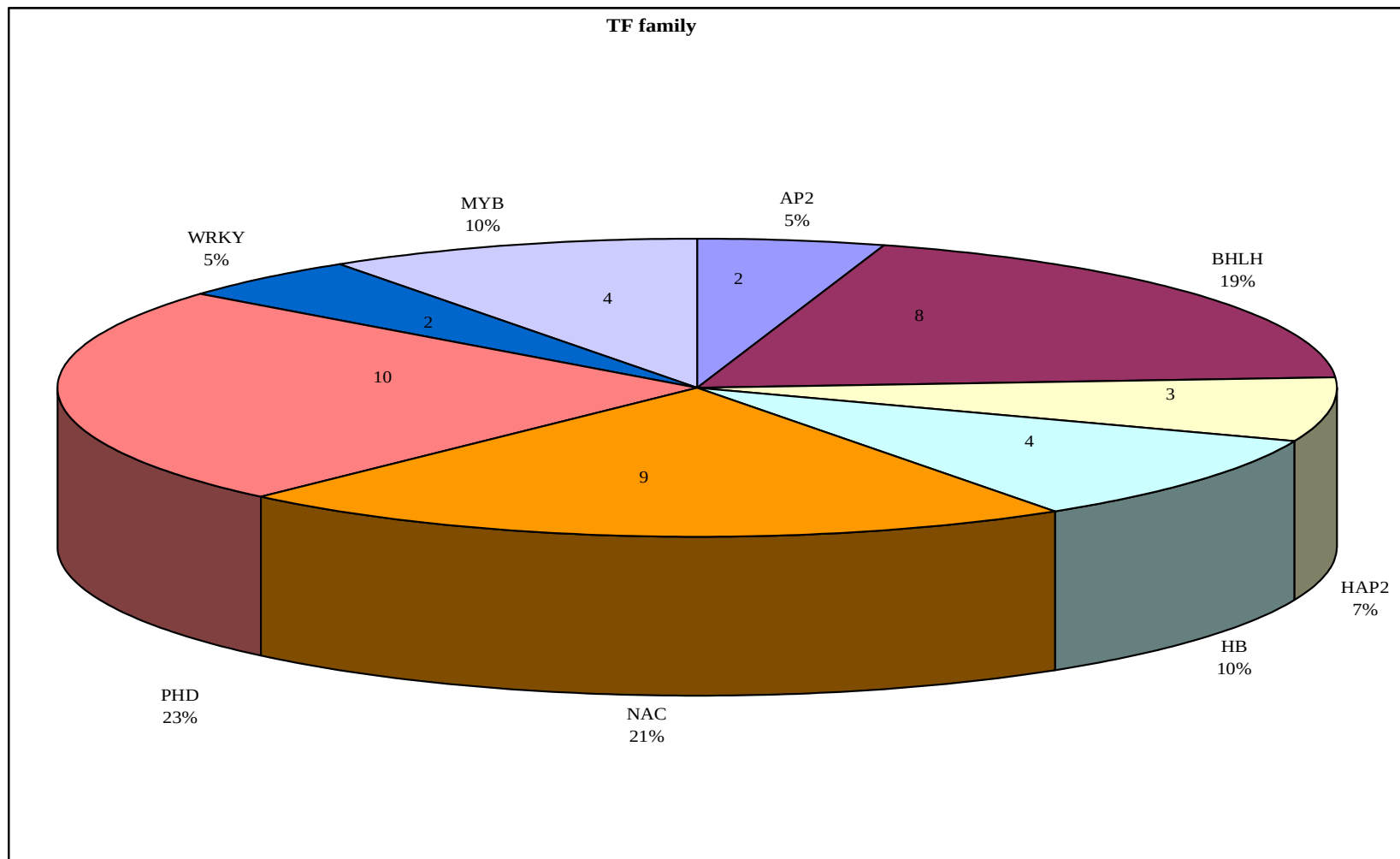


Fig.14. Group of transcription factor genes belonging to different family which showed no detectable gene expression in E36-1 and SPV 86 at 75 and 90 DAS

Amplification plot

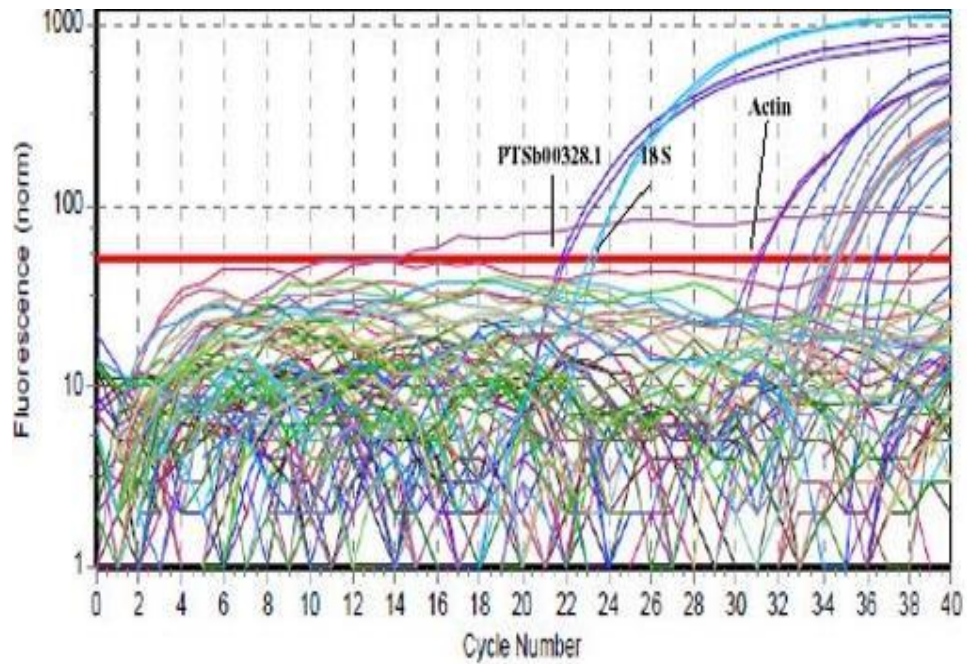


Fig.15a. Cycle threshold value (Ct) of target transcription factor WRKY (PTsb00328.1) And internal reference gene (18S and Actin) observed in infected E36-1 at 75 DAS

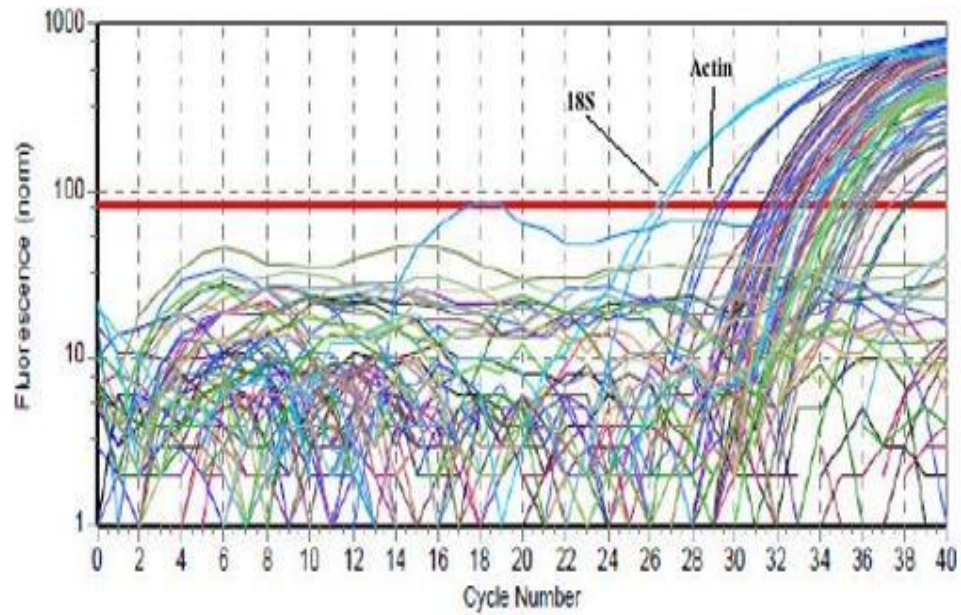


Fig.15b. Cycle threshold value (Ct) internal reference gene (18S and Actin) observed in mock inoculated SPV 86 at 75 DAS

Table 27: Fold change in expression of members of transcription factor genes in SPV 86 (susceptible) and E 36-1 (resistant) genotypes at 75 days after sowing under charcoal rot challenge infestation as revealed by real time PCR quantification

Sl. No.	Database ID	TF family	Normalized Ct value						Fold change in expression	
			SPV 86			E 36-1			SPV 86	E 36-1
			Δ Ct (control)	Δ Ct (infected)	t test	Δ Ct (control)	Δ Ct (infected)	t test		
1.	PTSb00019.1	AP2	2.255	0.8	0.0118	2.73	-1.565	0.0003	2.506	15.05
2.	PTSb00022.1	AP2	6.095	4.5	0.0300	4.01	0.435	0.0094	2.596	8.91
3.	PTSb00024.1	AP2	6.955	5	0.0300	4.23	1.435	0.0161	3.435	5.84
4.	PTSb00033.1	ARF	4.665	3.3	0.0400	4.18	2.435	0.0180	2.556	3.32
5.	PTSb00127.1	HAP2	11.04	8.9	0.0030	-	-	-	3.949	-
6.	PTSb00130.1	HAP5	7.16	4.1	0.0020	-	-	-	7.926	-
7.	PTSb00131.1	HAP5	6.84	5.26	0.0200	-	-	-	2.711	-
8.	PTSb00132.1	HAP5	9.84	7.9	0.0000	-	-	-	3.474	-
9.	PTSb00179.1	HMG	4.04	0.8	0.0030	5.74	-0.415	0.0006	9.296	69.33
10.	PTSb00182.1	HMG	8.84	7.7	0.0100	-	-	-	2.079	-
11.	PTSb00185.1	HMG	6.74	3.9	0.0400	6.40	2.385	0.0007	5.143	10.16
12.	PTSb00211.1	MADS	10.31	8.9	0.0052	-	-	-	2.291	-
13.	PTSb00214.1	MADS	7.31	5.2	0.0030	-	-	-	3.416	-
14.	PTSb00220.1	MADS	9.31	7.3	0.0060	-	-	-	3.228	-
15.	PTSb00229.1	MYB-related	8.11	6.9	0.0040	-	-	-	2.089	-
16.	PTSb00234.1	MYB-related	10.11	7.8	0.0101	-	-	-	3.888	-

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Sl. No.	Database ID	TF family	Normalized Ct test (75 days after sowing)						Fold change in expression	
			SPV 86			E 36-1			SPV 86	E 36-1
			Δ Ct (control)	Δ Ct (infected)	t test	Δ Ct (control)	Δ Ct (infected)	t test		
17.	PTSb00289.1	PHD	9.95	8.7	0.0012	-	-	-	2.248	-
18.	PTSb00331.1	WRKY	5.05	1.2	0.0023	1.49	-5.26	0.0007	8.857	51.00
19.	PTSb00349.1	bHLH	4.255	2.1	0.0145	6.23	2.435	0.0004	3.898	10.98
20.	PTSb00374.1	bZIP	5.74	4	0.0050	-	-	-	2.781	-
21.	PTSb00218.1	MADS	-	-	-	5.63	0.23	0.0001	-	26.30
22.	PTSb00232.1	MYB-related	-	-	-	4.51	-1.37	0.0002	-	31.70
23.	PTSb00328.1	WRKY	-	-	-	3.795	-3.26	0.0030	-	86.67
24.	PTSb00359.1	bHLH	-	-	-	2.49	1.135	0.0369	-	2.27
25.	PTSb00374.2	bZIP	-	-	-	3.945	0.885	0.0019	-	6.04
26.	PTSb00180.1	HMG	-	-	-	5.835	4.085	0.0147	-	3.36
27.	PTSb00088.1	YABBY	-	-	-	5.60	4.44	0.061	-	2.23
28.	PTSb00023.1	AP2	-	-	-	4.13	2.44	0.057	-	2.83
29.	PTSb00021.1	AP2	-	-	-	2.43	0.14	0.009	-	4.02
30.	PTSb00398.1	bZIP	-	-	-	8.05	5.39	0.009	-	6.32
31.	PTSb00136.1	HAP5	-	-	-	6.27	0.53	0.000	-	6.34
32.	PTSb00352.1	bHLH	-	-	-	6.91	6.44	0.024	-	2.39
33.	PTSb00219.1	MADS	-	-	-	9.17	8.53	0.040	-	2.45

Δ Ct = (Ct of target gene – Ct of reference gene)

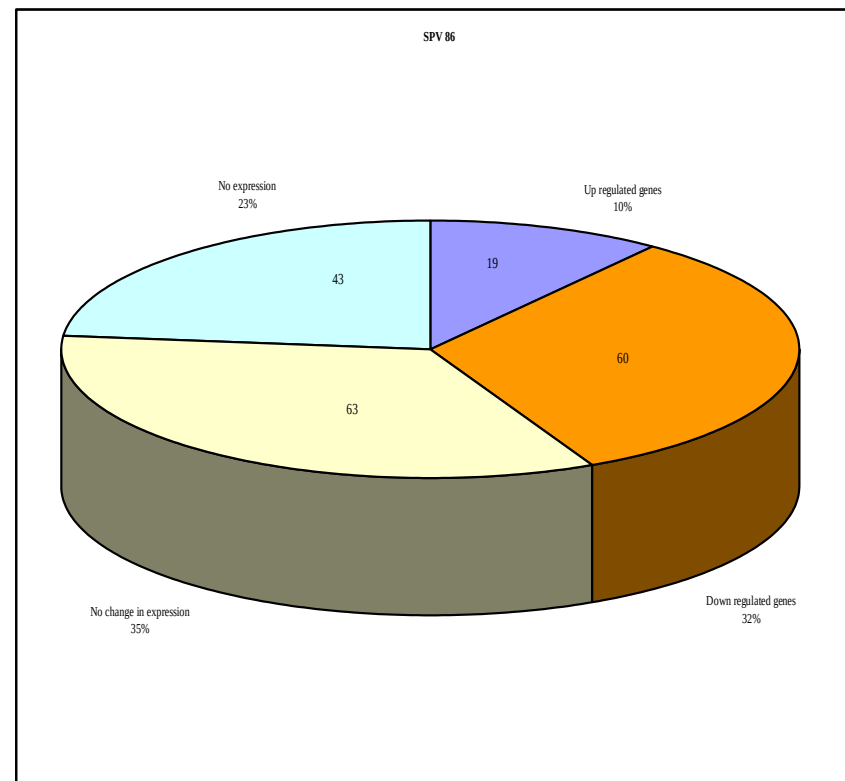
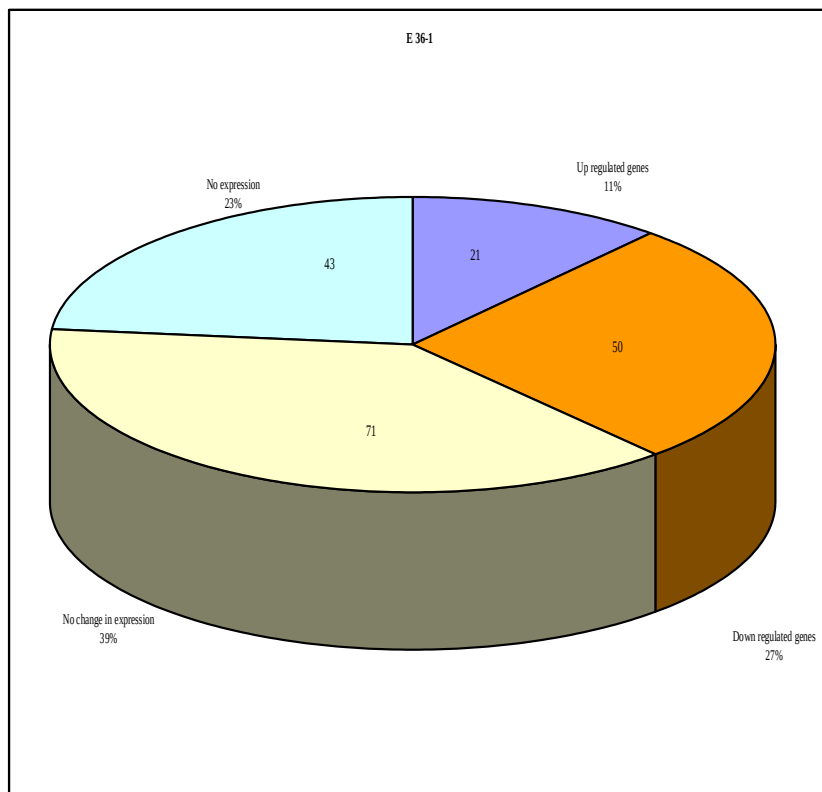


Fig.16. Fold change expression of 185 transcription factor genes at 75 DAS

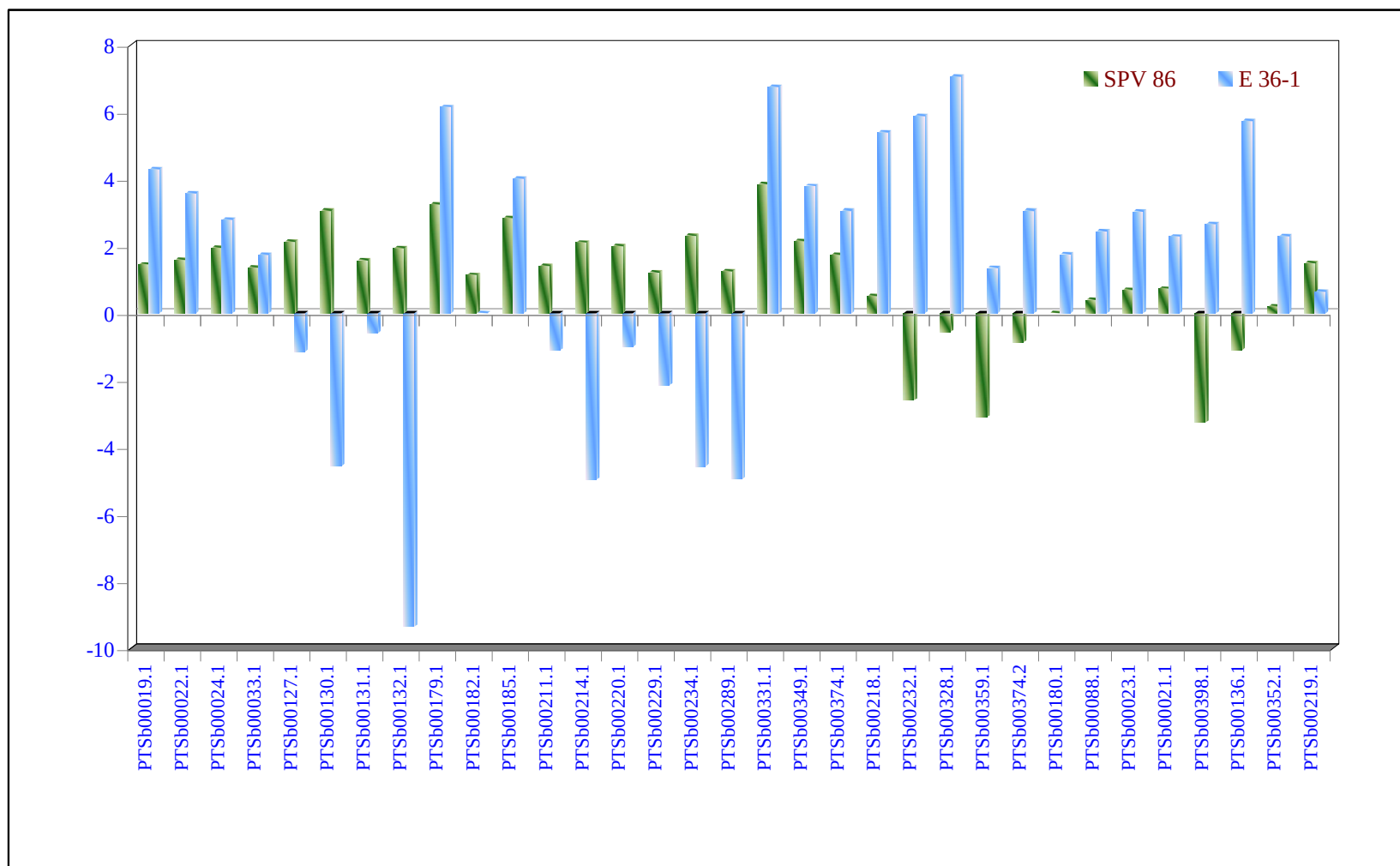


Fig.17.. Relative change in up and down regulation of transcription factor genes under charcoal rot disease manifestation in resistant and susceptible genotypes at 75 DAS

recorded down regulation and 71 genes showed no change in expression levels between treatments. The up regulation levels varied within a wide range of fold expression values between 2.23 and 86.67 (Table 27, Fig 16 and 17). The most highly expressed genes were from WRKY related transcription factor followed by MYB and bZIP related family. A set of 14.1% (21) TF genes were up regulated, 14 genes (PTSb00088.1, PTSb00023.1, PTSb00021.1, PTSb00398.1, PTSb00136.1, PTSb00352.1, PTSb00219.1, PTSb00022.1, PTSb00024.1, PTSb00033.1, PTSb00179.1, PTSb00359.1, PTSb00374.2, PTSb00180.1) recorded fold change expression within 2-10, 6 genes viz., PTSb00019.1(AP 2), PTSb00185.1(HMG), PTSb00349.1 (bHLH), PTSb00218.1(MADS), PTSb00232.1(MYB) recorded >10 fold expression while two genes belonging to WRKY family viz., PTSb00328.1 and PTSb00331.1 showed more than 50 fold expression compared to mock inoculated control E36-1.

TF genes expression in SPV 86 at 75 DAS

The expression data of 142 TF during pathogen manifestation (target) and mock inoculated (control) condition in SPV 86 (susceptible) at 75 DAS was analyzed. The threshold Ct value for target genes in target sample were in the range of 25.03 to 40 while in control sample they were in range of 29.9 to 40 with mean of 27.5 Ct value for internal reference genes (Actin and 18S). Out of 142 transcription factor belonging to 18 different family there was up regulation in 19 genes, while 60 genes showed down regulation, 63 genes were not showing any change in expression between treatments (Table 27 and Fig. 19 and 20). The highly expressed genes were from HMG, WRKY and HAP 5 related family. A set of 13.3 % (19) TF genes were up regulated. All the 19 up regulated genes showed fold expression within range of 2.08 to 9.3 of which majority of TF belong to four TF family viz., MADS (PTSb00211.1, PTSb00214.1, PTSb00220.1), HAP (PTSb00127.1, PTSb00130.1, PTSb00131.1, PTSb00132.1), AP2 (PTSb00019.1, PTSb00022.1, PTSb00024.1, PTSb00033.1), HMG (PTSb00179.1, PTSb00182.1, PTSb00185.1) and MYB (PTSb00229.1, PTSb00234.1).

TF genes expression in E36-1 at 90 DAS

The second set of plant sample were collected at 90 DAS with pathogen infected and mock inoculated (control) condition from resistant cultivar (E36-1). Same set 142 transcription factors were used to study the gene expression pattern. The threshold Ct value for target genes in target sample were in the range of 21.1 to 40 while in control sample they were in range of 27.1 to 40 with mean of 27.3 Ct value for internal reference genes (Actin and 18S). Out of 142 transcription factor belonging to 18 different family there was up regulation in 54 genes (38 %), 46 genes showed down regulation, 42 genes showed no change in expression. The observed fold change expression ranged from 2.25 to 182.25. Out of 54 up regulated TF genes, 29 genes expressed within 2 to 10 folds, while 19 genes expressed within 10- 50 fold viz., HMG (PTSb00191.1, PTSb00185.1, PTSb00180.1, PTSb00188.1, PTSb00184.1), AP2 (PTSb00018.1, PTSb00022.1, PTSb00024.1, PTSb00020.1), bHLH (PTSb00370.1, PTSb00349.1), ARF (PTSb00032.1, PTSb00033.1), bZIP (PTSb00393.1, PTSb00389.1), MADS (PTSb00218.1), HAPs, (PTSb00130.1) and WRKY (PTSb00328.1) showed expression level >50. The predominantly expressing gene was from WRKY family (PTSb00331.1) followed by AP2 (PTSb00019.1) and bZIP (PTSb00374.1), for which observed fold expression were 182.25, 99.37 and 86.35, respectively.

TF genes expression in SPV 86 at 90 DAS

The expression data of 142 TF genes was analyzed using samples obtained at 90 DAS with pathogen infected and mock inoculated (control) condition from susceptible cultivar (SPV 86). The threshold Ct value for target genes in target sample were in the range of 25.3.1 to 40 while in control sample they were in range of 27.8 to 40 with mean of 27.53 Ct value for internal reference genes (Actin and 18S). Out of 142 transcription factor belonging to 18 different family there was up regulation in 48 (33%) genes, 50 genes showed down regulation, 44 genes showed no change in expression while 51 genes were not expressed. The observed fold expression was ranged from 2.54 to 70.2. 24. Most of the up regulated genes (28) expressed within 2 to 50 fold compared to control. The 20 genes belonged to 11 different family viz., bHLH (PTSb00350.1, PTSb00353.1, PTSb00358.1, PTSb00352.1,

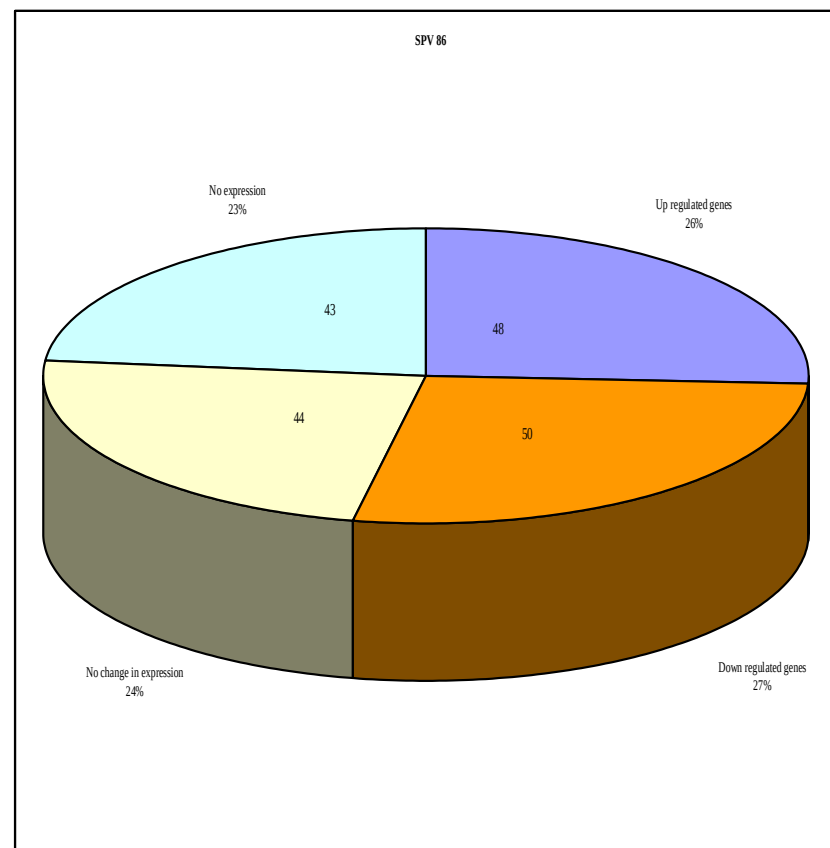
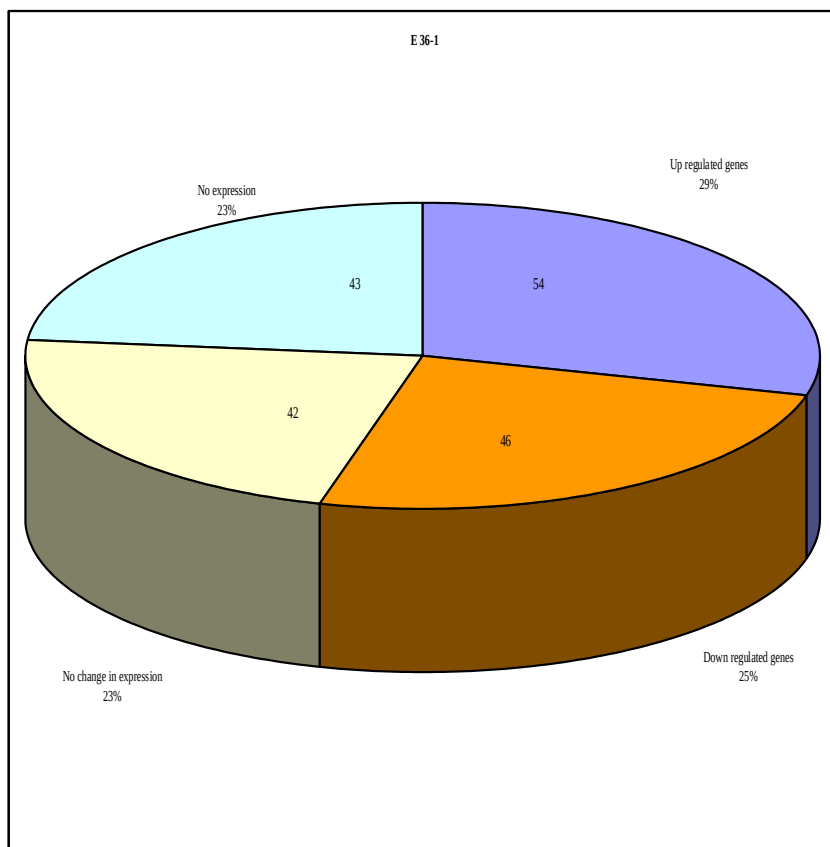


Fig..18. Fold change expression of 185 transcription factor genes at 90 DAS

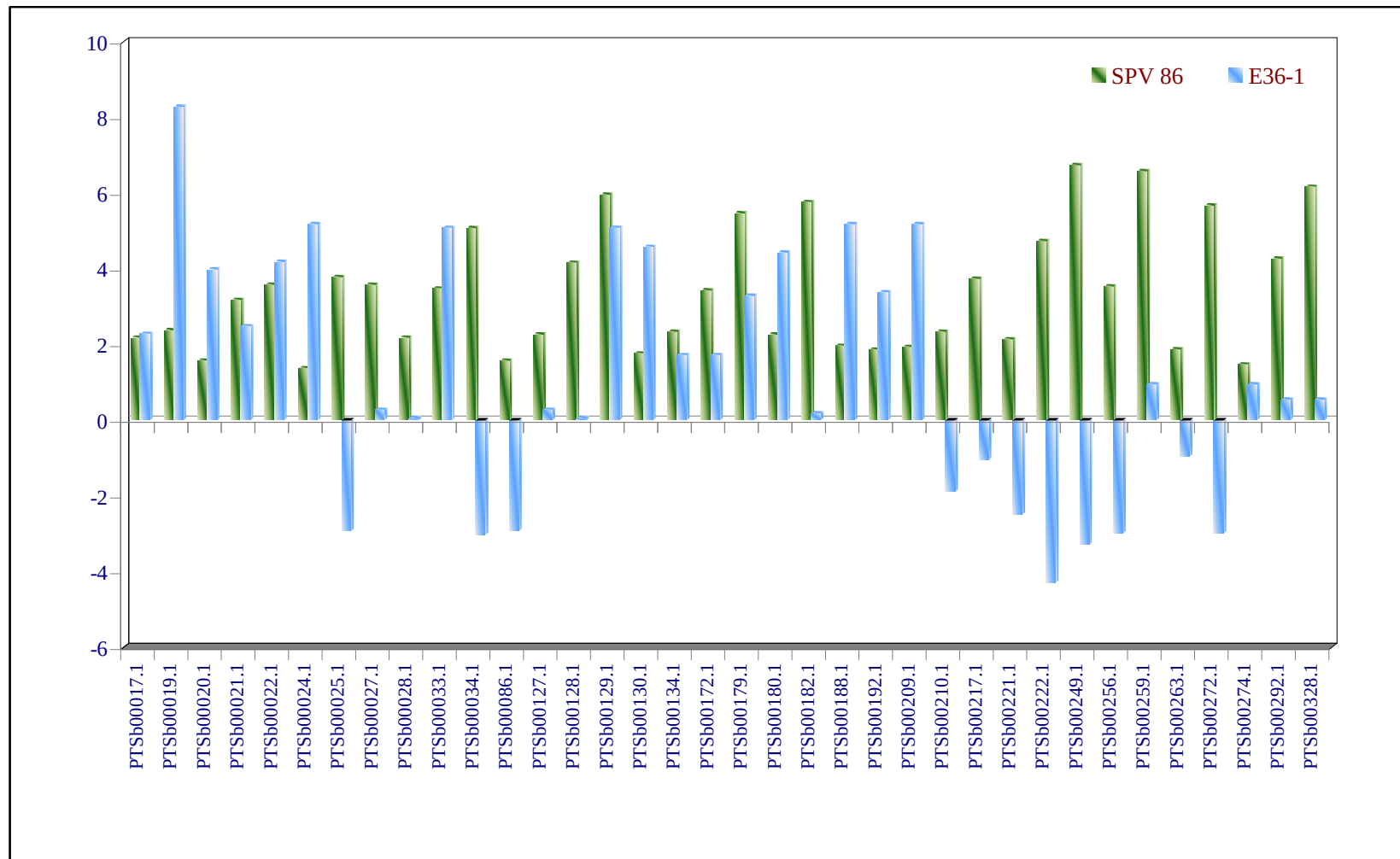


Fig.19.Relative change in up and down regulation of transcription factor genes under charcoal rot disease manifestation in resistant and susceptible genotypes at 90 DAS

Fig.19 Contd.....

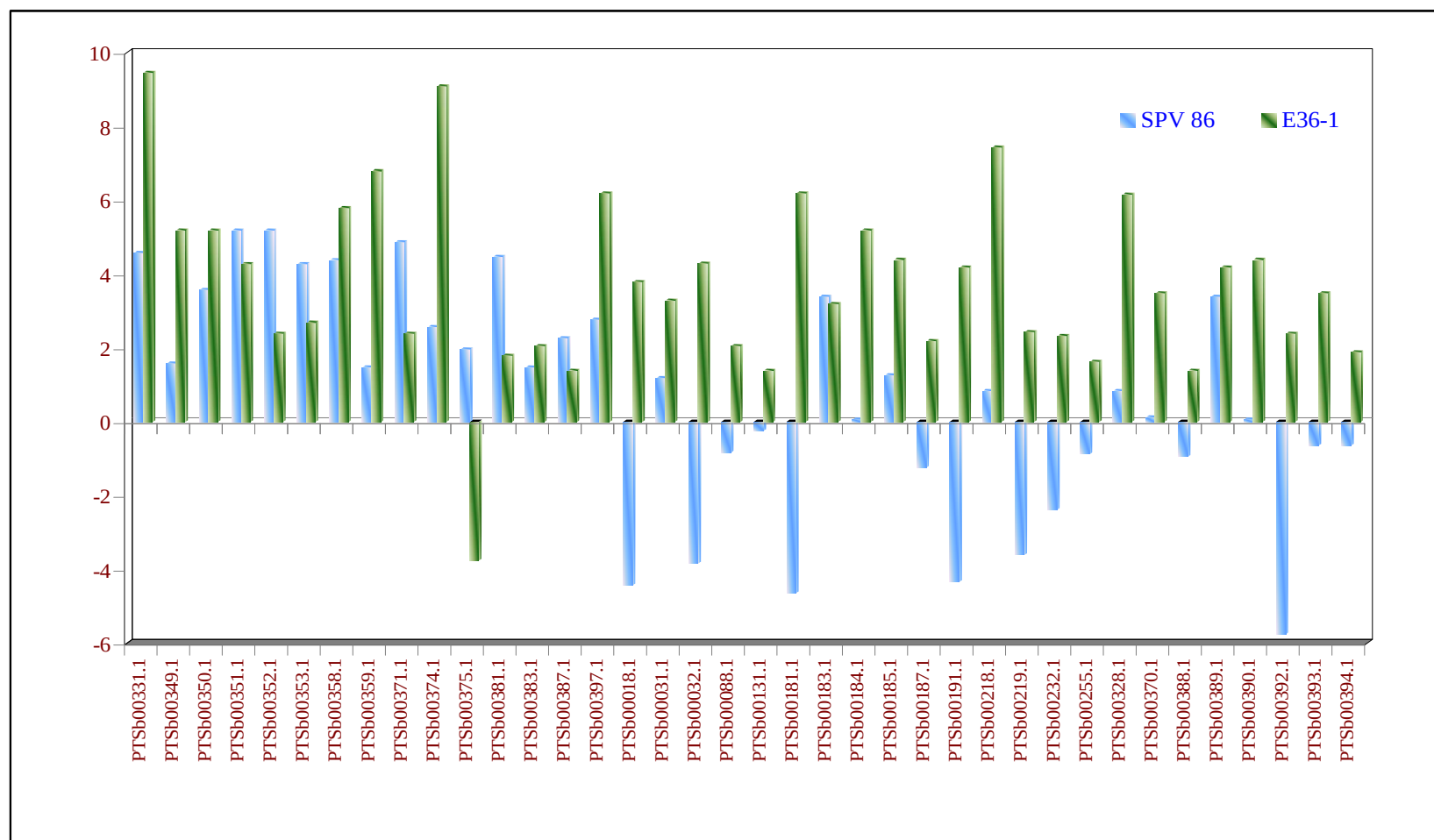


Table 28: Fold change in expression of members of transcription factor genes in SPV 86 (susceptible) and E 36-1 (resistant) genotypes at 90 days after sowing under charcoal rot challenge infestation as revealed by real time PCR quantitation

Sl. No.	Database ID	TF Family	Normalized Ct value						Fold change in expression	
			SPV 86			E 36-1			SPV 86	E 36-1
			Δ Ct (Control)	Δ Ct (Infected)	t test	Δ Ct (Control)	Δ Ct (Infected)	t test		
1.	PTSb00017.1	AP2	7.255	5.075	0.035566	7.73	5.445	0.0010	3.85	3.90
2.	PTSb00019.1	AP2	3.255	0.875	0.003041	7.83	0.54	0.2578	4.49	99.37
3.	PTSb00020.1	AP2	8.855	7.275	0.001599	9.53	5.545	0.0066	2.71	12.40
4.	PTSb00021.1	AP2	6.255	3.075	0.035566	2.73	0.245	0.0010	6.75	4.44
5.	PTSb00022.1	AP2	6.655	3.075	0.003041	5.73	1.545	0.0005	9.12	13.26
6.	PTSb00024.1	AP2	4.255	2.875	0.001599	7.73	2.545	0.0002	2.39	22.37
7.	PTSb00025.1	AP2	8.855	5.075	0.035566	-	-	-	12.02	-
8.	PTSb00027.1	AP2	5.255	1.675	0.003041	-	-	-	11.96	-
9.	PTSb00028.1	AP2	5.255	3.075	0.001599	-	-	-	3.83	-
10.	PTSb00033.1	ARF	5.355	1.875	0.035566	8.73	3.645	0.0013	10.94	32.97
11.	PTSb00034.1	ARF	5.155	0.075	0.003041	-	-	-	25.01	-
12.	PTSb00086.1	YABBY	8.65	7.075	0.001599	-	-	-	2.70	-
13.	PTSb00127.1	HAP2	11.04	8.775	0.035566	-	-	-	4.28	-
14.	PTSb00128.1	HAP2	9.24	5.075	0.003041	-	-	-	17.94	-
15.	PTSb00129.1	HAP2	11.04	5.075	0.001599	-	-	-	62.47	-
16.	PTSb00130.1	HAP5	8.14	6.375	0.035566	8.13	3.545	0.0031	3.42	25.17
17.	PTSb00134.1	HAP5	6.51	4.175	0.003041	1.27	-0.455	0.0001	4.37	2.97
18.	PTSb00172.1	HB	6.31	2.875	0.001599	6.27	4.545	0.003	7.53	2.69
19.	PTSb00179.1	HMG	7.34	1.875	0.035566	4.63	1.34	0.00124	42.98	9.59
20.	PTSb00180.1	HMG	6.14	3.875	0.003041	7.83	3.40	0.02366	4.81	20.89

Contd.....

Sl. No.	Database ID	TF Family	Normalized Ct value						Fold change in expression	
			SPV 86			E 36-1			SPV 86	E 36-1
			Δ Ct (Control)	Δ Ct (Infected)	t test	Δ Ct (Control)	Δ Ct (Infected)	t test		
21.	PTSb00182.1	HMG	11.04	5.275	0.001599	-	-	-	40.46	-
22.	PTSb00188.1	HMG	7.04	5.075	0.035566	9.73	4.545	0.004	3.53	27.23
23.	PTSb00192.1	HMG	4.94	3.075	0.003041	5.93	2.545	0.002	3.19	8.07
24.	PTSb00209.1	MADS	6.81	4.875	0.001599	-	-	-	3.82	-
25.	PTSb00210.1	MADS	7.11	4.775	0.035566	-	-	-	4.99	-
26.	PTSb00217.1	MADS	10.01	6.275	0.003041	-	-	-	10.99	-
27.	PTSb00221.1	MADS	8.21	6.075	0.001599	-	-	-	3.89	-
28.	PTSb00222.1	MADS	6.51	1.775	0.035566	-	-	-	26.63	-
29.	PTSb00249.1	MYB	11.41	4.675	0.003041	-	-	-	70.22	-
30.	PTSb00256.1	MYB	6.41	2.875	0.001599	-	-	-	9.31	-
31.	PTSb00259.1	NAC	8.65	2.075	0.035566	-	-	-	61.29	-
32.	PTSb00263.1	NAC	4.95	3.075	0.003041	-	-	-	3.05	-
33.	PTSb00272.1	NAC	11.85	6.175	0.001599	-	-	-	35.96	-
34.	PTSb00274.1	NAC	7.15	5.675	0.035566	-	-	-	2.56	-
35.	PTSb00292.1	PHD	7.15	2.875	0.003041	-	-	-	12.94	-
36.	PTSb00328.1	WRKY	9.05	2.875	0.001599	-	-	-	49.68	-
37.	PTSb00331.1	WRKY	9.65	5.075	0.035566	2.595	-6.455	0.0014	14.55	182.05
38.	PTSb00349.1	bHLH	2.255	0.675	0.003041	8.73	3.545	0.0003	2.71	26.39
39.	PTSb00350.1	bHLH	3.355	-0.225	0.001599	-	-	-	10.17	-
40.	PTSb00351.1	bHLH	8.355	3.175	0.035566	-	-	-	36.25	-
41.	PTSb00352.1	bHLH	6.255	1.075	0.003041	10.23	7.845	0.0057	36.26	5.22
42.	PTSb00353.1	bHLH	7.355	3.075	0.001599	0.23	-2.455	0.0005	15.60	5.60
43.	PTSb00358.1	bHLH	6.455	2.075	0.035566	-	-	-	20.37	-
44.	PTSb00359.1	bHLH	2.255	0.775	0.003041	7.03	0.245	0.0003	2.45	60.36

Contd.....

Sl. No.	Database ID	TF Family	Normalized Ct value						Fold change in expression	
			SPV 86			E 36-1			SPV 86	E 36-1
			Δ Ct (Control)	Δ Ct (Infected)	t test	Δ Ct (Control)	Δ Ct (Infected)	t test		
45.	PTSb00371.1	bHLH	9.04	4.175	0.001599	12.63	10.245	0.0049	17.45	4.06
46.	PTSb00374.1	bZIP	4.24	1.675	0.035566	7.03	-0.555	0.001	4.52	86.35
47.	PTSb00375.1	bZIP	8.14	6.175	0.003041	-	-	-	3.42	-
48.	PTSb00381.1	bZIP	10.84	6.375	0.001599	10.33	8.545	0.002	16.75	3.09
49.	PTSb00383.1	bZIP	9.84	8.375	0.035566	-	-	-	2.76	-
50.	PTSb00387.1	bZIP	3.14	0.875	0.003041	-	-	-	4.81	-
51.	PTSb00397.1	bZIP	11.04	8.275	0.001599	-	-	-	5.73	-
52.	PTSb00018.1	AP2	-	-	-	9.73	5.945	0.00036	-	10.63
53.	PTSb00031.1	ARF	-	-	-	7.73	4.445	0.00135	-	8.18
54.	PTSb00032.1	ARF	-	-	-	1.73	-2.555	0.0003	-	19.50
55.	PTSb00088.1	YABBY	-	-	-	5.595	3.545	0.00014	-	4.03
56.	PTSb00131.1	HAP5	-	-	-	3.73	2.345	0.40790	-	2.41
57.	PTSb00181.1	HMG	-	-	-	7.73	1.545	0.03418	-	72.76
58.	PTSb00183.1	HMG	7.93	4.545	-	5.73	2.545	0.002	-	7.47
59.	PTSb00184.1	HMG	-	-	-	8.73	3.545	0.0001	-	34.53
60.	PTSb00185.1	HMG	-	-	-	9.93	5.545	0.0004	-	12.53
61.	PTSb00187.1	HMG	-	-	-	5.73	3.545	0.0005	-	3.81
62.	PTSb00191.1	HMG	-	-	-	7.73	3.545	0.002	-	11.53
63.	PTSb00218.1	MADS	-	-	-	3.07	-2.055	0.0024	-	25.01
64.	PTSb00219.1	MADS	-	-	-	9.17	6.745	0.07740	-	4.10
65.	PTSb00232.1	MYB-related	-	-	-	0.27	-2.055	0.003	-	3.92
66.	PTSb00255.1	MYB	-	-	-	5.27	3.645	0.0002	-	3.09
67.	PTSb00328.1	WRKY	-	-	-	3.795	-2.355	0.01014	-	48.90
68.	PTSb00370.1	bHLH	-	-	-	1.33	-2.155	0.0049	-	11.08

Contd.....

Sl. No.	Database ID	TF Family	Normalized Ct value						Fold change in expression	
			SPV 86			E 36-1			SPV 86	E 36-1
			Δ Ct (Control)	Δ Ct (Infected)	t test	Δ Ct (Control)	Δ Ct (Infected)	t test		
69.	PTSb00388.1	bZIP	-	-	-	6.93	5.545	0.001	-	2.40
70.	PTSb00389.1	bZIP	-	-	-	9.33	5.145	0.02	-	13.73
71.	PTSb00390.1	bZIP	-	-	-	5.93	1.545	0.03	-	20.89
72.	PTSb00392.1	bZIP	-	-	-	5.93	3.545	0.002	-	5.20
73.	PTSb00393.1	bZIP	-	-	-	7.93	4.445	0.01032	-	12.40
74.	PTSb00394.1	bZIP	-	-	-	8.73	6.845	0.023	-	3.63

Δ Ct = (Ct

Table 29: Transcription factor genes differentially upregulated in resistant and susceptible genotypes under charcoal rot disease manifestation

Stage	Genotype	2-10		10-50		50-100		>100	
		Database ID	TF Family	Database ID	TF Family	Database ID	TF Family	Database	TF Family
75 DAS	E 36-1	PTSb00088.1	YABBY	PTSb00019.1	AP2	PTSb00328.1	WRKY		
		PTSb00023.1	AP2	PTSb00185.1	HMG	PTSb00331.1	WRKY		
		PTSb00021.1	AP2	PTSb00349.1	bHLH				
		PTSb00398.1	bZIP	PTSb00218.1	MADS				
		PTSb00136.1	HAP5	PTSb00232.1	MYB-related				
		PTSb00352.1	bHLH						
		PTSb00219.1	MADS						
		PTSb00022.1	AP2						
		PTSb00024.1	AP2						
		PTSb00033.1	ARF						
		PTSb00179.1	HMG						
		PTSb00359.1	bHLH						
		PTSb00374.2	bZIP						
		PTSb00180.1	HMG						
	SPV 86	PTSb00019.1	AP2						
		PTSb00022.1	AP2						
		PTSb00024.1	AP2						
		PTSb00033.1	ARF						
		PTSb00127.1	HAP2						
		PTSb00130.1	HAP5						
		PTSb00131.1	HAP5						
		PTSb00132.1	HAP5						
		PTSb00179.1	HMG						
		PTSb00182.1	HMG						
		PTSb00185.1	HMG						
		PTSb00211.1	MADS						
		PTSb00214.1	MADS						
		PTSb00220.1	MADS						
		PTSb00229.1	MYB-related						
		PTSb00234.1	MYB-related						
		PTSb00289.1	PHD						
		PTSb00331.1	WRKY						
		PTSb00349.1	bHLH						
		PTSb00374.1	bZIP						
90 DAS	E 36-1	PTSb00388.1	bZIP	PTSb00018.1	AP2	PTSb00371.1	bHLH	PTSb00331.1	WRKY
		PTSb00131.1	HAP5	PTSb00370.1	bHLH	PTSb00374.1	HMG		
		PTSb00172.1	HB	PTSb00191.1	HMG	PTSb00348.1	bZIP		
		PTSb00134.1	HAP5	PTSb00020.1	AP2	PTSb000019.1			
		PTSb00381.1	bZIP	PTSb00393.1	bZIP	1	AP2		
		PTSb00255.1	MYB	PTSb00185.1	HMG				
		PTSb00394.1	bZIP	PTSb00022.1	AP2				
		PTSb00187.1	HMG	PTSb00389.1	bZIP				

Contd.....

Stage	Genotype	2-10		10-50		50-100		>100	
		Database ID	TF Family	Database ID	TF Family	Database ID	TF Family	Database	TF Family
		PTSb00017.1	AP2	PTSb00032.1	ARF				
		PTSb00232.1	MYB-related	PTSb00180.1	HMG				
		PTSb00088.1	YABBY	PTSb00390.1	bZIP				
		PTSb00371.1	bHLH	PTSb00024.1	AP2				
		PTSb00219.1	MADS	PTSb00218.1	MADS				
		PTSb00021.1	AP2	PTSb00130.1	HAP5				
		PTSb00392.1	bZIP	PTSb00349.1	bHLH				
		PTSb00352.1	bHLH	PTSb00188.1	HMG				
		PTSb00353.1	bHLH	PTSb00033.1	ARF				
		PTSb00183.1	HMG	PTSb00184.1	HMG				
		PTSb00192.1	HMG	PTSb00328.1	WRKY				
		PTSb00031.1	ARF						
		PTSb00179.1	HMG						
	SPV 86	PTSb00024.1	AP2	PTSb00350.1	bHLH	PTSb00259.1	NAC		
		PTSb00359.1	bHLH	PTSb00033.1	ARF	PTSb00129.1	HAP2		
		PTSb00274.1	NAC	PTSb00217.1	MADS	PTSb00249.1	MYB		
		PTSb00086.1	YABBY	PTSb00027.1	AP2				
		PTSb00020.1	AP2	PTSb00025.1	AP2				
		PTSb00349.1	bHLH	PTSb00292.1	PHD				
		PTSb00383.1	bZIP	PTSb00331.1	WRKY				
		PTSb00263.1	NAC	PTSb00353.1	bHLH				
		PTSb00192.1	HMG	PTSb00381.1	bZIP				
		PTSb00130.1	HAP5	PTSb00371.1	bHLH				
		PTSb00375.1	bZIP	PTSb00128.1	HAP2				
		PTSb00188.1	HMG	PTSb00358.1	bHLH				
		PTSb00209.1	MADS	PTSb00034.1	ARF				
		PTSb00028.1	AP2	PTSb00222.1	MADS				
		PTSb00017.1	AP2	PTSb00272.1	NAC				
		PTSb00221.1	MADS	PTSb00351.1	bHLH				
		PTSb00127.1	HAP2	PTSb00352.1	bHLH				
		PTSb00134.1	HAP5	PTSb00182.1	HMG				
		PTSb00019.1	AP2	PTSb00179.1	HMG				
		PTSb00374.1	bZIP	PTSb00328.1	WRKY				
		PTSb00180.1	HMG						
		PTSb00387.1	bZIP						
		PTSb00210.1	MADS						
		PTSb00397.1	bZIP						
		PTSb00021.1	AP2						
		PTSb00172.1	HB						
		PTSb00022.1	AP2						
		PTSb00256.1	MYB						

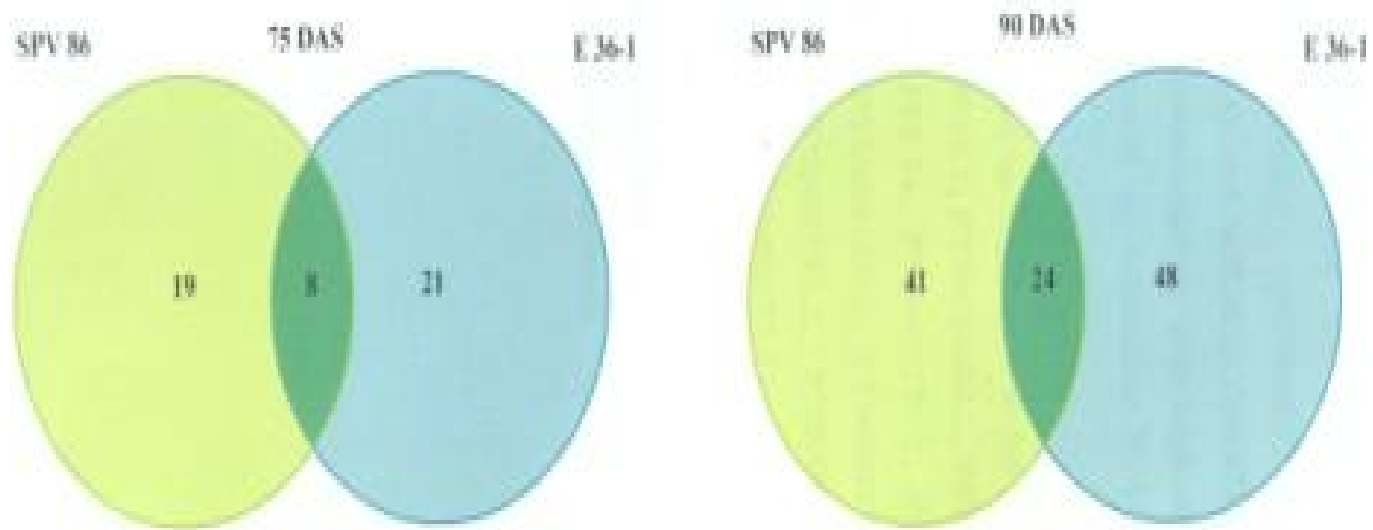


Fig. 20: Venn diagram showing the up regulated TF genes in both resistant (E 36-1) and susceptible (SPV 86) cultivar along with common set of upregulated genes

Fig.20. Venn diagram showing the up regulated TF genes in both resistant (E36-1) and susceptible (SPV86) cultivar along with common set of upregulated genes

PTSb00351.1, PTSb00371.1), HMG (PTSb00182.1, PTSb00179.1), MADS (PTSb00217.1, PTSb00222.1), MADS (PTSb00027.1, PTSb00025.1), WRKY (PTSb00331.1, PTSb00328.1), ARF (PTSb00033.1), PHD (PTSb00292.1), bZIP (PTSb00381.1), HAP2 (PTSb00128.1, NAC (PTSb00272.1) and ARF (PTSb00034.1), while for only three genes PTSb00259.1 (NAC), PTSb00129.1 (HAP2) and PTSb00249.1 (NAC) fold expression more than 50 was observed (Table 28, Fig 18 and 19).

Result indicated that the expression of selected transcription factors were almost same in control and infected samples of susceptible cultivar (SPV 86) at both the time point (75 and 90 DAS). However, expression of some transcription factor especially AP2 related were up regulated during 90 DAS in both control and infected samples of SPV 86 and E36-1 (Table 29). In resistant cultivar most of transcription factor were highly expressed compared to control at both the time point (75 and 90 DAS). There was very high expression for WRKY, MYB and BZIP related transcription factor at both time points however during 90 DAS there was comparatively low expression of all the genes that were up regulated at 75 DAS with few genes showing high gene expression only during 90 DAS.

Eight TF genes representing five TF family viz., AP2 (PTSb00019.1, PTSb00022.1, PTSb00024.1, WRKY (PTSb00331.1), HMG (PTSb00179.1, PTSb00185.1), ARF (PTSb00033.1) and bHLH (PTSb00349.1), were found commonly expressing in susceptible and resistant cultivar with high expression in resistant cultivar at 75 DAS, while 24 genes showed up regulation in both resistant and susceptible cultivar at 90 DAS (Fig. 20).

Common genes that showed up regulation at 90 DAS belonged to 9 TF family viz., AP2 (PTSb00017.1, PTSb00019.1, PTSb00020.1, PTSb00021.1, PTSb00022.1 and PTSb00024.1), HMG (PTSb00188.1, PTSb00179.1, PTSb00180.1, PTSb00192.1), ARF (PTSb00033.1), HAP5 (PTSb00129.1, PTSb00130.1, PTSb00134.1), bHLH (PTSb00349.1, PTSb00352.1, PTSb00353.1, PTSb00359.1, PTSb00371.1), bZIP (PTSb00374.1, PTSb00375.1), HB (PTSb00172.1)

Two transcription factors belonging to WRKY (PTSb00328.1 and PTSb00331.1) were conspicuously and highly up regulated at both time points in resistant cultivar compared to susceptible ones.

5. DISCUSSION

Charcoal rot is a soil borne disease caused by *Macrophomina phaseolina*. This disease is influenced by drought stress in terminal stages of the crop and causes furthermore yield losses including fodder quality. Drought tolerance in sorghum has been known to be positively associated charcoal rot tolerance both at phenotypic and genotypic level. Both charcoal rot resistance and drought tolerance in sorghum are polygenic in nature, involving a number of component traits with significant G x E interactions. Further, in sorghum, more than one drought tolerance mechanisms of field tolerance to drought has been reported where the pre-flowering drought tolerance is distinctly being different from that of post flowering drought tolerance, involving altogether different component traits. Genotypes that are pre-flowering drought tolerance were necessarily did not show any terminal or post flowering drought tolerance. The involvement of many genes in controlling complex traits each with very little individual effect is highly influenced by environment. Phenotypic selection for such traits will be generally difficult. Selection based on markers could theoretically facilitate the manipulation of such trait without affecting other important agronomic traits besides evaluating breeding progeny populations for the presence of gene/genes conditioning drought, charcoal rot tolerance or any other trait in sorghum.

Molecular markers have opened up an opportunity to dissect the complex traits and handle them much sophisticatedly than ever possible before. Despite concerted efforts over the last two decades of utilizing the existing cultivated source of resistance, the level of resistance achieved so is a limited for both drought and charcoal rot in sorghum. Recently Burrow *et al.* (2009) reported physiological and genetic study of the profuse wax trait of sorghum using a mutant population and analysed the molecular basis of profuse wax and cuticular features of sorghum through map location of *BLOOM-CUTICLE* (*BLMC*) by genetic mapping and identified candidate genes for the locus. They also characterized the genetic effects of mutation in *BLMC* on cuticular quantity, phenotypic response in the wild and biochemical composition of waxes using selected F3 families. The results from this study will pave the way for cloning sorghum genes which control profuse amount of cuticular wax and enhanced cuticular traits.

Marker assisted selection (MAS) under such situations can increase the efficiency of breeding efforts and the understanding of the complex nature of different traits (Knapp, 1998; Bouchez *et al.*, 2002).

MAS has the potential to greatly improve the efficiency of selection of quantitative traits. Since, the components of charcoal rot resistance are mostly quantitatively measured, it is important to analyse quantitative trait loci (QTL) from the view point of deeper understanding of the genetics and facilitating the breeding efforts. Molecular mapping and identification of genomic regions associated with charcoal rot resistance and yield would serve as basis to initiate marker assisted transfer of such genome regions into elite sorghum cultivars. The use of DNA based markers for genetic analysis and manipulation of important trait has become increasingly useful tool in plant breeding (Lande, 1990). However, their greatest potential appears to be in accelerating rate of gain from selection for desirable genotypes and in manipulation of QTL that condition complex economic trait.

The ultimate goal of QTL analysis is to develop tools that are useful for marker assisted selection in practical plant breeding programme aiming higher level of resistance through QTL introgression in agronomically elite genetic background. Case in focus here is the charcoal rot resistance in sorghum.

Plants are continually exposed to a vast array of pathogens, and are generally succeed in defending themselves. An important part of this process is the induction of defence gene products that hinder pathogen growth and delay or prevent disease development there on. The way in which plants do this is multi-factorial, and includes regulatory proteins known as transcription factors (TFs) which directly regulate the expression of defence-related genes. The expression and activity of these TFs can be differentially regulated by pathogen challenge. The regulation of plant defense responses is complex, with a number of TF families playing regulatory role. There is a considerable interest in identifying

and utilizing key transcription factor genes in plant defense for engineering increased resistance to plant pathogens in agriculture. The proteins encoded by the TF gene families have diverse functions throughout the plant life cycle, including regulation of development, responses to abiotic stresses such as drought and cold, as well as to biotic stresses such as fungal, bacterial and other plant pathogen infections (Rojo, 2003; Ulker and Somssich, 2004; Desveaux *et al.*, 2004; Gurr and Rushton, 2005).

In the present investigation, the RILs derived from the cross IS 22380 (susceptible) and E 36-1 (resistant) were characterized for the components of charcoal rot disease and related parameters. The parental polymorphism for both phenotypic traits and gene based markers was done first and later the genotypic data for individual RILs was generated to construct a genetic linkage map for this population. A series of phenotypic evaluation of RIP along with parents at Dharwad and Bijapur and Solapur over four successive years was done. The genetic architecture of charcoal rot disease resistance through QTL mapping was carried out and finally pinning-down the stable QTLs for marker assisted introgression of such QTLs for stalk rot resistance in sorghum. Further, in order to identify TFs potentially involved in plant defence, in particular the charcoal rot resistance in sorghum, the expression of 18 TF families comprising 185 genes (members) were selected from sorghum transcription database and were assayed for their levels of expression through a quantitative real time PCR method.

5.1 Genetic variability and character association

Understanding the genetic nature of charcoal rot resistance in sorghum, the phenotypic evaluation of mapping population derived from IS 22380 x E 36-1 cross for component traits of charcoal rot resistance and yield was carried out. The valuation was done in a sick plot situation creating required conditions for a better manifestation of the disease in terms of pathogen inoculums application. The field evaluations were carried out at Dharwad, Bijapur and solapur locations over four 2004, 2005, 2006, and 2007 *rabi* seasons. Genetic expectations of means and variance were obtained by pooled analysis within each location over the year of phenotypic data. RILs displayed considerable differences in their mean performance with respect to all the 15 traits investigated. Genetic variability among these RILs would be obvious, as this population has been developed from deliberately selected, phenotypically distinct parents for charcoal rot disease resistance including a number of other characters. Wide range of variation among line indicated the utility of RILs for genetic investigations. The pooled analysis has shown that the phenotypic coefficient of Variation (PCV) was higher than Genotypic Coefficient of Variation (GCV) for all the characters under study, which suggested that these traits had high environmental influence.

High values of PCV and GCV were evident for early seedling vigour, stem thickness, threshability, seed yield per plant, days to 50 per cent flowering and disease parameters, *viz.*, length of infection and per cent lodging, indicating that variation for these characters contributed markedly to the total variability. These results are in accordance with results obtained by Kumar and Singh (1986); Nimbalkar *et al.* (1988); Bapat *et al.* (1984); Berenje (1990); Khannur (1993); Birdar *et al.* (1996); Rao and Patil (1996); Can and Yoshida, (1999). Further, the findings are in accordance with the results of Punnuri (2004), Rajkumar *et al.* (2007), except for stem thickness, panicle length and plant height in the same population during previous years. The observations with respect to stem thickness, panicle length and plant height across four years and three locations indicated the greater influence of environment in this population.

PCV and GCV were moderate for panicle length, stem thickness and plant height. These results are in conformity with the reports of Liong. (1965); Kukadia *et al.*, (1983); Khannur (1993); Raut *et al.* (1999); Asthana *et al.* (1995); Reddy *et al.* (1996); Punnuri. (2004); Reddy (2006); Rajkumar *et al.* (2007). Though PCV was higher than the GCV for lignin at 75 DAS, lignin at 95 DAS, total phenol at 75 DAS, and total phenol at 95 DAS at both the location (Bijapur and Dharwad), however, the values were only moderately high for all these four biochemical trait. No similar reports were available for this RIL population or elsewhere in sorghum.

The range of variability was comparatively low for days to 50 per cent flowering and similar observation were also made by Lonc. (1969); Raja and Parikh. (1980); Desai *et al.*

(1980); Patel *et al.* (1980); Nimbalkar *et al.* (1988); Biradar *et al.* (1996) in sorghum germplasms and Punhuri (2004); Reddy (2006) and Rajkumar *et al.* (2007) in the same RIP. High variability is necessary for improvement through selection. Only one published report, with reference to this RIL population is available (Rajkumar *et al.*, 2007). However, one of the parents, E36-1 has been used both in breeding and mapping efforts for charcoal rot resistance, striga resistance and stay green (Hausmann, 2004) and drought tolerance studies both in India and abroad where as the behavior of IS 22380 is not known.

The co-efficient of variation reveals only the extent of variability present for different characters in the population. The effectiveness of selection for a trait depends on the relative level of genetic and non-genetic variation. Heritability is a useful quantitative parameter, which considers the role of heredity and environment determining the expression of a character (Allard, 1960). Effective selection can be achieved only when additive effects are substantial and environmental effects are small.

In the present study, high estimates of broad sense heritability were noticed for character *viz.*, stem thickness, panicle length, 1000 seed weight, plant height, seed yield per plant and two disease parameter namely, length of infection and per cent lodging. Similar reports were reported (Spikakov 1989; Rao and Patil 1996; Reddy *et al.*, 1996; Nguyen *et al.*, 1997; Punhuri, 2004; Reddy, 2006; Rajkumar *et al.*, 2007) have also made similar observations in sorghum. There was also high heritability for lignin at 75 DAS, lignin at 95 DAS, total phenol at 75 DAS and total phenol at 95 DAS. The high heritability observed for these traits indicates that it is less subjected to environmental influences. Moderate heritability estimates were noticed for early seedling vigour, days to 50 per cent flowering, ear head length, and number of internodes crossed by *M. phaseolina*. These observations were also in agreement with those studies reported by Giriraj and Gowda (1982) and Halli *et al.* (1983) in sorghum. In the same RIP of sorghum similar observations were made by Punhuri (2004); Reddy 2006 and Rajkumar *et al.*, (2007)

Presence of variability in the population is a prerequisite for selection to be effective, while heritability indicate how well this can be done. Expected genetic advance is a parameter that considers both variability and heritability and provides a realistic estimate of gain achievable following selection. In the present study, high heritability coupled with high genetic advance over per cent mean was observed for disease parameters *viz.*, length of infection and per cent lodging. Selection made through these characters would be effective as they are more likely to be controlled by additive gene effects. This kind of trend for the above traits is already known in sorghum (Bhat, 1975; Reddy *et al.* (1996); Punhuri, (2004); Reddy *et al.*, 2006).

Charcoal rot disease resistance is a complex character and its expression depends on interplay of several component characters. A correlation study can provide the role of component characters to the target phenotype. In the present investigation, both genotypic and phenotypic correlations were worked out for disease parameters and yield attributing traits, to assess the extent and nature of association between component traits of disease resistance and yield.

Among the charcoal rot parameters, a highly significant and positive association was found between per cent lodging and plant height, number of internodes crossed and length of infection, seed yield with 1000 seed weight and early seedling vigour with plant height at both phenotypic and genotypic level. On the contrary Lodging percent with seed yield and 1000 seed weight and panicle length with stem thickness, had negative association at genotypic and phenotypic level. Similar observations were made Esechia *et al.* (1977) and Rajkumar *et al.* (2007) in sorghum germplasm and this RIP, respectively.

There was significant positive correlation of lignin at 75 DAS with lignin at 95 DAS, total phenol at 75 DAS and total phenol at 95 DAS at both genotypic and phenotypic level. There was also significant and positive correlation at both genotypic and phenotypic level for total phenol at 75 DAS with total phenol at 95 DAS. On the contrary there was significant negative correlation with panicle length and total phenol at 75 DAS at genotypic and phenotypic level. The production of plant phenolic compounds which are thought to be part of the chemical defense of the plant is under the control of both genetic and environmental

factors. For instance an increase in the activities of phenolic related enzymes and the accumulation of phenolic compounds has been correlated with resistance of cereals to biotic stresses (Mohammadi and Kazemi, 2002). Leaves of sorghum resistant to fungi contained a higher content of total phenolics than leaves of susceptible ones upon pathogen challenge (Luthra *et al.*, 1988) insect damage (Woodhead and Bernays, 1978; Guinn and Eindenbock, 1982) will generally increase the synthesis of these compounds by the plants. These results have usually been obtained by determining the concentrations of total phenols or of different classes of compounds (e.g., Lignin, tannins and phenolic acids) in plant tissues.

Lignin provide a natural barrier to microorganisms (Bird, 1988). Production of lignin has been demonstrated to be particularly induced in plants in response to mechanical damage or infection by pathogens in numerous species including cucumber (*Cucumis sativus* L.), carrot [*Dacus carota* subsp. *sativus* (Hoffm.) Arcang], narcissus (*Narcissus peoticus* L.), radish (*Raphanus sativus* L.), melon (*cumis melo* L.), hop (*Humulus lupulus* L.), lignin plays a vital role in plant growth and development by improving water conduction through xylem tracheary elements, enhancing the strength of fibrous tissues, and limiting the spread of pathogens in and cell, plant tissues (Iiyama *et al.*, 1994). In general, crop yields are depressed by significant reductions in lignin content. Other negative effects observed in plants with lowered lignin contents include lodging and reduction of long-term survival of some perennial species. However, the interactions of genes involved in lignin metabolism with genetic background and the environment in which the low lignin crop is cultivated are substantial (Pedersen *et al.*, 2003).

Overall highly significant and positive association between plant height and per cent lodging with infection at both phenotypic and genotypic level was expected as the shorter plants are always resistant to lodging, irrespective of the disease. Occurrence of the disease at grain maturity in tall and productive plants will certainly promote lodging. A combination of medium height, thick stem, large ear head and resistance to charcoal rot disease are desirable in productive genotypes. Similar results were reported by Rajkumar *et al.* (2007). The RILs in the present study did not reveal such combination.

Characters like disease and insect resistance are quantitatively inherited in most of the systems. They are controlled by a number of factors and are dependent on many traits. The influence of these characters on each other can be known through the correlation studies. Genetic correlation between various plant characters arises because of linkage, pleiotropy due to developmental and functional relationships. Correlation studies are important, as biotechnological findings have to prove their merit at field level, where as biometrical genetics assisted analysis can only prove their worth.

5.2 Molecular mapping and QTL analysis

The development of molecular markers has resulted in renewed interest in genetic mapping. Genetic map construction efforts are greatly helped by availability of good permanent mapping populations such as RILs, a necessity for enriching the genetic map as more markers become available. Such mapping populations could be used by many researchers simultaneously, so that the mapping process is cumulative, towards the development of saturated molecular genetic map for any species. Similarly, RILs can be repeatedly phenotyped across environments, a facility extremely important in studying the quantitative traits.

A variety of DNA level markers such as RAPD, AFLP, SSR, EST-SSR, SNPs etc., have been used as genetic markers for mapping. EST-SSR, unlike a genomic marker, shows the position of functional part of the genome in relation to the others. Such markers are useful in providing functional association to the linked marker. The codominant and highly polymorphic nature of SSRs has increasingly made them the marker of choice for population genetics analyses and trait dissection. Unfortunately, the development of traditional 'anonymous' SSRs requires a substantial investment of both time and money, putting them out of reach for many researchers. Given that EST-based SSRs can be developed directly from existing sequence resources and can often be transferred from one species to another, EST databases are an attractive source of markers for the genetic analysis to understand the complex nature of the trait (Ellis and Burke, 2007; Chabane *et al.*, 2005).

Parental polymorphism

A total of 530 EST-SSR markers used to identify the parental polymorphism of which 473 (89.2 per cent) markers showed amplification. Out of 473 markers, 63 (13.3 per cent) of markers detected scorable polymorphism between the parents, E36-1 and IS 22380. Similar percent of polymorphism in N13 x E36-1 was reported by Sundaresh (2006). The EST-SSR primers have been reported to be less polymorphic compared with genomic SSRs in crop plants because of greater DNA sequence conservation in transcribed region (Cho *et al.*, 2000, Eujayl *et al.*, 2001 Rungis *et al.*, 2004 and Russell *et al.*, 2004). In an earlier study by Reddy (2006), 85 markers were used to construct the linkage map, we have integrated 63 new EST-SSR marker with 85 marker with total of 148 markers were used to construct a linkage map and QTL analysis in the present study. Molecular genetic markers represent one of the most powerful tools for the analysis of genomes and the association of heritable traits with underlying genetic variation. We could integrate gene based markers to the sorghum genetic map, potential EST-SSR and EST-SNP markers developed can be targets in molecular breeding programs for genetic improvement. This will also provide opportunities to understand the genetic mechanisms underlying different stress responses (Jaques and Edawrds, 2009)

Construction of linkage map

Rapid advances in molecular biology and in associated fronts of science 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 in these RILs by Reddy (2006). The length of the map was 657 cM with 78 markers on to the map with large gaps between the markers. So in order to saturate the map we have used the EST-SSR marker to genotype across the RIL population. Through in house effort several genic SSRs were identified in EST database of sorghum, a subset (530) of these were also used in the present study. A linkage distance of 20cM was considered as limit of resolution. The reason to select markers with linkage distance of about 20 cM as an interval was that a QTL in such a segment might contain many loci affecting the trait not necessarily in the same direction. Thus, more QTLs are likely to be detected when the alleles are in association than when they are in dispersion (Tanskley, 1993).

Linkage maps are available in sorghum for striga tolerance (Hausmann *et al.*, 2004) and stay green (Bhatramakki *et al.*, 2000) targeted populations. It has been reported that the QTLs for stay green, the trait that makes plant to remaining green for extended period and impart terminal drought tolerance and charcoal rot resistance 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 for charcoal rot resistance as well. Linkage map for population specifically developed for studying inheritance of charcoal rot incidence was available with 78 markers on all the linkage groups viz., LG-A, LG-B, LG-C, LG-D, LG-E, LG-F, LG-G, LG-H, LG-I and LG-J earlier developed by Reddy *et al.* (2008).

A total of 148 markers were used for linkage map construction with the help of MAPMAKER / EXP (3.0b). Out of 148 markers, 140 could be mapped on to linkage groups which we named as LG-A, LG-B, C, D, E, F, G, H, I and LG J (spanned a length of 2905 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); Hausmann *et al.* (2004); Reddy *et al.* (2008) spanned a distance of 1406 k, 1580 R, 599 H and 630 cM, respectively. Non-association of eight markers may be because of more linkage distance and additional anchor marker required to map them.

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 *txp302* and *txp350* was 7.9 cM on linkage group A (Bhattaramakki *et al.*, 2000). In this population the map distance between the same markers was 7.3 cM. The map distance between the marker, *txp63*, *txp211*, *txp8*, and SvPEPCAA on linkage groups B in case Bhattaramakki *et al.* (2000) was also far apart, (> 180 cM), while in the present study they are located on same linkage group very close to each other at 72.2 cM apart. Similarly, distance between *txp201* and *txp348* on linkage group B was 70 cM in map of Haussmann *et al.* (2004), but in the present study they were much closer to each other 9.5 cM on linkage group B.

The markers *txp343* and *txp12* located in D linkage group was more (4.4 cM) than that observed by Bhattaramakki *et al.* (2000). The markers *txp67*, *txp230* and *txp258* 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 Bhattaramakki *et al.* (2000). The markers *txp295*, SbAGEo3, *txp204*, *Corn73* and *txp92* were mapped on to linkage group E over a total length of 53.6 cM and while *txp325*, *txp324* and *txp338* 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 Bhattaramakki *et al.* (2000), Taramino *et al.* (1997) and Haussmann *et al.* (2004).

The minimum and maximum distance between two individual markers were 0.5 cM (SvPEPCAA and G16 on linkage group B) and 90 cM (*xiabt47* and *xiabt51* on linkage group C), respectively. Majority of the markers were sparsely mapped indicating that the QTLs detected need some more tightly linked marker. It was not possible to establish relationship for four RAPD (AB4, AT9, G17, M17 and H3) and three SSR markers (*txp290* and *txp354*). Similar observations have been made by Mahalaxmi *et al.* (2002) and Paterson *et al.* (2003) in comparative mapping efforts of sorghum for SSR markers. This may be because of small population (93 RILs) and limited genome coverage by polymorphic markers used. Hence, it is possible that as and when more markers added on to linkage maps, unlinked markers may get positioned.

5.2.1 QTL mapping

Genetic linkage maps are fundamental for localisation of genes or more generally genomic regions conferring resistance and/or tolerance to biotic and abiotic stresses. Development of highly saturated linkage map is required to accelerate the mapping of both agronomically important major genes and QTL in any crop species, and there efficient use in marker assisted breeding.

Analysis of co-segregation pattern of the markers loci with that of phenotypic information is important for detecting the genomic regions of important character (QTL). Assessing co-segregation of the markers (based on recombination frequency of marker loci) and phenotype gives the information on the location of a QTL between these flanking markers. If the proportion of recombinants with higher or and lower phenotypic values is less, tight linkage of between flanking markers and QTL is indicated.

Mapping QTLs for agronomically important character is to identify genomic regions with significant effects, positive or negative, in the progeny of a given cross. This can be extended to other crosses and to populations, and the allelic status of QTLs along with linked marker status can be found for a given set of genotypes and also populations.

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 genomic 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.

Linkage map constructed in the present study for the 93 individuals of the RIL population of cross IS 22380 x E 36-1 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.

A common feature of QTL analysis is that QTL effects depend on environment. The QTLs detected in one environment but not in other environments are considered as location specific QTLs. Identification of QTLs that pop-up in most of the environments would be important for practical breeding applications. Hence, stability of the QTLs is important for MAS. In the present investigation, the QTLs commonly detected at all the three locations viz, Dharwad, Bijapur and Solapur locations for all the four years (2004, 2005, 2006 and 2007) were considered as stable.

Charcoal rot resistance parameters

Two QTLs were found stable for the component trait –number of internodes crossed by the fungus, stably expressing at all the locations and over years. The QTLs were found on same linkage group B. The QTL on linkage group B positioned at 324.0-340.0cM was flanked by the marker *txp* 297 and *xiabt* 273 with phenotypic variance of 5.9-19.29, this QTL was found co localized with the QTL for stem thickness. The other QTL on linkage group B was flanked by genic SSRs (*xiabt* 92 - *xiabt* 62), with phenotypic variance of 8.8 -18.9 and additive effect of 3.5-8.8 .The additive effect indicated that the allele contributing for these QTL were contributed from E 36-1. Similar results were reported by Reddy *et al.* (2008) with change in marker types. In our study the QTL on Linkage group B was flanked by one EST-SSR which was absent in the report published by Reddy *et al.* (2008). The functional annotation (BLASTx) of this EST SSR revealed that there was no predicted function available for the DNA sequences harbouring those markers, as of now.

Three QTLs were detected for length of infection which were stably expressing at all the locations over the seasons. Three QTL were located on linkage group A (one QTL) and B (two QTLs).Two QTLs on linkage group B were flanked with the marker *xiabt* 275 - *xiabt*241 and *txp* 201 - *xiabt* 378 with phenotypic variance of 8.0 - 16.1 and 9.8 - 17.7, respectively with peak LOD value of 2.1-3.5 and 2.1-3.9. The QTL on linkage group A was flanked with the marker AC13 - *xiabt*224 with phenotypic variance of 8.9-17.9. For all the three QTLs the allele for additive effect was contributed from E36-1. The similar report of QTL on linkage group A was reported by Rajkumar (2004) and Reddy *et al.* (2008). Earlier report did not use EST-SSR for mapping which was used in the present study for increasing the mapping density and to find the probable candidate genes for disease resistance. The QTL on linkage group A was flanked with EST-SSR which showed the similarity with binding/catalytic/coenzyme binding protein. The QTL on linkage group B flanked by the marker EST-SSR (*xiabt*241) showed the similarity with Cytochrome P 450 while the QTL flanked by the marker *xiabt* 378 showed similarity with MYB protein. This protein is known to be involved in disease resistance mechanism.

Four QTLs were stably expressed for lodging per cent across all seasons and location. These were located on three different linkage group (D, I and J). For the QTL on linkage group J (*txp* 338 - *xiabt*420) the additive effect (-1.1-7.62) was contributed from the IS 22380 allele while for all the other three QTLs on linkage group D and LG-I the allele was contributed from E36-1. Similar result were reported in the same RILs population on linkage group D by Reddy *et al.* (2008) but not on the linkage group I and J which were additionally detected in this study. The QTL on linkage group J flanked by EST–SSR was showing putative function with phytosulfokines 5 precursor protein

There is a common genomic region (LG-I) flanked by marker *txp*274 – *xiabt*29, associated with QTL for lodging per cent, phenol content at 75 DAS and lignin content at 95

DAS on linkage group H two QTLs governing total phenol at 75 DAS and 95 DAS share same genomic region. It was proved genetically that the phenol content has role to play in the disease resistance as the QTL for charcoal rot resistance trait (lodging per cent) and phenol content were co-localised to the same genomic loci. These results were corroborated with the result of phenol content in resistant and susceptible genotypes in which there was a significantly higher phenol content at 75 and 95 DAS in the resistant cultivar than in susceptible cultivar. Hence, these stable QTLs would for further used in breeding efforts.

For early seedling vigour, two QTLs were common in all the locations and seasons which were located on linkage group B and D. Similar result was reported by Rajkumar (2004) and Reddy *et al.* (2008). The allele contributing to additive effect was from IS22380 parent for both the QTLs. On linkage group B, the QTL was positioned between flanking marker *txp297* and *xiabt 73* with phenotypic variance of 5.8 - 13.3 and Peak lod value of 2.3- 3.8. The QTL on linkage group D was bracketed by geneic SSRs (*xiabt9* - *xiabt77*) with 10.8 - 16.11 phenotypic variance and LOD value of 2.1-4.9. Though the QTLs were flanked by the EST-SSR marker for two QTL region putative function was not available for these EST-SSR as of today

Two QTL were found stable for plant height in all the locations and season. The QTL on linkage group C was flanked by marker *Corn 24* and *txp 210* with a phenotypic variance of 9.8-15.6 and peak LOD score of 2.3-4.8. The allele contributing to additive effect came from IS 22380 parent. The QTL on linkage group G was flanked by genic SSRs (*xiabt 81* and *xiabt 9*) with phenotypic variance of 7.29-18.9 with additive effect of 1.8 - 5.8. No such stable QTL were reported earlier in this population however they were reported in N13 x E36-1 population by Sundresh (2006) and Gebre (2009)

Two QTLs were detected for days to flowering, one each on linkage group B and F consistently for all the season. The allele contributing for early flowering was contributed from IS 22380. The QTL on linkage group B was flanked by AC12 and *xiabt 80* with additive effect of 1.4-7.0 and phenotypic variance of 5.8 - 15.9 while the QTL on linkage group F (*xiabt 78-xiabt 83*) showed the peak LOD score of 2.1 to 3.8 with phenotypic variance of 6.89 - 15.8. The QTL on F linkage group flanked by EST-SSR showed similarity with ring H2 finger protein at position 72cM while at position 104 cM the EST-SSR showed similarity with eukaryotic translation initiation factor 3 sub protein. For stem thickness two stable QTLs were in all the season, one QTL on linkage group B (*txp 297* and *xiabt 73*) with phenotypic variance of 9.54-20.4 showed co localization with QTLs for number of internodes crossed by the fungus. The QTL on linkage group F was flanked by the marker AK 10 and BH 13 with phenotypic variance of 8.9-16.5 with wider range of additive effect (1.61-7.1). The allele contributing for stem thickness was contributed from E 36-1 similar result were obtained by Rajkumar (2004) and Reddy *et al.* (2008).

For panicle length one QTL showed stable expression and which was positioned on linkage group I. The QTL on linkage group I was bracketed by marker *txp 176* and *txtp 312* with phenotypic variance of 7.9-18.1 and peak LOD value of 2.0-4.4. The additive effect of - 1.8 -5.6 was recorded which indicates the allele contributed from E 36-1 for this trait. Three QTLs for 1000 seed weight were stable in there expression at all the locations over the years. Two QTLs were located on linkage group B while one QTL was located on linkage group E (*xiabt 27-xiabt49*). For all the QTLs the additive effect indicated that the allele for this trait was contributed from E 36-1. One of the QTL on linkage group E was flanked with EST-SSR marker showing similarity with AAL2 like protein

Three QTLs were stably expressed for seed yield per plant at all the three locations over four seasons. The QTLs were located on linkage group C (two QTLs) and G (one QTL). Two QTLs on linkage group C were flanked by marker *Corn24-txp201* and *txp160-xiabt 85* with phenotypic variance of 4.4-17.8 and 9.6-17.9, respectively with peak LOD score of 2.1-3.2 and 2.3-2.9. The additive effect for all the three QTLs indicated that the allele from E36-1 was mostly contributing for seed yield. The QTL on linkage group C at 32 cM position was flanked with EST-SSR marker which showed similarity with glucan endo-1,3-beta-glucosidase 7 precursor protein.

For most of the traits the allele from E36-1 was contributing favourably except for lodging per cent where the one QTLs on linkage group J were contributed from susceptible parent while two QTLs on linkage group D and I was contributed from resistant parent (E36-1). Similar results were reported by Reddy *et al.* (2008).

5.3 Marker assisted introgression of stable QTLs

The use of DNA based marker for genetic analysis and manipulating important trait has become increasingly useful tool in plant breeding. However, their greatest potential appears to be in accelerating rate of gain from selection for desirable genotypes and in manipulation of QTL that condition complex economic trait. MAS has the potential to greatly improve the efficiency of selection of quantitative traits. Since, the components of charcoal rot resistance mostly quantitatively measured, it is important to analyse QTL from the point of view of genetics and breeding.

The experiment included: (i) detection of stable QTL for charcoal rot resistance trait in a cross between two elite lines, (ii) Initiation of marker-assisted introgression of the favourable alleles for three QTLs from E36-1 parent into the genetic background of recurrent parent M35-1 and SPV 86. Three QTLs, with major effect, confirmed stably across multiple environments were chosen for marker-assisted selection in this study (see section 4.3). Targeted QTLs, were one each for lodging percent (*Cr1*), number of internodes crossed by the fungus (*Cr2*) and length of infection (*Cr3*). These three QTLs collectively accumulated for 43 % of phenotypic variation. Selection of backcross progeny/progenies with the target QTL through the tightly-linked flanking markers minimized linkage drag. Selecting backcross progenies (that have already been selected for the target trait) with 'background' markers is important. In other words, markers can be used to select against the donor genome, which may accelerate the recovery of the recurrent parent genome. In principal these aspects were followed in the present case of transfer of charcoal rot resistance QTLs to recurrent parents. Marker-assisted introgression of desirable alleles may have several advantages over introgression using phenotypic information only. For the allele to be introgressed, marked chromosome segments ensure that the correct segment of donor genome is incorporated in to the recipient line. For alleles acting in a non-additive manner, especially those involved in inter-locus (epistatic) interactions, using markers may be the only way to ensure a successful trait introgression programme (Groen and Smith, 1995 and Basten *et al.*, 1997).

Charcoal rot susceptible SPV 86 and widely cultivated M35-1 cultivars were used in order to improve their charcoal rot resistance. Both cultivars are known for their agronomical eliteness and good grain quality. At the time of initial backcrossing stage foreground and limited background selection of the marker for all the three QTL regions was done. Backcrossing of SPV 86 x E36-1 and M35-1 x E36-1 derived progenies, it was ensured using the SSR and EST-SSR markers flanking the target QTL regions, the allele of donor parent in both the crosses are retained in heterozygous state. Similar, approaches have been made previously for QTL transferring for stay green trait from donor B35 to recurrent parents, Isiap Dorado (Central America origin) and R16 (India origin) by Punna Ramu (2005). Single copy or low copy markers with defined map locations, such as genic SSR and SSR are very much ideal for this process. At BC₁F₁ and BC₂F₁ generations of backcrossing phase recipient enrichment was done through background selection along with the favourable donor QTL allele at all the three QTL region at high enough frequency such that they can be selected in following backcrossing generation and that the same could be fixed through one selfing generation.

In BC₁F₁ generation for each QTL we have used two flanking markers (SSR or EST – SSRs) which were found sufficient to track the donor allele in carrier chromosome of recipient genome. Similar study was done in maize by Bouchez *et al.* 2002 where they reported the marker-assisted introgression of favourable alleles at three QTL for earliness and grain yield. In each backcrossing programme one plant was backcrossed with recurrent parent (M35-1 and SPV 86) which fulfilled all the marker status, a plant which showed heterozygous marker status for all the six polymorphic marker which were flanking the target QTL region. Similarly, in BC₂F₁ progenies which showed heterozygous for target QTL region along with recurrent parent allele outside the target QTL flanking markers.

Foreground selection in BC₁F₁

In M 35-1 and SPV 86 derived backcross 400 and 390 progenies, respectively, were screened for the entire six foreground polymorphic marker generating 2285 and 2033 marker data points, respectively. As per the computational study of Hospital and Charcosset (1997), with this population size, the risk of not obtaining at least 1 individual carrying donor alleles at all foreground selection markers controlling the QTL segments was below 1% (minimum population size at 1%: 115 individuals). In present study also such a large population was used to get more number of progenies satisfying the required condition (heterozygous for all the polymorphic markers flanking the three QTL region). This is also important in getting synchrony for further desired crossing (backcrossing). Similar strategy has also been advocated by Bouchez *et al.*, 2002, who also practiced the same in maize.

Foreground and limited background selection during BC₂F₁

One of the major problems during introgression programme is the linkage drag around the target QTL, because this segment hosts most of the unwanted donor genes still segregating in the population after a few generations (Hospital 2001; Naveira and Barbadilla 1992; Stam and Zeven 1981; Young and Tanksley, 1989). This reduction was achieved by selecting for individuals that are heterozygous at the target locus (foreground selection) and homozygous for recipient-type alleles at two markers flanking the target locus on either side of the target region (limited background selection). Hence, to overcome the linkage drag, strong selection with marker outside the QTL region on carrier chromosome as that of recipient type. In order to minimize the linkage drag around the QTL region we carried out limited background selection along with the foreground selection was done. According to simulation study by user interactive software "Popmin" (Friedrick, 2002), one need a population of 455 to find the double recombinant at BC₂ generation.

Two rounds of selection (foreground and limited background) was carried out in SPV 86 and M 35-1 derived progenies. First each progeny were screened with 6 polymorphic markers for foreground selection. Thereafter, a second level of selection was performed to avoid the other genome region from donor parent from QTL carrying linkage group B and I, *i.e* limited background selection was exercised so as to take care of linkage drag around target QTL position. Two flanking markers outside each targeted QTL region were used for identifying the backcross progenies having the recurrent parent allele at both the markers termed as double homozygote at that particular locus. A total of 526 and 509 progenies were screened with 12 polymorphic marker generating 5989 and 6002 scorable marker data points, respectively. The progenies which showed heterozygosity ("H") and double homozygote ("B") for each of the three QTL regions in both the crosses were selected. Foreground and limited background selection for all the background progenies simultaneously rather than sequentially to maximize our chances of getting a single progeny was done. This is in contrast to Frisch and Melchinger, 2001, where they carried out only foreground selection using sequential approach. Once this was done the progenies that satisfied the banding pattern for all the twelve primers. For this kind of selection sequential selection would not give correct picture to select the progenies satisfying the foreground and limited background selection.

5.4 Expressional analyses of TF genes in charcoal rot manifestation

Regulation of gene expression is vital for variety of essential processes in plants including growth, development, differentiation, metabolic regulation and adaptation to biotic and abiotic stresses). Transcription is the first step in the expression of any gene plays a central role in the regulation of the expression of genes transcription appears to be controlled by numerous transcription factors that mediate the effects of intercellular and extracellular signals (Verma 1992).. Therefore, the functional analysis of transcription factor genes is essential for an understanding of role of genes in manifesting the diseases and there on possible ways to approach the diagnosis and control strategies. The regulation of plant defense responses is complex, with a number of transcription factor families playing roles (Singh *et al.*, 2002). There is considerable interest in identifying and utilizing key transcription

factors in plant defense for engineering increased resistance to plant pathogens in agriculture crops (Gurr and Rushton, 2005).

Based on the available information about various transcription factors and their annotated functions in model systems such as *Arabidopsis thaliana*, *Zea mays* and *Oryza sativa*, a set of 18 important families having 185 members were selected for their expression analysis on the basis of their direct and indirect role in biotic and abiotic stress.

In order to choose the best genes for normalisation of the data, stably expressing genes were selected using an algorithm, Normfinder. The Normfinder is a statistical algorithm that determines the gene stability measure (M) of all the genes under investigation based on the geometric averaging of multiple control genes and mean pair wise variation (V) of a gene from all other control genes in a given set of samples, hence an increase in the suitability of particular gene as a control gene. A low M value is indicative of a more stable expression ((Vandesompele *et al.*, 2002), in the present study out of four genes (actin (AC1), β -tubulin, 18S-rRNA, and elongation factor-1 α) analysed the genes 18S and actin were found to be the best combination of the reference genes as their expression stability value (M) was 0.235 (calculated by pair wise variation (V). Analysing two reference genes which show a stable expression in different tissues and environments was sufficient in qRT-PCR experiment for normalisation as reported by Ruth *et al.* (2008). In all these experimental situation a TF gene was called significantly up regulated when P value <0.05 and fold change <0.5 or >2 was recorded at 75 and 90 DAS in resistant and susceptible cultivar ,similar kind of analysis was done in *Arabidopsis* as reported by Mark *et al.*, 2007 and Nagabhushana *et al.*, 2007)

Out of 185 transcription factor genes 43 genes were not showing any detectable level of fluorescence signal in any of the time point of sampling (75 DAS and 95 DAS) in resistant and susceptible cultivar. These 43 genes largely belong to the families BHLH (9), PHD (10) and NAC (9) families failed to express in situations tested. The possible reason could be that these transcription factor genes may not have any role in this particular biotic stress related mechanism. At 75 DAS in resistant (E36-1) cultivar, upregulation in 21(14.1%) TF genes was observed of which 14 genes (PTSb00088.1, PTSb00023.1, PTSb00021.1, PTSb00398.1, PTSb00136.1, PTSb00352.1, PTSb00219.1, PTSb00022.1, PTSb00024.1, PTSb00033.1, PTSb00179.1, PTSb00359.1, PTSb00374.2, PTSb00180.1 recorded fold change expression between 2 to 10, 6 genes viz., PTSb00019.1(AP2), PTSb00185.1(HMG), PTSb00349.1(bHLH), PTSb00218.1 (MADS), PTSb00232.1(MYB) recorded >10 fold expression while two genes belonging to WRKY family viz., PTSb00328.1 and PTSb00331.1 showed more than 50 fold expression compared to mock inoculated control E 36-1.

TF genes showing detectable expression levels belonged to 18 different families. There was an up regulation of 21 genes and down regulation of 50 genes while 71 genes showed no change in their expression levels. The up regulation varied with range of fold expression values from 2.23 to 86.67 at 75 DAS in resistant (E 36-1) cultivar. The TF gene expressing much elevated levels was from WRKY followed by MYB and bZIP family.

Similarly, at the same time point, in susceptible (SPV 86) cultivar there was up regulation of 19 TF genes, down regulation of 60 genes and a set of 63 genes showed no change in their expression levels. Folds of up regulation varied and ranged between 2.08 and 9.3. Significantly highly expressed gene were from HMG, WRKY and HAP 5 TF families. related family. Marc *et al.*, (2007) used both quantitative polymerase chain reaction and the Affymetrix *Arabidopsis* whole-genome array and analyzed the expression patterns of *Arabidopsis thaliana* TF and ubiquitin-ligase genes in response to purified *chitooctaose* at different treatment times and finally reported four TF families that were significantly over represented in a list of 118 TF genes that responded to chitin treatment in *Arabidopsis*. These included the AP2/ET-reponsive element binding protein (AP2/ERE), C2H2 zinc finger, MYB and HMG domain transcription factor and WRKY domain transcription factor families.

It is not clear if these genotype-specific differences in transcript abundance of transcription factor genes contribute to charcoal rot resistance in E 36-1 cultivar. These results point toward future experiments that could uncover the mechanisms responsible for charcoal rot resistance in E 36-1 and for the susceptibility of SPV 86 cultivar.

It was found that expression level in mock inoculated control samples of resistant and susceptible cultivar during 90 DAS and 75 DAS were almost same in expression level except for a few genes including the one coding for AP2 protein. AP2 gene showed more expression at 90 DAS in both the cultivar. This high expression of AP2 family at latter stage of crop may be an indication of its direct role (Dimosthenis *et al.*, 2001; Aharoni *et al.*, 2004).

The genes coding for WRKY related protein (PTSb00328.1 and PTSb00331.1) were found showing significantly up regulating at 75 and 90 DAS in resistant cultivar compared to susceptible ones, indicating the possible role in charcoal rot resistance. Similar host gene transcription to modulate defence was previously reported (Eulgem, 2005; Shahid *et al.*, 2005; Zhang and Wang 2005). These result would pave a way to confirm the actual involvement of these genes in resistance for *M. phaseolina* through other alternate tools.

The two transcription factor belonging to WRKY family could be potential target for charcoal rot resistance along with AP2 transcription factor, these genes can be used for candidate gene for charcoal rot resistance. The approach described here has allowed the identification of a vast number of genes involved in the response of the sorghum plant to *M. phaseolina* infection. A significant proportion of these genes resembled genes described as being involved directly in plant defence. A comprehensive analysis of both induced and repressed genes in sorghum during pathogen infections will lead to a better understanding of the molecular processes involved in infection and resistance and will contribute to the development of future biotechnological strategies for resistance

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. Finding gene content by aligning the sorghum genome sequence with the functional marker flanking the QTL for disease resistance.
4. Advancing the backcross progenies to know the worth of the QTLs introgressed.
5. Comparative mapping to see the systemic relationship between different cereals using functional marker.
6. To validate the up regulated TFs using northern blots and use the selected TFs in disease management through genetic engineering effort.

6. SUMMARY AND CONCLUSION

Sorghum (*Sorghum bicolor* (L.) Moench.) is an important staple food crop for more than 300 million people and feed for cattle living in semi-arid tropics of the world. Charcoal rot disease caused by a weak, soil borne fungus *Macrophomina phaseolina* (Tassi) Goid is an economically important disease of sorghum grown during post rainy season (*rabi*) in India. Classical breeding efforts through hybridization and selection are hindered by complex nature of the disease. Further, charcoal rot disease manifest vis-a-vis moisture stress during post flowering dry swells complicate the traditional approach of breeding for resistance to this disease. Conventional breeding supplemented by marker-assisted selection could help in improving level of resistance owing to the precision and neutrality of DNA markers in such complex situations. Construction of genetic linkage map and identification of stable QTLs for charcoal rot resistance and yield components would not only help to understand the genetic nature of disease but also would pave a way for efficient marker assisted introgression of QTLs for disease resistance. Underrating the network of genes and transcription factors possibly associated with charcoal rot resistance would go a long way in this endeavor. Following are the outcomes of the investigation carried out in the present study of molecular mapping, QTL analysis, marker assisted backcrossing and expressional analysis of transcription factor genes for charcoal rot resistance in sorghum.

1. A set of 93 RILs derived from the cross IS 22380 x E 36-1 were evaluated under sick plot condition at Dharwad, Bijapur and Solapur locations for charcoal rot incidence and yield components over four years. Artificial inoculation of virulent strains of *M. phaseolina* was done at critical stage of the crop to ensure proper disease manifestation.
2. Pooled ANOVA of data of four seasons each at Dharwad, Bijapur and Solapur locations revealed highly significant difference among individual RILs and parents for all the 15 traits studied.
3. PCV was higher over GCV indicating the significance of G x E at all the locations. High values of PCV and GCV were recorded for early seedling vigour, stem thickness, threshability, seed yield per plant, days to 50 per cent flowering and disease parameters, viz., length of infection and per cent lodging, indicating that variation for these characters contributed markedly to the total variability. However, the PCV and GCV values were moderate for panicle length, stem thickness and plant height.
4. Though PCV was higher than the GCV for lignin at 75 DAS, lignin at 95 DAS, total phenol at 75 DAS, and total phenol at 95 DAS at both the location (Bijapur and Dharwad); the values were moderately high for all these four biochemical trait which are directly or indirectly associated with disease resistance.
5. There was a significant difference between resistance and susceptible genotype groups for total phenol and lignin content indicating their possible role of these compounds in disease resistance.
6. High heritability were recorded for stem thickness, panicle length, 1000 seed weight, plant height, seed yield per plant and disease parameter viz., length of infection and per cent lodging for lignin at 75 DAS, lignin at 95 DAS, total phenol at 75 DAS and total phenol at 95 DAS. Moderate heritability estimates were noticed for early seedling vigour, days to 50 per cent flowering, ear head length, number and number of internodes crossed by *M. phaseolina*.
7. Correlations revealed highly significant and positive association between plant height and percent lodging at both phenotypic and genotypic level, which indicate shorter plants, are less likely to lodge. Lodging per cent with seed yield and 1000 seed weight showed negative association at genotypic and phenotypic level.
8. Parental screening with 530 genic SSR markers lead to the identification of 63 polymorphic markers between the parents, accounting for 13.3 per cent polymorphism. About 89.2% primers amplified and yielded expected amplicon size.

9. Genotyping of individual gene based markers across RILs was carried out either on 3.5 per cent agarose or 6% PAGE, silver stained to realise the amplicons. The segregation pattern of all the 63 marker loci in RILs was compared and checked for their fitness to expected 1:1 ratio. The calculated χ^2 values were compared with table value at 1 and 5 per cent confidence level for each marker locus. All marker loci were segregated in 1:1 ratio.
10. MAPMAKER / EXP, assigned 140 markers to ten linkage group which were named LG-A, LG-B, LG-C, LG-D, LG-E, LG-F, LG-G, LG-H, LG-I and LG-J in reference with earlier maps. The number of markers mapped per linkage group ranged from six to thirty three. The linkage map thus constructed had a total length of 2905 cM covered. The relative order of marker remained the same as that of other reported maps in sorghum.
11. Linkage distances in terms of centimorgan (cM) values were calculated using Kosambi mapping function
12. Out of 63 EST-SSR mapped on to different linkage group the functional annotation was available only for 27 (42.1%) EST-SSR while for remaining 38 (60.3%) EST-SSR no putative function could be assigned'
13. On linkage group A only two markers showed similarity with the known proteins, Ferredoxin-NADP reductase (*xiabt* 37) and catalytic coenzyme binding protein (*xiabt*224). In linkage group B, seven markers were assigned with putative function with the possible role in disease resistance, transcription initiation, fatty acid metabolism and photosynthesis. In linkage group C, three markers showed putative function viz., *xiabt*20 (RPT-2 like protein), *xiabt* 47 (NADP-dependent malic enzyme and *xiabt* 85 (glucan endo-1, 3-beta-glucosidase 7 precursor
14. In linkage group D, three markers *xiabt* 389 (NADPH toxin reductase), *xiabt*1 (Inositolphosphorylceramide-B-hydroxylase) and *xiabt* 61(receptor protein kinase) showed functional similarity with known protein. The marker on linkage group E (*xiabt* 49) showed similarity with AATL2 like protein while the markers on linkage group F showed similarity with xyloglucan endotransglucosylase (*xiabt* 30) and ring H2 finger protein (*xiabt*78). Eukaryotic translation initiation factor protein showed similarity with the marker (*xiabt* 83) on linkage group F
15. QTL analysis over locations and seasons indicated the presence two QTLs were stably expressing and which were found on same linkage group B. The QTL on linkage group B positioned at 324.0 - 340.0cM was flanked by the marker *txp* 297 and *xiabt* 273 with phenotypic variance of 5.9 - 19.29, this QTL was found co localized with the QTL for stem thickness. The other QTL on linkage group B was flanked by genic SSRs (*xiabt* 92 - *xiabt* 62), with phenotypic variance of 8.8 - 18.9 and additive effect of 3.5-8.8 .The additive effect indicated that the allele contributing for these QTL were contributed from E 36-1.
16. For length of spread of infection three QTLs were detected for this trait which was stably expressed at all the locations over the seasons. Three QTL were located on linkage group A (one QTL) and B (two QTLs).Two QTLs on linkage group B were flanked with the marker *xiabt* 275 - *xiabt*241 and *txp* 201 - *xiabt* 378 with phenotypic variance of 8.0-16.1 and 9.8-17.7, respectively with peak LOD value of 2.1 - 3.5 and 2.1 - 3.9. The QTL on linkage group A was flanked with EST-SSR which showed the similarity with binding/catalytic/coenzyme binding protein. The QTL on linkage group B flanked by the marker EST SSR (*xiabt*241) showed the similarity with Cytochrome P 450 while the QTL flanked by the marker *xiabt* 378 showed similarity with MYB protein. This protein is known to be involved in disease resistance mechanism.
17. For lodging per cent four QTL were stably expressed for all the season and location These were located on three different linkage group (LG-D, LG-I and LG-J). For QTL on linkage group J (*txp* 338-*xiabt* 420) the additive effect (-1.1-7.62) was contributed from the IS 22380 allele while for all the other three QTLs on linkage group D and LG-I the allele was contributed from E36-1. The QTL on linkage group J flanked by EST-SSR was showing putative function with phytosulfokines 5 precursor protein

18. There was a co-localization of genomic region (LG-I) flanked by marker *xtxp 274–xiabt 29*, associated with QTL for lodging per cent, phenol content at 75 DAS and lignin content at 95 DAS on linkage group H. Two QTLs governing total phenol at 75 DAS and 95 DAS share same genomic region. It was also proved genetically that the phenol content has role to play in the disease resistance as the QTL for charcoal rot resistance trait (lodging per cent) and phenol content were co-localised to the same genomic loci.
19. For marker assisted introgression three stable QTLs for charcoal rot resistance trait *viz.*, number of internode crossed, length of infection and percent lodging were used. These collectively contributed 43 % of phenotypic variance for the disease resistance. For all these genomic loci the resistant parent (E 36-1) was contributing favorably. The donor parent E36-1 was crossed with susceptible parent SPV 86 and agronomically elite but charcoal rot susceptible cultivar M 35-1.
20. Foreground selection in BC₁F₁ was employed in M 35-1 and SPV 86 derived backcross progenies which were screened for all the six polymorphic marker generating 2285 and 2033 marker data points, respectively. A single progeny satisfying the heterozygous condition for all the QTL flanking marker was used for backcrossing at advance for BC₂F₁ generation
21. Foreground and limited background selection during BC₂F₁ was employed in M35-1 and SPV 86 derived backcross progenies. A total of 526 and 509 progenies were screened with 12 polymorphic marker generating 5989 and 6002 scorable marker data points, respectively. Progeny showed heterozygosity (“H”) and double homozygote (“B”) for each of the three QTL region in both the crosses were selected and advanced to BC₃F₁ generation.
22. A total of 185 transcription factor genes from 18 different family were used to study the differential expression under charcoal rot manifestation in susceptible (SPV 86) and resistant (E36-1) cultivar at two different time points *viz.*, 75 DAS and 90 DAS.
23. Out of 185 TF genes from 18 different family, 43 genes were not showing any detectable expression in either resistant or susceptible cultivar at both the time points *viz.*, 75 DAS and 90 DAS.
24. At 75 DAS in resistant cultivar (E36-1), 14.1% (21) TF genes were upregulated, 14 genes (PTSb00088.1, PTSb00023.1, PTSb00021.1, PTSb00398.1, PTSb00136.1, PTSb00352.1, PTSb00219.1, PTSb00022.1, PTSb00024.1, PTSb00033.1, PTSb00179.1, PTSb00359.1, PTSb00374.2, PTSb00180.1 recorded fold change expression within 2-10, 6 genes *viz.*, PTSb00019.1(AP 2), PTSb00185.1(HMG), PTSb00349.1(bHLH), PTSb00218.1(MADS), PTSb00232.1(MYB) recorded >10 fold expression while two genes belonging to WRKY family *viz.*, PTSb00328.1 and PTSb00331.1 showed more than 50 fold expression compared to mock inoculated control E36-1, A set of 50 genes showed down regulation and 71 genes showed no change in expression.
25. At 75 DAS in susceptible cultivar (SPV 86), 13.3 % (19) TF genes were upregulated. All the 19 up regulated genes showed fold expression within range of 2.08 to 9.3 of which majority of TF belong to four TF family *viz.*, MADS (PTSb00211.1, PTSb00214.1, PTSb00220.1), HAP (PTSb00127.1, PTSb00130.1, PTSb00131.1, PTSb00132.1), AP2 (PTSb00019.1, PTSb00022.1, PTSb00024.1, PTSb00033.1), HMG (PTSb00179.1, PTSb00182.1, PTSb00185.1) and MYB (PTSb00229.1, PTSb00234.1).
26. At 95 DAS in resistant variety (E 36-1), out of 142 TFs up regulation of 54 genes (38 %) was noticed, followed by 46 genes down regulation, 42 genes no change in expression. The observed fold change expression ranged from 2.25 to 182.25. Out of 54 upregulated TF genes, 29 genes expressed within 2 to 10 folds, while 19 genes expressed within 10-50 fold. The predominant genes belong to WRKY (PTSb00331.1) followed by AP2 (PTSb00019.1) and bZIP (PTSb00374.1) families recorded 182.25, 99.37 and 86.35, respectively.

27. Eight TF genes representing five TF family viz., AP2 (PTSb00019.1, PTSb00022.1, PTSb00024.1 WRKY (PTSb00331.1), HMG (PTSb00179.1, PTSb00185.1) ARF (PTSb00033.1) and bHLH (PTSb00349.1), were found commonly expressing in susceptible and resistant cultivars. Two transcription factor belonging to WRKY member (PTSb00328.1 and PTSb00331.1) were conspicuously and highly up regulated at both time (75 and 95 DAS) points in resistant cultivar compared to susceptible ones.

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Appendix

I: Pedigree information of the RILs 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		87.	2507	IS 22380 × E 36-1
38.	2459	IS 22380 × E 36-1		88.	2508	IS 22380 × E 36-1
39.	2460	IS 22380 × E 36-1		89.	2509	IS 22380 × E 36-1
40.	2461	IS 22380 × E 36-1		90.	2510	IS 22380 × E 36-1
41.	2462	IS 22380 × E 36-1		91.	2511	IS 22380 × E 36-1
42.	2463	E 36-1 (Parent-2)		92.	2512	IS 22380 × E 36-1
43.	2464	IS 22380 × E 36-1		93.	2513	IS 22380 × E 36-1
44.	2465	IS 22380 × E 36-1			Parent-1	IS 22380
45.	2466	IS 22380 × E 36-1			Parent-2	E 36-1
46.	2467	IS 22380 × E 36-1				
47.	2468	IS 22380 × E 36-1		.		
48.	2469	IS 22380 × E 36-1				
49.	2470	IS 22380 × E 36-1				

Appendix II: List of stock solutions used in experiment

1. ExtraCtion buffer	:100 mM Tris HCl, pH 8.0
	50 mM EDTA, pH 8.0
	500 mM NaCl
2. 20 % SDS	:20 g of sodium doedecyl sulphate (sodium lauryl sulphate)
	100 ml of dH ₂ O.
	Should not be autoclaved
3. 5 M potassium acetate	: 60.0 ml 5 M potassium acetate
	11.5 ml Glacial acetic acid
	28.5 ml H ₂ O
4. Isopropanol Ammonium Acetate mixture	:Three volumes of 10 M Iso-propanol
	One volume of 10 M ammonium acetate
5. T ₁₀ E ₁	: Tris 10 mM containing 1 mM EDTA
6. RNase (10 mg/ml)	: Dissolve Rnase in water, place in a tube in a boiling water bath for 10 minutes. Allow this to cool on a bench and store at –20 °C
7. 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.
8. Ethanol (70 %)	: Absolute alcohol (70 ml) Distill water (30 ml)
9. NaCl 5 M	: Dissolve 292.2 g NaCl in 750 ml water. Make up to 1 litre with water, filter and autoclave.
10. Phenol /chloroform	: Mix equal volumes of the buffered phenol and chloroform : isoamyl alcohol (24:1). Store at 4 °C
11. Sodium acetate (2.5 M, pH 5.2)	: Dissolve 340.2 g sodium acetate in 500 ml of water adjust pH to 5.2 with glacial acetic acid and make up the volume to 1 litre and autoclave.
12. Tris HCl (1 M, pH 8.0)	: Dissolve 121.1 g Tris in 800 ml of water. Adjust pH 8.0 with conc. HCl. Make up the volume to 1 L and autoclave
13. Ethidium bromide (10 mg/ml)	: Dissolve 100 mg ethidium bromide in 10 ml of distil water, warp tube in aluminium foil and store at 4 °C. <u>Caution</u> : Ethidum bromide is extremely mutagenic
14. 4 % Acrylamide solution (1000 ml)	: 450 ml double distilled water 100 ml 5× TBE 420 g Urea

	100 ml, 40 % Acrylamide/bisacrylamide (19:1) (w/w) 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. <i>Contd...</i> Then acrylamide solution is combined with other ingredients in a storage container. <u>Note</u> : Acrylamide is a neuro toxic
15. 5× TBE	: 540 g Tris base 46 g EDTA 276 g boric acid dH₂O 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
16. 0.5 % Acetic acid in 95% ethanol	: 1 ml glacial acetic acid 199 ml 95% ethanol Aliquot and store at room temperature
17. 10 % APS (ammonium persulfate)	: 1 g ammonium persulfate 10 ml H₂O Should be stored at –20 °C if the APS will be used within the few weeks, store in light-tight bottle at 4 °C
18. TEMED (N, N, N', N' – tetra methyl ethylene diamine)	: Store at 4 °C
19. Binding solution	: Bind silane (γ - methacryloxy profile trimethoxysilane) M-6514 sigma industries 4 µl of bind silane + 1000 µl of 0.5 % acetic acid in 95% ethanol
20. 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)
21. Loading dye/tracking dye (10×)	: Sucrose 167 mg Bromophenol blue 4.2 mg Water 1 ml
22. 3× SSR dye (3× STR loading solution)	: 5 M NaOH 0.2 ml 95 % formamide 95 ml Bromophenol blue 50 mg xylene cyanol 50 mg water make upto 100 ml <u>Note</u> : Formamide is a toxic compound
23. 100 bp DNA ladder	: 10 µl of 100 bp marker (genetics) 95 µl of 3× SSR dye 95 µl sterile dH₂O

Contd...

24. Fix/stop solution (10% acetic acid)	: 200 ml glacial acetic acid in 1800 ml dH ₂ O
25. Impregnate solution	: 10 % glycerol + 10 % glacial acetic acid
26. Staining solution	: 2 g silver nitrate 3 ml 37 % formaldehyde 2000 ml ddH ₂ O Should be stored at room temperature in a cabinet or other dark storage space <u>Note</u> : Formaldehyde is a potential carcinogen
27. Developer solution	: 60 g sodium carbonate 3 ml 37 % formaldehyde 400 µl sodium thiosulphate (10 mg/ml) 2000 ml dH ₂ O This solution must be prepared fresh for each use Prepared the solution by dissolving the sodium carbonate in water and chilling to about 10 °C by placing on ice or in freezer Just before use add the formaldehyde and sodium thiosulphate
29. Loading dye (6×)	: 0.25 Bromophenol blue 40 % (W/V) sucrose in water Started at 4°C
30. 50× TAE (Tris-Acetate EDTA) (Miniatis <i>et al.</i> , 1982)	: 242 g/l Tris base and 57.1 ml Glacial acetic acid 100 ml 0.5 M EDTA (pH 8.0) 1000 ml Distilled water
31. Saturated aqueous bromophenol blue solution	: 16.00
EDTA (0.5M; pH: 8.0)	: 80.00
Formaldehyde	: 720.00
Glycerol (100 %)	: 2000.00
Formamide	: 3048.00
10× FA gel buffer	: 4000.00
Rnase free water	: 100.00
Total	: 10 ml
32. Recipe for 1.2 per cent formaldehyde agarose gel (60 ml)	
Agarose	: 720 mg
Formaldehyde	: 1 ml
Ethidium bromide (10 mg/ml)	: 3 µl
1× MOPs buffer	: 60 ml

33. 10x MOPs eleCtrophoresis buffer	
MOPs	: 41.8 g
Sodium Acetate (DEPC-treated)	: 20 ml
EDTA (DEPC-treated; pH: 8.0)	: 20 ml
Total volume 1000 ml with DEPC treated water	
34. Recipe for 1 per cent Agarose gel (40 ml)	
Agarose	400 mg
1x TAE	40 ml
EtBr (10 mg/ml)	2 μ l

Appendix III: List of genic SSR primer used in the present investigation for genetic map construction using RILs derived from the cross IS 22380 x E 36-1

Sl. No	Gene ID	Primer	Forward sequence	Reverse sequence
1	31332334	<i>xiabt 2</i>	GCCCACCAAATACAAGAACC	CTTTGCGGCACTAAAACACA
2	45949294	<i>xiabt 21</i>	GCAATGGAGTCTTGGACGATG	GAAACCAGAACCGAACCTGA
3	30973845	<i>xiabt 380</i>	CTTCCCCATAGCCAGAATCA	CCCGTCGAAGCACTCACTATA
4	31334597	<i>xiabt 37</i>	AACCGGATGGGTTGCTAATC	TTGATCTGGGGATGGAGAAG
5	17886525	<i>xiabt 57</i>	TTTCCGTTGTTCCCTTCCTTG	TGCTAATCGGCGAGTTTCTTC
6	30978605	<i>xiabt 67</i>	CTCCCTCCCTCCGCTCTC	GGTCCGTCGTCCTTACCC
7	9850502	<i>xiabt 224</i>	CAAGCAGCAGAGGAGCATT	GCTTGATGAGCTCCCTTACG
8	30942962	<i>xiabt 75</i>	CCTTCTCTTCTCCCAATCC	GAGGCAGAAGCCTCCTACAA
9	9305677	<i>xiabt 69</i>	TGCACAAGAGGCAGGATATG	AGCCCATGAGAAAAAGCTCA
10	13390776	<i>xiabt 71</i>	TCAGGAAATACGGGAACGAG	GACCTAAGGCATACCGCTGA
11	5042862	<i>xiabt 434</i>	AGCTAGTCGGGCAGTAACCA	CCGTAGAAGCACAACTGCAC
12	30932238	<i>xiabt 92</i>	ATCTCGCCTTCCCTTTCTTC	AAGTAGACGCGGTCGTTGAC
13	18060131	<i>xiabt 97</i>	GAGCAGTGAGGAAAGGCAAG	AAGGGAGGAGCGATAAAGGA
14	45961441	<i>xiabt 7</i>	CACCATCAGAAAGCAAAGCA	AAGCAAAGACGAGCAAGGAA
15	18052712	<i>xiabt 31</i>	TGACAGGATCGTGAGCAAAG	TCCGGTCCCATTATTAGTGC
16	30974381	<i>xiabt 378</i>	GAGCAGCGGATAAGTTCGAG	TAACCACATGGCCCGTTC
17	31347844	<i>xiabt 60</i>	CCATCCAATCCAGGTCCTAA	GATCTCGGCGACTACTTTGC
18	30949145	<i>xiabt 87</i>	CCTAGGGCTGTGCCTACTCA	ATCAGGCAAAAAGGTTGAGC
19	45965503	<i>xiabt 62</i>	CAGAGAAAAAGGAGCCGAAA	AGAAGGTGGAGCTGGAGACA
20	45992006	<i>xiabt 243</i>	GCCCACCAAATACAAGAACC	CTTTGCGGCACTAAAACACA
21	45994116	<i>xiabt 65</i>	AAGGGAGGAGAGCAGAGAGC	CTCCTCTGCCTCTGCCTCTA
22	34442563	<i>xiabt 66</i>	TAAATTTGTGCGCTCGCTTC	GACTGGAGGGTAGGGTCTGG
23	12775306	<i>xiabt 73</i>	CACCTCACCTCGATGATGC	GGAAAGCTGAAGTGGTCCAA
24	30948481	<i>xiabt 74</i>	GCTGAAATTCCCAATCCTC	GAAGAAGGTCGGTCCTGGATG
25	45996483	<i>xiabt 275</i>	AAGGATCCTCCTCCGAGAAC	CACCGTCCACATCTTCTCCTC
26	13784591	<i>xiabt 76</i>	ACCCCTTCTTGTTACCAC	GTGCTGCTGGCCGTAGTAGTA
27	12775835	<i>xiabt 80</i>	TCGTCTACGCCTGGAAGAAC	CAAGAACATTGCTCGCTGAA
28	14569915	<i>xiabt 82</i>	GCTTCCGTCACACCTCCTC	TTGTGGGAAATTGGGATGATA
29	45986595	<i>xiabt 241</i>	GCCCACCAAATACAAGAACC	TTTGTGGCACGAAAACACATA
30	31334672	<i>xiabt 20</i>	GTCTCTCCTCCCTTCCCTTC	CAAGCGCAACAGTGAAAGAA
31	33109119	<i>xiabt 47</i>	CGGTCTTCTGTAGGTGCTG	CAAATCACCAAGTCCCTGCTG
32	34509336	<i>xiabt 51</i>	GTGCGTCGAGTTGAAGAAG	AACATAACCCGTCACCATGC
33	9852958	<i>xiabt 53</i>	TTGTGAGTCAGGGGAAGAGC	GATCCATCATGTGGCATGTG
34	31346189	<i>xiabt 85</i>	GCCACCTCTGTGACTCTTCC	CTCTCCGTCATCTCCAGAGC
35	12618782	<i>xiabt 96</i>	GAGGTGGAGCAGAGCAGAAAG	CAGATGCTGAGAACGGGAGTA
36	30937893	<i>xiabt 389</i>	GCACGAGAACAGCAGATTA	AATCCATCGCACACATCAGTA
37	57815147	<i>xiabt 1</i>	CACGCGAACAACATCTCAAC	gAATGGATTAGGGCACGAGTA
38	30964510	<i>xiabt 22</i>	CCACCATCACCACCACATTA	CTCGGTCTCTTGCTTGTGTG

39	14328763	<i>xiabt 77</i>	CGTCCTCGTCTCCTTCGTC	GCATG TTCAGGAGAGCATCA
40	31384321	<i>xiabt 61</i>	GTACGACCGCATGCTCAAG	ACGATGTCGACGAACTTGC
41	57804841	<i>xiabt 63</i>	CTGCCAGACGGTGCTCAG	TGGTGATTGGAGGAAGAAGG
Sl. No	Gene ID	Primer	Forward sequence	Reverse sequence
				<i>Contd...</i>
42	18062597	<i>xiabt 01</i>	CTGCAGTACGTGCACATCAA	ACCACGACCGTTGGATAGTC
43	30942986	<i>xiabt 79</i>	TTCCATTCCATCCTCGTCTC	ATGGAGAGGTTGGTCAGCAC
44	30976670	<i>xiabt 374</i>	TGGAAAGAAGAAAGAGGAACG	GGAGTTGGAATGCGAGGTC
45	11679325	<i>xiabt 13</i>	CACGCGAACAACATCTCAAC	CAATGGATTAGGGCAGAGTA
46	34441344	<i>xiabt 27</i>	CTCTCTCCTCCCTTCCCTTC	CAAGCGCAACAGTGAAAGAA
47	34509854	<i>xiabt 49</i>	ATAGTTGGCGTGCTCCTGAC	GCCATCATCCATCCATATCC
48	7536147	<i>xiabt 30</i>	CTACTCCAGCCTGTGGAACG	AGGTCCAGCTCCTGGTTGTA
49	7218421	<i>xiabt 78</i>	CGGAGCTAAGCTAGCCAGAA	TGGCAGACGTAGAGATGGAG
50	30936043	<i>xiabt 83</i>	TCTGACCGAGCCCTAAACAC	GAAGGGGACAGACGAGGAG
51	45968710	<i>xiabt 9</i>	CGGGAGGTTTCAGGAACAAGA	AGGTGGTTTCAGTGTGTTTTG
52	30932211	<i>xiabt 46</i>	GCTGAAACCTGAAAGGTTGG	GGAATTAACATCGGCACCTC
53	9856009	<i>xiabt 81</i>	CTCCATCATCATCGTCATCG	CTCGGAGACGGAGACAGC
54	45974008	<i>xiabt 72</i>	CCGCCTACTCCTCCGACTAC	CCGCCCTCTTCTTCTTCTTC
55	13064958	<i>xiabt 5</i>	AGGGGTAAACCACCCTATCG	AGTCACTCTTCCTCGCCAAA
56	33108946	<i>xiabt 29</i>	GAATGAATCAGCAACGGACA	TTGTGGCTACGATTTTGCAG
57	34509330	<i>xiabt 64</i>	GAAGCGATTTGTGCCCTCTA	GTATGGAGGACGCCATGACTA
58	13585195	<i>xiabt 70</i>	GCGTGTGCTGTCTCTCTCTG	CATTTGCCCTTG TAGCCAATC
59	9301623	<i>xiabt 86</i>	GATGAGCTCCAGTCGAGACC	CGCTATTCAGAGCCATCAGA
60	30970132	<i>xiabt 94</i>	GCCACAAGAGCACACCACTA	CTCTCCTCCCTTCCTTGGAC
61	31332570	<i>xiabt 45</i>	GGGGAGGTTTCAGGAACAAGA	CGGTGGTTTCAGTGTGTTTTG
62	57820833	<i>xiabt 68</i>	CGCCACCTAAACCCTAGCTG	ATCTGGAACGCTTGGACATC
63	11680215	<i>xiabt 420</i>	ACATGCATGCTTGGAAGTTG	CTCTAGCATGGACCTGCACA

Appendix IV: Test of significance for segregation of all polymorphic DNA markers-nuclear SSR, genic SSR and RAPD in RILs of IS 22380 × E36-1

Sl. No.	Marker	P ₁ allele	P ₂ allele	χ^2
1	<i>xtxp9</i>	44	46	0.044**
2	SbAGB02	49	44	0.268**
3	<i>xtxp318</i>	47	45	0.043**
4	<i>xtxp63</i>	47	45	0.043**
5	<i>xtxp211</i>	43	49	0.392**
6	x txp8	45	47	0.043**
7	x txp343	45	46	0.011**
8	<i>xtxp12</i>	40	53	1.816**
9	<i>xtxp295</i>	39	49	1.136**
10	SbAGE03	49	42	0.538**
11	<i>xtxp67</i>	38	50	1.942**
12	<i>xtxp230</i>	44	49	0.263**
13	<i>xtxp258</i>	37	50	1.942**
14	SbAGH04	46	47	0.01**
15	<i>xtxp354</i>	41	49	0.711**
16	SbAGA01	48	47	0.01**
17	<i>xtxp274</i>	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	G19	44	51	0.515**
31	H3	48	47	0.01**
32	L18	43	52	0.852**
33	M3	50	45	0.263**
34	M10	55	40	2.368**
35	corn61	47	46	0.011**
36	<i>xtxp267</i>	42	49	0.538**
37	<i>xtxp316</i>	40	49	0.910**
38	corn74	39	53	2.130**
39	<i>xtxp201</i>	44	46	0.044**
40	<i>xtxp348</i>	42	50	0.696**
41	<i>xtxp303</i>	46	47	0.011**
42	corn59	49	44	0.269**
43	corn24	45	45	0.000**
44	<i>xtxp210</i>	40	51	1.330**
45	<i>xtxp353</i>	44	48	0.174**
46	<i>xtxp159</i>	41	52	1.301**
47	<i>xtxp358</i>	38	53	2.473**
48	<i>xtxp204</i>	39	51	1.600**

Contd.....

Sl. No.	Marker	P ₁ allele	P ₂ allele	χ^2
49	<i>txp284</i>	43	50	0.527**
50	<i>Pepc</i>	39	52	1.857**
51	<i>txp254</i>	43	48	0.275**
52	<i>txp217</i>	40	50	1.111**
53	<i>txp329</i>	39	54	2.419**
54	<i>txp248</i>	44	49	0.269**
55	<i>txp96</i>	41	46	0.287**
56	<i>txp176</i>	38	51	1.899**
57	<i>txp312</i>	47	44	0.099**
58	<i>txp325</i>	40	50	1.111**
59	<i>txp324</i>	42	46	0.182**
60	<i>txp338</i>	44	49	0.269**
61	<i>txp273</i>	43	49	0.391**
62	<i>txp327</i>	41	52	1.301**
63	<i>txp48</i>	40	53	1.817**
64	<i>corn73</i>	40	47	0.563**
65	<i>txp71</i>	46	44	0.044**
66	<i>txp92</i>	47	44	0.099**
67	<i>txp160</i>	37	52	2.528**
68	<i>txp213</i>	51	41	1.087**
69	<i>txp297</i>	42	45	0.103**
70	<i>txp302</i>	47	45	0.043**
71	<i>xiabt2</i>	37	51	2.227**
72	<i>xiabt9</i>	43	49	0.391**
73	<i>txp298</i>	42	50	0.696**
74	<i>xiabt20</i>	39	54	2.419**
75	<i>txp183</i>	41	52	1.301**
76	<i>txp45</i>	39	54	2.419**
77	<i>xiabt1</i>	47	46	0.011**
78	<i>M9</i>	37	54	3.176**
79	<i>txp290</i>	38	55	3.108**
80	<i>txp350</i>	41	52	1.301**
81	<i>xiabt46</i>	48	40	0.01**
82	<i>xiabt29</i>	40	49	0.19**
83	<i>xiabt05</i>	38	39	0.01**
84	<i>xiabt32</i>	44	40	0.19**
85	<i>xiabt13</i>	38	54	3.4**
86	<i>xiabt49</i>	42	43	0.01**
87	<i>xiabt01</i>	41	51	1.08**
88	<i>xiabt22</i>	44	49	0.26**
89	<i>xiabt82</i>	35	53	3.68**
90	<i>xiabt83</i>	45	48	0.09**
91	<i>xiabt45</i>	37	56	3.80**
92	<i>xiabt47</i>	45	48	0.09**
93	<i>xiabt85</i>	43	42	0.01**
94	<i>xiabt96</i>	38	49	1.39**
95	<i>xiabt72</i>	47	46	0.1**
96	<i>xiabt27</i>	44	47	0.09**
97	<i>xiabt94</i>	44	47	0.09**
98	<i>xiabt21</i>	50	43	0.52**
99	<i>xiabt22</i>	43	48	0.27**
100	<i>xiabt51</i>	38	54	3.4**

Sl. No.	Marker	P ₁ allele	P ₂ allele	χ^2
101	<i>xiab</i> t224	42	49	0.53**
102	<i>xiab</i> t434	40	51	1.32**
103	<i>xiab</i> t380	45	48	0.09**
104	<i>xiab</i> t378	40	50	0.53**
105	<i>xiab</i> t243	39	55	2.72**
106	<i>xiab</i> t275	37	54	0.87**
107	<i>xiab</i> t241	39	55	2.72**
108	<i>xiab</i> t420	42	51	0.72**
109	<i>xiab</i> t389	55	39	1.6**
110	<i>xiab</i> t374	40	48	3.1**
111	<i>xiab</i> t30	40	51	3**
112	<i>xiab</i> t60	38	55	0.72**
113	<i>xiab</i> t80	55	38	0.63**
114	<i>xiab</i> t07	40	48	0.05**
115	<i>xiab</i> t57	42	35	1.16**
116	<i>xiab</i> t92	41	39	0.68**
117	<i>xiab</i> t97	48	38	2.36**
118	<i>xiab</i> t37	43	50	3.8**
119	<i>xiab</i> t31	40	53	0.72**
120	<i>xiab</i> t53	37	55	0.91**
121	<i>xiab</i> t61	45	46	0.011**
123	<i>xiab</i> t63	40	53	1.816**
124	<i>xiab</i> t62	37	54	0.87**
125	<i>xiab</i> t65	39	55	2.72**
126	<i>xiab</i> t66	38	55	0.72**
127	<i>xiab</i> t67	40	55	2.041**
128	<i>xiab</i> t69	42	49	0.53**
129	<i>xiab</i> t68	37	56	3.80**
130	<i>xiab</i> t64	47	44	0.099**
131	<i>xiab</i> t71	46	49	0.094**
132	<i>xiab</i> t73	35	53	3.68**
133	<i>xiab</i> t74	42	47	0.103**
134	<i>xiab</i> t75	42	49	0.53**
135	<i>xiab</i> t76	37	54	0.87**
136	<i>xiab</i> t77	45	46	0.011**
137	<i>xiab</i> t78	41	54	0.085**
138	<i>xiab</i> t79	40	53	1.8**
139	<i>xiab</i> t81	45	48	0.093**
140	<i>xiab</i> t86	43	50	3.8**
141	<i>xiab</i> t87	37	54	0.81**

P<0.01> : 3.81

Appendix V: Normalized Ct values of 185 TF genes in mock inoculated (control) and inoculated (treated) samples of E 36-1 (resistant) and SPV 86 (susceptible) at 75 and 90 DAS

Sl. No.	Database ID	TF Member	CS ₁ Ct	CS ₁ ΔCt	S ₁ Ct	S ₁ ΔCt	T value	CS ₂ Ct	CS ₂ ΔCt	S ₂ Ct	S ₂ ΔCt	T value	CE ₁ Ct	CE ₁ ΔCt	E ₁ Ct	E ₁ ΔCt	T value	CE ₂ Ct	CE ₂ ΔCt	E ₂ Ct	E ₂ ΔCt	T value	S ₁ /CS ₁ Ratio	S ₂ /CS ₂ RATIO	E ₁ /CE ₁ RATIO	E ₂ /CE ₂ RATIO
1	PTSb00013.1	AP2	30.10	2.26	31.00	4.00	0.309	29.90	2.06	32.10	5.08	0.0552	33.10	1.73	29.10	1.44	0.302	29.90	2.53	29.40	1.85	0.9013	0.37	0.17	1.19	1.51
2	PTSb00014.1	AP2	40.00	12.16	40.00	13.00	0.033	40.00	12.16	40.00	12.98	0.4122	40.00	8.63	40.00	12.34	0.000	40.00	12.63	40.00	12.45	0.5298	1.00	1.00	1.00	1.00
3	PTSb00015.1	AP2	40.00	12.16	40.00	13.00	0.033	40.00	12.16	40.00	12.98	0.4122	40.00	8.63	40.00	12.34	0.000	40.00	12.63	40.00	12.45	0.5298	1.00	1.00	1.00	1.00
4	PTSb00016.1	AP2	31.20	3.36	30.80	3.80	0.043	28.20	0.36	29.70	2.68	0.3174	33.30	1.93	31.40	3.74	0.003	27.80	0.43	28.10	0.55	0.0729	0.76	0.23	0.31	0.93
5	PTSb00017.1	AP2	34.47	6.63	33.80	6.80	0.129	35.10	7.26	32.10	5.08	0.0117	34.80	3.43	33.70	6.04	0.056	35.10	7.73	33.00	5.45	0.0010	0.86	3.85	0.20	3.90
6	PTSb00018.1	AP2	32.10	4.26	35.80	8.80	0.001	33.10	5.26	36.70	9.68	0.0224	36.85	5.48	33.70	6.04	0.403	37.10	9.73	33.50	5.95	0.0004	0.06	0.06	0.74	10.63
7	PTSb00019.1	AP2	30.10	2.26	27.80	0.80	0.012	31.10	3.26	27.90	0.88	0.0245	34.10	2.73	26.10	-1.57	0.000	35.20	7.83	28.10	0.55	0.2578	2.51	4.49	15.05	99.37
8	PTSb00020.1	AP2	35.97	8.13	34.60	7.60	0.195	36.70	8.86	34.30	7.28	0.0115	35.10	3.73	32.10	4.44	0.007	36.90	9.53	33.10	5.55	0.0066	1.37	2.71	0.64	12.40
9	PTSb00021.1	AP2	33.45	5.61	31.87	4.87	0.053	34.10	6.26	30.10	3.08	0.0002	33.80	2.43	27.80	0.14	0.009	30.10	2.73	27.80	0.25	0.0011	1.60	6.75	4.02	4.44
10	PTSb00022.1	AP2	33.94	6.10	31.50	4.50	0.028	34.50	6.66	30.10	3.08	0.0016	35.38	4.01	28.10	0.44	0.001	33.10	5.73	29.10	1.55	0.0005	2.60	9.12	8.91	13.26
11	PTSb00023.1	AP2	32.00	4.16	31.80	4.80	0.024	33.20	5.36	33.30	6.28	0.9147	35.50	4.13	30.10	2.44	0.057	35.10	7.73	30.10	2.55	0.0002	0.70	0.58	2.83	22.37
12	PTSb00024.1	AP2	34.80	6.96	32.00	5.00	0.034	32.10	4.26	29.90	2.88	0.0161	35.60	4.23	29.10	1.44	0.016	34.10	6.73	28.80	1.25	0.0001	3.44	2.39	5.84	31.90
13	PTSb00025.1	AP2	34.00	6.16	33.60	6.60	0.055	36.70	8.86	32.10	5.08	0.0003	35.80	4.43	34.10	6.44	0.006	32.10	4.73	35.20	7.65	0.0140	0.75	12.02	0.27	0.15
14	PTSb00026.1	AP2	31.51	3.67	31.80	4.80	0.808	34.20	6.36	33.20	6.18	0.0452	32.60	1.23	32.80	5.14	0.004	30.10	2.73	33.10	5.55	0.0022	0.53	1.11	0.10	0.19
15	PTSb00027.1	AP2	32.48	4.64	31.30	4.30	0.064	33.10	5.26	28.70	1.68	0.0014	31.10	-0.27	28.90	1.24	0.047	29.90	2.53	29.80	2.25	0.2441	1.26	11.96	0.35	1.22
16	PTSb00028.1	AP2	32.53	4.69	32.39	5.39	0.232	33.10	5.26	30.10	3.08	0.0038	33.30	1.93	30.10	2.44	0.937	31.10	3.73	31.30	3.75	0.7064	0.64	3.83	0.71	1.03
17	PTSb00029.1	ARF	40.00	12.16	40.00	13.00	0.033	40.00	12.16	40.00	12.98	0.4122	32.10	0.73	40.00	12.34	0.000	33.20	5.83	40.00	12.45	0.0000	1.00	1.00	1.00	1.00
18	PTSb00030.1	ARF	29.88	2.04	36.70	9.70	0.001	31.10	3.26	38.10	11.08	0.0002	29.80	-1.57	31.00	3.34	0.000	30.10	2.73	32.00	4.45	0.0008	0.01	0.01	0.04	0.32
19	PTSb00031.1	ARF	34.28	6.44	33.00	6.00	0.002	33.20	5.36	31.20	4.18	0.0355	34.20	2.83	31.10	3.44	0.066	35.10	7.73	32.00	4.45	0.0014	1.32	2.13	0.68	8.18
20	PTSb00032.1	ARF	27.03	-0.81	33.80	6.80	0.001	27.20	-0.65	30.20	3.18	0.0014	28.70	-2.67	24.10	-3.57	0.431	29.10	1.73	25.00	-2.56	0.0000	0.01	0.07	1.86	19.50
21	PTSb00033.1	ARF	32.51	4.67	30.30	3.30	0.072	33.20	5.36	28.90	1.88	0.0033	35.55	4.18	30.10	2.44	0.018	36.10	8.73	31.20	3.65	0.0001	2.56	10.94	3.32	32.97
22	PTSb00034.1	ARF	34.33	6.49	32.90	5.90	0.078	33.00	5.16	27.10	0.08	0.0000	30.10	-1.27	34.60	6.94	0.000	32.10	4.73	35.30	7.75	0.0146	1.45	25.01	0.01	0.15
23	PTSb00035.1	ARF	32.06	4.22	31.20	4.20	0.089	32.10	4.26	30.10	3.08	0.0736	31.20	-0.17	32.10	4.44	0.065	32.10	4.73	34.10	6.55	0.0113	1.01	2.09	0.06	0.32
24	PTSb00036.1	ARF	35.00	7.16	33.80	6.80	0.099	32.10	4.26	30.30	3.28	0.0003	35.31	3.94	34.80	7.14	0.011	35.00	7.63	35.10	7.55	0.1072	1.27	1.92	0.12	1.06
25	PTSb00037.2	ARF	37.10	9.26	36.70	9.70	0.021	37.90	10.06	32.10	5.08	0.0004	33.20	1.83	36.80	9.14	0.000	35.10	7.73	36.70	9.15	0.9794	0.77	18.67	0.01	0.44
26	PTSb00349.1	bHLH	32.10	4.26	29.10	2.10	0.015	30.10	2.26	27.70	0.68	0.0020	37.60	6.23	30.10	2.44	0.000	36.10	8.73	31.10	3.55	0.0003	3.90	2.71	10.98	26.39
27	PTSb00350.1	bHLH	33.00	5.16	31.70	4.70	0.100	31.20	3.36	26.80	-0.22	0.0008	33.10	1.73	32.60	4.94	0.000	32.40	5.03	33.10	5.55	0.3182	1.34	10.17	0.13	0.72
28	PTSb00351.1	bHLH	33.20	5.36	31.90	4.90	0.030	36.20	8.36	30.20	3.18	0.0001	34.20	2.83	33.10	5.44	0.041	34.40	7.03	34.30	6.75	0.8252	1.37	36.25	0.16	1.22
29	PTSb00352.1	bHLH	33.00	5.16	31.90	4.90	0.013	34.10	6.26	28.10	1.08	0.0001	38.28	6.91	34.10	6.44	0.024	37.60	10.23	35.40	7.85	0.0057	1.19	36.26	1.39	5.22
30	PTSb00353.1	bHLH	33.80	5.96	29.90	2.90	0.026	35.20	7.36	30.10	3.08	0.0001	28.90	-2.47	26.10	-1.57	0.064	27.60	0.23	25.10	-2.46	0.0006	0.09	15.60	0.56	5.60
31	PTSb00354.1	bHLH	31.90	4.06	40.00	13.00	0.000	30.10	2.26	40.00	12.98	0.0000	30.10	-1.27	40.00	12.34	0.000	31.30	3.93	40.00	12.45	0.0000	1.00	1.00	1.00	1.00
32	PTSb00355.1	bHLH	40.00	12.16	40.00	13.00	0.033	40.00	12.16	40.00	12.98	0.4122	40.00	8.63	40.00	12.34	0.000	40.00	12.63	40.00	12.45	0.5298	1.00	1.00	1.00	1.00
33	PTSb00356.1	bHLH	40.00	12.16	40.00	13.00	0.033	40.00	12.16	40.00	12.98	0.4122	40.00	8.63	40.00	12.34	0.000	40.00	12.63	40.00	12.45	0.5298	1.00	1.00	1.00	1.00
34	PTSb00357.1	bHLH	40.00	12.16	40.00	13.00	0.033	40.00	12.16	40.00	12.98	0.4122	40.00	8.63	40.00	12.34	0.000	40.00	12.63	40.00	12.45	0.5298	1.00	1.00	1.00	1.00
35	PTSb00358.1	bHLH	32.00	4.16	31.10	4.10	0.031	34.30	6.46	29.10	2.08	0.0012	30.10	-1.27	31.30	3.64	0.000	32.20	4.83	32.20	4.65	0.2852	1.04	20.37	0.03	1.14
36	PTSb00359.1	bHLH	30.64	2.80	32.90	5.90	0.004	30.10	2.26	27.80	0.78	0.0177	33.86	2.49	28.80	1.14	0.037	34.40	7.03	27.80	0.25	0.0004	0.15	2.45	2.27	60.36
37	PTSb00360.1	bHLH	34.42	6.58	33.90	6.90	0.216	32.10	4.26	30.10	3.08	0.0028	32.80	1.43	33.20	5.54	0.001	33.30	5.93	35.40	7.85	0.0479	0.81	2.14	0.07	0.29
38	PTSb00361.1	bHLH	40.00	12.16	39.90	12.90	0.047	40.00	12.16	37.80	10.78	0.0045	40.00	8.63	40.00	12.34	0.000	40.00	12.63	40.00	12.45	0.5298	1.00	1.00	1.00	1.00
39	PTSb00363.1	bHLH	40.00	12.16	39.90	12.90	0.196	40.00	12.16	38.10	11.08	0.0082	40.00	8.63	40.00	12.34	0.000	40.00	12.63	40.00	12.45	0.5298	0.65	1.89	0.11	1.11
40	PTSb00364.1	bHLH	32.44	4.60	31.70	4.70	0.117	28.90	1.06	29.90	2.88	0.9925	33.81	-0.56	30.10	2.44	0.004	26.70	-0.67	31.20	3.65	0.0005	0.94	0.32	0.15	0.07
41	PTSb00365.1	bHLH	40.00	12.16	40.00	13.00	0.033	40.00	12.16	40.00	12.98	0.4122	40.00	8.63	40.00	12.34	0.000	40.00	12.63	40.00	12.45	0.5298	1.00	1.00	1.00	1.00
42	PTSb00366.1	bHLH	40.00	12.16	40.00	13.00	0.033	33.00	5.16	40.00	12.98	0.0000	33.14	1.77	40.00	12.34	0.000	34.40	7.03	40.00	12.45	0.0001	1.00	1.00	1.00	1.00
43	PTSb00367.1	bHLH	39.90	12.06	40.00	13.00	0.048	40.00	12.16	40.00	12.98	0.4122	30.10	-1.27	40.00	12.34	0.000	31.10	3.73	40.00	12.45	0.0006	1.00	1.00	1.00	1.00
44	PTSb00368.1	bHLH	40.00	12.16	40.00	13.00	0.033	40.00	12.16	40.00	12.98	0.4122	40.00	8.63	40.00	12.34	0.000	40.00	12.63	40.00	12.45	0.5298	1.00	1.00	1.00	1.00
45	PTSb00369.1	bHLH	40.00	12.16	40.00	13.00	0.033	40.00	12.16	40.00	12.98	0.4122	40.00	8.63	40.00	12.3										

46	PTSb00370.1	bHLH	30.72	2.88	32.00	5.00	0.971	32.10	4.26	31.10	4.08	0.0446	28.70	-2.67	27.80	0.14	0.010	28.70	1.33	25.40	-2.16	0.0050	0.23	1.13	0.15	11.08
47	PTSb00371.1	bHLH	40.00	11.04	37.00	10.00	0.001	38.00	9.04	31.20	4.18	0.0001	40.00	9.15	38.90	14.19	0.000	40.00	12.63	37.80	10.25	0.0010	1.84	17.45	0.05	4.06
48	PTSb00372.1	bHLH	32.52	3.56	33.70	6.70	0.222	32.10	3.14	30.10	3.08	0.0640	33.52	2.67	30.10	5.39	0.004	31.10	3.73	31.20	3.65	0.4878	0.11	1.05	0.15	1.06
49	PTSb00373.1	bZIP	31.80	2.84	31.90	4.90	0.891	32.30	3.34	29.90	2.88	0.0531	33.10	2.25	27.70	2.99	0.427	32.20	4.83	28.80	1.25	0.0200	0.30	1.31	0.65	8.23
50	PTSb00374.1	bZIP	34.70	5.74	31.00	4.00	0.005	33.20	4.24	28.70	1.68	0.0023	34.80	3.95	25.60	0.89	0.002	34.40	7.03	27.00	-0.56	0.0300	2.78	4.52	6.04	86.35
51	PTSb00375.1	bZIP	36.70	7.74	35.60	8.60	0.030	37.10	8.14	33.20	6.18	0.0039	30.10	-0.75	34.50	9.79	0.000	32.20	4.83	36.10	8.55	0.0078	0.58	3.42	0.00	0.10
52	PTSb00376.1	bZIP	35.90	6.94	33.00	6.00	0.816	34.30	5.34	38.10	11.08	0.0506	28.90	-1.96	34.50	9.79	0.000	30.10	2.73	35.40	7.85	0.0353	1.71	0.04	0.00	0.05
53	PTSb00378.1	bZIP	37.90	8.94	35.00	8.00	0.090	35.70	6.74	33.20	6.18	0.3848	32.10	1.25	33.40	8.69	0.000	33.20	5.83	34.40	6.85	0.2745	1.81	1.43	0.01	0.53
54	PTSb00379.1	bZIP	38.00	9.04	37.00	10.00	0.169	37.90	8.94	38.20	11.18	0.3001	33.90	3.05	35.80	11.09	0.000	34.40	7.03	36.00	8.45	0.2817	0.57	0.27	0.01	0.44
55	PTSb00381.1	bZIP	36.80	7.84	33.70	6.70	0.228	39.80	10.84	33.40	6.38	0.0035	38.70	7.85	35.50	10.79	0.000	37.70	10.33	36.10	8.55	0.0020	2.05	16.75	0.16	3.09
56	PTSb00382.1	bZIP	35.30	6.34	36.80	9.80	0.000	35.70	6.74	35.40	8.38	0.3071	36.10	5.25	30.10	5.39	0.211	33.10	5.73	28.20	0.65	0.0103	0.09	0.32	0.91	33.94
57	PTSb00383.1	bZIP	37.40	8.44	34.90	7.90	0.081	38.80	9.84	35.40	8.38	0.0356	37.10	6.25	32.20	7.49	0.010	35.30	7.93	32.10	4.55	0.0230	1.45	2.76	0.42	10.45
58	PTSb00385.1	bZIP	32.20	3.24	29.90	2.90	0.026	33.10	4.14	30.10	3.08	0.0452	31.20	0.34	30.10	5.39	0.000	32.10	4.73	31.10	3.55	0.0410	1.24	1.96	0.04	2.11
59	PTSb00386.1	bZIP	35.60	6.64	34.50	7.50	0.175	37.70	8.74	36.10	9.08	0.1506	35.80	4.95	33.20	8.49	0.000	36.30	8.93	34.00	6.45	0.0320	0.58	0.81	0.10	4.93
60	PTSb00387.1	bZIP	31.10	2.14	28.90	1.90	0.616	32.10	3.14	27.90	0.88	0.0030	30.10	-0.75	29.30	4.59	0.000	31.20	3.83	28.10	0.55	0.0032	1.18	4.81	0.02	9.75
61	PTSb00388.1	bZIP	35.70	6.74	33.00	6.00	0.016	35.90	6.94	34.90	7.88	0.0070	33.10	2.25	31.90	7.19	0.000	34.30	6.93	33.10	5.55	0.0400	1.60	0.55	0.04	2.40
62	PTSb00389.1	bZIP	35.50	6.54	34.20	7.20	0.438	36.20	7.24	36.80	9.78	0.0784	35.50	4.65	33.20	8.49	0.005	36.70	9.33	32.70	5.15	0.0200	0.66	0.20	0.09	13.73
63	PTSb00390.1	bZIP	32.90	3.94	31.40	4.40	0.825	33.60	4.64	34.80	7.78	0.6176	32.90	2.05	30.10	5.39	0.001	33.30	5.93	29.10	1.55	0.0100	0.73	0.11	0.10	20.89
64	PTSb00391.1	bZIP	39.90	10.94	40.00	13.00	0.655	40.00	11.04	40.00	12.98	0.0010	37.52	6.67	40.00	15.29	0.000	40.00	12.63	40.00	12.45	0.6597	1.00	1.00	1.00	1.00
65	PTSb00392.1	bZIP	34.90	5.94	32.10	5.10	0.770	35.10	6.14	38.90	11.88	0.0102	35.34	4.49	30.10	5.39	0.045	33.30	5.93	31.10	3.55	0.0034	1.79	0.02	0.54	5.20
66	PTSb00393.1	bZIP	34.68	5.72	31.90	4.90	0.642	34.10	5.14	32.80	5.78	0.0030	34.37	3.52	32.10	7.39	0.004	35.30	7.93	32.00	4.45	0.0041	1.81	0.63	0.06	12.40
67	PTSb00394.1	bZIP	35.74	6.78	35.80	8.80	0.190	36.70	7.74	36.70	9.68	0.6000	36.70	5.85	34.60	9.89	0.000	36.10	8.73	34.40	6.85	0.0030	0.25	0.27	0.06	3.63
68	PTSb00395.1	bZIP	40.00	11.04	38.00	11.00	0.016	40.00	11.04	37.10	10.08	0.0054	40.00	9.15	40.00	15.29	0.000	40.00	12.63	40.00	12.45	0.6597	1.03	1.84	0.02	1.12
69	PTSb00396.1	bZIP	32.00	3.04	29.90	2.90	0.162	34.70	5.74	33.10	6.08	0.2835	35.88	5.03	35.10	10.39	0.000	35.10	7.73	36.30	8.75	0.4033	1.10	0.80	0.03	0.50
70	PTSb00397.1	bZIP	33.00	4.04	34.20	7.20	0.005	40.00	11.04	35.30	8.28	0.0016	40.00	9.15	40.00	15.29	0.000	40.00	12.63	40.00	12.45	0.6597	0.14	5.73	1.00	1.00
71	PTSb00398.1	bZIP	33.70	4.74	35.00	8.00	0.437	33.90	4.94	29.80	2.78	0.0166	38.90	8.05	30.10	5.39	0.009	37.30	9.93	31.10	3.55	0.0308	0.10	4.49	6.32	83.67
72	PTSb00127.1	HAP2	40.00	11.04	35.90	8.90	0.003	40.00	11.04	35.80	8.78	0.0018	40.00	9.15	40.00	15.29	0.000	40.00	12.63	40.00	12.45	0.6597	3.95	4.28	0.02	1.13
73	PTSb00128.1	HAP2	37.00	8.04	34.90	7.90	0.009	38.20	9.24	32.10	5.08	0.0021	37.95	12.10	37.80	13.09	0.096	35.50	8.13	35.30	7.75	0.8700	1.10	17.94	0.50	1.31
74	PTSb00129.1	HAP2	40.00	11.04	37.70	10.70	0.003	40.00	11.04	32.10	5.08	0.0000	40.00	9.15	40.00	15.29	0.000	40.00	12.63	40.00	12.45	0.6597	1.27	62.47	0.01	1.14
75	PTSb00179.1	HMG	33.00	4.04	27.80	0.80	0.003	36.30	7.34	28.90	1.88	0.0017	36.60	5.75	27.30	2.59	0.000	32.00	4.63	28.90	1.35	0.0012	9.30	42.98	8.80	9.59
76	PTSb00180.1	HMG	35.90	6.94	34.00	7.00	0.144	35.10	6.14	30.90	3.88	0.0061	36.69	5.84	28.80	4.09	0.015	35.20	7.83	31.00	3.45	0.0237	0.96	4.81	3.36	20.89
77	PTSb00181.1	HMG	32.24	3.28	33.20	6.20	0.256	32.00	3.04	34.70	7.68	0.0024	34.83	3.98	30.10	5.39	0.001	35.10	7.73	29.10	1.55	0.0342	0.13	0.04	0.38	72.76
78	PTSb00182.1	HMG	37.80	8.84	34.70	7.70	0.012	40.00	11.04	32.30	5.28	0.0000	40.00	9.15	40.00	15.29	0.000	40.00	12.63	40.00	12.45	0.0010	2.08	40.46	0.02	1.13
79	PTSb00183.1	HMG	31.43	2.47	31.10	4.10	0.075	32.10	3.14	38.30	11.28	0.0002	32.12	1.27	29.90	5.19	0.000	33.10	5.73	30.10	2.55	0.0020	0.36	0.01	0.08	7.47
80	PTSb00184.1	HMG	32.38	3.42	33.70	6.70	0.433	33.20	4.24	31.20	4.18	0.1230	35.18	4.33	30.10	5.39	0.001	36.10	8.73	31.10	3.55	0.0001	0.11	1.05	0.48	34.53
81	PTSb00185.1	HMG	35.70	6.74	30.90	3.90	0.040	35.00	6.04	31.80	4.78	0.0238	37.26	6.41	33.10	2.39	0.001	37.30	9.93	33.10	5.55	0.0004	5.14	2.07	10.16	12.53
82	PTSb00186.1	HMG	31.90	2.94	30.10	3.10	0.931	32.10	3.14	31.30	4.28	0.3164	29.80	-1.06	29.10	4.39	0.000	31.10	3.73	29.80	2.25	0.4781	0.91	0.51	0.04	2.39
83	PTSb00187.1	HMG	34.98	6.02	32.70	5.70	0.558	35.10	6.14	34.40	7.38	0.0090	32.70	1.85	30.20	5.49	0.001	33.10	5.73	31.10	3.55	0.0005	1.26	0.46	0.11	3.81
84	PTSb00188.1	HMG	35.90	6.94	33.10	6.10	0.578	36.00	7.04	32.10	5.08	0.0021	38.70	7.85	33.10	8.39	0.059	37.10	9.73	32.10	4.55	0.0040	1.78	3.53	0.74	27.23
85	PTSb00189.1	HMG	40.00	11.04	40.00	13.00	0.751	40.00	11.04	40.00	12.98	0.0010	40.00	9.15	40.00	15.29	0.000	40.00	12.63	40.00	12.45	0.6597	1.00	1.00	1.00	1.00
86	PTSb00190.1	HMG	40.00	11.04	40.00	13.00	0.688	40.00	11.04	40.00	12.98	0.0010	40.00	9.15	40.00	15.29	0.000	40.00	12.63	40.00	12.45	0.6597	1.00	1.00	1.00	1.00
87	PTSb00191.1	HMG	33.07	4.11	33.80	6.80	0.546	34.30	5.34	36.70	9.68	0.0017	34.32	3.47	30.10	5.39	0.009	35.10	7.73	31.10	3.55	0.0020	0.21	0.08	0.33	11.53
88	PTSb00192.1	HMG	33.80	4.84	32.80	5.80	0.162	33.90	4.94	30.10	3.08	0.0651	34.29	3.44	31.10	6.39	0.012	33.30	5.93	30.10	2.55	0.0300	0.57	3.19	0.16	8.07
89	PTSb00130.1	HAP5	36.12	7.16	31.10	4.10	0.002	37.10	8.14	33.40	6.38	0.0047	34.79	3.94	33.20	8.49	0.001	35.50	8.13	31.10	3.55	0.0030	7.93	3.42	0.04	25.17
90	PTSb00131.1	HAP5	35.80	6.84	32.26	5.26	0.020	36.10	7.14	34.40	7.38	0.1506	35.70	4.85	30.20	5.49	0.007	31.10	3.73	29.90	2.35	0.0002	2.71	0.86	0.67	2.40
91	PTSb00132.1	HAP5	38.80	9.84	34.90	7.90	0.001	37.90	8.94	37.80	10.78	0.0526	33.50	2.65	36.70	11.99	0.000	32.10	4.73	35.10	7.55	0.1240	3.47	0.31	0.00	0.16
92	PTSb00133.1	HAP5	34.82	5.86	32.80	5.80	0.415	35.10	6.14	34.70	7.68	0.0052	31.70	0.84	34.60	9.89	0.000	32.10	4.73	35.50						

95	PTSb00136.1	HAP5	34.60	6.01	34.10	7.10	0.249	33.20	4.61	30.10	3.08	0.0394	37.10	6.27	31.10	0.53	0.000	35.50	4.67	33.30	5.75	0.8354	0.51	2.61	36.34	0.51
96	PTSb00137.1	HAP5	34.80	6.21	34.40	7.40	0.755	37.20	8.61	35.40	8.38	0.1993	35.10	4.27	33.20	8.63	0.004	34.30	3.47	34.40	6.85	0.3279	0.47	1.16	0.06	0.11
97	PTSb00138.1	HAP5	32.00	3.41	27.80	0.80	0.236	37.40	8.81	29.80	2.78	0.0008	39.10	8.27	34.30	9.73	0.604	38.10	7.27	35.40	7.85	0.6322	5.27	46.61	0.39	0.69
98	PTSb00139.1	HAP5	33.00	4.41	30.00	3.00	0.623	33.10	4.51	30.10	3.08	0.4172	32.10	1.27	29.90	5.33	0.002	33.20	2.37	31.30	3.75	0.4705	2.30	2.33	0.09	0.44
99	PTSb00171.1	HB	37.60	9.01	35.00	8.00	0.555	35.10	6.51	32.10	5.08	0.0620	36.30	5.47	35.40	10.83	0.002	35.40	4.57	36.40	8.85	0.2244	1.81	2.32	0.04	0.08
100	PTSb00172.1	HB	34.23	5.64	31.90	4.90	0.972	34.90	6.31	29.90	2.88	0.0044	36.49	5.66	31.80	7.23	0.000	37.10	6.27	32.10	4.55	0.1428	1.59	7.53	0.41	2.69
101	PTSb00173.1	HB	38.90	10.31	37.80	10.80	0.410	37.70	9.11	38.90	11.88	0.0053	35.40	4.57	33.80	9.23	0.002	35.50	4.67	33.00	5.45	0.9382	0.73	0.16	0.05	0.60
102	PTSb00174.1	HB	40.00	11.41	40.00	13.00	0.071	40.00	11.41	40.00	12.98	0.0022	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	1.00	1.00	1.00	1.00
103	PTSb00175.1	HB	40.00	11.41	40.00	13.00	0.719	40.00	11.41	40.00	12.98	0.0022	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	1.00	1.00	1.00	1.00
104	PTSb00176.1	HB	40.00	11.41	40.00	13.00	0.677	40.00	11.41	40.00	12.98	0.0022	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	1.00	1.00	1.00	1.00
105	PTSb00177.1	HB	40.00	11.41	40.00	13.00	0.756	40.00	11.41	40.00	12.98	0.0022	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	1.00	1.00	1.00	1.00
106	PTSb00178.1	HB	40.00	11.41	40.00	13.00	0.788	40.00	11.41	40.00	12.98	0.0022	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	1.00	1.00	1.00	1.00
107	PTSb00249.1	MYB	35.00	6.41	32.90	5.90	0.003	40.00	11.41	31.70	4.68	0.0000	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	1.33	70.22	0.02	0.14
108	PTSb00250.1	MYB	33.54	4.95	32.10	5.10	0.885	26.80	-1.79	29.10	2.08	0.0082	35.77	4.94	32.10	7.53	0.000	27.30	-3.53	28.80	1.25	0.3756	0.91	0.08	0.19	0.05
109	PTSb00251.1	MYB	35.69	7.10	33.48	6.48	0.735	37.20	8.61	35.60	8.58	0.6191	32.80	1.97	33.20	8.63	0.000	32.80	1.97	34.10	6.55	0.2352	1.42	1.02	0.01	0.06
110	PTSb00254.1	MYB	36.10	7.51	33.90	6.90	0.233	27.90	-0.69	27.80	0.78	0.0169	36.50	5.67	35.40	10.83	0.002	26.20	-4.63	26.60	-0.95	0.2499	1.54	0.39	0.04	0.10
111	PTSb00255.1	MYB	34.40	5.81	32.10	5.10	0.705	35.80	7.21	35.10	8.08	0.0433	35.70	4.87	30.10	5.53	0.780	36.10	5.27	31.20	3.65	0.0002	1.58	0.55	0.62	3.09
112	PTSb00256.1	MYB	32.10	3.51	31.90	4.90	0.088	35.00	6.41	29.90	2.88	0.0018	31.20	0.37	32.10	7.53	0.000	33.10	2.27	33.40	5.85	0.3975	0.42	9.31	0.01	0.10
113	PTSb00257.1	MYB	38.90	10.31	37.90	10.90	0.055	37.10	8.51	34.70	7.68	0.0774	36.01	5.18	36.70	12.13	0.006	35.10	4.27	35.30	7.75	0.3773	0.75	1.63	0.02	0.12
114	PTSb00207.1	MADS	36.90	8.31	37.80	10.80	0.155	35.10	6.51	37.90	10.88	0.0004	34.70	3.87	34.30	9.73	0.000	33.30	2.47	35.10	7.55	0.1497	0.22	0.07	0.03	0.04
115	PTSb00208.1	MADS	40.00	11.41	37.80	10.80	0.149	40.00	11.41	37.90	10.88	0.2133	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	1.46	1.40	0.02	0.13
116	PTSb00209.1	MADS	34.50	5.91	33.00	6.00	0.891	35.40	6.81	31.90	4.88	0.0258	30.10	-0.73	33.10	8.53	0.000	32.10	1.27	32.10	4.55	0.4223	0.91	3.82	0.00	0.11
117	PTSb00210.1	MADS	35.80	7.21	35.60	8.60	0.016	35.70	7.11	31.80	4.78	0.0634	33.90	3.07	30.30	5.73	0.010	34.10	3.27	32.70	5.15	0.0080	0.38	4.99	0.16	0.28
118	PTSb00211.1	MADS	38.90	10.31	35.90	8.90	0.005	36.30	7.71	36.70	9.68	0.2091	37.80	6.97	35.60	11.03	0.002	35.70	4.87	36.70	9.15	0.3529	2.29	0.32	0.09	0.08
119	PTSb00212.1	MADS	35.40	6.81	33.10	6.10	0.199	35.80	7.21	34.80	7.78	0.8701	33.90	3.07	30.10	5.53	0.190	33.30	2.47	31.10	3.55	0.5598	1.57	0.76	0.22	0.49
120	PTSb00213.1	MADS	40.00	11.41	40.00	13.00	0.833	40.00	11.41	40.00	12.98	0.0022	40.00	9.17	36.60	12.03	0.676	40.00	9.17	37.70	10.15	0.5382	0.36	0.37	0.16	0.53
121	PTSb00214.1	MADS	35.90	7.31	32.20	5.20	0.003	37.20	8.61	35.80	8.78	0.4689	33.10	2.27	31.80	7.23	0.003	32.10	1.27	34.30	6.75	0.1541	3.42	0.91	0.06	0.04
122	PTSb00217.1	MADS	35.10	6.51	33.00	6.00	0.052	38.60	10.01	33.30	6.28	0.0078	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	1.39	10.99	0.02	0.12
123	PTSb00218.1	MADS	32.00	3.41	29.90	2.90	0.003	32.20	3.61	29.80	2.78	0.0077	36.46	5.63	25.00	0.43	0.000	33.90	3.07	25.00	-2.56	0.0025	1.38	1.69	26.10	25.50
124	PTSb00219.1	MADS	36.89	8.30	34.40	7.40	0.953	33.80	5.21	35.20	8.18	0.0145	40.00	9.17	33.10	8.53	0.040	40.00	9.17	34.30	6.75	0.0774	1.69	0.18	1.45	4.10
125	PTSb00220.1	MADS	37.90	9.31	34.30	7.30	0.006	28.90	0.31	26.10	-0.92	0.6027	34.99	4.16	34.20	9.63	0.000	28.20	-2.63	35.10	7.55	0.0118	3.23	2.05	0.04	0.00
126	PTSb00221.1	MADS	37.41	8.82	36.70	9.70	0.566	36.80	8.21	33.10	6.08	0.0510	35.10	4.27	35.30	10.73	0.000	37.10	6.27	36.30	8.75	0.7027	0.57	3.89	0.02	0.21
127	PTSb00222.1	MADS	35.00	6.41	32.80	5.80	0.323	35.10	6.51	28.80	1.78	0.0004	36.70	5.87	34.60	10.03	0.000	34.10	3.27	35.10	7.55	0.2034	1.53	26.63	0.06	0.05
128	PTSb00223.1	MADS	40.00	11.41	40.00	13.00	0.081	40.00	11.41	40.00	12.98	0.0022	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	1.00	1.00	1.00	1.00
129	PTSb00222.1	MADS	40.00	11.41	40.00	13.00	0.075	40.00	11.41	40.00	12.98	0.0022	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	0.39	0.40	0.03	0.15
130	PTSb00224.1	MYB-related	40.00	11.41	40.00	13.00	0.071	40.00	11.41	40.00	12.98	0.0022	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	1.00	1.00	1.00	1.00
131	PTSb00225.1	MYB-related	40.00	11.41	40.00	13.00	0.788	40.00	11.41	40.00	12.98	0.0022	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	1.00	1.00	1.00	1.00
132	PTSb00226.1	MYB-related	40.00	11.41	39.80	12.80	0.455	40.00	11.41	39.80	12.78	0.4101	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	0.41	0.42	0.02	0.12
133	PTSb00227.1	MYB-related	35.80	7.21	39.90	12.90	0.006	34.10	5.51	40.00	12.98	0.0000	36.18	5.35	30.10	5.53	0.287	32.20	1.37	31.10	3.55	0.8575	1.00	1.00	1.00	1.00
134	PTSb00228.1	MYB-related	40.00	11.41	40.00	13.00	0.875	40.00	11.41	40.00	12.98	0.0022	40.00	9.17	40.00	15.43	0.000	40.00	9.17	40.00	12.45	0.3529	1.00	1.00	1.00	1.00
135	PTSb00229.1	MYB-related	36.70	8.11	33.90	6.90	0.846	35.10	6.51	33.50	6.48	0.0634	36.32	5.49	32.20	7.63	0.000	33.20	2.37	34.30	6.75	0.3483	2.09	1.02	0.28	0.07
136	PTSb00232.1	MYB-related	37.80	9.21	38.80	11.80	0.004	37.00	8.41	37.90	10.88	0.0072	35.34	4.51	23.20	-1.37	0.000	31.10	0.27	25.50	-2.06	0.0030	0.22	0.23	31.70	3.92

137	PTSb00233.1	MYB-related MYB-	39.80	11.21	37.70	10.70	0.374	36.20	7.61	38.20	11.18	0.0011	33.67	2.84	33.70	9.13	0.000	33.67	2.84	35.50	7.95	0.0894	1.35	0.12	0.02	0.05
138	PTSb00234.1	related	38.70	10.11	34.80	7.80	0.010	36.90	8.31	36.10	9.08	0.4996	37.80	6.97	36.10	11.53	0.000	35.10	4.27	38.10	10.55	0.0838	3.89	0.64	0.07	0.03
139	PTSb00258.1	NAC	37.70	9.55	38.90	11.90	0.954	30.10	1.95	28.90	1.88	0.3585	37.60	6.10	31.10	5.74	0.196	28.10	-3.41	27.10	-0.45	0.8642	0.20	1.05	1.28	0.13
140	PTSb00259.1	NAC	35.10	6.95	34.80	7.80	0.317	36.80	8.65	29.10	2.08	0.0001	36.50	5.00	35.60	10.24	0.000	37.10	5.60	32.20	4.65	0.2510	0.59	61.29	0.04	1.81
141	PTSb00260.1	NAC	37.60	9.45	37.80	10.80	0.295	38.60	10.45	38.70	11.68	0.5313	35.20	3.70	33.20	7.84	0.001	34.20	2.70	35.50	7.95	0.2294	0.42	0.45	0.07	0.03
142	PTSb00261.1	NAC	33.80	5.65	33.30	6.30	0.474	35.10	6.95	34.30	7.28	0.7219	36.10	4.60	30.10	4.74	0.923	35.10	3.60	31.20	3.65	0.1404	0.68	0.83	0.92	0.97
143	PTSb00262.1	NAC	34.70	6.55	32.90	5.90	0.909	28.50	0.35	27.10	0.08	0.1247	34.84	3.34	33.10	7.74	0.000	24.10	-7.41	29.20	1.65	0.0217	1.51	1.19	0.06	0.00
144	PTSb00263.1	NAC	31.80	3.65	29.90	2.90	0.557	33.10	4.95	30.10	3.08	0.4179	33.10	1.60	29.90	4.54	0.095	34.10	2.60	31.10	3.55	0.3364	1.56	3.05	0.17	0.57
145	PTSb00264.1	NAC	32.00	3.85	31.90	4.90	0.423	34.30	6.15	32.10	5.08	0.1839	32.10	0.60	30.10	4.74	0.001	31.10	-0.40	31.10	3.55	0.2876	0.48	2.12	0.06	0.06
146	PTSb00265.1	NAC	40.00	11.85	40.00	13.00	0.065	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
147	PTSb00266.1	NAC	40.00	11.85	40.00	13.00	0.592	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
148	PTSb00267.1	NAC	40.00	11.85	40.00	13.00	0.749	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
149	PTSb00268.1	NAC	40.00	11.85	40.00	13.00	0.677	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
150	PTSb00269.1	NAC	40.00	11.85	40.00	13.00	0.650	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
151	PTSb00270.1	NAC	40.00	11.85	40.00	13.00	0.666	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
152	PTSb00271.1	NAC	40.00	11.85	40.00	13.00	0.065	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
153	PTSb00272.1	NAC	40.00	11.85	38.00	11.00	0.242	40.00	11.85	33.20	6.18	0.0002	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.71	35.96	0.02	0.08
154	PTSb00273.1	NAC	35.20	7.05	34.80	7.80	0.386	33.20	5.05	31.30	4.28	0.3256	35.50	4.00	32.10	6.74	0.001	30.10	-1.41	34.30	6.75	0.0198	0.61	1.67	0.16	0.00
155	PTSb00274.1	NAC	36.10	7.95	33.80	6.80	0.557	35.30	7.15	32.70	5.68	0.0448	34.09	2.59	33.90	8.54	0.000	35.10	3.60	35.50	7.95	0.2407	1.99	2.56	0.02	0.07
156	PTSb00275.1	NAC	34.00	5.85	33.30	6.30	0.346	34.30	6.15	34.80	7.78	0.2850	34.35	2.85	34.10	8.74	0.000	34.70	3.20	32.10	4.55	0.8757	0.79	0.38	0.03	0.43
157	PTSb00276.1	NAC	34.90	6.75	37.80	10.80	0.003	35.90	7.75	38.30	11.28	0.0079	34.87	3.37	31.30	5.94	0.000	33.10	1.60	35.40	7.85	0.0927	0.08	0.11	0.21	0.02
158	PTSb00281.1	PHD	40.00	11.85	40.00	13.00	0.065	40.00	11.85	40.00	12.98	0.0060	35.23	3.73	40.00	14.64	0.000	36.60	5.10	40.00	12.45	0.0328	0.91	0.91	0.39	0.53
159	PTSb00282.1	PHD	40.00	11.85	40.00	13.00	0.065	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
160	PTSb00283.1	PHD	40.00	11.85	40.00	13.00	0.846	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
161	PTSb00284.1	PHD	40.00	11.85	40.00	13.00	0.702	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
162	PTSb00285.1	PHD	40.00	11.85	40.00	13.00	0.570	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
163	PTSb00286.1	PHD	40.00	11.85	40.00	13.00	0.570	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
164	PTSb00287.1	PHD	40.00	11.85	40.00	13.00	0.167	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
165	PTSb00288.1	PHD	40.00	11.85	40.00	13.00	0.749	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
166	PTSb00289.1	PHD	38.10	9.95	35.70	8.70	0.001	37.30	9.15	36.70	9.68	0.8763	34.40	2.90	33.20	7.84	0.000	35.70	4.20	31.10	3.55	0.3807	2.25	0.70	0.03	1.59
167	PTSb00290.1	PHD	31.90	3.75	34.20	7.20	0.083	33.40	5.25	34.20	7.18	0.0402	34.30	2.80	30.10	4.74	0.009	35.10	3.60	30.10	2.55	0.1606	0.11	0.29	0.28	2.00
168	PTSb00291.1	PHD	32.10	3.95	31.10	4.10	0.732	32.40	4.25	33.20	6.18	0.0021	35.30	3.80	31.10	5.74	0.024	33.50	2.00	32.20	4.65	0.8162	0.90	0.30	0.29	0.19
169	PTSb00292.1	PHD	31.90	3.75	31.10	4.10	0.103	35.30	7.15	29.90	2.88	0.0036	35.46	3.96	31.10	5.74	0.016	34.30	2.80	29.80	2.25	0.1494	0.84	12.94	0.36	1.36
170	PTSb00293.1	PHD	40.00	11.85	39.20	12.20	0.918	40.00	11.85	38.20	11.18	0.1181	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	0.82	1.53	0.02	0.08
171	PTSb00294.1	PHD	35.30	7.15	34.80	7.80	0.250	36.40	8.25	30.70	3.68	0.0030	35.70	4.20	30.30	4.94	0.026	36.10	4.60	31.10	3.55	0.1437	0.64	23.29	0.60	2.06
172	PTSb00295.1	PHD	40.00	11.85	40.00	13.00	0.065	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
173	PTSb00333.1	WIRLY	40.00	11.85	40.00	13.00	0.077	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
174	PTSb00334.1	WIRLY	32.10	3.95	33.20	6.20	0.683	34.30	6.15	34.30	7.28	0.3065	29.10	-2.41	30.10	4.74	0.000	30.10	-1.41	33.30	5.75	0.0936	0.24	0.49	0.01	0.01
175	PTSb00326.1	WRKY	32.80	4.65	30.90	3.90	0.492	33.30	5.15	35.30	8.28	0.0048	27.10	-4.41	30.10	4.74	0.000	27.00	-4.51	31.10	3.55	0.0281	1.62	0.15	0.00	0.01
176	PTSb00327.1	WRKY	40.00	11.85	40.00	13.00	0.594	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
177	PTSb00328.1	WRKY	35.40	7.25	34.80	7.80	0.300	37.20	9.05	29.90	2.88	0.0002	35.30	3.80	22.10	-3.26	0.000	35.30	3.80	25.20	-2.36	0.0101	0.71	49.68	86.67	48.90
178	PTSb00329.1	WRKY	40.00	11.85	40.00	13.00	0.666	40.00	11.85	40.00	12.98	0.0060	40.00	8.50	40.00	14.64	0.000	40.00	8.50	40.00	12.45	0.3352	1.00	1.00	1.00	1.00
179	PTSb00330.1	WRKY	33.80	5.65	39.80	12.80	0.001	36.10	7.95	39.80	12.78	0.0002	33.30	1.80	30.10	4.74	0.001	32.10	0.60	34.40	6.85	0.1817	0.01	0.04	0.15	0.02
180	PTSb00331.1	WRKY	33.20	5.05	28.20	1.20	0.002	37.80	9.65	32.10	5.08	0.0015	33.00	1.50	20.10	-5.26	0.001	34.10	2.60	21.10	-6.46	0.0014	8.86	14.55	51.00	182.05
181	PTSb00086.1	YABBY	35.80	7.65	33.90	6.90	0.256	36.80	8.65	34.10	7.08	0.1065	30.10	-1.41	25.70	0.34	0.344	30.00	-1.51	29.20	1.65	0.9748	1.54	2.70	0.33	0.14
182	PTSb00087.1	YABBY	31.80	3.65	31.90	4.90	0.055	32.10	3.95	32.10	5.08	0.0173	29.10	-2.41	30.10	4.74	0.000	28.10	-3.41	31.10	3.55	0.0610	0.47	0.50	0.01	0.01
183	PTSb00088.1																									

184	PTSb00223.1	MBF	37.70	9.55	37.80	10.80	0.639	28.90	0.75	31.10	4.08	0.0017	34.80	3.30	31.70	6.34	0.021	27.10	-4.41	28.30	0.75	0.1594	0.43	0.10	0.13	0.03
CS ₁ _Ct – Normalized Ct values for mock inoculated (control) SPV 86 at 75 DAS											CE ₁ _Ct – Normalized Ct values for mock inoculated (control) E 36-1 at 75 DAS															
E ₁ /CE ₁ – Fold change expression in E 36-1 at 75 DAS											S ₁ _Ct – Normalized Ct values for inoculated (treated) SPV 86 at 75 DAS															
E ₁ _Ct – Normalized Ct values for inoculated (treated) SPV 86 at 75 DAS											S ₂ /CS ₂ – Fold change expression in SPV-86 at 95 DAS															
CS ₂ _Ct – Normalized Ct values for mock inoculated (control) SPV 86 at 95 DAS											CE ₂ _Ct – Normalized Ct values for mock inoculated (control) E 36-1 at 95 DAS															
S ₁ /CS ₁ – Fold change expression in SPV-86 at 75 DAS											S ₂ _Ct – Normalized Ct values for inoculated (treated) SPV 86 at 95 DAS															
E ₂ _Ct – Normalized Ct values for inoculate (treated) E 36-1 at 95 DAS											E ₂ /CE ₂ – Fold change expression in E 36-1 at 95 DAS															
ΔCt = Ct of target gene - Ct of reference gene																										

Appendix VI: List of Transcription FaCtor (TF) genes used in qRT-PCR

Sl. No.	Database ID	Assay ID	5' Primers Sequence 3'	Tm	Product size
1	PTSb00127.1	H001_F	TTTGAAACCGGACTCGTAGC	60.2 °C	139 bp
		H001_R	CATATGGCATTGGACACCTG	59.8 °C	
2	PTSb00128.1	H002_F	AGCTAATCAGGCAGGCAGTC	59.6 °C	140 bp
		H002_R	ACAACAACCGACTGACACCA	60.0 °C	
3	PTSb00129.1	H003_F	TGTGTTCAATCTCGGAGTGC	59.8 °C	128 bp
		H003_R	CTCGCAGGAGGAGTCAAGTC	60.1 °C	
4	PTSb00249.1	M004_F	GGTTGAACACAGCAAAGCAG	59.5 °C	105 bp
		M004_R	AGATGGCTCAAGTTGCCAAG	60.4 °C	
5	PTSb00250.1	M005_F	TGCTGAGCGTAAATGGACTG	60.0 °C	141 bp
		M005_R	GGCTTCATTCTGCTCCAAAG	59.8 °C	
6	PTSb00251.1	M006_F	AGGATGAAACACTGCGGAAG	60.3 °C	106 bp
		M006_R	CAGCGATGCAAACATTGAAC	60.3 °C	
7	PTSb00254.1	M007_F	AACGAGAATGTGTGCAGCAG	60.1 °C	110 bp
		M007_R	TGTGGTGCCATCTTGATCTC	59.6 °C	
8	PTSb00255.1	M008_F	AAGTACGGAATCCGCAAATG	60.0 °C	105 bp
		M008_R	GTGGTTGTGCCATCTTTCAC	59.0 °C	
9	PTSb00256.1	M009_F	ACGGACAAAGTTGGGCTATG	60.0 °C	126 bp
		M009_R	CATCTTCTTCAGGGCTCCAG	59.9 °C	
10	PTSb00257.1	M010_F	AGCAGCATCAACACCAACAC	59.8 °C	102 bp
		M010_R	GAAACTTGGAGAAGGCAACG	59.9 °C	
11	PTSb00010.1	A011_F	AGTGAAGTTGAAGCGGCAAG	60.6 °C	134 bp
		A011_R	ATTGCCTCAGTTTCGGTGTC	60.1 °C	
12	PTSb00011.1	A012_F	GCAAGAAAGCCAAGGTGAAC	59.9 °C	102 bp
		A012_R	TCTCCTGGGATGCAATCTTC	60.2 °C	
13	PTSb00012.1	A013_F	CAAGAAGAAAGGCAGGCAAG	60.1 °C	130 bp
		A013_R	GTAATCCTCCACAGGGAAGTTG	59.9 °C	
14	PTSb00013.1	A014_F	TAGGGCATACGATGCTGAAG	58.9 °C	104 bp
		A014_R	CAGTTGGCTTTGCAGTTGAC	59.5 °C	
15	PTSb00014.1	A015_F	TCCATCGGAGGTTATGAAGC	60.0 °C	142 bp
		A015_R	TAATCACCAGGGGGAAG	59.0 °C	
16	PTSb00015.1	A016_F	TGCTGCTGCTATGGTTTCTG	60.2 °C	126 bp
		A016_R	TCCATCCCGAACAGATAGTTG	59.9 °C	
17	PTSb00016.1	A017_F	TGCTGGCATCTAGTGACCG	59.9 °C	133 bp
		A017_R	ACGTGGACACTTCCCAAGTC	60.0 °C	
18	PTSb00017.1	A018_F	GCCCATCAAGTTTCTTCGAG	59.8 °C	125 bp
		A018_R	TCAGTTCTGGCAGTGTTTGC	60.0 °C	
19	PTSb00018.1	A019_F	GCCAAATGCCATTATTCC	60.1 °C	120 bp
		A019_R	ATTCTCCCATGCCAAGTCTG	60.1 °C	
20	PTSb00019.1	A020_F	AGTCCTGAGGAAGCTGCAAG	59.7 °C	135 bp
		A020_R	GGGTGCTTTAGCAGAAGCTG	60.2 °C	

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Sl. No.	Database ID	Assay ID	5' Primers Sequence 3'	Tm	Product size
21	PTSb00020.1	A021_F	TTATCAAGCCGGCAGTCAAC	61.2 °C	147 bp
		A021_R	AGGTTCATGAATGGGAGCAG	60.1 °C	
22	PTSb00021.1	A022_F	TGACTCGCAACACGATAACC	59.7 °C	111 bp
		A022_R	CATTGGCCATCTCAGTATCG	59.1 °C	
23	PTSb00022.1	A023_F	TGACCGAAGCAACTATGTCTG	59.9 °C	134 bp
		A023_R	ATGTCTGGCCAAATCTGCTC	60.2 °C	
24	PTSb00023.1	A024_F	TCTCCGGCTGAGGAGATAGA	60.0 °C	108 bp
		A024_R	ACCCAGTCATCCTCTTGGTG	60.0 °C	
25	PTSb00024.1	A025_F	TCAGAGATCCTCGCAAAGGT	60.0 °C	142 bp
		A025_R	ATCGTCGAAACCTCATCTGG	60.1 °C	
26	PTSb00025.1	A026_F	GCCAGTTGAATCCGCTACAT	60.1 °C	127 bp
		A026_R	TTCCAATCATAGACGCACCA	60.1 °C	
27	PTSb00026.1	A027_F	GGGCAGCTGAAATCAGAGAC	60.0 °C	119 bp
		A027_R	TTTCTTTCCACGGATCTTGC	60.2 °C	
28	PTSb00027.1	A028_F	AGCTAGGGCATACGATGCTG	60.4 °C	112 bp
		A028_R	TGAATCAGTTGGCTTTGCAG	60.0 °C	
29	PTSb00028.1	A029_F	GATTATGATGCCCTGTTGC	60.3 °C	128 bp
		A029_R	CAAACACGAACACCTTCTG	59.2 °C	
30	PTSb00324.1	T030_F	TCCCAAATCCTTGACCTCAG	60.0 °C	129 bp
		T030_R	TGGCTATCAACCCCACTTC	59.9 °C	
31	PTSb00179.1	H031_F	ACCCCAATGTGAAGCAAGTC	60.0 °C	110 bp
		H031_R	CGTCATCCATCACTCGTCAC	60.1 °C	
32	PTSb00180.1	H032_F	ACCCCAATGTGAAGCAAGTC	60.0 °C	108 bp
		H032_R	TGAGCTTCTCGGCTTAGAG	59.9 °C	
33	PTSb00181.1	H033_F	TGACGATGGCTCAAAGTCTG	60.0 °C	120 bp
		H033_R	CAAGCCGGTAAGCTAGTTGG	59.9 °C	
34	PTSb00182.1	H034_F	AACAAGTCCTCCGCCAAG	58.8 °C	100 bp
		H034_R	TCCTGAAGTCGTCCATGAAG	58.8 °C	
35	PTSb00183.1	H035_F	CATGCTTTGTCCAACAATGG	60.0 °C	149 bp
		H035_R	ACAAGGAGCGCTATCCGTTA	59.9 °C	
36	PTSb00184.1	H036_F	GATGATGATGAGCAGGATGG	59.0 °C	109 bp
		H036_R	CAGCACATTGGAGGCTAAAC	58.4 °C	
37	PTSb00185.1	H037_F	TGATGTAGCGCTCCTTGTTG	60.0 °C	135 bp
		H037_R	CACAATGTTAGCCGATGACG	60.1 °C	
38	PTSb00186.1	H038_F	CCATTGCTGCCTACAACAAG	59.3 °C	142 bp
		H038_R	ATCGAGGATGCATGTGGAC	59.4 °C	
39	PTSb00187.1	H039_F	ATAACGGATAGCGCTCCTTG	59.3 °C	136 bp
		H039_R	CAATGTTAGCCGATGACCAC	59.0 °C	
40	PTSb00188.1	H040_F	CTACATGTTCTTCGCCAACG	59.3 °C	111 bp
		H040_R	TCGTTAAGAGCCTTCCATCG	60.3 °C	
41	PTSb00189.1	H041_F	TGGACGCCTACAACAACAAG	59.8 °C	117 bp
		H041_R	AACGACAGATGACGCATGAC	59.7 °C	

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Sl. No.	Database ID	Assay ID	5' Primers Sequence 3'	Tm	Product size
42	PTSb00190.1	H042_F	ACCCCAATGTGAAGCAAGTC	60.0 °C	108 bp
		H042_R	TGAGCTTCTCGGGCTTAGAG	59.9 °C	
43	PTSb00191.1	H043_F	AGAAGCACCCCAATGTGAAG	60.1 °C	146 bp
		H043_R	GTAGGCGTCCATCTTCTTGG	59.7 °C	
44	PTSb00192.1	H044_F	GGAAGCAAGCAGTTCAAAGC	60.1 °C	130 bp
		H044_R	CATGAACGGTGTCAATTGCTC	60.1 °C	
45	PTSb00333.1	W045_F	CTGAATGGGAAAGGTGAAGC	59.7 °C	106 bp
		W045_R	ATGGCACACTGTTCAAGCAC	59.8 °C	
46	PTSb00334.1	W046_F	CCAAGGGAGAGTTTGTCTGTC	59.8 °C	146 bp
		W046_R	TCGCCATTCGTACTCTGATG	59.8 °C	
47	PTSb00130.1	H047_F	CCCCATGGGCTACTACTACG	59.5 °C	148 bp
		H047_R	AGTTATTGGGTGGCAACAGG	59.9 °C	
48	PTSb00131.1	H048_F	ACGAGATCATGCCTCAAAGC	60.4 °C	125 bp
		H048_R	ATCTGGCATAGGCCAATCTG	60.1 °C	
49	PTSb00132.1	H049_F	ATGCGGTGAAGGATCGTAAG	60.1 °C	133 bp
		H049_R	ATCGCCTTCAAAGCATCTTC	59.4 °C	
50	PTSb00133.1	H050_F	GGGCAAGATTGCTTTAGCTG	60.0 °C	130 bp
		H050_R	CAAATGGGAAGAACCCACAG	60.3 °C	
51	PTSb00134.1	H051_F	TGGGGAATTCGCTTGTAGTC	60.1 °C	107 bp
		H051_R	TCGAGGAGGAATAGCAGTGG	60.4 °C	
52	PTSb00135.1	H052_F	TGAGAGTGCAGTGAGCGAAG	60.5 °C	107 bp
		H052_R	CAACCTGGTGGTGATGTGAG	60.0 °C	
53	PTSb00136.1	H053_F	CATCGCCCTGTTATCGTTTC	60.5 °C	114 bp
		H053_R	GGAGGCCCATAGATCACAAG	59.5 °C	
54	PTSb00207.1	M054_F	CCAGGATTATCTGAAGTTGAGG	57.8 °C	136 bp
		M054_R	CTTCAGGGATGTCTCTATTGG	57.8 °C	
55	PTSb00208.1	M055_F	GCAAAAGAGGAGCAGATTGG	60.0 °C	107 bp
		M055_R	TGGCTTTCTTGCAAGTTGTG	60.0 °C	
56	PTSb00209.1	M056_F	ATGCCCAGCAATTCTACCAG	60.1 °C	127 bp
		M056_R	AGCTGCTTCAGCTCCTTGAG	60.0 °C	
57	PTSb00210.1	M057_F	ATCCAACAAATCACCGATCC	59.6 °C	101 bp
		M057_R	ATGCCGCACAAGAGAGAGA	60.0 °C	
58	PTSb00211.1	M058_F	ACAGAAGGAAC TTGCGGAGA	60.0 °C	116 bp
		M058_R	GAAGGAGGACGATGATGAGC	59.8 °C	
59	PTSb00212.1	M059_F	GTACAAGAAGGCAAACAGTGACAAC	61.3 °C	119 bp
		M059_R	AGTTCTGCAAGTTAGTGATCTGCTG	61.3 °C	
60	PTSb00213.1	M060_F	CAAGCTCCCCATCACTGTCA	62.3 °C	115 bp
		M060_R	TCTGTTGATGCCTGGGTCAG	62.3 °C	
61	PTSb00214.1	M061_F	CGATGAACATGATGGGAGTG	59.9 °C	121 bp
		M061_R	TGCAGCTGATGGGAGTAGTG	60.0 °C	
62	PTSb00217.1	M062_F	TAGAAGAAAGCTGGAGGAGACC	59.1 °C	146 bp
		M062_R	CTTCCAGGGGATGAAAGAAC	58.6 °C	

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Sl. No.	Database ID	Assay ID	5' Primers Sequence 3'	Tm	Product size
63	PTSb00218.1	M063_F	TCGGACTGGTCATCTTCTCC	60.2 °C	130 bp
		M063_R	AGTTCTGTGTTGGGGTTTGC	60.0 °C	
64	PTSb00219.1	M064_F	AATGTGCTGCGACTGTCTTG	60.1 °C	116 bp
		M064_R	TACCCAATATGCAGGGAAGG	59.8 °C	
65	PTSb00220.1	M065_F	CTGACGACTGCTCCGATACA	60.0 °C	121 bp
		M065_R	CGGAGAGGGCAAGTAATCAG	59.8 °C	
66	PTSb00221.1	M066_F	CAGCAGCTACACGGCAATAA	60.0 °C	141 bp
		M066_R	AGTTCGATCTCATGGCAACC	60.1 °C	
67	PTSb00222.1	-M067_F	GCATCCCGTATCAAGGAAGA	60.0 °C	133 bp
		-M067_R	GAACCTGAGGATGGGCTCTG	59.8 °C	
68	PTSb00029.1	A068_F	GGAAGCATGCCAGCTAACTC	60.0 °C	124 bp
		A068_R	GGACGCAGAATAGCAGAAGG	60.0 °C	
69	PTSb00030.1	A069_F	GAGGGTGACGAATCTCCTGA	60.2 °C	146 bp
		A069_R	GGACGCAGAATAGCAGAAGG	60.0 °C	
70	PTSb00031.1	A070_F	AGTGGAGAAGCCTGCTGGTA	60.0 °C	120 bp
		A070_R	CGGACAGAGAATGAGCAACA	60.0 °C	
71	PTSb00032.1	A071_F	CGAGCCCTTCAGAGTTCATC	60.0 °C	105 bp
		A071_R	CCTCTTCCCCTTCAAACCTC	60.0 °C	
72	PTSb00033.1	A072_F	GAAGCAGCCAGAGGAGAATG	60.1 °C	117 bp
		A072_R	CTGGTGTCAGAGGCTGTCAA	60.0 °C	
73	PTSb00034.1	A073_F	CCTTCGAGGTGAAGATGGAG	59.8 °C	102 bp
		A073_R	TGGCTTGAGCACTGGTTATG	59.9 °C	
74	PTSb00035.1	A074_F	CTCAAAATGGCGTTCCTTA	60.1 °C	113 bp
		A074_R	GGTTCACAGGAGATGGGCTA	60.1 °C	
75	PTSb00036.1	-A075_F	ATGGGATGAACCTGACTTGC	59.9 °C	101 bp
		-A075_R	GAGAAAATGGTCCCAGGTGA	59.9 °C	
76	PTSb00326.1	W076_F	TCCAATGAACACACCTTGA	59.9 °C	130 bp
		W076_R	TAGAAGCAGCGCTGACAGAA	60.0 °C	
77	PTSb00327.1	W077_F	GATGGCTTCAACTGGAGGAA	60.2 °C	121 bp
		W077_R	CAGGGAAATGCTCAACCTTC	59.7 °C	
78	PTSb00328.1	W078_F	ATGGCAACAGCAACAATGA	60.1 °C	113 bp
		W078_R	TTCCGCCAGTTATAGCCATC	60.1 °C	
79	PTSb00329.1	W079_F	TCAGAACGAGATCAGAGGTGGA	62.3 °C	145 bp
		W079_R	CTCTCCACCCGCTTCTTCAC	62.3 °C	
80	PTSb00330.1	W080_F	GCTATGACTCGTCAACTTCACA	58.0 °C	126 bp
		W080_R	TAACAGACGTGCAGCAATCTC	58.0 °C	
81	PTSb00331.1	W081_F	GTGGCAAATGGAGTTTGTATC	57.1 °C	120 bp
		W081_R	AGGCAATCTCTATGCAATG	57.1 °C	
82	PTSb00332.1	W082_F	TCCACCACCAGTACCATTGA	59.8 °C	123 bp
		W082_R	ATACTTGCGCCACCGATAAC	60.0 °C	
83	PTSb00171.1	-H083_F	GACACGGACGCTCTGTTGTA	59.9 °C	130 bp
		-H083_R	CGTGGTTTACTGGACCCATC	60.2 °C	

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Sl. No.	Database ID	Assay ID	5' Primers Sequence 3'	Tm	Product size
84	PTSb00172.1	H084_F	TGCATGTCATGAGTGCAGAA	60.0 °C	122 bp
		H084_R	AACGATGCTGCTGATCATTG	59.8 °C	
85	PTSb00173.1	H085_F	TCAACAGCTCAGGCAACAAC	60.0 °C	138 bp
		H085_R	TACGGGTGAAGGAAATGCTC	60.1 °C	
86	PTSb00174.1	H086_F	AACAGCGAGCTTTCCAACAG	60.6 °C	129 bp
		H086_R	TTGGATACGGGTGAAGGAAG	59.9 °C	
87	PTSb00175.1	H087_F	CAGCACTGGCAAAGTCTCAA	60.2 °C	120 bp
		H087_R	AGCTCTCGTGCCAGACTCTC	59.9 °C	
88	PTSb00176.1	H088_F	GAAAGGGAAGCTACCGAAGG	60.2 °C	105 bp
		H088_R	GCTGCAAGCCTCACCTTATC	60.0 °C	
89	PTSb00177.1	H089_F	AGTACGGCACGCCTCAAG	60.0 °C	130 bp
		H089_R	GAGGAGAGGGCAAGTGTGAG	60.0 °C	
90	PTSb00178.1	H090_F	GAGCTTCGAGGAAGAGAACAAG	59.8 °C	132 bp
		H090_R	TCGAGCTGCTTAGTCTTCCAG	59.9 °C	
91	PTSb00086.1	Y091_F	TTGATGCTGAGACCATGTGCG	60.8 °C	107 bp
		Y091_R	GCAGGTGTTGTTGATGCTTG	60.3 °C	
92	PTSb00087.1	Y092_F	CATTTGGAGCTCGGTTCTTC	59.8 °C	149 bp
		Y092_R	AACCTGTTATACGCCGATGG	59.8 °C	
93	PTSb00088.1	Y093_F	GCAAGCCTCGTCAAGAAATC	60.0 °C	102 bp
		Y093_R	ACACGTTGCCTCTTCTCTGG	60.4 °C	
94	PTSb00223.1	Y094_F	AGGGAAGACAAGGCAAACCTG	59.3 °C	124 bp
		Y094_R	CAAGCAGCAAGCATTACAGG	59.6 °C	
95	PTSb00258.1	N095_F	TGCAGAAGCAGCTCCATATC	59.1 °C	108 bp
		N095_R	CGATCGATCTCAAGAAGCAC	58.5 °C	
96	PTSb00259.1	N096_F	TTGACCTCTACCGCATTGAG	58.9 °C	103 bp
		N096_R	TACTTGCGGTCTTGTAGCTG	60.4 °C	
97	PTSb00260.1	N097_F	GATCCATGGGAACTGAATGG	60.1 °C	120 bp
		N097_R	CTCGTCTGCACAAACAGAGC	59.8 °C	
98	PTSb00261.1	-N098_F	CCCAGTACCTGCTTGCACTC	60.9 °C	145 bp
		-N098_R	AAGCGTCCATCTTCTGAACC	59.3 °C	
99	PTSb00262.1	N099_F	TCGAATCATGCTGCTACACC	59.8 °C	105 bp
		N099_R	CAGTTCAATGCCACCATTTG	60.0 °C	
100	PTSb00263.1	N100_F	GCAGGATCCACAAGAAGAGC	60.0 °C	136 bp
		N100_R	TGCAACTGGAAGTGGAACTG	59.9 °C	
101	PTSb00264.1	N101_F	ATCGAGCTCATCAACCTCGT	59.8 °C	106 bp
		N101_R	GGTCTCTCGGCACGTAGAAG	60.0 °C	
102	PTSb00265.1	N102_F	AGCTGGATCATGAACGAGTACC	60.5 °C	121 bp
		N102_R	TGGTGCTGAGGTGAAAGTTG	59.9 °C	
103	PTSb00266.1	N103_F	TGGTGGCATGAAGAAGAC	59.8 °C	138 bp
		N103_R	ACTCATCCCTTGTGGAGGTG	60.0 °C	
104	PTSb00267.1	N104_F	ATTTCCAGTTGTCTGGACCAG	60.0 °C	102 bp
		N104_R	AGTCGTTTCATGCTGTTGTGCG	59.9 °C	

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Sl. No.	Database ID	Assay ID	5' Primers Sequence 3'	Tm	Product size
105	PTSb00268.1	N105_F	GTCAACATCTACCAGTGCAACC	59.5 °C	118 bp
		N105_R	GAAACGAGCAGTACGTTTTGG	59.8 °C	
106	PTSb00269.1	N106_F	TGGTCGTCCACTACCTCAAG	58.7 °C	115 bp
		N106_R	AAGGTCGCCTTATCAGGTAGC	59.8 °C	
107	PTSb00270.1	N107_F	ACAGCACAAGACCAAACAGG	58.8 °C	102 bp
		N107_R	TCAAGCCTATGCACTTGCTC	59.2 °C	
108	PTSb00271.1	N108_F	ACACTCATCCGCAGAACTG	58.9 °C	136 bp
		N108_R	CAACGGACATCAGCATCAAC	60.1 °C	
109	PTSb00272.1	N109_F	ATCGGCGAATCAACTACCAG	60.1 °C	108 bp
		N109_R	ACTCATGCATCACCCAGTTG	59.6 °C	
110	PTSb00273.1	N110_F	TTCCCTTGAGTCTTGCCAAC	60.2 °C	149 bp
		N110_R	TAGCAAGGCAATCCAACCTC	60.2 °C	
111	PTSb00274.1	N111_F	TCCTTTGATGGCCTCTTCAG	60.3 °C	129 bp
		N111_R	TGATTCTCTCCAGCACAAAG	59.0 °C	
112	PTSb00275.1	N112_F	AACTGATCGGCTTGACTTGG	60.3 °C	149 bp
		N112_R	CGAATCCGACCTTCTTCTTG	59.8 °C	
113	PTSb00276.1	N113_F	GTTGCCAAGGAGTTTGAAG	59.7 °C	106 bp
		N113_R	CATCATCTTCTCTTCGTCTCC	60.2 °C	
114	PTSb00349.1	B114_F	AAACGATGCTGACAGACGTG	59.9 °C	140 bp
		B114_R	ATTCTTTGAGTCGCCGTAGG	59.3 °C	
115	PTSb00350.1	B115_F	AGCTGATGATGCCCTTTGTC	60.2 °C	125 bp
		B115_R	AGTCGACCGAAGCAATTACG	60.3 °C	
116	PTSb00351.1	B116_F	CAATTGGATCATGGGAGGAC	60.1 °C	130 bp
		B116_R	TTCGCCACACCAAAGTGTAG	59.8 °C	
117	PTSb00352.1	B117_F	TGTTCCCAGCAGTCGTTATC	58.7 °C	125 bp
		B117_R	ACAGCTCACGCTCACAAATG	60.1 °C	
118	PTSb00353.1	B118_F	ATGCCGTGTGCACTATTGAG	59.7 °C	102 bp
		B118_R	AGTCTTCATCGTCGGAATCG	60.2 °C	
119	PTSb00354.1	B119_F	GGGAGTTGAAGCATTTCAG	59.7 °C	150 bp
		B119_R	TCATAGCCACTGCCAATCTG	59.8 °C	
120	PTSb00355.1	B120_F	ATCCACTGAATGCTGCGAAC	61.2 °C	125 bp
		B120_R	TCCATAAACATGGGGTCCTG	60.6 °C	
121	PTSb00356.1	B121_F	AAGCAAGTGAAGAGGCGATG	60.5 °C	110 bp
		B121_R	AACCTTCCCTTCCATTCTGG	60.3 °C	
122	PTSb00357.1	B122_F	AAAGGCGCAATAGGTCCATC	61.3 °C	104 bp
		B122_R	ATCAAGAATGGCTGGTCGAG	60.2 °C	
123	PTSb00358.1	B123_F	ACCACAAGGTCTTGGAATGC	60.0 °C	103 bp
		B123_R	AGATGGGAATGGCTGTATGC	59.9 °C	
124	PTSb00359.1	B124_F	GGATAATCACGATGCTGACG	59.1 °C	105 bp
		B124_R	CAGCACTGGAACACCATTG	60.2 °C	
125	PTSb00360.1	B125_F	GTCAAACCTCCGGAACCATC	59.4 °C	119 bp
		B125_R	ACTTGAATGGCCAACTGGAG	60.1 °C	

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Sl. No.	Database ID	Assay ID	5' Primers Sequence 3'	Tm	Product size
126	PTSb00361.1	B126_F	ATCTTGTGCCCAACATGGAC	60.8 °C	121 bp
		B126_R	CAACAGTTTGCTTGGTCGTG	60.3 °C	
127	PTSb00363.1	B127_F	ACGCTCAAGACCACATCATC	58.7 °C	133 bp
		B127_R	TACTTGGTCGCGTCTGAAAG	59.1 °C	
128	PTSb00364.1	B128_F	GCAAGGCGAGATTCTTGTTT	60.0 °C	134 bp
		B128_R	GCACAACTGTCCATTGTTG	60.0 °C	
129	PTSb00365.1	B129_F	TCCTCGCTCCAAGATTTGAC	60.3 °C	135 bp
		B129_R	CATTGATCTTGCTGCGTCTG	60.6 °C	
130	PTSb00366.1	B130_F	ACTTTGGTTCGGGTGCTATG	60.0 °C	140 bp
		B130_R	AGGCAAAGCATTCTGTAGGC	59.5 °C	
131	PTSb00367.1	B131_F	GCAACAACATCCAGCTTCAG	59.4 °C	112 bp
		B131_R	TTATCTCGCTTGGAGGAAGG	59.4 °C	
132	PTSb00368.1	B132_F	TTCTCCCCTTTGCCAGTATG	60.1 °C	127 bp
		B132_R	AAGCTTTTCTGGGAGCAGTG	60.6 °C	
133	PTSb00369.1	B133_F	AACACCTGGGGTCTTTTCATC	58.8 °C	148 bp
		B133_R	ACACAAAGTTGGGGCTGAAG	60.1 °C	
134	PTSb00370.1	B134_F	ATGCCTGGAGAACAACCTCG	60.3 °C	101 bp
		B134_R	GACTTATCCAATCGGCCTTG	59.5 °C	
135	PTSb00371.1	B135_F	TGCAGAAGCAGGTGGATAAC	58.9 °C	142 bp
		B135_R	TGAACCATGATCTCCGTGTC	59.5 °C	
136	PTSb00372.1	B136_F	GGTTGCAATAAGGTGGTTGG	60.2 °C	104 bp
		B136_R	GTGGCCAATTTCATGGACAG	61.3 °C	
137	PTSb00373.1	B137_F	ACCTCATCCACCCAATTCTG	59.8 °C	143 bp
		B137_R	GCCCTGATTCACACAAACAC	59.0 °C	
138	PTSb00374.1	B138_F	TTACGCAAGCAGCAAGAGTG	60.3 °C	110 bp
		B138_R	TCTTGGCAAGCCTTCTTGAG	60.6 °C	
139	PTSb00375.1	B139_F	TGACATGGTTGATGGCTACG	60.5 °C	135 bp
		B139_R	CACGCTTCAAACAACACCTG	60.3 °C	
140	PTSb00376.1	B140_F	CTTCTGGAAACGTGGCAAAC	60.7 °C	144 bp
		B140_R	CTTGTCGGATTGTCAGGATG	59.1 °C	
141	PTSb00378.1	B141_F	ACCATGAGCAAGGAATCAGC	60.2 °C	127 bp
		B141_R	GCTGCGATGGAAAGATGTTT	60.8 °C	
142	PTSb00379.1	B142_F	AATCATCCGGACACAAGGAG	59.9 °C	132 bp
		B142_R	ATGAAGACCTCGTCCAGTCG	60.3 °C	
143	PTSb00381.1	B143_F	CGTGAAAGGAGGAAGCAGTC	60.0 °C	122 bp
		B143_R	CGGAGTGATGTGTTCTCTGC	59.4 °C	
144	PTSb00382.1	B144_F	AACCCACAGGACGTGAAGAG	60.2 °C	149 bp
		B144_R	AGCTGCTTAAAGAGCGATGC	59.9 °C	
145	PTSb00383.1	B145_F	AGCAGGTGCGAAATGTTGGAG	60.3 °C	111 bp
		B145_R	ACGGACCTGTCAATGTCCTC	60.0 °C	
146	PTSb00385.1	B146_F	AGGAAGCGAAGGAAGGAGTC	60.0 °C	115 bp
		B146_R	GCTTGTTCTGGTCCTTCAGC	60.0 °C	

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Sl. No.	Database ID	Assay ID	5' Primers Sequence 3'	Tm	Product size
147	PTSb00386.1	B147_F	AACAGCGGACCATACTGACC	60.0 °C	147 bp
		B147_R	GGTTCTGCTGCTCGTAGACC	60.0 °C	
148	PTSb00387.1	B148_F	CCCAGCAGAGCAATGGATAC	60.6 °C	127 bp
		B148_R	ACGTTGAGCCAACTCCTCAC	60.3 °C	
149	PTSb00388.1	B149_F	AATCACCTCGAGCCCCTAAC	60.5 °C	137 bp
		B149_R	GATCCAGCCAAAGTCTCTGC	60.0 °C	
150	PTSb00389.1	B150_F	GGCAGAGGACTCAGTGAAGC	60.1 °C	139 bp
		B150_R	GTCATCTTGATGGGGACAG	60.3 °C	
151	PTSb00390.1	B151_F	TTCGACCAGGAGCGAAATAC	60.2 °C	108 bp
		B151_R	CCAATATTCGACCTCCATCG	60.3 °C	
152	PTSb00391.1	B152_F	AGCAGTGAAACCCAGAGATAC	59.6 °C	142 bp
		B152_R	AGCTTGCAAGAGGCTGAAAG	59.9 °C	
153	PTSb00392.1	B153_F	TCTGAAATCGGCAGGCTAAC	60.4 °C	130 bp
		B153_R	AGGATGTGACTGCTGTGTGC	59.9 °C	
154	PTSb00393.1	B154_F	AAGTCACCTTGGGTTCCCTTG	59.0 °C	128 bp
		B154_R	CACAGCAACGATGCATACAG	58.9 °C	
155	PTSb00394.1	B155_F	GAACATAGCCGAGGAAGCTG	60.0 °C	139 bp
		B155_R	ATCTTCAATGGCGACAGAGG	60.2 °C	
156	PTSb00395.1	B156_F	GGATTGTCGAGAGGCCATAG	59.7 °C	120 bp
		B156_R	GATCGTAGGTGGCGCTATTC	59.7 °C	
157	PTSb00396.1	B157_F	ATTGAGCCAAGCTCAGTCG	60.5 °C	124 bp
		B157_R	ATTGCCCATGCTATTTCAGC	60.1 °C	
158	PTSb00397.1	B158_F	TGACATCGAGAGCATTGAGG	59.9 °C	137 bp
		B158_R	GGGTTCCAGTTCTTGGTACG	59.4 °C	
159	PTSb00281.1	P160_F	CCAACAGATGCACCTGAGTC	59.3 °C	120 bp
		P160_R	ACCCCTTTTCATGTTGCAGAG	60.1 °C	
160	PTSb00282.1	P162_F	ATTGATGCTCCACAGGAAGG	60.0 °C	135 bp
		P162_R	TCTCCTTATCACCCGTCGAG	60.2 °C	
161	PTSb00283.1	P163_F	CGGGGAGTATGTCTCTGGTG	60.5 °C	145 bp
		P163_R	GGTGGCCTCATTCTCGTTAG	59.7 °C	
162	PTSb00284.1	P164_F	CACAGGTGGTGCAATGAAAC	60.0 °C	124 bp
		P164_R	TCTGCTCAGTCCATCAATCG	59.9 °C	
163	PTSb00285.1	P165_F	GATGCCCATTTGTGTAAGCAG	59.2 °C	111 bp
		P165_R	AAACCTGATCACGCTCTTCG	60.4 °C	
164	PTSb00286.1	P166_F	ATGCTGCATTGGACAGGAAG	61.2 °C	113 bp
		P166_R	TGCAACATTGTCAGGACCAC	60.6 °C	
165	PTSb00287.1	P167_F	ACTGGTTCATCCATCTTGC	59.9 °C	140 bp
		P167_R	GGGCTCAGAAATTTGGTGAAG	59.7 °C	
166	PTSb00288.1	P168_F	GCTACTACTGCCGCTCCAAC	60.0 °C	123 bp
		P168_R	CTGCTCCGTGGGTACCTTAG	59.8 °C	
167	PTSb00289.1	P169_F	CACATGGCATTATGGGTCAG	59.8 °C	132 bp
		P169_R	CCTGGCTAGCTTCAATGTCC	59.8 °C	

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Sl. No.	Database ID	Assay ID	5' Primers Sequence 3'	Tm	Product size
168	PTSb00290.1	P170_F	GTGGATGGGCAAGACATTG	59.9 °C	142 bp
		P170_R	CATCGCCACAAATACCACAG	60.0 °C	
169	PTSb00291.1	P171_F	TTATGGTGTGACGGGAAAGC	60.0 °C	109 bp
		P171_R	CAAAGCACCACTTTTCACAG	59.3 °C	
170	PTSb00292.1	P172_F	TCTGCAAAGTCTGGGAATC	60.2 °C	130 bp
		P172_R	GGGCAATACCAGTTTCCAAG	59.4 °C	
171	PTSb00293.1	P173_F	TCGAGCGAAAGAACAGCAC	60.3 °C	150 bp
		P173_R	TGCACCTCGATCTTTCCTG	60.0 °C	
172	PTSb00294.1	P174_F	AATGGATCTGTGCTGACC	60.1 °C	124 bp
		P174_R	TCAGACATTGGGTGCAACTG	60.7 °C	
173	PTSb00295.1	P175_F	TAAATCAGCTGGGGTTACCG	60.0 °C	116 bp
		P175_R	AGCATTTCTCCTTCCCCATC	60.4 °C	
174	PTSb00224.1	M176_F	ATGGTCGTCTTCTCCACGAC	60.1 °C	130 bp
		M176_R	ATCGAACAGACTTGGGATCG	60.1 °C	
175	PTSb00225.1	M177_F	CAGCGAAGCCTGAGAAGAAG	60.4 °C	113 bp
		M177_R	TGCTGCACTTAGCTTCATGG	60.2 °C	
176	PTSb00226.1	M178_F	TCACCTTCCTCTCGATGGTC	60.2 °C	113 bp
		M178_R	AGTGATGGTGTACGGCTTCC	60.0 °C	
177	PTSb00227.1	M179_F	AATGGCCACAACCTCAGAAC	60.0 °C	108 bp
		M179_R	CCCAAACAGCCTCAAGCTAC	59.9 °C	
178	PTSb00228.1	M180_F	CCAAGGTAGAAGCGAGCAAC	60.0 °C	120 bp
		M180_R	AAGAGGCGGACAGTGACATAC	60.3 °C	
179	PTSb00229.1	M181_F	CGTCATTACGCAGACCACAC	60.2 °C	100 bp
		M181_R	CACTTCCTTGCCCTTCTACG	59.9 °C	
180	PTSb00232.1	M182_F	AGTGCCCTGCTACGGTTATG	60.2 °C	112 bp
		M182_R	GACGTTTAATGGGGGTGTTG	60.1 °C	
181	PTSb00233.1	M183_F	CGCTTTCCATCAAGCTCAAC	60.9 °C	122 bp
		M183_R	GACAGCGGAGTTGAAAGAG	60.0 °C	
182	PTSb00234.1	M184_F	GCCGAATGGTTCAGATATGG	60.3 °C	110 bp
		M184_R	AGTCTGCAAGTCGCCGTAAC	60.5 °C	
183	PTSb00235.1	M185_F	AGCTGTTCCAGCCTCTCAAG	59.7 °C	117 bp
		M185_R	TCCAAATCCTCCTCTGATGG	60.0 °C	
184	PTSb00236.1	M186_F	GAGGCTCTGGTGAGGAAATG	59.8 °C	138 bp
		M186_R	CTATACGCTGCCATGCTCTG	59.6 °C	
185	PTSb00237.1	M187_F	TCCAGGGTGAGTTCAGAAGG	60.2 °C	142 bp
		M187_R	CTGCTGCTGACAGACCAAAC	59.6 °C	
186	ACTIN	A188_F	TGGCATCTCTCAGCACATTC	60.0 °C	103 bp
		A188_R	GGGCGGAAAGAATTAGAAGC	60.2 °C	
187	18S rRNA	R190_F	GCCTTTTGAAGCACTTTTAC	60.0 °C	109 bp
		R190_R	AACCAAACCTCCATGCTCAC	60.0 °C	

GENOME-WIDE MOLECULAR MAPPING, INTROGRESSION OF STABLE QTLs AND EXPRESSIONAL QUANTITATION OF TRANSCRIPTION FACTOR GENES IN CHARCOAL ROT MANIFESTATION IN *Sorghum bicolor* (L) Moench

AYYANAGOUDA M. PATIL 2009

Dr. B. FAKRUDIN
Major advisor

ABSTRACT

Recombinant inbred lines derived from the cross IS22380 (susceptible) and E36-1 (resistant) were characterized for the morphological and biochemical components of charcoal rot resistance and yield related traits at three locations over four *rabi* seasons revealed significant differences among RILs and GxE interactions. A significant association among relevant traits was noticed at phenotypic and genotypic level with high heritability estimates. A total of 141 polymorphic DNA markers (48/275 SSRs, 65/533 EST-SSRs, 28/410 RAPD) were genotyped to construct a genetic linkage map spanning 2905 cM and used for Composite Interval Mapping of QTLs. Stable QTLs were detected lodging percent (*xtxp176–xtxp312* (CR1) number of internodes crossed by the fungus (*xtxp297-xiabt273* (CR2) and length of infection (*xtxp275-xtxp241*(CR3): the additive effect at all these loci was contributed by E36-1. Two major QTLs of plant height and three QTLs of plant yield accounted for 38% and 41% phenotypic variance respectively. On LG-I, the genomic region flanked by *xtxp274–xiabt29* harbored a common QTL. Three stable QTLs, CR1, CR2 and CR3 collectively contributing 43% of phenotypic variation were introgressed into M35-1 and SPV86 backgrounds: at BC₁F₁ with 2285 and 2033 marker and at BC₂F₁ 5989 and 6002 marker points in M35-1 and SPV86 backgrounds were screened to identify progenies harboring all the three QTLs.

Expressional quantitation of 185 transcription factor genes was done in charcoal rot challenged and control tissue of E36-1 and SPV86 genotypes at 75 DAS and 90 DAS where 142 genes up regulated in both genotypes while eight genes of five family viz., AP2 (PTSb00019.1, PTSb00022.1, PTSb00024.1 WRKY (PTSb00331.1), HMG (PTSb00179.1, PTSb00185.1) ARF (PTSb00033.1) and bHLH (PTSb00349.1) were up regulated in pathogen challenged tissues. Two genes of WRKY family (PTSb00328.1 and PTSb00331.1) were significantly up regulated in resistant genotype alone: these understandings would help in developing strategies for resistance breeding in sorghum.