

**GENETIC MAPPING AND QTL ANALYSIS FOR
DISEASE RESISTANCE, YIELD AND QUALITY IN
GROUNDNUT (*Arachis hypogaea* L.)**

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ABBREVIATIONS

QTL	: Quantitative Trait Loci
LOD	: Log of odd ratio
cM	: centiMorgan
μ l	: Microlitter
U	: Unit
pM	: Picomoles
mM	: Micromoles
ng	: Nanogram
PAGE	: Polyacrylamide gel electrophoresis
LG	: Linkage group
R^2	: Phenotypic variance
RIL	: Recombinant Inbred Line
SSD	: Single seed descent
LLS	: Late leaf spot
LLN	: Leaf length
LWD	: Leaf width
Plht	: Plant height
PBR	: Number of primary branches
PY/P	: Pod yield per plant
NOP/P	: Number of pods per plant
HSW	: 100 seed weight
SH%	: Shelling percent
a^0	: Additive effect
BSA	: Bulk segregant analysis
SCAR	: Sequence characterized amplified regions
SSR	: Simple sequence repeats
CAPS	: Cleaved amplified polymorphic sequence
MAS	: Marker assisted selection
O/L	: Oleic/linoleic
SMA	: Single marker analysis
SD	: Segregation distortion
DAS	: Days after sowing
Rust-SI	: Rust stage I at 80 DAS
Rust-SII	: Rust stage II at 90 DAS
LLS-SI	: LLS stage I at 70 DAS
LLS-SII	: LLS stage II at 90 DAS

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), also known as peanut, is an important oilseed crop in tropical and subtropical regions of the world. *Arachis hypogaea* is believed to have originated in South America via hybridization of two diploid wild species (*A. duranensis* and *A. ipaensis*) followed by rare spontaneous duplication of the chromosomes (Halward *et al.*, 1991; Young *et al.*, 1996). The cultivated tetraploid groundnut ($2n = 4x = 40$) is a member of genus *Arachis* and belongs to the family *Leguminosae*, subfamily *Fabaceae*, tribe *Aeschynomeneae*, subtribe *Stylosanthenae* (Krapovickas and Gregory, 1994). Based on the differences in the branching pattern and presence of reproductive node on the main stem, the species has been classified into two subspecies, *hypogaea* and *fastigiata* (Krapovickas and Rigoni, 1960). Further each subspecies has been divided into two botanical varieties viz. subsp. *hypogaea* into var. *hypogaea* (virginia) and var. *hirsuta* and subsp. *fastigiata* into var. *fastigiata* (valencia), var. *vulgaris* (spanish), var. *peruviana* and var. *aequatoriana* (Krapovickas and Gregory, 1994).

Cultivated groundnut is grown in six continents but mainly in Asia, America and Africa in over 100 countries with a world production of 38.0 m t from an area of 24.0 m ha (FAO, 2012). Groundnut is an economically important crop because of its oil, confectionary and dietetic value which is providing over 30 essential nutrients and phytonutrients. China, India and USA are the major producers of groundnut. Though, India is a leading producer of groundnut the productivity is very low (about 0.92 t ha⁻¹) as compared to China (3.31 t ha⁻¹). In India low productivity of the groundnut is attributed to the damage caused by biotic and abiotic stresses. Among the biotic stresses, two fungal diseases viz. late leaf spot (LLS) caused by *Cercosporidium personatum* and rust caused by *Puccinia arachidis* are widespread in most of the tropical countries and severely affect the crop productivity. Moreover, co-occurrence of both the diseases makes it unmanageable leading to yield losses up to 50–70% (Subrahmanyam *et al.*, 1984) as well as adversely affecting the quality of the produce. Despite the fact that several fungicides are available to control these diseases, host-plant resistance is considered as the best approach to manage the resistance genes and to overcome hazardous effect of fungicides.

Classical genetic studies on LLS and rust resistance suggest that, resistance to these fungal diseases is complex and polygenic in nature and most likely controlled by recessive genes (Nevill 1982; Green and Wynne 1986; Dwivedi *et al.*, 2002; Paramshivam *et al.*, 1990 and Motagi, 2001) and their selection becomes difficult in the segregating generations. Because of its inbreeding nature of groundnut crop the most commonly used breeding methods are pedigree selection, bulk pedigree selection and single seed descent. Though, immense achievement has been seen in identifying the accessions from landraces and wild relatives with desirable traits like resistance to diseases and pests, high oil content and O/L ratio. But their utilization in transferring the trait in the genetic background of cultivated types is coupled with linkage drag of undesirable traits which is unmanageable. Hence, backcross breeding has not been extensively utilized in groundnut because of the complex inheritance pattern of the traits (Knauff and Wynne, 1995). Moreover, conventional plant breeding strategies had restricted the success in enhancing resistance against LLS and rust due to lack of genetic information, complexity of the genome and most of the time the two diseases interfere with each other because of its co-occurrence.

Molecular markers as an alternative approach and its integration with conventional breeding have seen a momentous advancement in crop improvement programs (Varshney *et al.*, 2005; Varshney *et al.*, 2006). Molecular markers often follows expected Mendelian segregation, they are neutral, occur throughout the genome, not influenced by the environment, co-dominant and monitored in any tissue and stage of the plant. An approach based on Marker-assisted selection (MAS) using tightly linked markers offers an effective and efficient breeding tool for detecting, tracking, retaining, combining, and pyramiding disease resistance genes (Kelly and Miklas, 1998 and 1999). Efficiency of conventional breeding can be complemented with the application of MAS, especially in the case of low heritable traits, where phenotypic selection is difficult, expensive, lack accuracy or precision (Crouch, 2001). MAS can be a prospective approach for the introgression of resistance from different genetic backgrounds of wild relatives and for fastest recovery of the recurrent parent genome by using foreground and background selection approach.

Though MAS is a potential approach complimenting the conventional breeding, the prerequisite to initiate any molecular breeding program is the availability of sufficient genomic resources in terms of appropriate molecular markers, dense genetic maps, tightly linked markers or significant QTLs contributing for the trait of interest.

In groundnut several studies have been reported earlier in establishing the genetic relationship between the cultivars/accessions using RFLPs, RAPDs, and AFLPs (Kochert *et al.*, 1991, Halward *et al.*, 1992, Paik-Ro *et al.*, 1992, and Stalker *et al.*, 1994). A very low level of polymorphism was observed and it has become a giant bottleneck hampering the strategies of molecular breeding in cultivated groundnut. Markers developed based on “simple sequence repeats” are considered to be most efficient and breeder friendly DNA markers because they are uniformly distributed throughout the genome, multi-allelic, co-dominant and transferable between the populations (Gupta and Varshney, 2000).

Later, a large number of SSR markers have been developed by several groups during last 10 years (Hopkins *et al.*, 1999; He *et al.*, 2003; Ferguson *et al.*, 2004; Moretzsohn *et al.*, 2005; Mace *et al.*, 2007; Cuc *et al.*, 2008). As a result, development of trait specific mapping populations has been developed with respect to drought tolerance, diseases and quality traits (Pandey *et al.*, 2012). With these efforts, linkage mapping and QTL detection had been possible using SSR markers in cultivated groundnut (Varshney *et al.*, 2009a; Khedikar *et al.*, 2010; Hong *et al.*, 2010; Ravi *et al.*, 2011; Sarvamangala *et al.*, 2011) and also using BC₁F₁ population developed from a cross between cultivated × synthetic amphidiploid (Foncéca *et al.*, 2009).

Though, genetic maps and QTL studies has been initiated in groundnut it is still in its infancy due to narrow genetic base, lack of sufficient polymorphic markers and it has forced to develop partial genetic maps or maps with low marker density (Khedikar *et al.*, 2010; Sarvamangala *et al.*, 2011; Hong *et al.*, 2010). In this regard development and identification of polymorphic SSR markers is still in progress and has enabled the development of new set of SSR markers from different sources (Liang *et al.*, 2009b; Nagy *et al.*, 2010; Yuan *et al.*, 2010; Song *et al.*, 2010; Koilkonda *et al.*, 2012) and approximately, more than 7000 SSR markers are now accessible in the public domain (Pandey *et al.*, 2012). And genetic mapping based on single mapping population are in a way forward towards the development of dense genetic maps (Qin *et al.*, 2012, Gautami *et al.*, 2012a, Wang *et al.*, 2012, Shirasawa *et al.*, 2012b, 2013).

The aberrant status of genetic mapping prevailing in groundnut has made the researchers to think in the direction of developing consensus genetic maps. In turn consensus map provides a platform to map larger number of loci onto a single map. This is particularly imperative when attempting to map specific genes/candidate genes of interest that are unlikely to segregate specifically within single mapping population. In recent years the approach of consensus mapping has been successful in wheat (Khlestkina *et al.*, 2009), soybean (Song *et al.*, 2004; Choi *et al.*, 2007), barley (Diab 2006, Marcel 2007; Varshney *et al.*, 2007), cowpea (Muchero *et al.*, 2009), and sorghum (Mace *et al.*, 2009). Recent reports of consensus mapping in groundnut have been reported (Gautami *et al.*, 2012 a and b; Shirasawa *et al.*, 2013) where in the marker density on these consensus maps has increased immensely.

With these strategies the rationale of effective QTL mapping, identification of chromosomal regions significantly contributing for disease resistance, quality and yield related traits has been reported recently with partial linkage maps based on TAG 24 × GPBD 4 (RIL-4) and TG 26 × GPBD 4 (RIL-5) recombinant inbred line mapping populations (Khedikar *et al.*, 2010 (RIL-4) and Sarvamangala *et al.*, 2011 (RIL-5)). Considering the status of genetic mapping studies done earlier on these two populations it was determined to saturate both the genetic maps using more number of SSR markers and to facilitate comprehensive QTL analysis to define proper genomic regions contributing for LLS, rust, quality and yield attributing traits. Hence, the present study was undertaken with the following objectives:

Objectives

1. Saturation of genetic maps based on TAG 24 × GPBD 4 (RIL-4) and TG 26 × GPBD 4 (RIL-5) mapping populations
2. Development of consensus map based on the two RIL mapping populations
3. Comprehensive QTL analysis for disease resistance, yield and quality traits
4. Validation of rust resistance linked markers using diverse germplasm

REVIEW OF LITERATURE

Conventional breeding approaches have been successful in development and release of several improved cultivars not just for increased yield but also for resistance/tolerance to biotic and abiotic stresses. Thus, offering protection against losses and choice for consumer preferred traits in groundnut. However, the progress has been limited in crop improvement approaches employed for breeding trait specific genotypes in groundnut.

Recent advancements in application of molecular marker technology have enabled tremendous progress towards reaching the goals of targeted breeding programs. And molecular markers have helped the breeders in understanding the genetic architecture of the crop plants in terms of their genome and its organization. During the process of phenotyping and QTL identification it is important to understand interaction effect between genotype and environment ($G \times E$ interactions). The information on $G \times E$ interaction effect will be of importance in designing the breeding strategies using molecular markers. Consequently, the research in groundnut genomics in the last decade has witnessed the deployment of molecular breeding approaches to complement the ongoing groundnut improvement programs in USA, China, India, and Japan (Janila *et al.*, 2013). Literature related to above said points are collected keeping the view of main objectives of the study and they are presented under the following headings.

2.1 Variability for morphological, quality and yield traits in RIL-4 and RIL-5 population

2.2 Status of markers studies in groundnut

2.3 Genetic linkage map studies in groundnut

2.4 Consensus map construction

2.5 Marker trait association studies in groundnut

2.6 Status of QTL studies in groundnut

2.7 Status of genetic mapping in RIL-4 and RIL-5 population

2.8 Need for saturating the genetic maps

2.1 Variability for morphological, yield and quality traits in RIL-4 and RIL-5 population

Among the various oil seed crops, groundnut is a unique commercial crop and has been aptly described as “nature’s” master piece of food value. Pod yield in groundnut, a quantitatively inherited complex trait, is the result of number of cumulative, duplicate and dominant genes and highly influenced by $G \times E$ interaction. This necessitates a thorough knowledge of variability owing to genetic factors. Further, a comprehensive knowledge on the interrelationship between yield determining characters and their association for enhancement of yield is of paramount importance in crop improvement.

2.1.1 Variability for morphological and yield traits in RIL-4 and RIL-5 population

Analysis of variance for twelve agronomic traits revealed significant differences among the genotypes for all the traits except days to maturity, pods per plant and yield per plant. Estimates of components of variability revealed moderate to high level of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for plant height, primary branches, shelling percentage, 100 seed weight and yield per plant. Heritability was very high for plant height, pods per plant and shelling percentage and moderate to high for leaf length, leaf width, primary branches and plant yield per plant. In general, high genetic advance over percent of mean (GAM) was observed for LLS and rust at different stages and environments. Similarly, high GAM was evident for plant height, yield and it was low to moderate for other agronomic traits. Mostly normal distribution was observed for agronomic traits showing their quantitative nature of inheritance (Khedikar, 2008). Further evaluation of the RIL-4 population in another experiment revealed a little wider difference between PCV and GCV among the yield and yield component traits across the seasons of evaluation, thus indicating the prevalence of the environmental influence on the expression of these traits (Mukri, 2011).

In RIL-5 population the magnitude of variation as revealed by PCV was very high for all the traits except for shelling per cent. But lower heritability and GAM indicated lower heritable variation for most of these traits except plant height and 100-seed weight, which recorded moderate to high

heritability. For number of branches, number of pods per plant and pod yield per plant heritability was low to moderate coupled with very low GAM. While, higher heritability had led to moderate GAM for plant height and 100-seed weight (Sarvamangala, 2009).

2.1.2 Variability for quality traits in RIL-4 and RIL-5 population

Analysis of variance for quality traits in RIL-5 population revealed significant variation among the RILs, seasons and RILs $\times$ Season interaction for all the traits. Genetic variability components was low to moderate magnitude of variation and GAM coupled with very high heritability for protein but lower magnitude of variation with higher heritability and lower GAM for oil content. However, among the oil quality parameters, oleic and linoleic acids, O/L ratio exhibited low to moderate magnitude of variation with very high heritability (Sarvamangala, 2009).

Similarly, pooled analysis across environments in RIL-4 population revealed significant differences among the RILs, environments and there interaction effect on the nutritional quality. The phenotypic and genotypic variation explained by the population was narrow within the season. However, PCV and GCV was moderate for oil content, palmitic acid, behenic acid, O/L ratio and percent saturated fatty acid. There was a low genotypic coefficient of variation for oleic acid and linoleic acid. The nutritional quality traits recorded high heritability coupled with high GAM in the population. Overall variability studies in RIL-4 population revealed the possible chances of improvement for protein content, oleic acid content, linoleic acid content, stearic acid, arachidic acid content and O/L ratio (Mukri, 2011).

2.1.3 Association studies in RIL-4 and RIL-5 population

Association analysis revealed strong correlation among morphological (leaf length, leaf width, plant height and primary branches) and yield traits (pods per plant, yield per plant, plot yield, hundred seed weight and shelling percentage) and also between some morphological and yield traits revealing genetic association due to linkage and / or pleiotropy. Transgressive segregants for all the agronomic traits were observed indicating the contribution of favorable alleles by both the parents (Khedikar, 2008). Further evaluation of the same population and association studies between 100 kernel weight and days to 50 percent flowering, plant height indicated negative impact of vegetative growth and increased biomass on yield. The shelling percentage as one of the yield determining factor associated positively with 100 kernel yield, kernel yield per plant and pod yield per plant. Pod yield per plant associated negatively with days to 50 percent flowering suggesting delayed crop growth may reduce the yield (Mukri, 2011). Similar studies in RIL-5 population revealed significant positive correlation of pod yield with plant height, number of pods per plant and 100-seed weight thus indicating importance of these traits for enhancing the productivity in the population (Sarvamangala, 2009)

2.2 Status of markers studies in groundnut

Quantitative nature of inheritance of foliar diseases under study makes selection very difficult in breeding high yielding resistant varieties. Defoliating, partial and polygenic nature of LLS makes the identification of resistant and susceptible lines cumbersome through conventional screening techniques (Leal-Bertioli *et al.*, 2009). Recent trends in the area of crop genomics applied to breeding have seen significant progress in its application and its integration in breeding programs (Varshney *et al.*, 2005). In parallel several studies have established the relevance of molecular markers and marker-assisted selection (MAS) to improve the efficiency of conventional breeding especially in case of low heritable traits, where phenotypic selection is expensive, difficult or lacks accuracy (Varshney *et al.*, 2006).

In the earlier genetic relationship studies RFLP played an important role in assessing the extent of variability (Halward *et al.*, 1991, Kochert *et al.*, 1991, Paik-Ro *et al.*, 1992 and Stalker *et al.*, 1995,) in *Arachis* section (*A. hypogaea*, *A. monticola*, *A. batizocoi*, *A. cardenasii*, *A. duranensis* and *A. glandulifera*) and found that *A. monticola* was more closely related to *A. hypogaea* subspecies *hypogaea* than to subspecies *fastigiata*. Using RFLP technique no variations were observed between *Arachis hypogaea* and *Arachis monticola* and concluded that the cultivated groundnut resulted from the cross between *Arachis duranensis* and *Arachis ipaensis* (Kochert *et al.*, 1996). Based on the chloroplast analysis it was reported that *Arachis duranensis* as the female progenitor of cultivated groundnut. Further genomic relationship between AA genome, BB genomes and AABB genome species were also assessed using RFLP technique (Gimenes *et al.*, 2002).

Next to RFLP, several marker studies have been initiated using RAPD for the genetic diversity studies and to assess the genetic relationship (Lanham *et al.*, 1992, Hilu and Stalker 1995,

Bhagwat *et al.*, 1997, Subramaniam *et al.*, 2000, Santos *et al.*, 2001 and Dwivedi *et al.*, 2001) among the accessions of groundnut. Further they have been utilized in relating the possible donor for the cultivated tetraploid groundnut. Similarly in the due course of time they found that AFLP approach was more efficient than RFLP and RAPD. With this, studies involving AFLPs were conducted to assess the genetic relationship among the groundnut accessions (He and Prakash 1997, Gimenes *et al.*, 2002, Herselman, 2003 and Milla *et al.*, 2005).

Microsatellites or simple sequence repeat (SSR) markers are considered as markers of choice for breeding applications and extensively used in several crop species (Gupta and Varshney 2000). Initially with the availability of few SSR markers several genetic diversity and assessment of genetic relationship studies was done (Luo *et al.*, 2005; He *et al.*, 2005; Bravo *et al.*, 2006; Kottapalli *et al.*, 2007; Tang *et al.*, 2007; Upadhyaya *et al.*, 2007; Varshney *et al.*, 2009b) using germplasm accessions, core collections and composite collections of groundnut.

During last 10 years, a large number of SSR markers have been developed by several groups (e.g., Hopkins *et al.*, 1999; He *et al.*, 2003; Ferguson *et al.*, 2004; Moretzsohn *et al.*, 2005; Mace *et al.*, 2007; Cuc *et al.*, 2008; Gautami *et al.*, 2009). A set of 946 novel and highly informative SSR markers (199 SSRs with >0.50 PIC) were identified with an anticipation of usefulness of SSR markers in groundnut breeding (Pandey *et al.*, 2011). Further, recently with the availability of transcript sequence data using next generation sequencing technologies, development of the new set of SSR markers has been reported by several research groups in peanut community (Liang *et al.*, 2009b; Nagy *et al.*, 2010; Yuan *et al.*, 2010; Song *et al.*, 2010; Koilkonda *et al.*, 2012). SSR markers have also been developed using BAC (bacterial artificial chromosome)-end sequences (Wang *et al.*, 2012). However, in peanut extensive characterization of *AhMITE1* has been done for determining their use as DNA markers to understand the phylogeny, genetics, genomics and breeding (Shirasawa *et al.*, 2012a).

In the recent days, though there is access to lot of SSR markers in the public domain, the major bottlenecks for slow progress in molecular mapping in groundnut is low level of genetic diversity present in germplasm of cultivated groundnut and non-availability of large number of polymorphic markers. However, genome mapping and trait mapping was in its infancy in groundnut till 2010. For instance, the first SSR-based genetic map based on recombinant inbred line (RIL) mapping population has been developed very recently (Varshney *et al.*, 2009a) and in succession trait mapping studies have been initiated in cultivated groundnut (Herselman *et al.*, 2004; Varshney *et al.*, 2009a).

2.3 Genetic linkage map studies in groundnut

A linkage map may be thought of as a 'road map' of the chromosomes derived from two different parents. Linkage maps indicate the position and relative genetic distances between markers along chromosomes. The most important use of linkage maps is to identify chromosomal locations containing genes and QTLs associated with traits of interest. Construction of genetic linkage map is necessary to apply marker assisted selection tool in crop improvement programme. Though, a low level of polymorphism has been reported in cultivated groundnut. Attempts have been made to develop linkage maps both in diploid and tetraploid species for understanding the genome architecture of groundnut.

2.3.1 Genetic maps based on A genome

Genetic linkage map was developed for the first time based on a F₂ (87 individual) population derived from the interspecific hybridization of two related diploid species in the section *Arachis* (*Arachis stenosperma* × *Arachis cardenasii*). RFLP markers from both genomic and c-DNA clones of groundnut *Arachis hypogaea* cv.GK7 were used to develop the genetic map. A total of 117 loci were mapped on 11 linkage groups (LGs) with a total map distance of 1400 cM was covered with a 20 cM resolution (Halward *et al.*, 1993). Later another map based on F₂ population of *A. kuhlmannii* × *A. digoi*, was developed comprising of 102 AFLP markers mapped on 12 LGs with a genome coverage of 1068.1 cM (Milla, 2003).

Nevertheless, construction/development of genetic maps was continued and a genetic map based on backcross population [*Arachis stenosperma* × (*Arachis stenosperma* × *A. cardenasii*)] was reported, where in 39 shared RFLPs were used to place 167 RAPD loci on the RFLP map. The RAPDs covered a total genetic length of 800 cM and were mapped on to 11 LGs (Garcia *et al.*, 2005).

Moretzsohn *et al.* (2005) constructed a linkage map based on microsatellites using an F₂ population obtained from a cross between two diploid wild species with AA genome (*Arachis*

durocnesis and *A. stenosperma*). A total of 170 marker loci were mapped on to 11 linkage groups covering 1,230.89 cM of total map distance with an average distance of 7.24 cM between markers. Saturation of the same $F_{2.5}$ AF_5 derived RIL population with a total of 437 new polymorphic loci, together with 160 loci previously mapped has enabled the development of high density genetic linkage map of A genome diploid species. With this a total of 597 loci were mapped onto 10 LGs spanning around 544 cM of the A genome (Shirasawa *et al.*, 2013).

Earlier genetic map developed based on F_2 population derived from a cross between *A. duranensis* accession and *A. stenosperma* was saturated with 165 new markers. With this a total of 369 markers were mapped on to 10 LGs spanning around 2540.1 cM. These markers included 188 microsatellites, 80 legume anchor markers, 46 AFLPs, 32 NBS profiling, 17 SNP, four RGA-RFLP and two SCAR markers (Leal-Bertioli *et al.*, 2009). And recently based on *A. duranensis* $\times$ *A. duranensis* derived population a high density genetic map has been developed with 1054 SNP markers. In addition, 598 EST-SSR markers identified in *A. hypogaea* assemblies were included in the map along with 37 disease resistance gene candidate (RGC) and 35 other previously published markers. In total, 1724 markers were mapped over 10 LGs with genome coverage of 1081.3 cM (Nagy *et al.*, 2012).

2.3.2 Genetic maps based on B genome

Gobbi *et al.* (2006) constructed B genome map of groundnut based on F_2 population of 93 individuals obtained from the cross between *Arachis ipaensis* (KG 30076) and *Arachis magna* (KG 30097). Eleven LGs were obtained from 94 polymorphic micro-satellite markers covering a total distance of 754.8 cM. The length of LGs ranged from 5.6 to 130 cM.

Based on, F_2 population obtained from the cross between the diploid *A. ipaensis* and the closely related *A. magna*, a genetic map was developed. The map has 10 linkage groups, with 149 loci spanning a total map distance of 1,294 cM (Moretzsohn *et al.*, 2009). Further saturation of earlier B genome map (Moretzsohn *et al.*, 2009) with new set of 680 polymorphic loci, along with 118 previously mapped loci, has led to mapping of 798 loci on 10 LGs covering 461 cM of B genome (Shirasawa *et al.*, 2013).

2.3.3 Genetic maps based on AB genome

Garcia *et al.* (1995) constructed a genetic linkage map consisting of one tetraploid (*A. hypogaea*, $2n = 4x = 40$) parent and other being diploid species (*A. cardenasii kra poickas* and W. C. Gregory, $2n = 2x = 20$) for the introgression of *A. cardenasii* chromosome segments. A total of 34 c-DNA RFLP probes and 45 RAPD primers introgressed chromosomal segments in one or more lines were used for map construction. The introgression segments covered 10 out of the 11 linkage groups, smallest of which were RFLP markers and the largest had 3-4 adjacent markers at a distance of 30-40 cM.

Burrow *et al.* (2001) constructed the first molecular map representing entire tetraploid genome of groundnut. To introduce variability from diploid wild species into tetraploid cultivated *A. hypogaea*, a synthetic amphidiploid TXAG-6 {(*Arachis batizacoi* K9484 $\times$ (*Arachis cardenasii* GKP10017 $\times$ *Arachis digoi* GKP10602)^{4x}} was used as a donor parent to generate a back cross (BC_1) population of 78 progeny. A total of 370 RFLP loci were mapped onto 23 LGs spanning 2210 cM which was slightly greater than twice the length of (1063 cM) the diploid map (Garcia *et al.*, 1995).

Identification of molecular markers linked to Aphid resistance to groundnut rosette disease was done with the partial AFLP based genetic linkage map for cultivated peanut with $F_{2.3}$ population developed from a cross using the aphid resistant parent ICG 12991 and the aphid susceptible male parent ICGV 93541. A total of 308 AFLP primers and 144 primer combinations were used to identify markers associated with the aphid resistance. Twenty putative markers were identified of which 12 mapped to five LGs covering a map distance of 139.4 cM (Herselman *et al.*, 2004).

First SSR based genetic linkage map in cultivated groundnut was constructed using 318 RILs obtained from a cross of TAG 24 $\times$ ICGV 86031. A total of 135 out of 150 SSR loci were mapped on 22 LGs with a total map distance of 1270.5 cM with an average intermarker distance of 9.4cM Varshney *et al.* (2009a). Additional marker data obtained on 65 loci were used to integrate into the same framework map (Varshney *et al.*, 2009a) and a total of 56 loci got integrated into different LGs and nine markers remained unlinked. The map was saturated with 191 loci mapped on to 22 LGs, covering a length of 1785.4 cM Ravi *et al.* (2011).

Further a map based on backcross (BC) populations involving 88 individuals of the BC_1F_1 population a total of two hundred ninety eight (298) loci were mapped on 21 LGs, spanning a total

map distance of 1843.7 cM with an average distance of 6.1 cM between adjacent markers (Foncéka *et al.*, 2009).

Three recombinant inbred lines (RILs) populations were developed from three crosses with one common female parental line Yueyou 13, a high yielding Spanish market type. The four parents were screened with 1044 primer pairs designed to amplify SSRs and 901 primer pairs produced clear PCR products. Of the 901 primer pairs, 146, 124 and 64 primer pairs (markers) were polymorphic in these populations, respectively, and used in genotyping these RIL populations. Individual LGs were constructed from each of the three populations. Where in 132 loci were mapped on 19 LGs spanning a total genetic distance of 684.9 cM based on Yueyou 13 × Zhenzhuhei population, in case of Yueyou 13 × Fu 95-5 population 109 loci mapped onto 21 LGs, spanning a total genetic distance of 540.69 cM and of the genetic map derived from Yueyou 13 × J11 population only forty-six markers were assigned to 13 LGs with a genome coverage of 401.7 cM (Hong *et al.*, 2010)

To understand the genetic basis and identification of QTL for drought tolerance, two new RIL mapping populations, namely ICGS 76 × CSMG 84-1 (RIL-2) and ICGS 44 × ICGS 76 (RIL-3), were used. After screening of 3,215 simple sequence repeat (SSR) markers on the parental genotypes of these populations, two new genetic maps were developed with 119 (RIL-2) and 82 (RIL-3) SSR loci (Gautami *et al.*, 2012a). Similarly a new population comprising 164 recombinant inbred lines derived from resistant (VG 9514) and susceptible (TAG 24) cultivated groundnut parents were screened for rust resistance in five environments. Subsequent genotyping of these lines with 109 SSR markers generated a genetic linkage map with 24 linkage groups, spanning around 882.9 cM with an average of 9.0 cM between neighbouring markers (Mondal *et al.*, 2012).

With the advancement in development of new mapping population and molecular markers a new set of 1,152 SSRs as well as about 4,000 published or unpublished SSRs were screened against two parents (Tifrunner and GT-C20) of a F₂ mapping population, generating 385 polymorphic loci. A genetic linkage map was constructed, consisting of 318 loci mapped onto 21 LGs and covering a total of 1,674.4 cM, with an average distance of 5.3 cM between adjacent loci. In addition two markers related to resistance gene homologs (RGH) were also mapped to two different groups (Wang *et al.*, 2012)

Lot of importance has been given for the development of improved dense genetic maps with more number of markers. Based on the cross derivative of 'Satonoka' and 'Kintoki' F₂ (SKF₂) population, a map was developed with a total of 1,114 loci mapped onto 21 LGs. The genetic linkage map spanned around 2,166.4 cM, with distances ranging from 44.1 cM to 199.8 cM. Similarly another genetic map based on 'Nakateyutaka' and 'YI-0311' F₂ (NYF₂) population was developed. In which 342 polymorphic loci were found to be polymorphic from 338 markers. The *ahFAD2A* and *ahFAD2B* genes were subjected to genotyping of the F₂ population together with the 342 polymorphic loci. A total of 326 segregating loci formed 19 LGs. The total length of the map was 1,332.9 cM, with individual LGs ranging from 2.9 cM to 167.3 cM. The average marker density was 4.3 cM, ranging from 1.7 cM to 23.9 cM. In the NYF₂ map, 7.7% of the marker loci showed significant segregation distortions (Shirasawa *et al.*, 2012b).

Genetic maps from Tifrunner × GT-C20 (T population, 248 RILs) and SunOleic 97R × NC94022 (S population, 352 RILs) were developed (Qin *et al.*, 2012). The T linkage map consisted of 239 loci mapped onto 26 linkage groups and covered 1,213.4 cM. Similarly The S linkage map consisted of 172 loci in 22 LGs, including two CAPS markers for *ahFAD2A* and *ahFAD2B*, and covering 920.7 cM. Further, the genetic map derived from SunOleic 97R × NC94022 was saturated with additional polymorphic marker loci. The saturated genetic map possesses 249 loci distributed on 23 LGs with total map distance of 3,433.5 cM (Qin *et al.*, 2012).

A new genetic map was constructed based on the mapping population developed from a cultivated (Runner IAC 886) and an artificial amphidiploids (*A. ipae* × *A. duranensis* 'V14167')^{4x}. A total of 1469 segregation loci were successfully mapped onto 20 LGs covering 1442 cM. The 20 LGs were classified into 10 pairs of A and B genomes, according to the DNA fragment size of mapped loci corresponding to 'K30076' or 'V14167'. The average marker density of the map was 1.0 cM, and segregation distortions were observed in 19% (285 loci) of the mapped loci AB genome tetraploids (Shirasawa *et al.*, 2013).

2.4 Consensus map construction

In order to have a dense genetic maps for the purpose of advanced strategies of molecular breeding, In recent years researchers has chosen the approach of consensus mapping and it has

been successful in developing dense genetic maps in the crops like wheat (Khlestkina *et al.*, 2009), soybean (Song *et al.*, 2004; Choi *et al.*, 2007; Hyten *et al.*, 2010), barley (Diab 2006, Marcel 2007; Varshney *et al.*, 2007), cowpea (Muchero *et al.*, 2009), and sorghum (Mace *et al.*, 2009). Keeping in mind large genome size of groundnut a few studies have been reported in cultivated groundnut which can enhance the use of molecular markers in breeding programs.

A composite linkage map based on common loci from three individual maps was developed with 175 SSR markers in 22 composite LGs. The total composite map length is 885.4 cM, with an average marker density of 5.8 cM (Hong *et al.*, 2010). The map consisted of 47 SSRs that have been used in the published AA genome maps, and could be used in comparative mapping studies.

With the availability of new genetic maps a comparative integrated map from two cultivated × cultivated RIL mapping populations was developed for mapping Tomato spotted wilt virus (TSWV) resistance trait in peanut. A total of 4,576 SSR markers were used for assessing parental polymorphisms and two cleaved amplified polymorphic sequence markers were also included to differentiate *ahFAD2A* alleles and *ahFAD2B* alleles. A total of 324 markers were anchored on this integrated map covering 1,352.1 cM with 21 LGs. Upon comparing with published maps several chromosomal rearrangements were observed. High consistence with cultivated peanut maps derived from different populations may support this integrated map as a reliable reference map for peanut whole genome sequencing assembling (Qin *et al.*, 2012).

Very recently a consensus map was developed by combining three individual genetic maps (TAG24 × ICGV 86031, ICGS 76 × CSMG 84-1 and ICGS 44 × ICGS 76) developed specifically for drought-tolerance-related traits at ICRISAT. A total of 293 SSR loci were mapped onto 20 LGs, spanning 2,840.8 cM providing an opportunity to understand the distribution of QTLs for drought-tolerance-related traits (Gautami *et al.*, 2012a). Further with the availability/access to the information on several other genetic maps in the public domain has led to the development of high density genetic map with 897 marker loci including 895 SSR and 2 cleaved amplified polymorphic sequence (CAPS) loci. They were distributed on 20 LGs (a01–a10 and b01–b10) spanning a map distance of 3, 863.6 cM with an average map density of 4.4 cM (Gautami *et al.*, 2012b).

Based on the 16 mapping populations a high density consensus map was developed covering a genomic region of 2651 cM with 3693 marker loci which was anchored to 20 consensus LGs corresponding to the A and B genomes. The integrated tetraploid consensus map and genome-specific diploid maps has facilitated the better understanding of genome architecture of *Arachis species* (Shirasawa *et al.*, 2013).

2.5 Marker trait association studies

The first practical use of MAS in groundnut was reported by Burow *et al.* (1996) for resistance to root knot nematode. Three RAPD markers *viz.*, RKN410, RKN440 and RKN229 linked with resistance to root knot nematode (*Meloidogyne arenaria*) were identified in the breeding populations derived from Florunner × TxAG-7. The cloned RAPD marker RKN440 was later mapped on LG1 and denoted as RF440 (Burow *et al.*, 2001). Choi *et al.* (1999) identified three RFLP loci (S1137, R2545, and R2430) linked to nematode resistance at distance of 4.2, 5.2 and 11.0 cM. Church *et al.* (2000) utilized two RFLP loci linked to a single gene of nematode resistance to select individuals homozygous for resistance in a segregating population.

Similarly RAPD markers were used to map two dominant genes *Mae* and *Mag* which confer resistance to the groundnut root knot nematode *Meloidogyne arenaria* race 1 (Garcia *et al.*, 1996). Bulk Segregant Analysis (BSA) was performed on the progenies using 450 RAPD primers. One RAPD marker Z3/265 was found to be linked to both *Mag* and *Mae* at a distance of 10 ± 2.5 cM and 1.4 ± 2.9 cM, respectively. Later the linked markers [RAPD markers *viz.*, Z3/265 (Garcia *et al.*, 1996) and *RKN440* (Burow *et al.*, 1996)] for nematode resistance were converted into SCAR (Sequence Characterized Amplified Region) marker (Chu *et al.*, 2007a).

Similar reports of marker trait association were reported, for resistance to *Cercosporidium arachidicola* sporulation, lesion diameter, defoliation and overall rating (Stalker and Mozingo, 2001), resistance to tomato spotted wilt virus (Milla, 2003), resistance to aphids (Herselman *et al.*, 2004), resistance to rust (Varma *et al.*, 2005; Mondal *et al.*, 2007) and resistance to *Aspergillus flavus* infection (Lei *et al.*, 2006).

Based on conventional genetics and breeding studies, two recessive alleles *ol₁* and *ol₂* were identified for high and low oleic acid genotypes (Lopez *et al.*, 2000). Later loss of function of oleoyl-

PC desaturase activity has been reported being solely responsible for the high O/L trait (Ray *et al.*, 1993) and two homeologous genes, *ahFAD2A* and *ahFAD2B* have been found to control the oleoyl-PC desaturase activity (Jung *et al.*, 2000a & b). CAPS markers were developed to differentiate mutant and wild-type *ahFAD2A* alleles (Chu *et al.*, 2007b). Further based on the previous study a simple PCR assay for detection of *FAD2* alleles on both genomes was developed by designing allele-specific primers and altering PCR annealing temperatures. This assay was successfully used for detecting *FAD2* alleles in peanut (Chen *et al.*, 2010).

2.6 QTL studies in Groundnut

QTL analysis for drought-tolerance-related-traits resulted in identification of 25 QTLs for transpiration, transpiration efficiency, specific leaf area and SPAD chlorophyll meter reading (SCMR) for the data obtained across 2 years, the phenotypic variation explained by these QTLs was in the range of 3.5–14.1%. This was the first report on the construction of the first genetic map for cultivated groundnut and demonstrates its utility for molecular mapping of QTLs controlling drought tolerance related traits as well as establishing relationships with diploid AA genome of groundnut and model legume genome species (Varshney *et al.*, 2009a).

QTL linkage analyses for height of main axis, pod weight/plant, number of mature pods/plant, stem diameter, number of total branches, number of fruiting branches, leaf length, leaf width, leaf length/width ratio, protein content, oil content, and resistance to *A. flavus* infection. A total of 42 QTLs have been detected that explained 7.0–24.1% of the total variation for different traits, and one to seven QTLs were identified for each of the traits. These QTLs were distributed on a total of 17 linkage groups. Of the 42 QTLs identified 25 QTLs were major with a PVE ranging from 10.20–24.10% and remaining 17 QTLs were minor QTLs as they explained <10% PVE (Liang *et al.*, 2009a).

Candidate genome regions that control disease resistance were identified and candidate disease resistance genes and QTLs against late leaf spot disease were placed on the genetic map of the A-genome of *Arachis*, which is based on microsatellite markers and legume anchor markers. These marker types are transferable within the genus *Arachis* and to other legumes respectively, enabling this map to be aligned to other *Arachis* maps and to maps of other legume crops including those with sequenced genomes. In total, 34 sequence-confirmed candidate disease resistance genes and five QTLs were mapped. Candidate genes and QTLs were distributed on all linkage groups except for the smallest, but the distribution was not even. Groupings of candidate genes and QTLs for late leaf spot resistance were apparent on the upper region of linkage group 4 and the lower region of linkage group 2, indicating that these regions are likely to control disease resistance (Leal-Bertioli *et al.*, 2009).

Segregation data obtained for these markers facilitated development of partial linkage map (14 linkage groups) with 56 SSR loci. QTL analysis using CIM on genotyping and phenotyping data yielded 11 QTLs for LLS in three environments and 12 QTLs for rust. Interestingly a major QTL associated with rust (QTLrust01), contributing 6.90–55.20% variation, was identified by both CIM and single marker analysis (SMA). However the inter-marker distance of the QTL identified with IPAHM 103 and pPGSseq19D6 was around 50 cM and needs to be saturated for further use in the molecular breeding program (Khedikar *et al.*, 2010).

A partial genetic linkage map comprising of 45 SSR loci on 8 linkage groups with an average inter-marker distance of 14.62cM was developed. QTL analysis based on single marker analysis (SMA) and composite interval mapping identified some candidate SSR markers associated with major QTLs as well as several minor QTLs for the nutritional traits. The identified QTLs for oil content (IPAHM103) and protein content (TC6H03) are considered as major QTLs. However, Validation of these major QTLs using a wider genetic background may provide the markers for molecular breeding for improving groundnut for nutritional traits (Sarvamangala *et al.*, 2011).

Genetic mapping and QTL analysis in 164 recombinant inbred lines derived from resistant (VG 9514) and susceptible (TAG 24) parents were screened for rust resistance across environments. Revealed a QTL flanked between pPGPseq4A05 and gi56931710 at map distances of 4.7 cM and 4.3 cM, respectively, in linkage group 2. The significant association of these two markers with the rust reaction was also confirmed by discriminant analysis. The informative SSR markers classified rust-resistant and susceptible groups with 99.97% correctness. The SSR markers pPGPseq4A05 and gi56931710 were able to identify all the susceptible genotypes from a set of 20 cultivated genotypes differing in rust reaction (Mondal *et al.*, 2012).

Shirasawa *et al.* (2012b) QTL analysis in the SKF2 population revealed a total of 23 significant QTLs for the 15 investigated traits. The phenotype variance explained by the QTLs ranged from 4.8 to 28.2%. These QTLs were distributed on a total of nine linkage groups. From among the 23 QTLs identified 17 (11.80-28.20% PVE) were found to be major and remaining six were minor QTLs with PVE ranging from 4.80-9.90 per cent.

2.7 Status of genetic mapping in RIL-4 and RIL-5 population

Two RIL mapping populations were developed at the University of Agricultural Sciences, Dharwad from the cross TAG 24 × GPBD 4 (RIL-4) and TG 26 × GPBD 4 (RIL-5). The RIL populations comprised of 266 and 146 RILs in RIL-4 and RIL-5, respectively. These two populations were segregating for LLS, rust, morphological traits, yield attributing traits, nutritional and oil quality traits.

Based on these two mapping populations partial genetic linkage maps were developed by Khedikar *et al.* (2010) based on TAG 24 × GPBD 4 (RIL-4) and Sarvamangala *et al.* (2011) based on TG26 × GPBD4 (RIL-5) population. A total of 59 markers were mapped on 13 linkage groups spanning around 462.24cM with an average marker interval of 15.25 cM in case of TAG 24 × GPBD 4 (RIL-4) population. Similarly another SSR based partial genetic linkage map based on TG 26 × GPBD 4 (RIL-5) population harbored 45 markers on 8 linkage groups; the genome coverage was spanning around 657.9 cM, with an average marker distance of 14.62 cM. Earlier phenotyping, for LLS and rust on both the (RIL-4 and RIL-5) populations was done during Kharif season of the years 2004, 2005, 2006, and 2007. Further based on these two mapping population phenotyping data was generated on oil content, protein content and nutritional quality traits during Kharif 2007 and summer 2008 for RIL-5 population (Sarvamangala *et al.*, 2011). On the other hand another study on oil content, protein content and nutritional quality traits based on RIL-4 population was done during Kharif 2008 and summer 2009 (Mukri, 2011)

2.8 Need for saturating the genetic maps

The most interesting perspective of application of the new markers will be to define and characterize previously published QTL in a better manner with respect to their position and inter-marker distance between the flanking markers which defines the QTL region. The saturation of the genetic maps will basically help in understanding genome organization and evolution, comparative genomics, mapping genes and quantitative trait loci, and associating genomic segments with phenotypic traits. As the two genetic maps developed earlier based on TAG 24 × GPBD 4 (RIL-4) (Khedikar *et al.*, 2010) and TG 26 × GPBD 4 (RIL-5) (Sarvamangala *et al.*, 2011) were partial genetic maps. With the efforts made earlier only 56 and 45 SSR loci were mapped onto 14 and 8 linkage groups in RIL-4 and RIL-5 population respectively. Interestingly a major QTL associated with rust (QTLrust01), contributing 6.90–55.20% variation, was identified by both CIM and single marker analysis (SMA). Though candidate SSR marker (IPAHM 103) linked with this QTL was validated in another mapping population (TG 26 × GPBD 4), the inter-marker distance of the QTL identified with IPAHM 103 and pPGSseq19D6 was around 50 cM and the genomic region was putative. In this view there is a need to saturate both the genetic maps using more number of SSR markers to enrich the present partial maps and further to facilitate comprehensive QTL analysis. It is necessary to enhance map saturation with new set of molecular markers localized in linkage groups associated to genomic regions involved in disease resistance and yield attributing traits.

MATERIAL AND METHODS

Present experiment mainly focuses on saturation of the genetic maps developed earlier in groundnut. And further identification of quantitative trait loci for resistance to diseases, morphological, yield attributing and quality traits.

3.1 Description of the material

3.1.1 Salient features of parents of mapping population

- ❖ TAG 24: A Spanish bunch type variety derived from TGS-2 × TGE-1 (Patil *et al.*, 1995). This is a popular high yielding cultivar which matures early with high harvest index, better partitioning coefficient and field tolerance to bud necrosis disease. As for as protein and oil quality parameters considered, it contains 29 percent protein, 45 percent oil and low O/L ratio. TGS-2 was derived from TG-187A × M-13 and TGE-1 from TG-9 × Tall, where TG-18A, TG-9 and Tall are either mutants or mutant derivatives traced to Spanish improved.
- ❖ TG 26: An improved Spanish bunch variety, derived from a cross of BARCG1 × TG 23 released in 1998 (Kale *et al.*, 1997). It is an early maturing (duration is 95-105 days), semi dwarf, erect variety with high pod growth rate, high harvest index, greater partitioning efficiency, tolerance to bud necrosis with high linoleic acid content but susceptible to LLS and rust (Kale *et al.*, 1997; Badigannavar *et al.*, 2002).
- ❖ GPBD 4: An improved Spanish bunch groundnut variety derived from KRG1 × CS 16 (ICGV86855) cross was developed at University of Agricultural Sciences, Dharwad (Gowda *et al.*, 2002). GPBD 4 is a second cycle product of interspecific hybridization with desirable combination of early maturity, high yield, high pod growth rate, desirable pod and kernel features, high oil and protein content, better Oleic/Linoleic (O/L) ratio, resistant to late leaf spot and rust. KRG 1 is an early maturing, Spanish bunch cultivar, susceptible to foliar diseases developed at regional research station, Raichur, Karnataka. The CS 16 is a Virginia bunch interspecific derivative of *Arachis hypogaea* × *A. cardenasii* (ICGV 86855), resistant to foliar diseases developed at ICRISAT, Patanacheru, India.

3.1.2 Development of mapping population

Mapping populations were developed by employing single seed descent (SSD) method from F₂. Two recombinant inbred line mapping populations were developed at the University of Agricultural Sciences, Dharwad from the cross TAG 24 × GPBD 4 (RIL-4) and TG 26 × GPBD 4 (RIL-5). Both TAG 24 and TG 26 were used as female parents in developing the populations. The RIL populations comprised of 266 RILs in case of RIL-4 and 146 RILs in RIL-5 population. These two populations were segregating for LLS, rust, for agronomic trait, nutritional and oil quality traits. The F₆₋₁₂ progenies were used for phenotyping and DNA isolated from F₇ progenies was used for genotyping. Earlier from these two mapping populations partial genetic linkage maps were developed by Khedikar *et al.* (2010) RIL-4 and Sarvamangala *et al.* (2011) RIL-5 with a few marker loci.

3.1.3 Germplasm comprising of resistant and susceptible accessions to rust

Germplasm comprising of a total of 64 (Table 1) accessions differing for rust resistance from a wide genetic background has been considered for validation of rust linked markers. The germplasm will be phenotyped for rust resistance across seasons and observations on rust will be recorded at 80 days (Rust-SI) and 90 days (Rust-SII) after sowing by using a modified 9-point scale (Subba rao *et al.*, 1990)

3.2 Experimental site

The present research work was conducted at Botanical garden of University of Agricultural Sciences, Dharwad for three years during Kharif 2008, 2009 and 2010. This location is situated in the Northern Transitional Tract of Karnataka at 15° 13', at latitude of 75° 07' E longitudes at an altitude of 678 m above mean sea level with an average rainfall of 800 mm.

3.2.1 Experimental layout

The experiment was laid out in randomized block design (RBD) with two replications. Each genotype was sown in 2.5 m rows with 30 cm and 10 cm inter and intra row spacing, respectively. All recommended package of practices was followed to raise good crop.

Table 1: List of Germplasm Accessions

Sl. No	Genotypes	Botanical Type	Pedigree
1	ICGV 93008	VB	[(Mani Pintar x (Robut 33-1 x NC Ac 2232)) x ICG 2320
2	D 39d(Purple & large)	SB	KRG 1 x CS 16 (ICGV 86855)
3	B 37c	SB	JL 24 x ICGV 87165
4	ICGV 90266	VB	[((J 11 x (M 13 x NC Ac 2214)) x ICG 2271]
5	R 8972	SB	ICGS 59 x NC Ac 2240
6	ICGV 92188	VB	[(Robut 33-1x (M 13 x NC Ac 2214))] x JL 24
7	ICGV 93023	VB	[(Robut 33-1x NC Ac 2214) x Cyto 213-2]
8	A 30b	VB	KRG 1 x ICGV 87165
9	ICGV 91177	VB	(F 334 A-B-14 xNC Ac 2232) x ((TMV 7 xFSB 7-2) x NC Ac 2214)
10	ICGV 86156		(Ah 2105 x Chico) x (TMV 10 X Chico)
11	MG 8	SB	Mutant 45 x GBFDS 272
12	IFDRVT 11		
13	GBFDS 272	VB	(A. hypogaea x A. carentenasii)
14	JG Thin shell	SB	JL 24 x GBFDS 272
15	ICG 99005	VB	TMV 2 x (A. hypogaea x A. batizocoi x A. duranensis)
16	ICGV 87165	VB	[A. hypogaea var. fastigiata (PI 261942) xA. carentenasii] CS 9
17	D 39d(GPBD 4)	SB	KRG 1 x CS 16 (ICGV 86855)
18	ICGV 86699	VB	[(A. batizocoi x A. duranensis) A. hypogaea (Cv.NC 2)] CS 29
19	VB 8	VB	EMS mutant of VL 1
20	ICGV 99003	VB	A. hypogaea x (A. duranensis)
21	D 39d (Purple & small)	SB	KRG 1 x CS 16 (ICGV 86855)
22	VL 2	VL	EMS mutant of Dharwad Early Runner (DER)
23	VL 1	VL	EMS mutant of Dharwad Early Runner (DER)
24	ICGV 87807	VL	[(MK 374 x Robut 33-1) x FESR 2
25	ICGV 87264	SB	Manfredi x NC Ac 17133RF
26	ICGV 88256	VB	(CS 9 x (Robut 33-1 xNC Ac 316)
27	Dh 73	SB	Dh 3-30 x ICGV 87264
28	ICGV 86155	VL	ICGS 30 x (TMV 10 x Chico F6)
29	ICGV 86590	VL	X14-4-B-19B x PI 259747
30	JLVR-1	VR	Spontaneous mutation from JL 24
31	JLVR 2	VR	Spontaneous mutation from JL 24
32	Dh 43	VR	Mardur Local
33	CHITRA	VR	Spanish 5B-1 x EC 1688
34	VB 9	VB	EMS mutant of VL 1
35	NC Ac 343	VB	NC Bunch x PI 1216067
36	KRG 1	SB	Selection from Argentina
37	M 28-2	SB	EMS mutant of Valencia 1 (VL 1)
38	R 9227	SB	(ICGS 7 x NC Ac 2214) x ICGV 86031
39	ICGV 86031	SB	F 334 A-B-14 x NC Ac 2214
40	R 8808	SB	ICGS 11 x Chico
41	R 9214	SB	(ICGS 7 x NC Ac 2214) x ICGV 86031
42	Dh 40 (Red seed)	SB	Dh 3-30 xTGE 2
43	Dh 40 (Tan)	SB	Dh 3-30 xTGE 2
44	ICGV 91180	VB	[(TMV 2 x FSB 7-2) x NC Ac 2232) x (F 334 A-B-14 x NC Ac 2214)]
45	K 134	SB	Kadiri 3 x JL 24
46	Mutant 110	SB	EMS mutant of Valencia 1 (VL 1)

Contd...

Sl. No	Genotypes	Botanical Type	Pedigree
47	Mutant 45	SB	EMS mutant of Valencia 1 (VL 1)
48	TMV 2	SB	Mass selection from "Gudhiatham bunch"
49	JL 24	SB	Selection from EC 94943
50	Dh 8	SB	Selection from RS 144
51	TAG 24	SB	TGS 2 x TGE 1
52	R 9251	SB	BARCG 1 x TG 23
53	ICGV 99004	SB	TMV 2 x (A. hypogaea x A. cardenasii)
54	ICGV 86158		(Ah 2105 x Chico) x (TMV 10 X Chico)
55	ICGV 87378		Kanto No 40
56	TG 41	SB	TG 28 x TG 22
57	VR 5	VR	5-azacytidine mutant of DER
58	ICGV 87921		(Robut 33-1 x NC Ac 2698) x (RMV 2 x Chico)
59	NC Ac 17090	VL	ICGV 1697 (Peru)
60	S 230	VR	Selection from Tandur Local
61	GIRNAR MOSAIC	SB	Spontaneous mutant of Girnar 1
62	GIRNAR	SB	X14-4-B-19B x NC Ac 17090
63	MN 1-28	VL	Mutant 28-2 x NC Ac 343
64	ICGV 91173	VB	[(NC Ac 343 x NC AC 2214) x ICG 5240]

3.3 Phenotyping for disease

3.3.1 Phenotyping for late leaf spot and rust

In, addition to the phenotyping done earlier (during the years of 2004, 2005, 2006, and 2007) for LLS and rust on both the (RIL-4 and RIL-5) populations. Further phenotyping was carried out in three additional environments/years during rainy season of 2008, 2009 and 2010 at University of Agricultural Sciences, Dharwad, India. Necessary agronomic practices, experimental design and artificial disease epiphytotics were created for both the diseases using “spreader row technique” as explained in Khedikar *et al.* (2010).

3.3.2 Production of rust and late leaf spot:

The artificial epiphytotic conditions were maintained for late leaf spot and Rust using ‘Spreader Row Technique’. Mutant-28-2 (Resistant to LLS but susceptible to rust) for Rust and TMV-2 for Late leaf spot were used as spreader row. The inoculum was produced and maintained separately on these cultivars for respective diseases. The infected leaves collected in the field for LLS and rust were soaked in water for half an hour to one hour. Then the LLS conidia and rust uredinospores were collected by rubbing the infected leaves in the water and used for inoculation on test material separately.

Artificial disease epiphytotics were created in separate screening experiments for the two diseases. Spreader rows were planted at every 10th row as well as border around the field to maintain the effective inoculum load. After every 50 rows, both the parents and respective spreader rows for both the diseases (TMV-2 for LLS and M-28-2 for rust) were planted in order to compare the scores of LLS and rust of parents with the RILs. Thirty five days after sowing, plants were inoculated uniformly in the evening with LLS/Rust for a week. The inoculum contained 20,000 conidia/uredinospores per ml water and mixed with Tween 80 (0.2 ml/1000ml of water) as a mild surfactant and atomized on the plants using knapsack sprayer. High humidity was maintained by irrigating the field in the night by furrow irrigation. Additional inoculum was provided by placing pots containing diseased plants at every 50 rows. The non-targeted diseases *i.e.*, Rust/LLS in the LLS and Rust experiments were controlled by spraying fungicide carbendazim (bavistin) 1g/litre and tridemorph (Calixin) 1ml/litre, respectively.

3.3.3 Disease scoring

Modified 9 point scale (1-9 score) was followed for recording observations on both the diseases as per Subbarao *et al.*, 1990 (Table 2a and 2b and Fig. 1a and 1b). Disease scoring for LLS was done at 70 days (LLS-SI) and 90 days after sowing (LLS-SII). Whereas, for rust scoring was done at 80 (Rust-SI) and 90 days (Rust-SII) after sowing by using modified 9-point scale for rust (Subbarao *et al.*, 1990).

3.4 Phenotyping for morphological and yield attributing traits

Observations were recorded under two broad category *i.e.*, morphological and yield attributing traits. Plant height (cm), primary branches, Leaf length (mm) and leaf width (mm) were considered under morphological traits. Whereas, number of pods per plant, pod yield per plant (g), shelling percent (%) and hundred seed weight (g) were grouped under yield attributing traits. Phenotyping was done from random plants selected from each RILs and parents of the mapping population in two replications. The following observations were recorded.

3.4.1 Morphological traits

1. Plant height (cm): The height of the plant was measured in centimeters (cm) from the ground level to the tip of the main stem at the time of harvest from 2 randomly selected representative plants.
2. Number of primary branches per plant: Number of primary branches (n+1) in each RIL line was recorded before the harvest.
3. Leaf length (mm): Measured on the third leaf, apical leaflet of the main stem when fully expanded.
4. Leaf width (mm): Measured on the third leaf fully expanded apical leaflet on the main stem at its widest point.

Table 2a: Modified 9-point scale used for field screening groundnut genotypes for resistance to rust disease

Disease score	Description	Disease severity (%) ψ
1	No disease	0
2	Pustules sparsely distributed, largely on lower leaves	1-5
3	Many pustules on lower leaves, necrosis evident, very few pustules on middle leaves	6-10
4	Number of pustules on lower and middle leaves, severe necrosis on lower leaves	11-20
5	Severe necrosis of lower and middle leaves, pustules may be present on top leaves but less severe	21-30
6	Extensive damage to lower leaves, middle leaves, necrotic with dense distribution of pustules, pustules on top leaves	31-40
7	Severe damage of lower and middle leaves, pustules densely distributed on top leaves	41-60
8	100 per cent damage to lower and middle leaves, pustules on top leaves, which are severely	61-80
9.	Almost all leaves withered, bare stems seen	81-100

ψ Percentage leaf area damaged by rust

Table 2b: Modified 9-point scale used for screening groundnut genotypes for resistance to late leaf spot disease

Disease score	Description	Disease severity (%) ψ
1	No disease	0
2	Lesions present largely on lower leaves; no defoliation	1-5
3	Lesions present largely on lower leaves, very few on middle leaves; defoliation of some leaf lets evident on lower leaves	6-10
4	Lesions present on lower and middle leaves but severe on lower leaves; defoliation of some leaf lets evident on lower leaves	11-20
5	Lesions present on lower and middle leaves, over 50 % of defoliation of lower leaves	21-30
6	Severe; eosins on lower and middle leaves; lesions present but less severe on top leaves; extensive defoliation of lower leaves; some defoliation on middle leaves	31-40
7	Lesions on all leaves but less severe on top leaves; defoliation of all lower and middle leaves	41-60
8	Defoliation of all lower and middle leaves; severe lesions on top leaves evident.	61-80
9	Almost all leaves defoliated, leaving bare stem; some leaflets my remain, but show severe leaf spot	81-100

ψ Percentage leaf area damaged by late leaf spot

(Subbarao *et al.*, 1990)

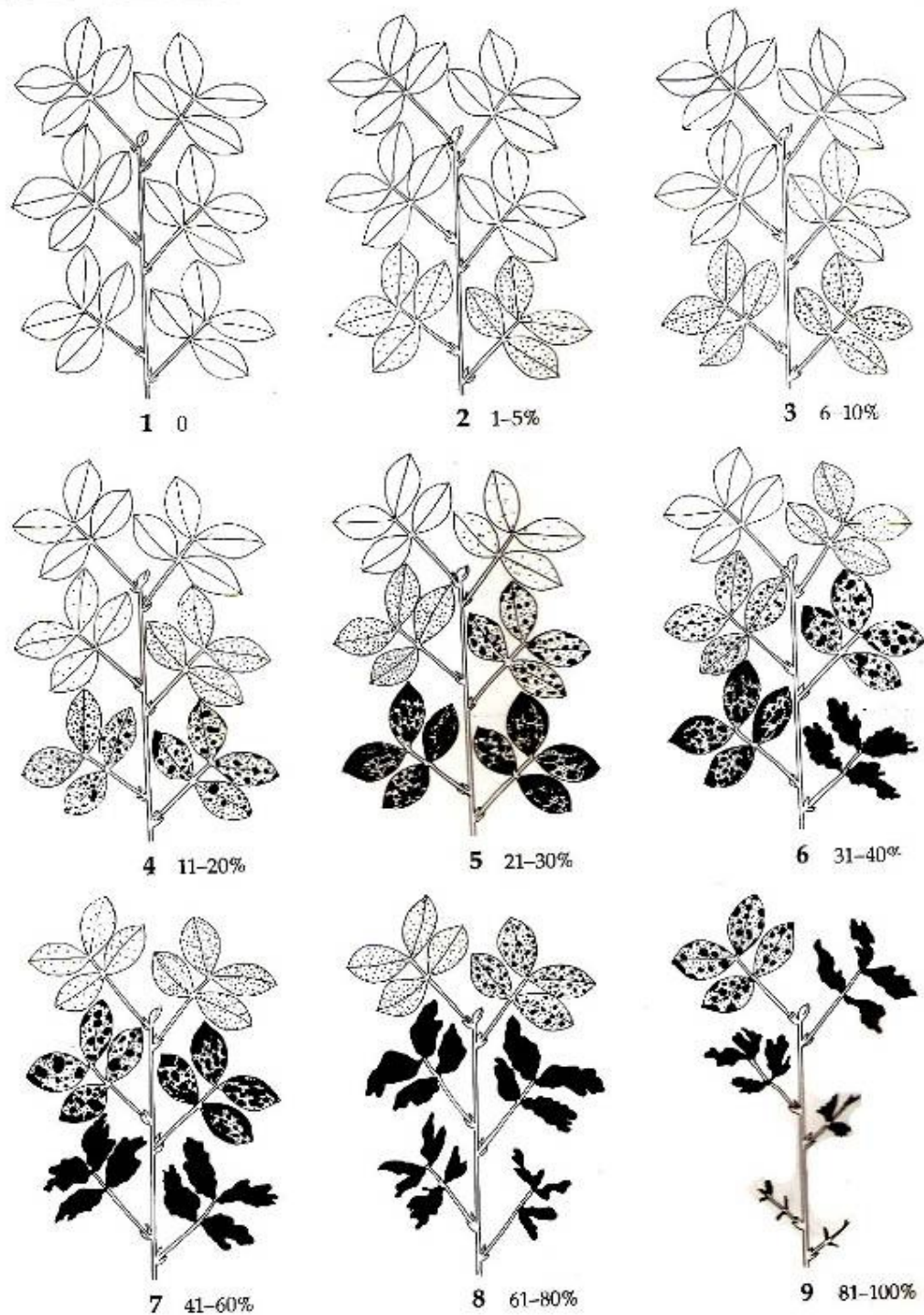


Fig. 1a: The modified 9-point scale for field evaluation of rust of groundnut

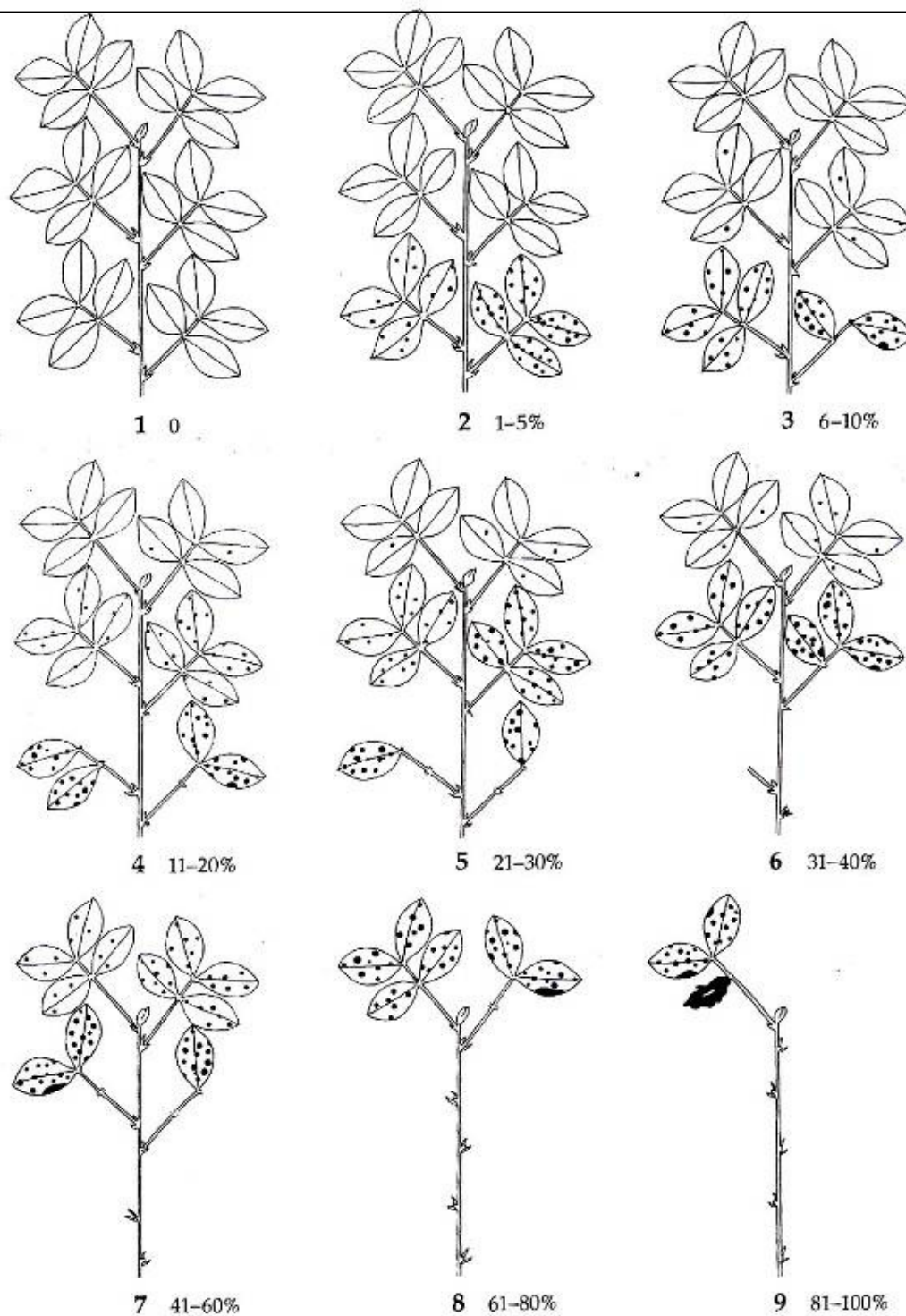


Fig. 1b: The modified 9-point scale for field evaluation of late leaf spot of groundnut

3.4.2 Yield attributing traits

1. Number of pods per plant: Number of pods per plant was counted at the time of harvest from two plants of each RIL in two replications.
2. Pod yield per plant (g): Mean dry weight of Pods from five plants of each RIL was recorded in grams.
3. Shelling Percent (%): 100g of dry pods of each RIL was shelled and shelling % was calculated as kernel weight (g) /pod weight (g) and expressed in percent.
4. Hundred Seed weight (g): Weight of well dried randomly selected 100 seeds was weighed in grams after the harvest from each RIL in two replications.

3.4.3 Quality traits

The data obtained from the studies of Sarvamangala (2009) and Mukri (2011) on quality parameters viz., oil content, protein content, oleic acid, linoleic acid and O/L ratio has been utilized only for QTL analysis for quality traits with new genetic maps developed upon saturation with additional markers.

3.5 Genotyping

3.5.1 DNA isolation of RILs and parents

DNA was extracted from fresh leaves of the parental genotypes and from their respective RILs using the modified cetyl trimethyl ammonium bromide (CTAB) extraction method, as described in Cuc *et al.* (2008). The DNA quality and quantity were checked on 0.8% agarose gels and DNA concentration was normalized to ~5 ng/ μ l for further work. Subsequently identified polymorphic markers were used to genotype the RILs of the respective populations. PCR reactions and separation of fragments of SSR markers was done essentially the same as given in Varshney *et al.* (2009a).

3.5.2 Quantification of DNA

Quality of the sample DNA stock was checked and quantified in 0.8 percent agarose gel with known concentration of uncut lambda DNA of 50 ng/ μ l, 100 ng/ μ l and 200 ng/ μ l. The DNA stocks of the samples were diluted accordingly to make it to required concentration of 5 ng/ μ l. Then the diluted DNA was confirmed with 5.0 ng/ μ l and 10 ng/ μ l uncut lambda in 0.8 percent agarose gel.

3.5.3 Amplification

Polymerase Chain Reactions (PCR) were performed by using a Touch - Down PCR profile as mentioned below and an amplification protocol appropriate for each pair of primers. DNA amplification was performed in 5 μ l reaction mixture using Gene Amp® PCR system 9700. The reaction mixture contained 5 ng/ μ l template DNA, 10 pM/ μ l SSR primers pair (Forward and Reverse), 25 mM MgCl₂ (Bioline), 2 mM DNTP's, 10X PCR buffer (Bioline), and 5U/ μ l Taq DNA polymerase (Bioline). PCR and separation of amplicons for *FAD2A* and *FAD2B* allele specific markers were done as explained in Chen *et al.* (2010).

Touch Down Profile

Steps	Temperatures ($^{\circ}$ C)	Time
Initial denaturation	95	5 min
Denaturation	94	45 sec
Annealing	65	45 sec
Primer extension	72	45 sec
Denaturation	94	45 sec
Annealing	59	45 sec
Primer extension	72	45 sec
Final extension	72	10 min
Store at	4	

3.5.4 Electrophoresis

Before loading PCR Products in the sequencing gel, amplification was checked on 1.4 percent agarose gel. For the separation of DNA fragments, non-denaturing polyacrylamide gel electrophoresis (PAGE) and capillary electrophoresis were used.

a) Non-Denaturing Polyacrylamide Gel Electrophoresis (PAGE)

After PCR amplification, 1 μ l of orange dye was added to 5 μ l reaction mixture. Then 2 μ l of this reaction mixture was loaded by using Hamilton syringe on each lane of 96-track of 6% non-denaturing PAGE and as the base pair marker, 100 bp DNA ladder was loaded on both the corners of the gel. Recipe for 6% gel consisted of 52.5 ml of distilled water, 7.5 ml of 10X TBE, 15 ml of acrylamide-methylbisacrylamide 29:1 (V/V), 100 μ l of TEMED and 450 μ l of ammonium per sulphate (APS). Electrophoresis was run at 900 volts for 2 hours in 0.5X TBE running buffer, using BIORAD sequencing gel unit.

PCR products were visualized by using silver staining protocol (Kolodny, 1984). Initially, the gel was rinsed with distilled water for 5 min with gentle shaking followed by soaking in 0.1% CTAB for 20 min (1.5 g in 1.5 litre of water) then kept in 0.3% liquid ammonia for 15 min (19.5 ml of 25% liquid ammonia solution in 1.5 litre of water) and later placed in 0.1% silver nitrate solution (1.5 g of silver nitrate + 6 ml of 1M NaOH in 1.5 litre of water and then titrated with ammonia solution till it became colorless) followed by rinsing in water for 1 min. After this gel was kept for developing in developer solution (22.5 g sodium carbonate and 400 μ l of formaldehyde in 1.5 litre of water) till the bands become conspicuous. The gel was kept in water for 5 min to stop the staining reaction and fixed in 3% glycerol.

After staining the gel, bands were scored as A, B, H, and O. Where, A represents homozygosity for the allele from female parent (TAG 24), B indicates homozygosity for the allele from male parent (GPBD 4) and H denotes heterozygote *i.e.* presence of A and B alleles and O represents off type (neither A nor B allele). A subjective score was given to each marker from 1 to 4 denoting the fragment amplification quality. Where, 1 indicates single and strong band, 2 represents single and weak band, 3 denotes multiple and strong bands and 4 indicates multiple and weak bands.

b) Capillary electrophoresis (ABI 3730 DNA sequencer)

After confirming the PCR amplification on 1.2 percent agarose gel, amplified products were separated by using capillary electrophoresis. For post PCR multiplexing, 1.2 μ l PCR products of FAM, HEX and TAMRA were mixed separately to a mix of 7 μ l of formamide, 0.05 μ l of Rox-500/Rox-400 size standard (For VIC, NED and PET labeled primers, 0.05 μ l of Liz-500 was used as a size standard) and 2.95 μ l of double distilled water (adjusted as per dye and number of primers used for multiplexing). The pooled PCR amplicons were denatured for 5 min at 94°C (Before placing plates containing samples were centrifuged at 900 rpm for 1 minute) and size fractionated using capillary electrophoresis on an ABI-3730 automatic DNA sequencer (Applied Biosystems, USA). Allele sizing (A, B, H and missing (-) peak patterns) of the electrophoretic data was carried out using GeneMapper[®] version4.

3.5.5 Parental polymorphism and genotyping in mapping population using SSRs

In addition to the markers reported in Khedikar *et al.* (2010) and Cholin *et al.* (2011), a set of novel EST-SSR markers (2,098) developed at University of Georgia (Nagy *et al.*, 2010) and genomic SSR markers (123) reported by Wang *et al.* (2007b) were used to screen parental polymorphism between the parental genotypes of the respective mapping population.

3.6 Statistical analysis

Data collected on each RIL in two replications at each environment was used to calculate mean, range, genetic parameters and correlation analysis as given below.

3.6.1 Mean and range

The mean and range of each character were calculated for each genotype at each location.

- Sum of observations of all plants
- i. Mean ($\bar{X}$) = $\frac{\text{Sum of observations of all plants}}{\text{Number of plants}}$
- ii. Range = The minimum and maximum values for each trait.

3.6.2 Analysis of variance (ANOVA)

The analysis of variance (ANOVA) for all the characters was carried out. The model of analysis of variance is given below.

Sl. No.	Source of variation	df	MSS	Expected MSS
1.	Replication	$r - 1$	M1	-
2.	Genotypes	$g - 1$	M2	$\sigma^2_e + \sigma^2_g$
3.	Error	$(r - 1)(g - 1)$	M3	σ^2_e
4.	Total	$(rg - 1)$		

3.6.3 Two way analysis of variance

The data obtained over locations was subjected to two way analysis of variance using the method outlined by Sundararaj *et al.* (1972). This was done for each character in order to find out the variation due to genotypes and environments to reveal the existence of genotype $\times$ environment interaction.

Sources of variation	Df	MSS	Expected MSS
Replication (r)	$(r - 1)$		
Environments (t)	$(t - 1)$	M1	$\sigma^2_e + r\sigma^2_{g \times e} + rg\sigma^2_E$
Genotypes (G)	$(g - 1)$	M2	$\sigma^2_e + r\sigma^2_{g \times e} + rt\sigma^2_G$
G $\times$ E interaction	$(g - 1)(t - 1)$	M3	$\sigma^2_e + r\sigma^2_{g \times e}$
Pooled Error	$gt(r - 1)$	M4	σ^2_e
Total	$t(rg - 1)$		

3.6.4 Estimation of genetic parameters

In order to assess and quantify the genetic variability among the genotypes for important yield contributing characters, the following parameters were estimated.

3.6.4.1 Estimation of variance components

Phenotypic and genotypic variance was estimated using the following formula (Singh and Chaudhary, 1979).

$$\text{Genotypic variance } (\sigma^2_g) = \frac{\text{MSS (genotype)} - \text{MSS (error)}}{\text{Number of replications}} = \frac{M2 - M3}{r}$$

Phenotypic variance (σ^2_p) =

$$= \sigma^2_g + \text{MSS error} = \frac{M2 - M3}{r} + M3$$

For pooled data,

$$\text{Genotypic variance } (\sigma^2_g) = \frac{\text{MSS (Genotypes)} - \text{MSS (G} \times \text{E)}}{rt}$$

$$\text{Phenotypic variance } (\sigma^2_p) = \sigma^2_g + \text{MSS error}$$

3.6.4.2 Coefficient of variability

Both genotypic and phenotypic coefficients of variability were computed as per the method suggested by Burton and Devane (1953).

a. Genotypic coefficient of variability (GCV)

$$GCV = \frac{\sigma_g}{\bar{X}} \times 100$$

b. Phenotypic coefficient of variability (PCV)

$$PCV = \frac{\sigma_p}{\bar{X}} \times 100$$

Where,

σ_g = Genotypic standard deviation

σ_p = Phenotypic standard deviation

$\bar{X}$ = General mean of the character.

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

0-10%	: Low
10-20%	: Moderate
20% and above	: High

3.6.4.3 Heritability (h^2)

Habitability in broad sense was computed as the ratio of genetic variance to the total phenotypic variance as suggested by Hanson *et al.* (1956) and expressed as percentage.

$$\text{Heritability } (h^2) = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where, σ^2_g = Genotypic variance; σ^2_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

3.6.4.4 Genetic advance (GA)

Genetic advance was estimated by using the formula given by Johnson *et al.* (1955).

$$GA = h^2 k \sigma_p$$

Where,

h^2 = Heritability in broad sense

k = Selection differential which is equal to 2.06 at 5% intensity for selection.

σ_p = Phenotypic standard deviation.

3.6.4.5 Genetic advance as percent of mean (GAM)

$$\text{GAM} = \frac{\text{GA}}{\bar{X}} \times 100$$

Where,

GA = Genetic advance

$\bar{X}$ = General mean of the character.

Genetic advance as percent of mean was categorized as low, moderate and high as given by Johnson *et al.* (1955).

It is as follows,

0-10%	: Low
10-20%	: Moderate
20% and above	: High

3.6.5 Correlation analysis

The correlation coefficients were worked out to determine the degree of association of a character with yield and also among the yield components. Such correlations were calculated for each location separately.

Genotypic and phenotypic correlations were computed by using the formula given below.

$$r_g = \frac{\text{Cov X Y}_g}{\sqrt{X^2_g \times Y^2_g}}$$

Where,

r_g = Genotypic correlation

Cov X Y_g = Genotypic covariance between the characters 'X' and 'Y'.

X^2_g and Y^2_g = Genotypic variance of the characters 'X' and 'Y' respectively.

$$r_p = \frac{\text{Cov X Y}_p}{\sqrt{X^2_p \times Y^2_p}}$$

Where,

r_p = Phenotypic correlation

Cov X Y_p = Phenotypic covariance between the characters 'X' and 'Y'.

X^2_p and Y^2_p = phenotypic variance of the characters 'X' and 'Y' respectively.

Genotypic and phenotypic correlation coefficients were compared against r values given in Fisher and Yates (1963) table at (n-2) degrees of freedom at the probability levels of 0.05 and 0.01 to test their significance.

3.7 Genotypic data analysis

3.7.1 Genetic map construction

Genotyping data generated on RILs of both mapping population was subjected to Chi square test to test the null hypothesis of expected 1:1 Mendelian segregation for each of the marker. Later markers showing Segregation Distortion (SD) were listed and percent of distorted markers was calculated. Due to less number of markers the distorted markers were also considered for linkage map construction.

Genotyping data obtained for all the polymorphic marker loci on both mapping populations (RIL-4 and RIL-5) were used for the linkage map construction using MAPMAKER/EXP V 3.0 (Lander *et al.*, 1987; Lincoln *et al.*, 1992). A minimum LOD score of 5.0 and maximum recombination fraction (θ) of 0.5 were set as threshold values for linkage group determination. Linkage groups were defined with "Sequence All" command. The most likely order within each linkage group was estimated by using three point analysis ("Three Point" command). Marker orders were confirmed by comparing the log likelihood of the possible orders using multipoint analysis ("Compare" command) and by permuting all the adjacent triple orders ("Ripple" command).

In the second step, LOD score was set to 3.0 in order to include new markers in the linkage groups. "Try" and "Build" commands were used to determine the exact position and order of the new marker on the linkage group. The new marker orders were again confirmed with the "Compare" and "Ripple" commands. Finally the best possible order in each linkage map was used for map construction. Recombination fraction was converted into map distances in centiMorgans (cM) using Kosambi mapping function (Kosambi 1944). The inter-marker distances calculated from MAPMAKER were used to construct linkage map by using MAPCHART version 2.2 (Voorrips 2006).

3.7.2 Consensus genetic map Construction

A consensus map was developed with JoinMap 4.0 (Van Ooijen 2006) using two individual linkage maps constructed based on the RIL-4 and RIL-5 mapping populations. The "Locus Genotype Frequency" function was used to calculate chi-square (χ^2) values for each marker to test for expected 1:1 segregation ratio. Markers were placed into linkage groups with the "LOD Groupings" and "Create Groups for Mapping" command using Kosambi's mapping function (Kosambi 1944). Calculation parameters were set for a minimum LOD of 5 and recombination fraction of 0.45. Marker order in groups was established using the "Calculate Map" command. Linkage groups with common markers on individual maps were merged to create a composite map using "Join-combine Groups for Map Integration" command and the order of the markers was fixed using the marker order from framework map obtained from mapmaker. Further marker order in groups was established using the "Calculate Map" command. Linkage maps were drawn using MapChart for Windows (Voorrips 2006).

3.7.3 Marker-Trait Association

3.7.3.1 Quantitative trait loci (QTL) analysis

Phenotypic data on LLS, rust, morphological, yield and quality related traits in each individual seasons and across the seasons were combined with genotypic data and linkage map in order to identify the QTLs associated. QTL analysis was conducted using WinQTL Cartographer, version 2.5 (Wang *et al.*, 2007a).

The replicated means of RILs for all the traits were used for QTL mapping in each individual season. For identification of QTLs, composite interval mapping (CIM) approach (Zeng 1994) was deployed using WinQTL Cartographer, version 2.5 (Wang *et al.*, 2007a). CIM was performed using the Model 6, scanning intervals of 2.0 cM between markers and putative QTLs with a window size of 10.0 cM. The number of marker cofactors for the background control was set by forward-backward stepwise regression. Automatically "Locate QTLs" option was used with minimum of 5 cM between QTLs to define a QTL region and if the peak's distance was less than 5.0 cM, then the highest peak was considered to locate QTLs. Permutations for 500 times were also done while determining the QTLs using WinQTL Cartographer, version 2.5 (Wang *et al.*, 2007a) using the option "permutations times" with 0.05 significance level.

The presence of putative QTL in an interval was tested by using a critical value for LOD threshold as determined by QTL CARTOGRAPHER using the Bonferroni chisquare approximation (Zeng, 1994) corresponding to genome wise type-I error of 0.25. As the mapping population comprised of RILs, the additive model "AA" was used for analysis in which additive $\times$ additive epistatic effects were included. The point at which the LOD score had the maximum value in the interval was taken as the estimated QTL position. The coefficient of determination also known as coefficient of variance (R^2) explained by the QTL was used as a measure of the magnitude of association and it is estimated as the square of the partial correlation coefficient. Estimates of the additive effect of each detected QTL, the total LOD score, the total proportion of phenotypic variance explained by all the detected QTLs were obtained by fitting a multiple linear regression model that simultaneously included all the detected QTLs for the traits in question. The LOD score was calculated from the F value for the multiple regressions (Haley and Knott, 1992) as

$$\text{LOD} = n/n (1 + p8F/Df) * 0.2171$$

Where,

p = number of parameters fitted (Haley and Knott, 1992)

F ratio = $\text{SSR (full)} - \text{SSR (red)} / \text{pMSE (full)}$

SSR (full) = Sum of square for regression with full model *i.e* with QTL and cofactors

SS (red) = Sum of square for regression with reduced model *i.e* without the QTL

MSE (full) = $\text{SSE} / \text{DEF} = \text{Residual mean square (full model)}$

pMSE = Number of estimated QTL effects

Df = Number of degrees of freedom for residual sum of square in multiple regression,

The percentage of phenotypic variance (R^2) explained by a QTL was estimated. This is based on the partial correlation of putative QTL with observed variable, adjusted for cofactors (Kendall and Stuart, 1961). In the simultaneous fit, the cofactors are ignored and only the putative QTLs initially detected and their estimated position were used in multiple regressions to obtain the final estimates of the additive effects and the percentage of phenotypic variation for the particular trait that could be explained by the QTLs. The additive effect was calculated as half the differences between genotypic values of two homozygotes (Falconer, 1989):

$$\text{Additive effect} = (\text{Parent 2} - \text{Parent 1}) / 2$$

3.7.3.2 Single marker analysis (SMA)

Single marker analysis was performed to validate the markers linked to the rust resistance based on the genotypic and phenotypic data on the germplasm comprising of 64 accessions, which is based on simple linear regression method (Haley and Knott, 1992). The germplasm consisted of susceptible and resistant accessions derived from different breeding strategies where in a total of 8 were identified from selection, 16 accessions derived through hybridization with the background of resistant NcAc accessions 2214, 2232 and 2240, 16 from hybridization followed by selection with diverse genetic background, 13 accessions were interspecific derivatives where in wild species has been utilized as the donors for resistance and 11 were derived from mutations.

EXPERIMENTAL RESULTS

Mapping population consisting of 266 (RIL-4) and 146 (RIL-5) RILs were considered for the present study. Phenotyping was done for diseases (rust and LLS), morphological (plant height, number of branches, leaf length and leaf width) and yield attributing traits (Number of pods per plant, pod yield per plant, shelling percent and hundred seed weight) at University of Agricultural Sciences, Dharwad. The populations were segregating for all the above traits. In addition to earlier phenotyping data for LLS and rust on both the populations (during 2004, 2005, 2006, and 2007), phenotyping was done during Kharif 2008, 2009 and 2010. Whereas, for morphological and yield traits phenotyping was carried out during Kharif 2007, 2008 and 2010. Genotypic data was generated using 208 (RIL-4) and 206 (RIL-5) polymorphic SSR markers at Applied Genomics Lab, ICRISAT and utilized for map development. Phenotypic data obtained on the two populations were analyzed for estimating genetic parameters, mean and range. QTL analysis was performed by combining the genotypic and phenotypic data generated on both populations. The experimental results of the present investigation have been divided in following sub headings.

4.1 Phenotypic data analysis

4.2 Genotypic data analysis

4.3 QTL analysis

4.4 Consensus QTL map for diseases, morphological, yield attributing and quality traits

4.5 Validation of rust resistance linked markers in germplasm

4.1 Phenotypic data analysis

4.1.1 Analysis of variance (ANOVA)

Analysis of variance for LLS and rust in both the populations revealed significant differences among the RILs in all the seasons/environments of phenotyping and at both the stages of disease scoring [Table 3 (RIL-4) and 4 (RIL-5)]. Similarly, ANOVA for morphological and yield attributing traits during Kharif 2007, 2008 and 2010 revealed significant differences among the RILs for plant height, leaf length, number of pods per plant, pod yield per plant, shelling percent and hundred seed weight in both RIL-4 (Table 5) and RIL-5 (Table 6) population. Significant differences were observed between RILs for leaf width and number of primary branches during 2008 and 2010 in RIL-5 population, but for the same traits variance component in RIL-4 population had a non significant difference among the RILs. The magnitude of coefficient of variation (CV @ 5 %) was less than the threshold level of acceptance (Table 3 and 4) in both the populations for both the diseases in all environments except for LLS (LLS-SI= 22.54%) during Kharif 2004 in RIL-4 (Table 3) population and in case of RIL-5 population a higher magnitude of CV was observed for LLS (Table 4) during Kharif 2004 (LLS-SI=22.98% and LLS-SII=26.52%) and during 2007E2 (LLS-SI=30.60). In general morphological and yield attributing traits exhibited a lower magnitude of coefficient of variation in all the three seasons of evaluation. However, for number of pods per plant and pod yield per plant a higher degree of CV was observed in both the populations during Kharif 2007 [Table 5 (RIL-4) and 6 (RIL-5)].

4.1.2 qPooled Analysis of variance (Pooled ANOVA)

4.1.2.1 Late leaf spot and rust

In view of estimating the genotype $\times$ environment interaction, pooled ANOVA was performed with the phenotypic data recorded across six seasons/environments for LLS in both the populations. The analysis revealed significant differences among the RILs at both the stages of disease scoring and also between the environments in RIL-4 (Table 7) and RIL-5 population (Table 8). Significant differences was also observed for variance due to G $\times$ E interaction which revealed the significant influence of environment and its interaction effect on the occurrence of disease.

Likewise, pooled ANOVA (Table 7) for rust across 6 environments in RIL-4 and 7 environments in RIL-5 population (Table 8) exhibited significant differences among the RILs, environments and G $\times$ E interaction at both the stages of disease scoring.

4.1.2.2 Morphological and yield attributing traits

Data recorded on morphological and yield attributing traits across 3 seasons/environments was subjected to pooled ANOVA for estimating the G $\times$ E interaction effect. Analysis revealed



Creation of artificial disease epiphytotics for LLS and rust using spreader row technique



RILs segregating for rust



RILs segregating for LLS

Plate 1: Artificial disease epiphytotics for LLS and rust and segregating pattern of RILs for disease

Table 3: ANOVA for Late leaf spot and rust in TAG 24 × GPBD 4 (RIL-4) population

Source	Season/ Environment	df	Late leaf spot		Source	Season/ Environment	df	Rust	
			LLS-SI	LLS-SII				Rust-SI	Rust-SII
Replication	<i>Kharif-2004</i>	1	2.42	3.95	Replication	<i>Kharif-2006</i>	1	1.91	1.36
	<i>Kharif-2005</i>		0.42	16.49		<i>Kharif-2007E1</i>		0.32	0.12
	<i>Kharif-2006</i>		10.22	24.67		<i>Kharif-2007E2</i>		109.26	38.69
	<i>Kharif-2008</i>		1.46	0.03		<i>Kharif-2008</i>		9.94	60.45
	<i>Kharif-2009</i>		18.66	0.23		<i>Kharif-2009</i>		2.84	28.69
	<i>Kharif-2010</i>		0.42	2.98		<i>Kharif-2010</i>		5.04	33.50
Genotypes	<i>Kharif-2004</i>	267	3.05**	3.69**	Genotypes	<i>Kharif-2006</i>	267	4.62**	5.79**
	<i>Kharif-2005</i>		3.69**	3.66**		<i>Kharif-2007E1</i>		1.42**	1.96**
	<i>Kharif-2006</i>		1.69**	3.45**		<i>Kharif-2007E2</i>		9.35**	8.99**
	<i>Kharif-2008</i>		6.93**	0.92**		<i>Kharif-2008</i>		2.11**	8.83**
	<i>Kharif-2009</i>		2.56**	4.75**		<i>Kharif-2009</i>		2.62**	4.22**
	<i>Kharif-2010</i>		1.23**	4.68**		<i>Kharif-2010</i>		0.81**	3.21**
Error	<i>Kharif-2004</i>	267	1.03	1.49	Error	<i>Kharif-2006</i>	267	0.29	0.39
	<i>Kharif-2005</i>		0.53	0.34		<i>Kharif-2007E1</i>		0.46	0.68
	<i>Kharif-2006</i>		0.45	0.73		<i>Kharif-2007E2</i>		0.96	0.94
	<i>Kharif-2008</i>		0.91	0.29		<i>Kharif-2008</i>		0.33	0.58
	<i>Kharif-2009</i>		0.74	0.92		<i>Kharif-2009</i>		0.61	0.95
	<i>Kharif-2010</i>		0.32	0.59		<i>Kharif-2010</i>		0.37	0.94
CV (%)	<i>Kharif-2004</i>		22.54	17.57	CV (%)	<i>Kharif-2006</i>		10.97	11.01
	<i>Kharif-2005</i>		13.55	9.08		<i>Kharif-2007E1</i>		15.54	15.37
	<i>Kharif-2006</i>		19.85	13.90		<i>Kharif-2007E2</i>		19.31	15.35
	<i>Kharif-2008</i>		16.04	17.26		<i>Kharif-2008</i>		15.43	12.77
	<i>Kharif-2009</i>		14.84	14.27		<i>Kharif-2009</i>		14.34	14.44
	<i>Kharif-2010</i>		11.98	12.67		<i>Kharif-2010</i>		13.65	17.24

* = significant at $P < 0.05$, ** = significant at $P < 0.01$, ns = not significant, LLS-SI: Stage I =70DAS, LLS-SII: Stage II =90DAS,

Rust-SI: Stage I =80DAS, Rust-SII: Stage II =90DAS, E1: Environment 1, E2: Environment 2, DAS: Days after sowing

Table 4: ANOVA for Late leaf spot and rust in TG 26 × GPBD 4 (RIL-5) population

Source	df	Season/ Environment	Late leaf spot		Source	df	Season/ Environment	Rust	
			LLS-SI	LLS-SII				Rust-SI	Rust-SII
Replication	1	Kharif-2004	0.49	2.65	Replication	1	Kharif-2004	0.08	0.34
		Kharif-2005	4.63	5.68			Kharif-2005	1.49	1.79
		Kharif-2007E2	99.95	9.85			Kharif-2007E1	0.12	6.25
		Kharif-2008	1.49	1.49			Kharif-2007E2	32.45	8.11
		Kharif-2009	1.64	13.41			Kharif-2008	0.05	3.46
		Kharif-2010	0.27	1.09			Kharif-2009	18.00	0.05
						Kharif-2010	0.01	0.76	
Genotypes	147	Kharif-2004	2.71**	2.79**	Genotypes	147	Kharif-2004	0.71**	1.32**
		Kharif-2005	4.05**	2.43**			Kharif-2005	1.57**	4.16**
		Kharif-2007E2	2.93**	1.41**			Kharif-2007E1	0.75**	1.68**
		Kharif-2008	0.68**	6.14**			Kharif-2007E2	7.72**	8.14**
		Kharif-2009	1.95**	4.31**			Kharif-2008	2.00**	7.98**
		Kharif-2010	0.68**	4.08**			Kharif-2009	2.77**	4.42**
						Kharif-2010	0.62**	3.47**	
Error	147	Kharif-2004	1.04	1.46	Error	147	Kharif-2004	0.25	0.28
		Kharif-2005	0.50	0.67			Kharif-2005	0.37	0.64
		Kharif-2007E2	1.71	0.78			Kharif-2007E1	0.26	0.46
		Kharif-2008	0.17	0.28			Kharif-2007E2	0.67	1.10
		Kharif-2009	0.56	0.72			Kharif-2008	0.22	0.21
		Kharif-2010	0.29	0.63			Kharif-2009	0.51	0.67
						Kharif-2010	0.31	0.73	
CV (%)		Kharif-2004	22.98	26.52	CV (%)		Kharif-2004	14.07	11.74
		Kharif-2005	12.68	13.36			Kharif-2005	20.37	19.78
		Kharif-2007E2	30.6	11.91			Kharif-2007E1	14.15	12.91
		Kharif-2008	14.69	11.37			Kharif-2007E2	16.81	16.32
		Kharif-2009	14.08	14.36			Kharif-2008	11.7	7.1
		Kharif-2010	11.76	13.51			Kharif-2009	12.48	11.35
						Kharif-2010	12.48	14.67	

* = significant at P < 0.05, ** = significant at P < 0.01, ns = not significant, LLS-SI: Stage I =70DAS, LLS-SII: Stage II =90DAS,

Rust-SI: Stage I =80DAS, Rust-SII: Stage II =90DAS, E1: Environment 1, E2: Environment 2, DAS: Days after sowing

Table 5: ANOVA for morphological and yield attributing traits in TAG 24 × GPBD 4 (RIL-4) population

Source	df	Environment /Season	Plant height (cm)	Number of primary branches	leaf length (mm)	Leaf width (mm)	Number of pods per plant	Pod yield per plant (g)	Shelling Percent (%)	Hundred seed weight (g)
Replication	1	<i>Kharif-2007</i>	3822.85	2.42	12.39	33.75	107.20	141.37	162.91	12.32
		<i>Kharif-2008</i>	2.25	20.77	154.75	21.36	47.46	131.73	1395.94	62.14
		<i>Kharif-2010</i>	361.98	0.29	747.55	167.77	1.09	44.44	0.78	104.79
Genotypes	267	<i>Kharif-2007</i>	31.89**	0.48**	68.66**	25.42**	41.39**	25.59**	48.66**	48.91**
		<i>Kharif-2008</i>	25.37**	1.76**	35.30**	7.75 ^{ns}	56.54**	47.73**	27.32**	43.32**
		<i>Kharif-2010</i>	26.10**	0.81 ^{ns}	3.11**	0.31**	8.74**	30.96**	31.43**	42.93**
Error	267	<i>Kharif-2007</i>	9.15	0.33	30.99	14.22	16.92	13.29	13.27	7.28
		<i>Kharif-2008</i>	9.01	1.08	21.20	6.93	5.08	15.47	13.96	9.54
		<i>Kharif-2010</i>	3.56	0.67	0.95	0.19	6.08	5.43	7.00	12.95
CV (%)		<i>Kharif-2007</i>	14.86	8.93	14.38	19.27	22.43	23.63	4.99	6.45
		<i>Kharif-2008</i>	9.71	15.70	9.51	12.20	7.74	18.32	5.22	8.34
		<i>Kharif-2010</i>	7.38	13.05	1.99	1.85	8.25	12.64	3.63	8.48

* = significant at $P < 0.05$, ** = significant at $P < 0.01$, ns = not significant

Table 6: ANOVA for morphological and yield attributing traits in TG 26 × GPBD 4 (RIL-5) population

Source	df	Environment /Season	Plant height (cm)	Number of primary branches	leaf length (mm)	Leaf width (mm)	Number of pods per plant	Pod yield per plant (g)	Shelling Percent (%)	Hundred seed weight (g)
Replication	1	<i>Kharif-2007</i>	79.76	5.82	18	3.79	0.02	8.41	71.76	38.66
		<i>Kharif-2008</i>	14.14	8.28	175.62	30.49	137.17	32.31	143.36	79.6
		<i>Kharif-2010</i>	11.22	0.86	1.09	32.78	84.34	19.72	2.77	34.46
Genotypes	147	<i>Kharif-2007</i>	39.59**	1.47**	84.21**	15.61**	38.12**	28.12**	20.33*	76.76**
		<i>Kharif-2008</i>	29.85**	1.46**	49.41**	11.93**	88.77**	83.06**	32.88**	45.24**
		<i>Kharif-2010</i>	22.54**	0.48**	34.59**	11.89**	29.73**	36.38**	23.46**	29.84**
Error	147	<i>Kharif-2007</i>	11.00	0.99	23.18	7.29	10.39	7.35	14.61	11.72
		<i>Kharif-2008</i>	11.88	0.99	17.11	5.26	19.28	14.41	17.50	8.22
		<i>Kharif-2010</i>	5.81	0.36	8.34	5.43	16.27	8.03	10.10	5.16
CV(%)		<i>Kharif-2007</i>	14.35	16.48	9.46	11.63	19.32	20.95	4.98	9.93
		<i>Kharif-2008</i>	11.11	14.45	7.8	9.88	13.69	15.92	5.83	8.03
		<i>Kharif-2010</i>	9.07	9.92	6.05	10.03	18.82	13.26	4.27	6.12

* = significant at P < 0.05, ** = significant at P < 0.01, ns = not significant

Table 7: Pooled ANOVA for Late leaf spot and rust in TAG 24 × GPBD 4 (RIL-4) population

Source	df	Late leaf spot		df	Rust	
		LLS-SI	LLS-SII		Rust-SI	Rust-SII
Replication	1	17.31	0.72	1	0.34	30.46
Environments	5	544.82**	139.30**	5	201.76**	136.69**
Genotypes	267	7.35**	15.65**	267	13.59**	22.61**
G × E Interaction	1335	1.16**	2.30**	1335	1.47**	2.08**
Pooled Error	1602	0.56	0.83	1602	0.50	0.75
CV (%)		16.56	14.46		15.21	14.53

* = significant at $P < 0.05$, ** = significant at $P < 0.01$, ns = not significant,
 LLS-SI: Stage I =70DAS, LLS-SII: Stage II =90DAS, DAS: Days after sowing
 Rust-SI: Stage I =80DAS, Rust-SII: Stage II =90DAS, DAS: Days after sowing

Table 8: Pooled ANOVA for Late leaf spot and rust in TG 26 × GPBD 4 (RIL-5) population

Source	df	Late leaf spot		df	Rust	
		LLS-SI	LLS-SII		Rust-SI	Rust-SII
Replication	1	10.88	18.85	1	0.66	6.05
Environments	5	283.24**	328.99**	6	245.59**	385.88**
Genotypes	147	5.92**	9.06**	147	9.53**	20.14**
G × E Interaction	735	1.42**	2.42**	882	1.10**	1.84**
Pooled Error	882	0.71	0.76	1029	0.37	0.59
CV (%)		18.75	15.11		14.58	13.45

* = significant at $P < 0.05$, ** = significant at $P < 0.01$, ns = not significant,
 LLS-SI: Stage I =70DAS, LLS-SII: Stage II =90DAS, DAS: Days after sowing
 Rust-SI: Stage I =80DAS, Rust-SII: Stage II =90DAS, DAS: Days after sowing

Table 9: Pooled ANOVA for morphological and yield attributing traits in TAG 24 × GPBD 4 (RIL-4) population

Source	df	Plant height (cm)	Number of primary branches	leaf length (mm)	Leaf width (mm)	Number of pods per plant	Pod yield per plant (g)	Shelling Percent (%)	100 seed weight (g)
Replication	1	2099.02	2.02	43.18	2.12	1.95	92.97	216.48	11.46
Environments	2	14924.90**	18.15**	18045.52**	2144.68**	22427.78**	4897.46**	292.73**	4750.82**
Genotypes	267	58.31**	1.76**	46.56**	12.35**	45.19**	53.54**	55.42**	87.02**
G × E Interaction	534	12.53**	0.64 ^{ns}	30.25**	10.56**	30.74**	25.37**	25.99**	24.07**
Pooled Error	801	7.24	0.69	17.71	7.11	9.36	11.39	11.41	9.92
CV (%)		10.5	12.94	9.27	12.36	11.86	18.3	4.66	7.79

* = significant at P < 0.05, ** = significant at P < 0.01, ns = not significant

Table 10: Pooled ANOVA for morphological and yield attributing traits in TG 26 × GPBD 4 (RIL-5) population

Source	df	Plant height (cm)	Number of primary branches	leaf length (mm)	Leaf width (mm)	Number of pods per plant	Pod yield per plant (g)	Shelling Percent (%)	Hundred seed weight (g)
Replication	1	85.77	0.06	33.70	28.83	144.93	17.41	1.13	3.34
Environments	2	4672.71**	74.06**	2124.84**	0.021 ^{ns}	18353.08**	9676.87**	1844.30**	515.87**
Genotypes	147	65.05**	1.38**	120.71**	27.20**	95.09**	79.57**	36.09**	108.50**
G × E Interaction	294	13.47**	1.01**	23.76**	6.12 ^{ns}	30.76**	33.99**	20.29**	21.67**
Pooled Error	441	9.57	0.78	16.21	5.99	15.31	9.93	14.07	8.36
CV (%)		11.49	13.96	7.97	10.54	16.72	16.26	5.05	8.09

* = significant at P < 0.05, ** = significant at P < 0.01, ns = not significant

significant differences among the RILs across environments and also between environments in both the population (RIL-4 and RIL-5) for all the traits [Table 9 (RIL-4) and 10 (RIL-5)]. Variance due to $G \times E$ interaction was significant for all other traits except for number of primary branches in RIL-4 (Table 9) and leaf width in case of RIL-5 population (Table 10).

4.1.3 Components of variation

4.1.3.1 Mean and range in parents and RILs for late leaf spot and rust

The mean disease score of parents of both RIL-4 and RIL-5 population is presented in Table 11 and 12, respectively. Both the susceptible parents (TAG 24 and TG 26) exhibited higher incidence of disease in all the environments as compared to the resistant parent GPBD 4. Mean values of RILs for LLS and rust in both the population were slightly inclined towards the susceptible parents. The range as exhibited by RILs for LLS and rust scores were within the limit of mean disease scores revealed by the parents. However, in some season/environments the disease scores of RILs for LLS and rust diseases were beyond the disease scores of resistant and susceptible parents indicating the presence of transgressive segregants. The disease incidence in both the population increased with the stages and was comparable with the parents of mapping population [Table 11 (RIL-4) and 12 (RIL-5)].

4.1.3.2 Estimates of variance components for late leaf spot and rust

Variance components estimated in all the environments for both the populations is summarized in Table 11. The magnitude of variation as revealed by GCV and PCV indicated ample variability for LLS and rust at both the stages of disease scoring in RIL-4 population. For LLS, GCV was moderate (14.48) to high (31.43) and PCV ranged from 18.80 (moderate) to 31.74 (high). Similarly for rust in RIL-4 population, it was moderate to high for both GCV (10.46-40.32) and PCV (17.19-44.70).

On the other hand, in case of RIL-5 population GCV estimates were low to high for LLS (7.59-36.83) and Rust (8.99-38.54). Whereas PCV was moderate to high ranging from, 14.12-38.54 for LLS and 15.39-42.05 for rust in RIL-5 population (Table 12).

Heritability estimates in broad sense were estimated in all the environments at both the stages of disease scoring for LLS and rust in RIL-4 and RIL-5 population. Heritability estimate in RIL-4 population was moderate to high for both LLS and rust coupled with high (20.29-56.74) genetic advance over percent of mean (GAM) for LLS and GAM was moderate (13.10) to high (74.91) for rust. Heritability for LLS ranged from 42.50 % (LLS-SII_2004) to 83.23 % (LLS-SII_2005). Similarly for rust it ranged from 36.98 % (Rust-SI-2010) to 88.09 % (Rust-SI-2006) Table 11.

In case of RIL-5 population heritability was low (26.42 %) to high (91.32 %) for LLS and moderate (34.16 %) to high (84.01 %) for rust (Table 12). Further, GAM was low to high for LLS (8.41-72.50) and rust (10.8-72.76) indicating the wide scope for improving disease resistance. In general, in both the populations GCV and PCV for LLS and rust was moderate to high at both the stages of disease scoring coupled with moderate to high heritability and GAM.

4.1.3.3 Mean performance of parents and RILs for morphological and yield attributing traits

Differential performance was exhibited by the parents of mapping population for plant height, leaf length, leaf width, number of pods per plant, pod yield per plant and hundred seed weight. It was comparable and on par among the parents of population for number of primary branches and shelling percent [Table 13 (RIL-4) and 14 (RIL-5)].

Mean of the RILs were within the parental limit for all the morphological and yield attributing traits. However, as compared to the parents (Table 13 and 14) RILs exhibited wider range of variation and the mean values were beyond the parents, indicating the presence of transgressive segregants towards both higher and lower value parents for all the traits [Table 13 (RIL-4) and 14 (RIL-5)].

4.1.3.4 Estimates of variance components for morphological and yield attributing traits

In RIL-4 population, the extent of variability as explained by GCV and PCV ranged from low to moderate for morphological and yield attributing traits. Among the morphological traits, plant height had a moderate (13.41) to high (22.24) PCV as compared to other traits viz., leaf length (2.90-18.23), leaf width (2.12-22.74) seed weight (11.17-13.89) exhibited ample amount of variability ranging from moderate to high PCV. In general GCV was found to be low to moderate for plant height (9.25-16.55), number of primary branches (4.26-15.63), leaf length (2.12-11.21) and leaf width (1.04-12.08). Similarly for yield attributing traits the variance components (Table 13) explained low to moderate

GCV for number of pods per plant (3.86-19.07), hundred seed weight (8.01-11.10) and pod yield per plant (11.75-19.37), whereas shelling % recorded low GCV and PCV in each of the season and across seasons.

Heritability estimates in RIL-4 population was moderate to high for plant height (47.57-75.99), pod yield per plant (30.18-70.15), shelling percent (30.06-63.58) and hundred seed weight (51.39-74.10). However, for other traits it was low to moderate/high as presented in Table 13. Wide range of genetic advance over percent of mean (GAM) was observed among the traits phenotyped in the population with low to high variability coupled with low to high heritability. From among the traits moderate to high GAM was observed for plant height (13.14-25.37), pod yield per plant (13.08-33.43) and hundred seed weight (11.83-19.33). For other traits, generally GAM was low in most of the environments however it was moderate to high in specific environments/season for individual traits (Table 13). The pooled analysis across the seasons/environments revealed a significant reduction in all the components of variation indicating role of $G \times E$ interaction effects on the traits.

In case of RIL-5 population (Table 14), the degree of variability explained by GCV and PCV was low to high. Among the morphological and yield attributing traits moderate to high PCV was observed for plant height (14.16-21.76), number of pods per plant (21.81-29.51) and pod yield per plant (21.60-32.55). Whereas, number of primary branches (10.72-18.39), leaf length (9.71-14.40), leaf width (12.62-14.57), shelling percent (5.45-7.00) and hundred seed weight (11.27-19.30) explained low to moderate PCV. As compared to PCV, GCV was moderate to high for number of pods per plant (12.10-22.31) and pod yield per plant (14.22-24.57). However, all other traits showed low to moderate GCV (Table 14) except shelling percent (2.18-3.87) which was low in all the seasons and also across the seasons.

The heritability estimates in RIL-5 population (Table 14) was moderate to high for number of pods per plant (29.28-64.31), pod yield per plant (43.34-70.43), hundred seed weight (63.37-73.52), leaf length (48.55-61.16) and plant height (43.06-59.01). While it was moderate for leaf width (36.29-38.76), low to moderate for shelling percent (15.76-39.77) and low for number of primary branches (7.29-19.62). GAM in RIL-5 population was moderate to high for plant height, number of pods per plant, pod yield per plant and hundred seed weight. It was moderate for leaf length and leaf width but low for shelling percent and number of primary branches (Table 14).

4.1.4 Association studies

4.1.4.1 Association among late leaf spot and rust

Phenotypic and genotypic correlation coefficient estimates (Table 15) between stages revealed a significant positive association for both the diseases (LLS and rust). However negative association was observed between LLS and rust during Kharif 2008, 2009 and 2010. Correlation coefficients between LLS-SII and rust at both the stages (80 and 90 DAS) across environments had a significant negative association (Table 15).

Similar trend of association was seen in RIL-5 population (Table 16) among the diseases and stages of scoring. Though, significant negative association was observed between LLS and rust in most of the seasons/environments in RIL-5 population, a contrasting positive association was observed between LLS and rust at both the stages (SI and SII) during Kharif 2004. The range of correlation coefficients varied from 0.124 to 0.998 in RIL-4 and 0.159 to 0.996 in RIL-5 population.

4.1.4.2 Association among morphological traits

Significant positive association was observed for plant height with other morphological traits viz., primary branches, leaf length and leaf width in both (RIL-4 and RIL-5) the population. On contrary, as revealed by correlation matrix number of primary branches had shown a significant negative association with leaf length and leaf width. However, it exhibited positive association with leaf width and leaf length during Kharif 2007 and 2010 in case of RIL-4 population (Table 17). Similarly, in RIL-5 population (Table 16) it was positively associated with leaf length during Kharif 2007 and across environments.

4.1.4.3 Association among yield attributing traits

Genotypic and phenotypic correlation matrix revealed a significant positive association among the yield attributing traits in both the population [Table 17 (RIL-4) and 18 (RIL-5)]. However, significant association was not observed for shelling percent with majority of the traits in both RIL-4 and RIL-5 population. Though positive associations were observed among the yield attributing traits, shelling

Table 11: Mean, range, estimates of variance components, broad sense heritability and genetic advance over percent of mean (GAM) for LLS and rust in TAG 24 × GPBD 4 (RIL-4) population

Characters / Environment	TAG 24	GPBD 4	Mean	Range		GCV	PCV	h ² _{b.s}	GAM
				Min.	Max.				
Late leaf spot									
LLS-SI_2004	7.00	1.50	4.50	1.50	7.00	22.36	31.74	49.60	32.43
LLS-SII_2004	9.00	3.00	6.95	1.50	9.00	15.10	23.17	42.50	20.29
LLS-SI_2005	7.00	1.50	5.38	2.00	7.00	23.34	26.99	74.77	41.58
LLS-SII_2005	7.50	2.00	6.38	2.50	8.50	20.22	22.16	83.23	37.99
LLS-SI_2006	4.50	1.00	3.39	1.50	5.50	23.24	30.56	57.84	36.41
LLS-SII_2006	8.50	3.50	6.15	2.50	9.00	18.95	23.49	65.04	31.48
LLS-SI_2008	7.50	3.00	3.35	2.00	5.00	16.82	23.23	52.40	25.08
LLS-SII_2008	9.00	3.50	5.52	2.50	9.00	31.43	35.86	76.82	56.74
LLS-SI_2009	8.00	3.00	5.81	3.50	8.50	16.43	22.13	55.08	25.12
LLS-SII_2009	9.00	3.00	6.73	4.00	9.00	20.57	25.03	67.50	34.81
LLS-SI_2010	7.00	3.00	4.68	3.50	6.50	14.48	18.80	59.31	22.97
LLS-SII_2010	9.00	3.00	6.05	4.00	9.00	23.63	26.82	77.66	42.90
LLS-SI_Pooled	6.83	2.17	4.52	2.67	5.92	22.49	27.93	64.84	37.30
LLS-SII_Pooled	8.67	3.00	6.30	3.67	8.42	23.69	27.75	72.85	41.64
Rust									
Rust-SI_2006	7.00	3.00	4.93	2.50	7.50	29.83	31.79	88.09	57.68
Rust-SII_2006	8.00	3.00	5.65	2.50	8.00	29.09	31.10	87.49	56.06
Rust-SI_2007E1	5.50	3.00	4.35	3.00	6.50	15.97	22.29	51.33	23.57
Rust-SII_2007E1	7.50	4.00	5.38	3.00	7.50	14.90	21.41	48.39	21.35
Rust-SI_2007E2	6.50	2.50	5.08	2.00	9.00	40.32	44.70	81.35	74.91
Rust-SII_2007E2	8.00	3.00	6.31	3.00	9.00	31.82	35.32	81.13	59.04
Rust-SI_2008	6.50	3.00	3.73	2.50	6.00	25.31	29.64	72.87	44.50
Rust-SII_2008	8.50	4.00	5.98	3.00	9.00	33.96	36.28	87.62	65.48
Rust-SI_2009	7.00	3.00	5.46	4.00	7.50	18.33	23.27	62.04	29.75
Rust-SII_2009	8.00	3.00	6.75	4.00	9.00	18.93	23.81	63.26	31.02
Rust-SI_2010	6.00	3.00	4.46	3.50	6.50	10.46	17.19	36.98	13.10
Rust-SII_2010	7.00	3.00	5.63	4.00	8.50	18.93	25.60	54.66	28.83
Rust-SI_Pooled	6.50	2.92	4.67	3.08	6.42	30.45	34.04	80.02	56.11
Rust-SII_Pooled	7.83	3.33	5.95	3.50	8.17	31.09	34.31	82.08	58.02

LLS-SI: Stage I =70DAS, LLS-SII: Stage II =90DAS, Rust-SI: Stage I =80DAS, Rust-SII: Stage II =90DAS, E1: Environment 1, E2: Environment 2, DAS: Days after sowing

Table 12: Mean, range, estimates of variance components, broad sense heritability and genetic advance over percent of mean (GAM) for LLS and rust in TG 26 × GPBD 4 (RIL-5) population

Characters / Environment	TG 26	GPBD 4	Mean	Range		GCV	PCV	h ² _{b.s}	GAM
				Min.	Max.				
Late leaf spot									
LLS-SI_2004	5.50	2.00	4.43	2.00	7.00	20.61	30.88	44.56	28.34
LLS-SII_2004	7.00	3.00	4.55	2.00	8.50	17.93	32.03	31.35	20.69
LLS-SI_2005	5.50	2.00	5.59	2.50	7.00	23.82	26.98	77.91	43.31
LLS-SII_2005	7.00	2.00	6.14	3.00	8.00	15.26	20.28	56.62	23.65
LLS-SI_2007	5.00	3.00	4.27	2.00	7.50	18.34	35.68	26.42	19.42
LLS-SII_2007	7.50	3.00	7.40	5.00	9.00	7.59	14.12	28.90	8.41
LLS-SI_2008	6.00	3.00	2.81	2.00	4.00	17.92	23.16	59.84	28.55
LLS-SII_2008	7.00	4.00	4.65	3.00	8.00	36.83	38.54	91.31	72.50
LLS-SI_2009	6.50	3.00	5.32	4.00	7.50	15.65	21.04	55.30	23.97
LLS-SII_2009	8.00	3.00	5.92	4.00	9.00	22.62	26.79	71.31	39.35
LLS-SI_2010	5.00	3.00	4.56	4.00	6.00	9.76	15.28	40.83	12.85
LLS-SII_2010	7.00	3.00	5.85	4.00	8.50	22.48	26.23	73.45	39.69
LLS-SI_Pooled	5.58	2.67	4.23	3.17	6.08	20.48	28.58	51.35	30.23
LLS-SII_Pooled	7.25	3.00	5.47	4.17	7.58	19.22	24.94	59.44	30.53
Rust									
Rust-SI_2004	6.00	3.00	3.58	2.50	5.00	13.33	19.40	47.23	18.87
Rust-SII_2004	8.50	4.00	4.53	3.00	6.00	15.87	19.75	64.59	26.28
Rust-SI_2005	7.00	3.00	3.00	2.00	5.00	25.75	32.85	61.47	41.59
Rust-SII_2005	8.50	3.00	4.06	2.00	7.50	32.66	38.17	73.18	57.55
Rust-SI_2007E1	6.50	3.00	3.59	2.50	5.00	13.77	19.74	48.67	19.79
Rust-SII_2007E1	8.00	4.00	5.24	3.00	7.00	14.90	19.71	57.13	23.20
Rust-SI_2007E2	7.00	2.00	4.87	2.00	8.00	38.54	42.05	84.01	72.76
Rust-SII_2007E2	8.00	3.00	6.42	3.00	9.00	29.23	33.48	76.23	52.57
Rust-SI_2008	7.00	3.00	4.05	2.50	6.00	23.28	26.05	79.87	42.86
Rust-SII_2008	8.50	4.00	6.52	3.50	9.00	30.22	31.04	94.76	60.60
Rust-SI_2009	7.00	3.00	5.71	4.00	8.00	18.62	22.41	69.05	31.88
Rust-SII_2009	8.00	3.00	7.23	4.00	9.00	18.93	22.07	73.56	33.44
Rust-SI_2010	7.00	3.00	4.43	4.00	6.00	8.99	15.39	34.16	10.83
Rust-SII_2010	9.00	3.00	5.83	4.00	8.50	20.07	24.87	65.14	33.37
Rust-SI_Pooled	6.79	2.86	4.18	2.86	5.50	28.38	31.91	79.13	52.01
Rust-SII_Pooled	8.36	3.43	5.69	3.57	7.57	30.69	33.51	83.88	57.90

LLS-SI: Stage I =70DAS, LLS-SII: Stage II =90DAS, Rust-SI: Stage I =80DAS, Rust-SII: Stage II =90DAS, E1: Environment 1, E2: Environment 2, DAS: Days after sowing

Table 13: Mean, range, estimates of variance components, broad sense heritability and genetic advance over percent of mean (GAM) for morphological and yield attributing traits in TAG 24 × GPBD 4 (RIL-4) population

Characters	TAG 24	GPBD 4	Mean	Range		GCV	PCV	$h^2_{b.s}$	GAM
/ Environment				Min.	Max.				
Plant height (cm)									
<i>Kharif-2007</i>	26.38	34.30	20.37	9.65	34.38	16.55	22.24	55.39	25.37
<i>Kharif-2008</i>	27.90	35.28	30.92	21.48	44.45	9.25	13.41	47.57	13.14
<i>Kharif-2010</i>	29.30	35.68	25.57	17.16	38.66	13.13	15.06	75.99	23.58
<i>Pooled</i>	27.86	35.08	25.62	16.90	37.42	10.78	15.05	51.30	15.91
Number of primary branches									
<i>Kharif-2007</i>	6.00	6.50	6.41	5.00	8.00	4.28	9.91	18.64	3.80
<i>Kharif-2008</i>	6.00	6.25	6.63	4.50	10.00	8.75	17.98	23.70	8.78
<i>Kharif-2010</i>	7.50	6.25	6.26	4.50	7.50	4.26	13.73	9.61	2.72
<i>Pooled</i>	6.50	6.33	6.43	4.83	8.33	6.70	14.16	22.38	5.17
Leaf length (mm)									
<i>Kharif-2007</i>	41.50	50.75	38.71	22.00	65.00	11.21	18.23	37.80	14.20
<i>Kharif-2008</i>	42.50	57.50	48.44	36.75	64.50	5.48	10.97	24.95	5.64
<i>Kharif-2010</i>	48.50	56.50	49.05	46.09	53.46	2.12	2.90	53.22	3.18
<i>Pooled</i>	44.17	54.92	45.40	38.72	59.98	3.63	9.96	13.30	2.73
Leaf width (mm)									
<i>Kharif-2007</i>	20.75	27.50	19.58	11.50	35.50	12.08	22.74	28.25	13.23
<i>Kharif-2008</i>	20.00	25.00	21.57	16.25	26.75	2.97	12.56	5.60	1.45
<i>Kharif-2010</i>	22.00	25.25	23.58	22.53	25.14	1.04	2.12	24.10	1.05
<i>Pooled</i>	20.92	25.92	21.58	18.12	28.07	2.53	12.62	4.02	1.04
Number of pods per plant									
<i>Kharif-2007</i>	27.75	35.00	18.34	6.59	36.25	19.07	29.44	41.96	25.45
<i>Kharif-2008</i>	30.50	35.25	29.14	15.50	44.75	17.41	19.05	83.50	32.77
<i>Kharif-2010</i>	23.50	32.00	29.91	22.50	35.74	3.86	9.10	17.96	3.37
<i>Pooled</i>	27.25	34.08	25.80	19.82	33.83	6.02	13.30	20.46	5.60
Pod yield per plant(g)									
<i>Kharif-2007</i>	23.02	29.19	15.42	6.60	25.00	16.09	28.59	31.65	18.64
<i>Kharif-2008</i>	24.67	28.33	21.47	9.58	32.78	18.71	26.18	51.05	27.53
<i>Kharif-2010</i>	20.39	29.14	18.44	9.44	32.95	19.37	23.13	70.15	33.43
<i>Pooled</i>	22.69	28.89	18.44	10.51	26.42	11.75	21.75	30.18	13.08
Shelling percent (%)									
<i>Kharif-2007</i>	70.00	74.25	72.96	43.50	83.00	5.77	7.63	57.14	8.98
<i>Kharif-2008</i>	75.75	74.25	71.65	57.50	79.75	3.61	6.34	32.37	4.23
<i>Kharif-2010</i>	71.25	78.00	72.89	61.00	83.00	4.80	6.01	63.58	7.88
<i>Pooled</i>	72.33	75.50	72.50	59.00	78.17	3.05	5.57	30.06	3.45
Hundred seed weight (g)									
<i>Kharif-2007</i>	41.64	46.83	41.85	31.00	60.00	10.90	12.66	74.10	19.33
<i>Kharif-2008</i>	40.50	44.75	37.02	27.00	54.50	11.10	13.89	63.90	18.28
<i>Kharif-2010</i>	40.50	42.50	42.46	31.00	56.00	9.12	12.45	53.64	13.76
<i>Pooled</i>	40.88	44.69	40.44	30.50	54.50	8.01	11.17	51.39	11.83

Table 14: Mean, range, estimates of variance components, broad sense heritability and genetic advance over percent of mean (GAM) for morphological and yield attributing traits in TG 26 × GPBD 4 (RIL-5) population

Characters / Environment	TG 26	GPBD 4	Mean	Range		GCV	PCV	h ² _{b.s}	GAM
				Min.	Max.				
Plant height (cm)									
Kharif-2007	15.90	29.45	23.11	11.90	32.53	16.36	21.76	56.50	25.33
Kharif-2008	24.50	39.65	31.04	20.30	39.53	9.66	14.72	43.06	13.05
Kharif-2010	25.25	38.20	26.59	16.59	37.50	10.88	14.16	59.01	17.21
Pooled	21.88	35.77	26.91	17.35	33.74	10.89	15.84	47.33	15.44
Number of primary branches									
Kharif-2007	4.75	4.50	6.02	4.50	8.50	8.14	18.39	19.62	7.43
Kharif-2008	6.00	5.75	6.91	5.00	9.00	7.00	16.05	19.01	6.28
Kharif-2010	5.00	7.00	6.06	5.00	7.00	4.06	10.72	14.32	3.16
Pooled	5.25	5.75	6.33	5.33	7.33	3.91	14.50	7.29	2.18
Leaf length (mm)									
Kharif-2007	42.50	57.50	50.89	37.50	64.25	10.86	14.40	56.83	16.86
Kharif-2008	47.25	62.25	53.02	40.75	65.50	7.58	10.88	48.55	10.88
Kharif-2010	48.50	60.00	47.70	37.50	59.25	7.60	9.71	61.16	12.24
Pooled	46.08	59.92	50.54	38.67	62.92	7.95	11.26	49.92	11.58
Leaf width (mm)									
Kharif-2007	20.00	25.00	23.22	17.50	32.75	8.78	14.57	36.29	10.89
Kharif-2008	20.75	28.25	23.23	18.00	29.50	7.86	12.62	38.76	10.08
Kharif-2010	23.00	28.75	23.24	17.75	29.00	7.73	12.66	37.26	9.72
Pooled	21.25	27.33	23.23	18.50	29.75	8.07	13.28	36.94	10.10
Number of pods per plant									
Kharif-2007	13.04	16.67	16.69	8.00	32.75	22.31	29.51	57.13	34.73
Kharif-2008	27.00	37.50	32.07	14.75	47.25	18.38	22.92	64.31	30.37
Kharif-2010	28.25	35.50	21.44	13.75	31.00	12.10	22.37	29.28	13.49
Pooled	22.76	29.89	23.40	14.83	33.25	13.99	21.81	41.18	18.50
Pod yield per plant (g)									
Kharif-2007	7.02	10.21	12.94	6.63	25.50	24.91	32.55	58.55	39.26
Kharif-2008	22.49	30.75	23.84	7.73	41.74	24.57	29.28	70.43	42.48
Kharif-2010	21.57	28.47	21.38	10.63	31.45	17.61	22.04	63.83	28.98
Pooled	17.03	23.14	19.39	9.91	28.30	14.22	21.60	43.34	19.28
Shelling percent (%)									
Kharif-2007	68.71	75.99	76.71	69.37	86.04	2.20	5.45	16.37	1.84
Kharif-2008	73.00	76.50	71.72	51.75	78.50	3.87	7.00	30.53	4.40
Kharif-2010	75.00	80.00	74.48	64.72	80.81	3.47	5.50	39.77	4.51
Pooled	72.24	77.50	74.30	66.03	79.07	2.18	5.50	15.76	1.79
Hundred seed weight (g)									
Kharif-2007	32.83	50.76	34.47	20.03	58.74	16.54	19.30	73.52	29.22
Kharif-2008	29.75	45.00	35.72	26.00	55.50	12.04	14.47	69.24	20.65
Kharif-2010	37.00	49.00	37.11	29.50	50.00	9.46	11.27	70.49	16.37
Pooled	33.19	48.25	35.77	27.54	50.58	10.64	13.36	63.37	17.44

Table 15: Genotypic and phenotypic correlation matrix for LLS and rust in TAG 24 × GPBD 4 (RIL-4) population

		<i>Kharif-2004</i>				<i>Kharif-2005</i>				<i>Kharif-2006</i>			
		LLS-SI	LLS-SII	Rust-SI	Rust-SII	LLS-SI	LLS-SII	Rust-SI	Rust-SII	LLS-SI	LLS-SII	Rust-SI	Rust-SII
LLS-SI	P	1	0.981**	-	-	1	0.965**	-	-	1	0.896**	0.112	0.072
	G	1	0.702**	-	-	1	0.818**	-	-	1	0.621**	0.074	0.039
LLS-SII	P		1	-	-		1	-	-		1	-0.083	-0.143*
	G		1	-	-		1	-	-		1	-0.059	-0.090
Rust-SI	P			1	-			1	-			1	0.931**
	G			1	-			1	-			1	0.885**
Rust-SII	P				1				1				1
	G				1				1				1
		<i>Kharif-2007E1</i>				<i>Kharif-2007E2</i>				<i>Kharif-2008</i>			
		LLS-SI	LLS-SII	Rust-SI	Rust-SII	LLS-SI	LLS-SII	Rust-SI	Rust-SII	LLS-SI	LLS-SII	Rust-SI	Rust-SII
LLS-SI	P	1	-	-	-	1	-	-	-	1	0.866**	-0.412**	-0.537**
	G	1	-	-	-	1	-	-	-	1	0.610**	-0.383**	-0.406**
LLS-SII	P		1	-	-		1	-	-		1	-0.750**	-0.840**
	G		1	-	-		1	-	-		1	-0.606**	-0.772**
Rust-SI	P			1	0.953**			1	0.998**			1	0.975**
	G			1	0.832**			1	0.978**			1	0.868**
Rust-SII	P				1				1				1
	G				1				1				1
		<i>Kharif-2009</i>				<i>Kharif-2010</i>				<i>POOLED</i>			
		LLS-SI	LLS-SII	Rust-SI	Rust-SII	LLS-SI	LLS-SII	Rust-SI	Rust-SII	LLS-SI	LLS-SII	Rust-SI	Rust-SII
LLS-SI	P	1	0.870**	-0.213**	-0.467**	1	0.924**	-0.006	-0.124*	1	0.963**	0.033	-0.056
	G	1	0.709**	-0.220**	-0.303**	1	0.702**	-0.091	-0.099	1	0.910**	0.027	-0.056
LLS-SII	P		1	-0.383**	-0.668**		1	-0.042	-0.173**		1	-0.208**	-0.285**
	G		1	-0.361**	-0.601**		1	-0.105	-0.194**		1	-0.195**	-0.284**
Rust-SI	P			1	0.846**			1	0.991**			1	0.996**
	G			1	0.689**			1	0.644**			1	0.973**
Rust-SII	P				1				1				1
	G				1				1				1

LLS-SI: Stage I =70DAS, LLS-SII: Stage II =90DAS, Rust-SI: Stage I =80DAS, Rust-SII: Stage II =90DAS, E1: Environment 1, E2: Environment 2, DAS: Days after sowing, * = significant at P < 0.05, ** = significant at P < 0.01
G=genotypic correlation, P= phenotypic correlation

Table 16: Genotypic and phenotypic correlation matrix for LLS and rust in TG 26 × GPBD 4 (RIL-5) population

		<i>Kharif-2004</i>				<i>Kharif-2005</i>				<i>Kharif-2007E1</i>			
		LLS-SI	LLS-SII	Rust-SI	Rust-SII	LLS-SI	LLS-SII	Rust-SI	Rust-SII	LLS-SI	LLS-SII	Rust-SI	Rust-SII
LLS-SI	P	1	0.718**	0.339**	0.250**	1	0.968**	-0.545**	-0.613**	1	-	-	-
	G	1	0.577**	0.175*	0.159*	1	0.793**	-0.403**	-0.497**	1	-	-	-
LLS-SII	P		1	0.512**	0.350**		1	-0.452**	-0.496**		1	-	-
	G		1	0.204*	0.179*		1	-0.297**	-0.417**		1	-	-
Rust-SI	P			1	0.946**			1	0.841**			1	0.894**
	G			1	0.787**			1	0.829**			1	0.707**
Rust-SII	P				1				1				1
	G				1				1				1
		<i>Kharif-2007E2</i>				<i>Kharif-2008</i>				<i>Kharif-2009</i>			
		LLS-SI	LLS-SII	Rust-SI	Rust-SII	LLS-SI	LLS-SII	Rust-SI	Rust-SII	LLS-SI	LLS-SII	Rust-SI	Rust-SII
LLS-SI	P	1	0.307**	-0.374**	-0.399**	1	0.656**	-0.315**	-0.448**	1	0.630**	-0.130	-0.262**
	G	1	0.469**	-0.211**	-0.240**	1	0.517**	-0.186*	-0.338**	1	0.528**	-0.204*	-0.256**
LLS-SII	P		1	0.239**	0.184*		1	-0.831**	-0.909**		1	-0.657**	-0.823**
	G		1	0.109	0.052		1	-0.703**	-0.855**		1	-0.402**	-0.506**
Rust-SI	P			1	0.996**			1	0.960**			1	0.921**
	G			1	0.902**			1	0.864**			1	0.808**
Rust-SII	P				1				1				1
	G				1				1				1
		<i>Kharif-2010</i>				<i>POOLED</i>							
		LLS-SI	LLS-SII	Rust-SI	Rust-SII	LLS-SI	LLS-SII	Rust-SI	Rust-SII				
LLS-SI	P	1	0.608**	0.222**	0.155	1	0.971**	-0.118	-0.185				
	G	1	0.531**	-0.081	0.094	1	0.863**	-0.122	-0.179				
LLS-SII	P		1	-0.059	-0.014		1	-0.422**	-0.460**				
	G		1	0.056	0.012		1	-0.374**	-0.429**				
Rust-SI	P			1	0.750**			1	0.995**				
	G			1	0.607**			1	0.966**				
Rust-SII	P				1				1				
	G				1				1				

LLS-SI: Stage I =70DAS, LLS-SII: Stage II =90DAS, Rust-SI: Stage I =80DAS, Rust-SII: Stage II =90DAS, E1: Environment 1, E2: Environment 2, DAS: Days after sowing, * = significant at P < 0.05, ** = significant at P < 0.01
G=genotypic correlation, P= phenotypic correlation

Table 17: Genotypic and phenotypic correlation matrix for disease, morphological and yield attributing traits in TAG 24 × GPBD 4 (RIL-4) population

Characters		Genotypic correlation matrix											
		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 ₁	Kharif-2007	1	0.277**	0.319**	0.326**	-	-	-0.083	-0.080	0.466**	0.444**	-0.210**	0.087
	Kharif-2008	1	0.051	0.166**	0.516**	-0.260**	-0.243**	0.043	0.051	0.237**	0.109	0.013	-0.026
	Kharif-2010	1	0.297**	0.405**	0.226**	-0.131*	-0.205**	0.101	-0.012	0.071	0.113*	-0.127*	-0.025
	Pooled	1	0.072	0.413**	0.402**	-0.293**	-0.333**	0.022	0.004	0.400**	0.318**	-0.174**	-0.001
X ₂	Kharif-2007	0.039	1	-0.124*	0.156**	-	-	-0.055	-0.061	0.132*	0.083	-0.001	-0.010
	Kharif-2008	-0.032	1	-0.268**	-0.540**	-0.125*	-0.051	-0.049	-0.102	0.332**	0.169**	-0.083	-0.101
	Kharif-2010	0.087	1	0.405**	0.096	-0.011	-0.039	0.117*	0.256**	-0.336**	0.077	0.056	-0.265**
	Pooled	0.051	1	-0.077	0.044	-0.192**	-0.181**	-0.017	-0.004	0.277**	0.076	-0.010	-0.058
X ₃	Kharif-2007	0.150**	-0.031	1	0.926**	-	-	-0.146*	-0.141*	0.184**	0.338**	0.073	0.265**
	Kharif-2008	0.158**	0.025	1	0.546**	-0.050	-0.102	-0.032	-0.007	0.059	0.077	-0.120*	0.324**
	Kharif-2010	0.319**	0.061	1	0.619**	-0.113*	-0.110	0.113*	0.003	-0.349**	0.071	-0.131*	0.145*
	Pooled	0.290**	-0.033	1	0.663**	-0.370**	-0.385**	-0.086	-0.058	0.052	0.296**	-0.154**	0.405**
X ₄	Kharif-2007	0.119*	-0.02	0.652**	1	-	-	-0.258**	-0.250**	0.333**	0.462**	0.137*	0.355**
	Kharif-2008	0.049	-0.003	0.392**	1	0.197**	0.152**	-0.147*	-0.241**	0.038	-0.386**	-0.191**	0.564**
	Kharif-2010	0.112	0.049	0.621**	1	-0.285**	-0.120*	-0.005	-0.051	-0.320**	0.053	0.058	0.170**
	Pooled	0.211**	0.013	0.590**	1	-0.597**	-0.444**	-0.224**	-0.189**	0.156**	0.433**	0.210**	0.480**
X ₅	Kharif-2007	-	-	-	-	1	-	-	-	-	-	-	-
	Kharif-2008	-0.094	-0.071	-0.046	-0.014	1	0.866**	-0.412**	-0.537**	-0.089	0.003	0.181**	-0.053
	Kharif-2010	-0.078	0.025	-0.045	-0.052	1	0.924**	-0.006	-0.124*	-0.196**	-0.228**	-0.117*	-0.136*
	Pooled	-0.207**	-0.114*	-0.191**	-0.232**	1	0.916**	-0.170**	-0.231**	-0.236**	-0.128*	-0.042	-0.026
X ₆	Kharif-2007	-	-	-	-	1	-	-	-	-	-	-	-
	Kharif-2008	-0.133*	-0.001	-0.053	-0.013	0.610**	1	-0.750**	-0.840**	0.093	0.073	0.063	0.072
	Kharif-2010	-0.159**	0.015	-0.092	-0.054	0.702**	1	-0.042	-0.173**	-0.243**	-0.291**	-0.129*	-0.100
	Pooled	-0.254**	-0.098	-0.210**	-0.179**	0.800**	1	-0.558**	-0.588**	-0.060	-0.043	0.078	0.154**
X ₇	Kharif-2007	-0.048	-0.032	-0.026	-0.051	-	-	1	0.998**	-0.368**	-0.623**	-0.343**	-0.295**
	Kharif-2008	0.017	-0.052	-0.042	-0.053	-0.383**	-0.606**	1	0.975**	-0.231**	-0.308**	-0.254**	-0.175**
	Kharif-2010	0.041	0.058	0.044	-0.022	-0.091	-0.105	1	0.991**	-0.649**	-0.284**	-0.020	-0.168**
	Pooled	0.011	-0.017	-0.058	-0.119*	-0.152**	-0.437**	1	0.932**	-0.483**	-0.432**	-0.241**	-0.265**
X ₈	Kharif-2007	-0.046	-0.034	-0.024	-0.034	-	-	0.978**	1	-0.370**	-0.609**	-0.352**	-0.302**
	Kharif-2008	0.038	-0.058	-0.016	-0.040	-0.406**	-0.772**	0.868**	1	-0.231**	-0.173**	-0.138*	-0.155**
	Kharif-2010	-0.01	0.024	-0.005	-0.010	-0.099	-0.194**	0.644**	1	-0.472**	-0.195**	-0.063	-0.232**
	Pooled	0.001	-0.013	-0.040	-0.098	-0.216**	-0.529**	0.963**	1	-0.450**	-0.389**	-0.256**	-0.295**
X ₉	Kharif-2007	0.257**	0.082	0.088	0.096	-	-	-0.229**	-0.212**	1	0.694**	0.124*	-0.009
	Kharif-2008	0.127**	0.168**	0.081	0.040	-0.032	0.058	-0.178**	-0.197**	1	0.315**	-0.013	-0.167**
	Kharif-2010	0.015	-0.117*	-0.076	-0.054	-0.108	-0.121*	-0.115*	-0.128*	1	0.224**	0.175**	-0.094
	Pooled	0.298**	0.183**	0.063	0.097	-0.144*	-0.031	-0.361**	-0.345**	1	0.536**	0.173**	-0.098
X ₁₀	Kharif-2007	0.212**	0.066	0.128*	0.109	-	-	-0.323**	-0.306**	0.755**	1	0.329**	0.522**
	Kharif-2008	0.091	0.029	0.056	-0.021	-0.025	0.056	-0.225**	-0.125*	0.188**	1	0.136*	0.194**
	Kharif-2010	0.091	0.06	0.044	0.028	-0.166**	-0.181**	-0.212**	-0.168**	0.127**	1	0.182**	0.114*
	Pooled	0.248**	0.062	0.185**	0.191**	-0.105	-0.037	-0.361**	-0.319**	0.458**	1	0.285**	0.340**
X ₁₁	Kharif-2007	-0.084	-0.029	-0.006	0.005	-	-	-0.271**	-0.269**	0.077	0.198**	1	0.221**
	Kharif-2008	0.024	-0.033	-0.067	-0.074	0.040	0.047	-0.063	-0.069	-0.022	0.096	1	0.202**
	Kharif-2010	-0.048	0.001	-0.089	-0.009	-0.031	-0.086	-0.048	-0.020	0.063	0.108	1	0.175**
	Pooled	-0.125**	-0.015	-0.092	0.071	-0.023	0.042	-0.216**	-0.215**	0.110	0.220**	1	0.217**
X ₁₂	Kharif-2007	0.044	0.021	0.144*	0.146*	-	-	-0.266**	-0.264**	-0.008	0.280**	0.204**	1
	Kharif-2008	-0.069	-0.021	0.163**	0.140*	-0.002	0.071	-0.134*	-0.129*	-0.110	0.114*	0.141*	1
	Kharif-2010	-0.001	-0.005	0.038	0.049	-0.069	-0.056	-0.094	-0.111	0.032	0.132*	0.139*	1
	Pooled	-0.003	-0.037	0.272**	0.259**	-0.027	0.102	-0.239**	-0.261**	-0.073	0.274**	0.196**	1
		Phenotypic correlation matrix											

X₁=Plant height (cm), X₂=Number of primary branches, X₃=Leaf length (mm), X₄=Leaf width (mm), X₅=LLS-SI (70DAS), X₆=LLS-SII (90DAS), X₇=Rust-SI (80DAS), X₈=Rust-SII (90DAS), X₉=Number of pods per plant, X₁₀=Pod yield per plant (g), X₁₁=Shelling percent (%) and X₁₂=Hundred seed weight (g)

* = significant at P < 0.05, ** = significant at P < 0.01

Above the diagonal is genotypic correlation

Below the diagonal is phenotypic correlation

Table 18: Genotypic and phenotypic correlation matrix for disease, morphological and yield attributing traits in TG 26 × GPBD 4 (RIL-5) population

Characters	Genotypic correlation matrix											
	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 ₁ Kharif-2007	1	0.662**	0.541**	0.502**	0.287**	-0.371**	-0.048	-0.102	0.546**	0.482**	0.016	0.167*
Kharif-2008	1	-0.161*	0.536**	0.525**	-0.232**	-0.162*	-0.080	-0.005	0.238**	0.454**	0.295**	0.278**
Kharif-2010	1	0.425**	0.551**	0.472**	-0.025	-0.143	-0.011	0.010	0.413**	0.274**	-0.150	0.140
Pooled	1	0.068	0.513**	0.473**	-0.049	-0.276**	-0.024	-0.021	0.350**	0.434**	-0.120	0.225**
X ₂ Kharif-2007	0.207*	1	0.798**	0.666**	-0.092	-0.222**	0.002	0.056	0.371**	0.145	-0.362	-0.092
Kharif-2008	-0.095	1	-0.218**	-0.355**	-0.291**	-0.188*	0.038	0.192	0.309**	-0.116	-0.074	-0.333**
Kharif-2010	0.068	1	-0.103	-0.305**	-0.004	-0.426**	-0.221**	-0.001	0.088	0.375**	0.282**	0.365**
Pooled	0.060	1	0.235**	-0.184*	-0.027	-0.041	0.128	0.237**	0.187*	0.117	0.170*	-0.155
X ₃ Kharif-2007	0.314**	0.240**	1	0.764**	0.103	-0.350**	-0.193*	-0.209**	0.206*	0.207*	0.219**	0.254**
Kharif-2008	0.387**	-0.115	1	0.862**	-0.069	0.014	-0.110	-0.140	0.173*	0.272**	0.239**	0.307**
Kharif-2010	0.382**	0.103	1	0.726**	0.002	-0.087	-0.100	-0.158	0.327**	0.356**	0.149	0.385**
Pooled	0.464**	0.148	1	0.791**	0.013	-0.089	-0.176*	-0.179*	0.112	0.321**	0.215**	0.342**
X ₄ Kharif-2007	0.231**	0.040	0.669**	1	-0.044	-0.448**	-0.299**	-0.401**	0.280**	0.419**	0.391**	0.317**
Kharif-2008	0.310**	-0.087	0.703**	1	-0.050	0.124	-0.282**	-0.280**	0.065	0.406**	0.349**	0.462**
Kharif-2010	0.285**	0.013	0.438**	1	-0.072	-0.164*	-0.412**	-0.373**	0.031	0.287**	0.042	0.437**
Pooled	0.406**	-0.088	0.733**	1	-0.032	-0.092	-0.320**	-0.342**	0.081	0.432**	0.258**	0.439**
X ₅ Kharif-2007	0.142	0.101	0.066	0.049	1	0.307**	-0.374**	-0.399**	0.426**	0.404**	-0.130	0.195*
Kharif-2008	-0.067	-0.074	0.019	0.009	1	0.656**	-0.315**	-0.448**	0.001	0.130	-0.050	0.047
Kharif-2010	0.047	-0.084	-0.051	-0.081	1	0.984**	0.222**	0.155	0.061	0.054	-0.152	-0.164*
Pooled	0.000	0.005	0.021	-0.014	1	0.995**	-0.303**	-0.322**	0.247**	0.204**	-0.160*	0.200*
X ₆ Kharif-2007	-0.140	-0.023	-0.207*	-0.145	0.469**	1	0.239**	0.184*	0.121	0.105	0.263**	-0.365**
Kharif-2008	-0.051	-0.102	0.039	0.099	0.517**	1	-0.831**	-0.909**	-0.001	0.190*	0.128	0.220**
Kharif-2010	-0.096	-0.128	-0.073	-0.089	0.531**	1	-0.059	-0.014	-0.112	-0.019	-0.089	-0.079
Pooled	-0.207**	-0.061	-0.080	-0.068	0.727**	1	-0.646**	-0.591**	0.146	0.162*	-0.023	0.122
X ₇ Kharif-2007	-0.003	0.036	-0.128	-0.169*	-0.211*	0.109	1	0.996**	-0.360**	-0.457**	-0.505**	-0.379**
Kharif-2008	0.011	0.025	-0.083	-0.148	-0.186*	-0.703**	1	0.960**	-0.136	-0.192*	-0.180*	-0.292**
Kharif-2010	0.024	-0.048	-0.030	-0.064	-0.081	0.056	1	0.942**	-0.353**	-0.215**	-0.088	-0.236**
Pooled	-0.016	0.069	-0.154	-0.261**	-0.252**	-0.478**	1	0.970**	-0.301**	-0.406**	-0.438**	-0.372**
X ₈ Kharif-2007	-0.020	0.031	-0.129	-0.150	-0.240**	0.052	0.902**	1	-0.403**	-0.482**	-0.453**	-0.379**
Kharif-2008	-0.024	0.103	-0.120	-0.190*	-0.338**	-0.855**	0.864**	1	-0.148	-0.232**	-0.244**	-0.235**
Kharif-2010	0.003	-0.024	-0.063	-0.180*	0.094	0.012	0.607**	1	-0.192*	-0.360**	-0.071	-0.331**
Pooled	-0.020	0.134	-0.161**	-0.290**	-0.264**	-0.506**	0.957**	1	-0.328**	-0.436**	-0.403**	-0.378**
X ₉ Kharif-2007	0.291**	0.017	0.074	0.039	0.125	-0.035	-0.259**	-0.270**	1	0.732**	0.071	0.044
Kharif-2008	0.148	0.034	0.119	0.095	-0.016	-0.014	-0.127	-0.116	1	0.297**	0.181*	-0.031
Kharif-2010	0.148	-0.035	0.111	0.069	0.029	0.044	-0.080	-0.111	1	0.359**	0.158*	0.153
Pooled	0.297**	0.09	0.107	0.077	0.161*	0.090	-0.255**	-0.277**	1	0.475**	0.067	0.093
X ₁₀ Kharif-2007	0.250**	-0.008	0.058	0.103	0.174*	0.040	-0.328**	-0.334**	0.792**	1	0.192*	0.525**
Kharif-2008	0.239**	-0.108	0.171**	0.280**	0.064	0.116	-0.162*	-0.183*	0.369**	1	0.216**	0.367**
Kharif-2010	0.174**	0.061	0.244**	0.182*	0.034	-0.008	-0.097	-0.198*	0.136	1	-0.307**	0.323**
Pooled	0.360**	0.029	0.261**	0.346**	0.152	0.124	-0.335**	-0.369**	0.450**	1	-0.033	0.645**
X ₁₁ Kharif-2007	-0.002	0.061	0.016	0.042	0.025	0.063	-0.189*	-0.148	0.014	0.068	1	0.333**
Kharif-2008	0.029	-0.070	0.045	0.102	-0.048	0.069	-0.071	-0.085	0.073	0.143	1	0.410**
Kharif-2010	-0.126	0.010	0.090	-0.031	-0.06	-0.051	-0.025	-0.010	0.054	-0.133	1	-0.140
Pooled	-0.076	0.059	0.138	0.158	-0.082	0.002	-0.293**	-0.269**	0.059	0.005	1	0.144
X ₁₂ Kharif-2007	0.154	-0.033	0.127	0.162*	0.075	-0.145	-0.300**	-0.280**	0.072	0.387**	0.068	1
Kharif-2008	0.133	-0.087	0.164**	0.208**	0.010	0.163*	-0.234**	-0.175*	0.028	0.323**	0.190*	1
Kharif-2010	0.041	0.118	0.260**	0.169*	-0.052	-0.039	-0.114	-0.229**	0.044	0.244**	-0.033	1
Pooled	0.195**	-0.084	0.292**	0.356**	0.133	0.083	-0.339**	-0.342**	0.078	0.535**	0.116	1
Phenotypic correlation matrix												

X₁=Plant height (cm), X₂=Number of primary branches, X₃=Leaf length (mm), X₄=Leaf width (mm), X₅=LLS-SI (70DAS), X₆=LLS-SII (90DAS), X₇=Rust-SI (80DAS), X₈=Rust-SII (90DAS), X₉=Number of pods per plant, X₁₀=Pod yield per plant (g), X₁₁=Shelling percent (%) and X₁₂=Hundred seed weight (g)
 * = significant at P < 0.05, ** = significant at P < 0.01

Above the diagonal is genotypic correlation

Below the diagonal is phenotypic correlation

percent in RIL-5 population exhibited a negative association with pod yield per plant during Kharif 2010 and also with number of pods per plant during Kharif 2008 (Table 18).

4.1.4.4 Association between morphological and yield attributing traits

Significant positive association was observed between morphological traits (plant height, leaf length, leaf width and number of primary branches) and yield attributing traits. Nonetheless, negative association was observed for shelling percent contributed by plant height, number of primary branches, leaf length and leaf width in case of RIL-4 population (Table 17). While contrast association was observed in case of RIL-5 population where in all the morphological characters were associated positively with shelling percent (Table 18). However, during Kharif 2008 number of primary branches exhibited significant negative association with shelling percent in RIL-5 population (Table 18).

4.1.4.5 Association between disease, morphological and yield attributing traits

In general a significant negative association was seen for all the morphological and yield attributing traits with late leaf spot and rust in both the population [Table 17 (RIL-4) and 18 (RIL-5)]. However, a contrasting association was observed in case of RIL-5 population where in late leaf spot at 70 days (LLS-SI) of scoring had shown a positive association with number of pods per plant and pod yield per plant during Kharif 2007 and across environments (Table 18). Varied degree of association was present between these traits indicating the importance of proper incidence of disease during the disease scoring stages for both LLS and rust.

4.2 Genotypic data analysis

4.2.1 Identification of polymorphic markers and genotyping of mapping populations

In addition to screening of 1,089 SSR markers on parents of RIL-4 (Khedikar *et al.*, 2010) and 1,043 SSR markers on the parents of RIL-5 (Sarvamangala *et al.*, 2011), 2,008 and 2,239 additional SSR markers were screened on the parents of RIL-4 and RIL-5 populations, respectively. Of the new SSR markers screened, a total of 139 and 151 markers were found polymorphic between the parents of RIL-4 and RIL-5 mapping populations respectively. Further, along with new SSR markers the allele specific primers for *FAD2A* and *FAD2B* (Chen *et al.*, 2010) were also used to check the polymorphism between the parents. Of the two alleles only one allele (*FAD2A*) was polymorphic and *FAD2B* was monomorphic between the parents of the respective population. The marker data generated (Mukri, 2011) based on CAPS marker for *ahFAD2A* on TAG 24 × GPBD 4 population was also included in the present study in RIL-4 population. After including the earlier polymorphic markers (Khedikar *et al.*, 2010; Sarvamangala *et al.*, 2011), a total of 208 and 206 markers were polymorphic between the parents of RIL-4 and RIL-5 populations, respectively (Table 19). Subsequently, genotyping data were generated for 208 SSR markers on 182 lines of RIL-4 and for 206 markers on 138 lines of RIL-5 (Plate 2 and 3). However, while genotyping the mapping population, segregation data were scored at two loci for three markers (GM1971, GM2724 and pPGSseq18A05) in case of RIL-4 and four markers (GM1971, GM2724, GM2589 and TC3H07) in RIL-5 population. As a result segregation data were obtained for a total of 211 and 210 SSR loci in RIL-4 and RIL-5 population.

In summary, a total of 3,101 SSR markers (1,089 markers in Khedikar *et al.*, 2010 and 2,008 SSRs in the present study) were screened on parents of RIL-4 population. Similarly a total of 3,284 SSR markers (1,043 markers in Sarvamangala *et al.*, 2011 and 2,240 in the present study) were screened on parental genotypes of RIL-5 population. Segregation data were obtained for 211 and 210 marker loci in RIL-4 and RIL-5 population (67 SSR loci-Khedikar *et al.*, 2010, 53 SSR loci-Sarvamangala *et al.*, 2011), while in present study a total of 141 and 152 marker loci for RIL-4 and RIL-5 population, respectively).

4.2.2 Development of improved genetic maps

Genotypic data obtained for all 211 (RIL-4) and 210 (RIL-5) marker loci for both mapping populations were subjected to chi-square (χ^2) test to estimate the segregation ratio. Out of 211 loci, a total of 167 (RIL-4) and 157 (RIL-5) marker loci showed the expected 1:1 segregation ratio ($P < 0.05$). The remaining 44 (20%) and 53 (28%) markers were found distorted in RIL-4 and RIL-5 population, respectively (Table 20; Appendix I).

As the number of polymorphic markers between the parents of mapping population were less and hence distorted markers were also considered for linkage mapping. Using the entire marker dataset in each of RIL population linkage mapping was done by using a minimum LOD score of 5.0 and a maximum recombination fraction (θ) of 0.37. As a result, an improved genetic map with 190

SSR loci mapped onto 20 LGs with genome coverage of 1963.40 cM was developed based on RIL-4 population. Similarly, a map comprising of 182 loci mapped on 21 LGs with 1982.90 cM map distance was developed based on RIL-5 population (Table 20; Fig. 2 and 3).

Both genetic maps were compared with the reference genetic map for cultivated groundnut species developed based on TAG 24 × ICGV 86031 mapping population (Varshney *et al.*, 2009a). A good congruence was found between the maps for common markers and for order of the markers with few exceptions. Eventually, names for the LGs of newly developed genetic maps were assigned according to the LGs of the reference map (TAG 24 × ICGV 86031) for cultivated groundnut based on the common markers observed and suffix “*” indicating LGs named in comparison with the reference genetic map (Varshney *et al.*, 2009a). In the case of genetic map based on RIL-5 population, the linkage group AhIII* was split into three fragments and hence named as AhIIIa*, AhIIIb* and AhIIIc* (Fig. 3).

Number of markers mapped per linkage group ranged from two (AhXX) to seventeen (AhIII*) and length of linkage groups ranged from 17.6 cM (AhXX) to 192.4 cM (AhXIV*) with a mean map distance of 98.17 cM in case of the genetic map based on RIL-4 population (Table 20 and 21). Similarly, in the case of the genetic map developed based on RIL-5, number of markers mapped per linkage group ranged from two (AhXX and AhXXI) to fifteen (AhXV and AhVII*) and length of linkage groups ranged from 14.7 cM (AhXIX) to 208.6 cM (AhXIV*) with a mean map distance of 99.15 cM (Table 20 and 21). Mean map density ranged from 4.22 (AhVIII) to 20.95 cM (AhXIX) in the genetic map based on RIL-4 and 4.54 (AhIIIc) to 17.38 cM (AhXIV) in the genetic map based on RIL-5 population (Table 21). The detailed features of these genetic maps have been specified in Table 20 and 21. Comparison of the two newly constructed genetic maps showed 144 common markers distributed on 18 LGs. The position and order of these common markers were similar in majority of the cases and was comparable with the available reference genetic map (Fig. 4).

4.2.3 Construction of a consensus map

As developed genetic maps are based on the populations (RIL-4 and RIL-5) that segregate for LLS and rust, a consensus map was developed by using 144 common markers distributed on 18 LGs on these genetic maps. In this context, the newly developed genetic map based on RIL-4 population with highest number (190) of mapped loci was used as a framework map. Subsequently, a consensus map was developed using the framework map as a fixed backbone onto which the unique loci of the genetic map developed based on RIL-5 population were added following the “neighbors” map approach described by Cone *et al.* (2002). The markers from corresponding LGs of the genetic map based on RIL-5 population were assigned onto linkage group of the framework map (Table 22; Fig. 4). As a result, the consensus map developed here has 227 SSR loci mapped on 20 LGs of which 18 LGs were integrated from both genetic maps using common markers and remaining two LGs were adopted as such from framework map (Table 20 and 22). The number of mapped loci on the consensus map ranged from two (AhXX) to seventeen (AhIII* and AhXVII*). The length of LGs ranged from 17.82 cM (AhXX) to 131.50 cM (AhXIII*) with a mean map distance of 59.94 cM and mean inter-locus gap distance of 8.14 cM (Table 21 and 22). The mean map density ranged from 2.40 (AhXVII)-29.04 (AhXIX) cM in the consensus map (Table 21).

4.3 QTL analysis

4.3.1 QTL analysis for late leaf spot and rust resistance

4.3.1.1 Identification of QTLs for LLS resistance

Phenotypic data obtained at both the stages (LLS-SI and LLS-SII) in all six environments for both the populations were analyzed together with mapping data for all mapped loci assigned to the genetic maps of respective mapping populations using WinQTL Cartographer, version 2.5 (Wang *et al.*, 2007a). A threshold LOD value of 2.5 was considered for identification. Automatically locate QTLs option was used with minimum of 5.0 cM between QTLs to define a QTL region and if the peak's distance is less than 5.0 cM, then the highest peak was considered to locate QTLs. As a result, a total of 13 QTLs were identified for LLS in RIL-4 and 15 QTLs in RIL-5 populations (Table 23 and 24; Fig. 5 and 6). The 13 QTLs identified in RIL-4 population were distributed on nine LGs (AhXII, AhXV, AhV, AhXIII, AhIX, AhVIII, AhXVIII, AhVI and AhVII). Among them five were major QTLs (QTL_{R4-LLS}01, QTL_{R4-LLS}02, QTL_{R4-LLS}03, QTL_{R4-LLS}04 and QTL_{R4-LLS}05) with a phenotypic variance explained (PVE) ranging from 10.27-67.98% (Table 23; Fig. 5). Similarly 15 QTLs identified in RIL-5 population were distributed on nine LGs (AhXV, AhVIII, AhV, AhI, AhXIV, AhXIII, AhX, AhVII and AhIIIc). Among these

QTLs, eight QTLs (QTL_{R5-LLS}01, QTL_{R5-LLS}02, QTL_{R5-LLS}03, QTL_{R5-LLS}04, QTL_{R5-LLS}05, QTL_{R5-LLS}06, QTL_{R5-LLS}07 and QTL_{R5-LLS}08) were major with PVE ranging from 7.58-63.17% (Table 24, Fig. 6). Though, QTL_{R5-LLS}01 and QTL_{R5-LLS}02 were major QTLs the PV explained was less than 10% in one of the environments hence the range was ranging from 7.58-49.64% and 9.79-22.46% respectively. The remaining seven QTLs in RIL-4 and eight QTLs in RIL-5 were considered as minor QTLs as they explained <10% PV (Collard *et al.*, 2005). The contribution of favourable alleles for LLS has come from the resistant parent GPBD 4.

In terms of stability of the QTLs, the QTLs identified in at least three seasons were considered as the “consistent” QTLs. For instance, of the 13 QTLs identified in RIL-4 population, one QTL (QTL_{R4-LLS}01 with 10.27-62.34% PVE) was found consistently across all six seasons, however, the QTL_{R4-LLS}05 (10.81-15.34% PVE) and QTL_{R4-LLS}09 (3.39-8.5%PVE) were identified in four and three seasons respectively (Table 23). The remaining 10 QTLs identified were found in less than three seasons and they were either specific to particular stage or seasons of disease screening. The QTLs QTL_{R4-LLS}02 and QTL_{R4-LLS}03 were identified only in two (rainy season 2008 and 2009) seasons with a PVE ranging from 10.83-67.98%. Nevertheless three QTLs QTL_{R4-LLS}04, QTL_{R4-LLS}06 and QTL_{R4-LLS}07 were present only in the year of 2008, 2006 and 2004 at both the stages of LLS respectively. The comparison of the QTLs for their contribution at different stages of disease scoring revealed three QTLs (QTL_{R4-LLS}03, QTL_{R4-LLS}05 and QTL_{R4-LLS}09) which were specific to LLS-SII. However, exception was observed for QTL_{R4-LLS}03 and QTL_{R4-LLS}05 for their appearance, wherein they also appeared for LLS-SI during Kharif 2009. In case of RIL-5 population, of the 15 QTLs identified, none of the QTLs were present in more than three seasons. However, two QTLs QTL_{R5-LLS}01 (7.58-49.64% PVE) and QTL_{R5-LLS}02 (9.79-22.46% PVE) were present in more than two seasons. Though, with high PVE none of the other QTLs were identified in more than two environments. They were either specific to one of the stage or for both the stages of disease scoring during a particular season (Table 24).

4.3.1.2 Identification of QTLs for rust resistance

For identification of QTLs for rust resistance, detailed QTL analysis was done in RIL-4 and RIL-5 using the corresponding genetic mapping data and extensive phenotyping data for rust obtained for the two scoring stages (80 days after sowing, referred as Rust-SI and 90 days after sowing referred as Rust-SII) under six (in case of RIL-4) and seven (in case of RIL-5) seasons/environments. As a result, a total of eight (RIL-4) and six (RIL-5) QTLs for rust resistance were identified from both the populations with the PVE ranging from 2.54-82.96% in RIL-4 and 2.89-78.96% in RIL-5 (Table 25 and 26; Fig. 5 and 6). The QTLs were distributed on six (AhXV, AhXVI, AhII, AhVII, AhI and AhXVIII) and four (AhXV, AhV, AhXX, and AhXIV) LGs in the respective populations (Fig 5 and 6). Among these, the number of major QTLs identified was three (10.68-82.96% PVE) in RIL-4 (Table 25; Fig. 5) and four QTLs (15.04-78.96% PVE) in RIL-5 population (Table 26; Fig. 6). And the remaining QTLs were minor QTLs as they explained <10% PV. As represented by the additive effect the favourable alleles for rust was contributed by the resistant parent GPBD 4.

Among the identified QTLs in RIL-4, three (QTL_{R4-Rust}01, QTL_{R4-Rust}02 and QTL_{R4-Rust}03) QTLs were consistently present in all the seasons, except for few stages such as Rust-SII_2007E2 and Rust-SI_2010 for QTL_{R4-Rust}01; Rust-SII_2007E1 for QTL_{R4-Rust}02; Rust-SII_2009 for QTL_{R4-Rust}03 (Table 25). Similarly in RIL-5, only two QTLs (QTL_{R5-Rust}01 and QTL_{R5-Rust}02) were present in more than three seasons and in both the stages of disease scoring. In a total of seven seasons of disease scoring QTL_{R5-Rust}01 was detected in all the seasons but QTL_{R5-Rust}02 was present only in six seasons as it was not detected during the year Kharif 2005. QTL_{R5-Rust}03 was specific to Rust-SI during 2005 and 2010 with a PV of 15.07-29.02% and QTL_{R5-Rust}04 was specific to Rust-SI during Kharif 2010 with a contribution of 30.16% PVE (Table 26). From both populations the remaining five (RIL-4) and two (RIL-5) QTLs were considered as minor QTLs as they explained <10% PV (Table 25 and 26).

4.3.2 QTLs for morphological traits

4.3.2.1 Plant height (cm):

A total of fourteen QTLs were identified for plant height in RIL-4 and 5 QTLs in RIL-5 population (Table 27 and 28; Fig. 7 and 8). Fourteen QTL identified in RIL-4 population were distributed on five LG (AhV*, AhXVIII*, AhVII*, AhX* and AhXII*). Similarly 5 QTLs in RIL-5 population were distributed on three LGs (AhV*, AhII* and AhX*). For plant height the PVE ranged from 4.39-13.55% in RIL-4 and 6.80-18.60% in RIL-5 population (Table 27 and 28).

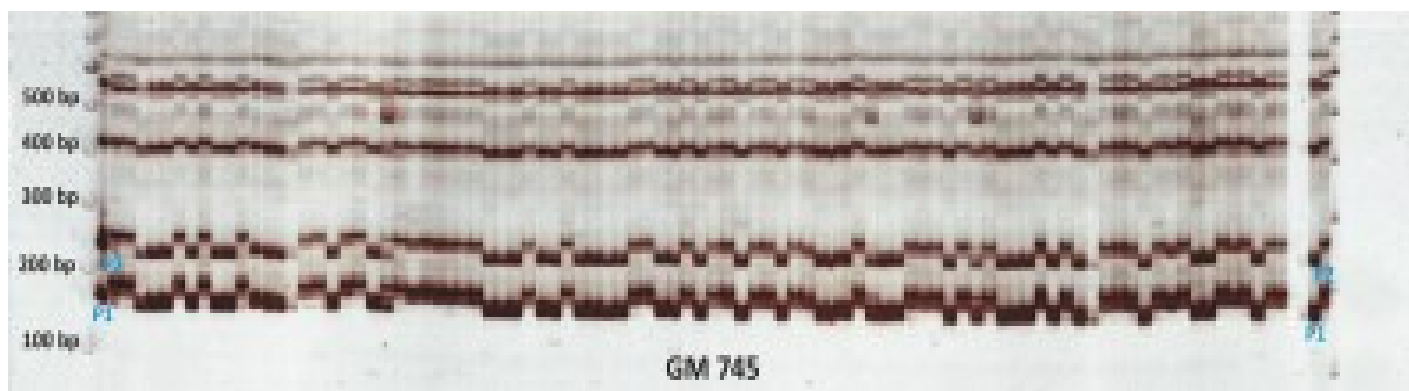
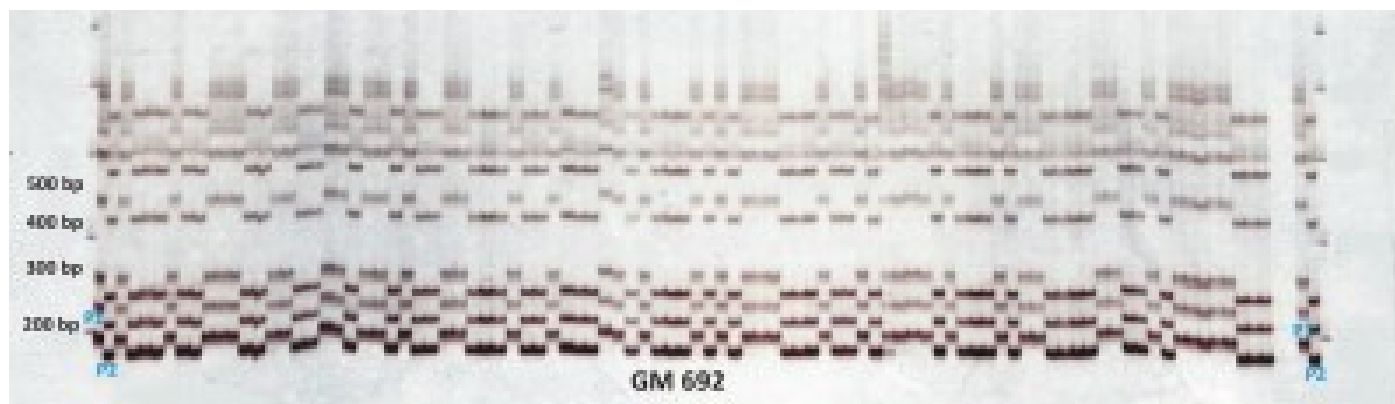
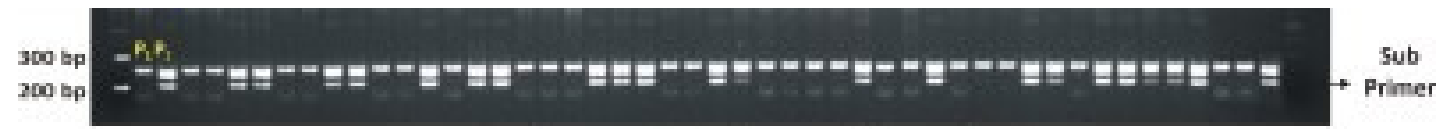


Plate 2: Genotyping of RILs in RIL-4 population (TAG 24 x GPBD 4)



Genotyping of RIL-4 population for *FAD2A* allele specific primer

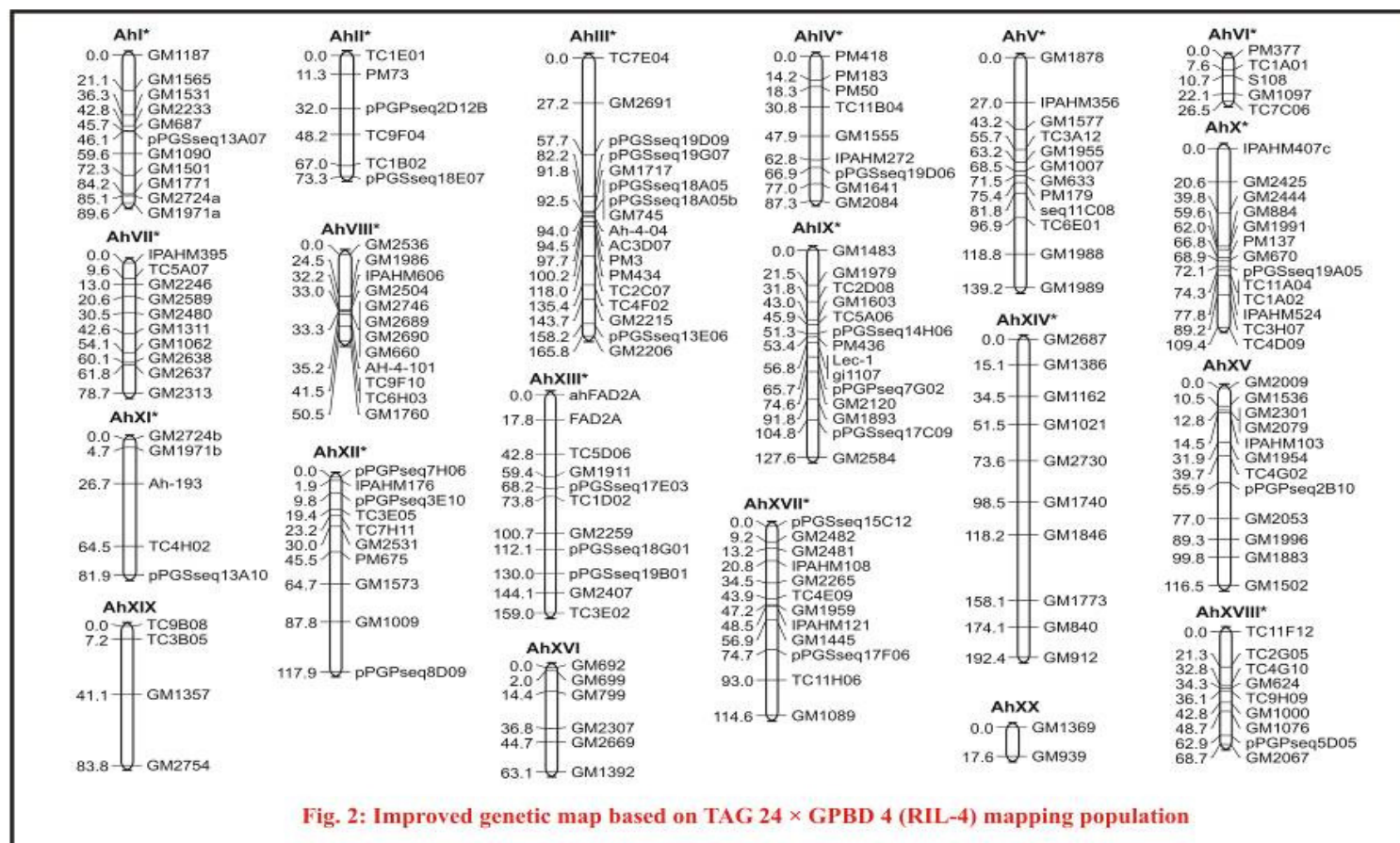


Genotyping of RIL-5 population for *FAD2A* allele specific primer

Plate 3: Genotyping of RILs for *FAD2A* allele specific primer in both the population

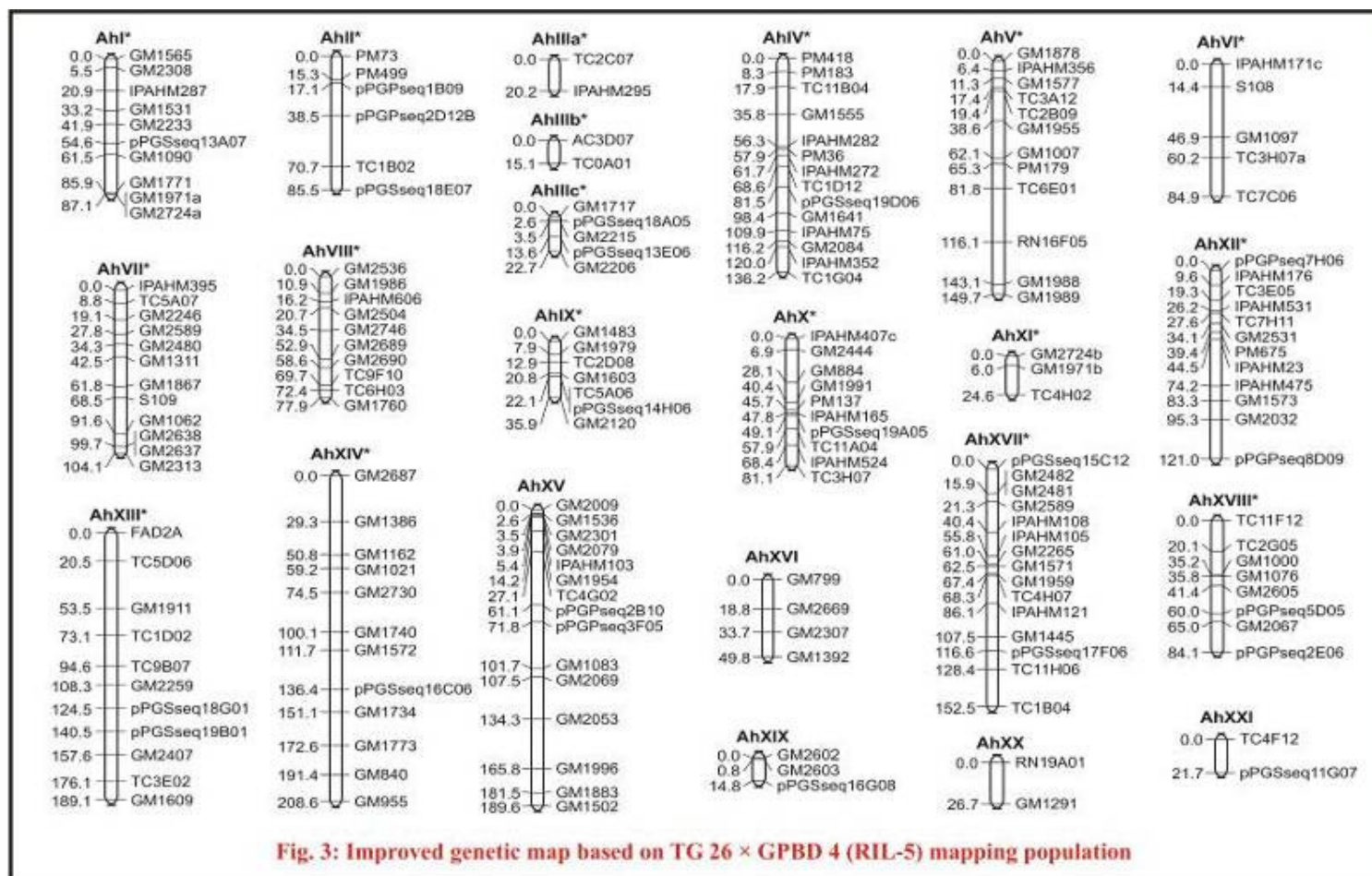
Table 19: Marker polymorphism in parental genotypes of two mapping populations

Marker name	Number of markers screened	Number of polymorphic markers	Percent polymorphism	Number of markers screened	Number of polymorphic markers	Percent polymorphism	Sources
	RIL-4			RIL-5			
pPGPseq, pPGSseq	226	25	11.5	226	23	10.12	Ferguson <i>et al.</i> 2004
Lup, Dal, Stylo, Ades, Amor, Chaet	51	0	0	79	0	0	Mace <i>et al.</i> 2007
IPAHM	104	10	9.62	170	21	12.35	Cuc <i>et al.</i> 2008
AC, Ah, gi, RN, TC, Seq	338	41	12.13	338	38	11.24	Moretzsohn <i>et al.</i> 2004; 2005
RN, RM	53	0	0	46	0	0	Proite <i>et al.</i> 2007
PM	59	11	18.64	158	9	5.69	He <i>et al.</i> 2003
GM	2098	115	5.53	2098	112	5.53	S J Knapp, University of Georgia (UGA), USA (Nagy <i>et al.</i> 2010)
Ah, Lec	26	3	11.54	26	0	0	Hopkins <i>et al.</i> 1999
Ap	18	0	0	18	0	0	Palmieri <i>et al.</i> 2002, 2005
S	123	1	0.81	123	2	1.63	Wang <i>et al.</i> 2007
Ah FAD2 allele (CAPS Marker)	2	1	100	0	0	0	Chu <i>et al.</i> 2009
FAD2allele specific marker	2	1	50	2	1	50	Chen <i>et al.</i> 2010
Total	3101	208	6.71	3284	206	6.27	



*Indicates the linkage groups having common markers and LGs assigned according to tetraploid reference map (Varshney *et al.*, 2009a)

Fig 2: Improved genetic map based on TAG 24 × GPBD 4 (RIL-4) mapping population



*Indicates the linkage groups having common markers and LGs assigned according to tetraploid reference map (Varshney *et al.*, 2009a)

Fig 3: Improved genetic map based on TG 26 × GPBD 4 (RIL-5) mapping population

Table 20: Features of individual genetic linkage maps and consensus map

Features / mapping populations	Individual genetic map		Consensus map
	RIL-4	RIL-5	
Number of RILs	266	146	-
Total number of linkage groups	20	21	20
Total number of polymorphic markers	211	210	266
Total number of mapped loci	190	182	227
Total number of unlinked markers	21	28	39
Total number of markers with expected segregation	167	157	175
Total number of distorted markers	44	53	91
Total number of distorted loci mapped	36	41	70
Maximum number of markers per group	17	15	17
Minimum number of markers per group	2	2	2
Total map distance (cM)	1,963.40	1,982.90	1,198.76
Average map distance (cM)	98.17	99.15	59.94
Average inter-locus gap distance (cM)	12.75	15.80	8.14

Table 21: Details of individual genetic maps and consensus maps

Genetic map for RIL-4 population					Genetic map for RIL-5 population					Consensus genetic map				
Linkage group	No. of mapped loci	Map length (cM)	Map density (cM)	Inter-locus gap distance (cM)	Linkage group	No. of mapped loci	Map length (cM)	Map density (cM)	Inter-locus gap distance (cM)	Linkage group	No. of mapped loci	Map length (cM)	Map density (cM)	Inter-locus gap distance (cM)
AhI*	11	89.60	8.15	8.97	AhI*	10	87.10	8.71	9.68	AhI*	13	52.91	4.07	4.41
AhII*	6	73.30	12.22	14.66	AhII*	6	85.50	14.25	17.10	AhII*	8	63.37	7.92	9.05
AhIII*	17	165.80	9.76	10.37	AhIIIa*	2	20.20	10.10	20.20	AhIII*	17	51.31	3.02	3.21
AhIV*	9	87.30	9.70	10.91	AhIIIb*	2	15.10	7.55	15.10	AhIV*	15	60.97	4.06	4.36
AhV*	12	139.20	11.61	12.66	AhIIIc*	5	22.70	4.54	5.68	AhV*	14	41.97	3.00	3.23
AhVI*	5	26.50	5.32	6.65	AhIV*	14	136.20	9.73	10.48	AhVI*	7	40.77	5.82	6.80
AhVII*	10	78.70	7.86	8.73	AhV*	12	149.70	12.48	13.61	AhVII*	12	54.30	4.53	4.94
AhVIII*	12	50.50	4.22	4.60	AhVI*	5	84.90	16.98	21.23	AhVIII*	12	38.67	3.22	3.52
AhIX*	14	127.60	9.11	9.82	AhVII*	12	104.10	8.69	9.48	AhIX*	14	86.51	6.18	6.65
AhX*	13	109.40	8.42	9.12	AhVIII*	10	77.90	7.77	8.63	AhX*	14	44.14	3.15	3.40
AhXI*	5	81.90	16.38	20.48	AhIX*	7	35.90	5.13	5.98	AhXI*	5	51.86	10.37	12.96
AhXII*	10	117.90	11.79	13.11	AhX*	10	81.10	8.11	9.01	AhXII*	14	67.57	4.83	5.20
AhXIII*	11	159.00	14.45	15.90	AhXI*	3	24.60	8.20	12.30	AhXIII*	13	131.50	10.12	10.96
AhXIV*	10	192.40	19.24	21.38	AhXII*	12	121.00	10.08	11.00	AhXIV*	14	72.14	5.15	5.55
AhXV	12	116.50	9.71	10.59	AhXIII*	10	189.10	17.19	18.91	AhXV	15	67.96	4.53	4.85
AhXVI	6	63.10	10.50	12.61	AhXIV*	12	208.60	17.38	18.96	AhXVI	6	48.62	8.10	9.72
AhXVII*	12	114.60	9.55	10.42	AhXV	15	189.60	12.62	13.52	AhXVII*	17	40.74	2.40	2.55
AhXVIII*	9	68.70	7.62	8.58	AhXVI	4	49.80	12.45	16.60	AhXVIII*	11	49.50	4.50	4.95
AhXIX	4	83.80	20.95	27.93	AhXVII*	15	152.50	10.17	10.89	AhXIX	4	116.16	29.04	38.72
AhXX	2	17.60	8.80	17.60	AhXVIII*	8	84.10	10.51	12.01	AhXX	2	17.82	8.91	17.82
					AhXIX	3	14.80	4.90	7.35					
					AhXX	2	26.70	13.35	26.70					
					AhXXI	2	21.70	10.85	21.70					
Total	190	1963.40	215.36	255.09	Total	182	1982.90	241.79	316.12	Total	227	1198.76	132.92	162.85
Mean	9.5	98.17	10.77	12.75	Mean	9.10	99.15	12.09	15.81	Mean	11.35	59.94	6.65	8.14

*Indicates the linkage groups having common markers and LGs assigned according to tetraploid reference map (Varshney *et al.* 2009a)

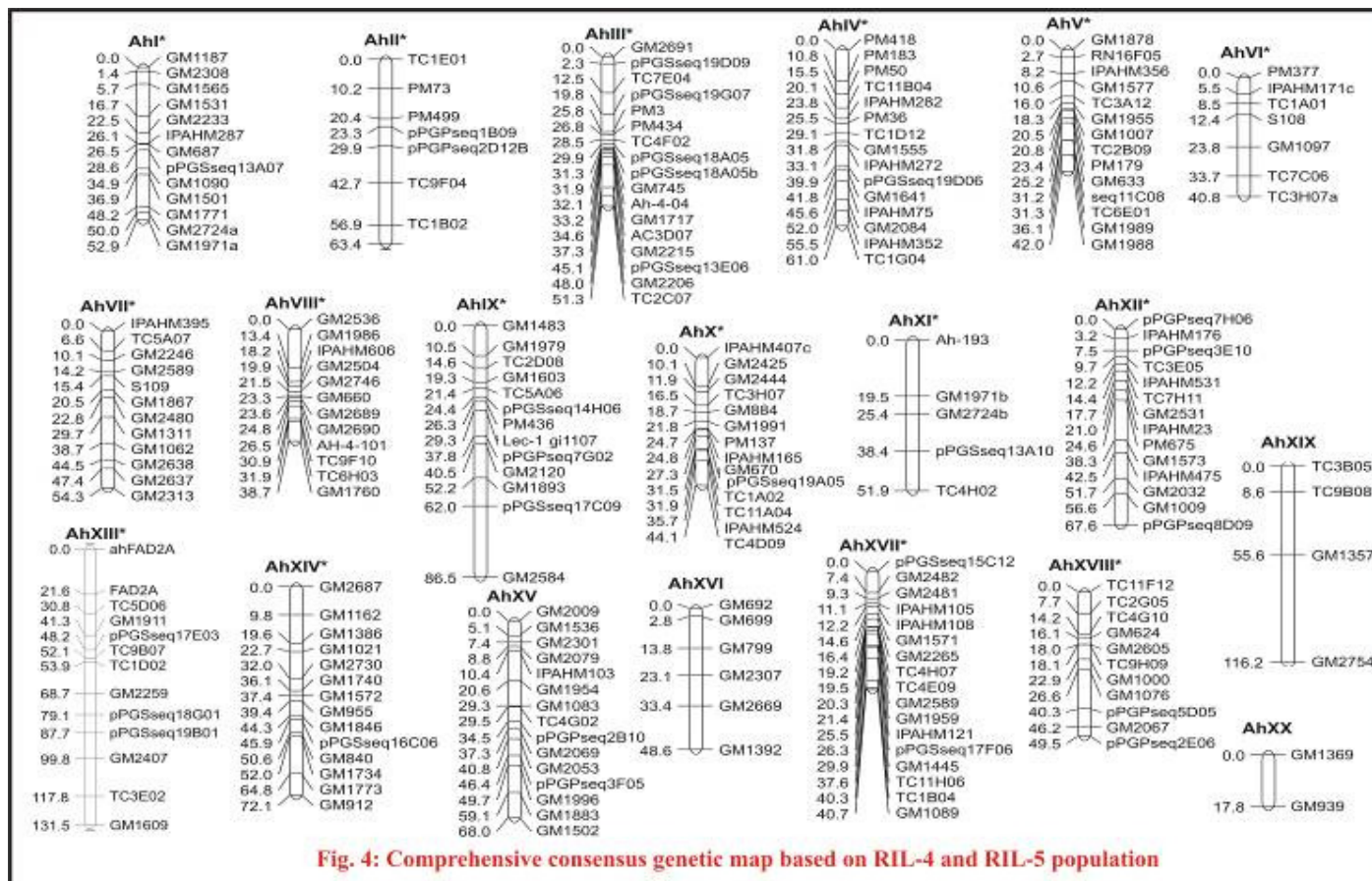


Fig. 4: Comprehensive consensus genetic map based on RIL-4 and RIL-5 population

*Indicates the linkage groups having common markers and LGs assigned according to tetraploid reference map (Varshney *et al.*, 2009a)

Fig 4: Comprehensive consensus genetic map based on RIL-4 and RIL-5 population

Table 22: Summary of consensus genetic linkage map of tetraploid groundnut based on two mapping populations

S. No.	Individual linkage groups		Consensus linkage groups		
	RIL-4	RIL-5	LGs	Loci	Length (cM)
1	AhI*	AhI*	AhI*	13	52.91
2	AhII*	AhII*	AhII*	8	63.37
3	AhIII*	AhIIIa,b,c*	AhIII*	17	51.31
4	AhIV*	AhIV*	AhIV*	15	60.97
5	AhV*	AhV*	AhV*	14	41.97
6	AhVI*	AhVI*	AhVI*	7	40.77
7	AhVII*	AhVII*	AhVII*	12	54.3
8	AhVIII*	AhVIII*	AhVIII*	12	38.67
9	AhIX*	AhIX*	AhIX*	14	86.51
10	AhX*	AhX*	AhX*	14	44.14
11	AhXI*	AhXI*	AhXI*	5	51.86
12	AhXII*	AhXII*	AhXII*	14	67.57
13	AhXIII*	AhXIII*	AhXIII*	13	131.50
14	AhXIV*	AhXIV*	AhXIV*	14	72.14
15	AhXV	AhXV	AhXV	15	67.96
16	AhXVI	AhXVI	AhXVI	6	48.62
17	AhXVII*	AhXVII*	AhXVII*	17	40.74
18	AhXVIII*	AhXVIII*	AhXVIII*	11	49.49
19	AhXIX	-	AhXIX	4	116.16
20	AhXX	-	AhXX	2	17.82
Total	20	18	20	227	1198.76

*Indicates the linkage groups having common markers and LGs assigned according to tetraploid reference map (Varshney *et al.* 2009a)

Table 23: Major and minor QTLs identified for Late leaf spot in TAG 24 × GPBD 4 (RIL-4) population

QTLs	Stage and season	Linkage Group	Position	Marker	LOD	R² (%)	a⁰
<i>QTL_{R4-LLS01}</i>	LLS-SI_2004	AhXII	86.71	<i>GM1573-GM1009/pPGPseq8D09</i>	4.82	10.57	0.39
	LLS-SII_2004	AhXII	84.71	<i>GM1573-GM1009/pPGPseq8D09</i>	5.12	12.60	0.47
	LLS-SI_2005	AhXII	87.81	<i>GM1573-GM1009/pPGPseq8D09</i>	10.19	21.70	0.62
	LLS-SII_2005	AhXII	86.71	<i>GM1573-GM1009/pPGPseq8D09</i>	9.55	23.29	0.64
	LLS-SI_2006	AhXII	86.71	<i>GM1573-GM1009/pPGPseq8D09</i>	10.12	23.64	0.61
	LLS-SII_2006	AhXII	87.81	<i>GM1573-GM1009/pPGPseq8D09</i>	9.78	21.90	0.61
	LLS-SI_2008	AhXII	86.71	<i>GM1573-GM1009/pPGPseq8D09</i>	7.39	16.06	0.26
	LLS-SII_2008	AhXII	89.81	<i>GM1573-GM1009/pPGPseq8D09</i>	5.08	10.27	0.57
	LLS-SI_2009	AhXII	84.71	<i>GM1573-GM1009/pPGPseq8D09</i>	4.12	12.77	0.39
	LLS-SII_2009	AhXII	93.81	<i>GM1573-GM1009/pPGPseq8D09</i>	6.09	23.87	0.74
	LLS-SI_2010	AhXII	91.81	<i>GM1573-GM1009/pPGPseq8D09</i>	8.73	26.80	0.39
	LLS-SII_2010	AhXII	97.81	<i>GM1573-GM1009/pPGPseq8D09</i>	5.72	62.34	1.23
	LLS-SI_Pooled mean	AhXII	93.81	<i>GM1573-GM1009/pPGPseq8D09</i>	13.32	50.54	0.51
	LLS-SII_Pooled mean	AhXII	95.81	<i>GM1573-GM1009/pPGPseq8D09</i>	10.94	51.39	0.87
<i>QTL_{R4-LLS02}</i>	LLS-SII_2008	AhXV	6.01	<i>GM2009-GM1536</i>	37.38	67.98	-1.53
	LLS-SII_2009	AhXV	4.01	<i>GM2009-GM1536</i>	5.28	12.49	-0.53
<i>QTL_{R4-LLS03}</i>	LLS-SII_2008	AhXV	12.51	<i>GM1536-GM2301/GM2079</i>	11.00	17.37	-1.09
	LLS-SI_2009	AhXV	12.81	<i>GM1536-GM2301/GM2079</i>	3.19	4.83	-0.24
	LLS-SII_2009	AhXV	12.51	<i>GM1536-GM2301/GM2079</i>	6.57	10.13	-0.48
<i>QTL_{R4-LLS04}</i>	LLS-SI_2008	AhXV	14.51	<i>IPAHM103-GM1954</i>	11.98	16.14	-0.25
	LLS-SII_2008	AhXV	20.51	<i>IPAHM103-GM1954</i>	19.86	42.66	-1.44

LLS-SI: Stage I =70DAS, LLS-SII: Stage II =90DAS, DAS: Days after sowing

R² (%) =% Phenotypic variance explained, **a⁰** =Additive effect, '-' positive alleles come from TAG 24, '+' positive alleles come from GPBD 4.

Cont....

QTLs	Stage and season	Linkage Group	Position	Marker	LOD	R² (%)	a⁰
<i>QTL_{R4-LLS05}</i>	LLS-SII_2005	AhV	41.01	<i>IPAHM356-GM1577</i>	4.81	12.42	0.47
	LLS-SII_2006	AhV	43.01	<i>IPAHM356-GM1577</i>	2.94	10.81	0.34
	LLS-SI_2009	AhV	43.21	<i>IPAHM356-GM1577</i>	4.84	11.05	0.37
	LLS-SI_Pooled mean	AhV	41.01	<i>IPAHM356-GM1577</i>	4.58	15.34	0.31
<i>QTL_{R4-LLS06}</i>	LLS-SI_2006	AhXIII	27.21	<i>GM1911-pPGSseq17E03</i>	3.22	5.37	0.29
	LLS-SII_2006	AhXIII	26.01	<i>GM1911-pPGSseq17E03</i>	3.09	5.63	0.31
<i>QTL_{R4-LLS07}</i>	LLS-SI_2004	AhIX	53.41	<i>PM436-Lec-1</i>	2.85	4.06	0.24
	LLS-SII_2004	AhIX	53.41	<i>PM436-Lec-1</i>	2.81	3.85	0.26
<i>QTL_{R4-LLS08}</i>	LLS-SI_2004	AhVIII	41.51	<i>TC6H03-GM1760</i>	2.72	3.76	0.23
	LLS-SII_2004	AhVIII	41.51	<i>TC6H03-GM1760</i>	2.55	3.38	0.24
<i>QTL_{R4-LLS09}</i>	LLS-SII_2005	AhXVIII	21.31	<i>TC11F12-TC2G05</i>	2.57	3.47	0.25
	LLS-SII_2006	AhXVIII	12.01	<i>TC11F12-TC2G05</i>	2.98	8.50	0.38
	LLS-SI_Pooled mean	AhXVIII	21.31	<i>TC11F12-TC2G05</i>	2.51	3.39	0.13
<i>QTL_{R4-LLS10}</i>	LLS-SII_Pooled mean	AhVI	12.71	<i>S108--GM1097</i>	2.57	4.72	0.26
<i>QTL_{R4-LLS11}</i>	LLS-SI_2005	AhV	63.21	<i>GM1955-GM1007</i>	2.81	4.64	0.29
<i>QTL_{R4-LLS12}</i>	LLS-SI_2005	AhV	75.41	<i>PM179-seq11C08</i>	3.13	4.11	0.27
<i>QTL_{R4-LLS13}</i>	LLS-SII_2005	AhVII	9.61	<i>TC5A07-GM2246</i>	3.04	4.01	0.27

LLS-SI: Stage I =70DAS, LLS-SII: Stage II =90DAS, DAS: Days after sowing

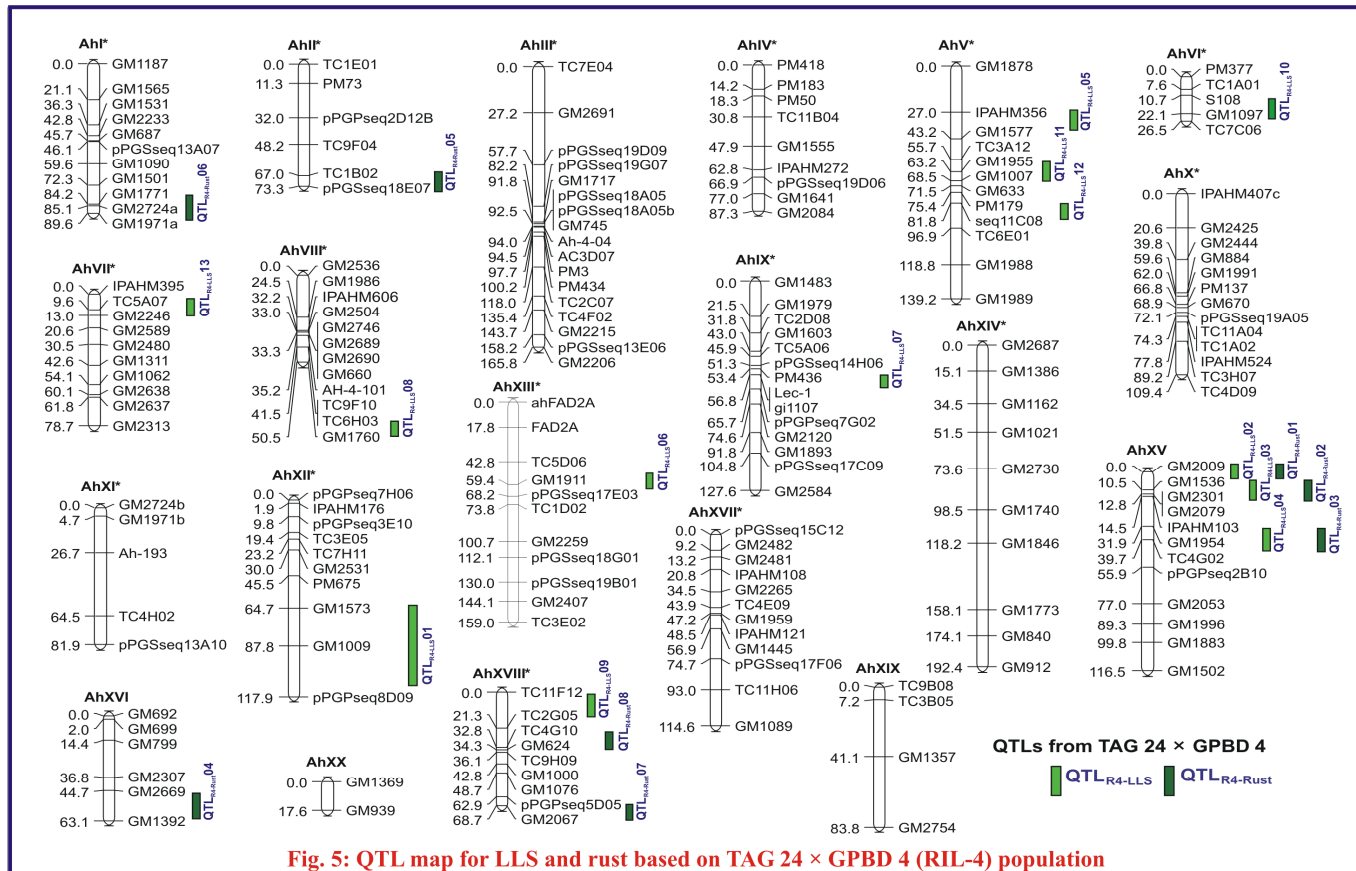
R² (%) =% Phenotypic variance explained, a⁰ =Additive effect, '-' positive alleles come from TAG 24, '+' positive alleles come from GPBD 4.

Table 24: Major and minor QTLs identified for Late leaf spot in TG 26 × GPBD 4 (RIL-5) population

QTLs	Stage and season	Linkage Group	Position	Marker	LOD	R² (%)	a⁰
<i>QTL_{R5-LLS01}</i>	LLS-SI_2008	AhXV	0.01	<i>GM2009-GM1536</i>	6.62	17.24	-0.22
	LLS-SII_2008	AhXV	0.01	<i>GM2009-GM1536</i>	26.10	49.64	-1.25
	LLS-SI_2009	AhXV	0.01	<i>GM2009-GM1536</i>	2.90	7.59	-0.27
	LLS-SII_2009	AhXV	0.01	<i>GM2009-GM1536</i>	12.15	28.47	-0.78
	LLS-SII_Pooled mean	AhXV	0.01	<i>GM2009-GM1536</i>	6.01	15.17	-0.33
<i>QTL_{R5-LLS02}</i>	LLS-SII_2005	AhVIII	28.71	<i>GM2504-GM2746</i>	2.83	9.79	0.33
	LLS-SI_2009	AhVIII	30.71	<i>GM2504-GM2746</i>	3.77	14.31	0.42
	LLS-SII_2009	AhVIII	26.71	<i>GM2504-GM2746</i>	4.36	14.96	0.65
	LLS-SI_Pooled mean	AhVIII	28.71	<i>GM2504-GM2746</i>	5.09	22.47	0.36
<i>QTL_{R5-LLS03}</i>	LLS-SII_2008	AhXV	9.41	<i>IPAHM103-GM1954</i>	17.16	63.17	-1.44
<i>QTL_{R5-LLS04}</i>	LLS-SII_2004	AhV	124.11	<i>RN16F05-GM1988</i>	3.55	16.17	-0.48
<i>QTL_{R5-LLS05}</i>	LLS-SI_2009	AhVIII	42.51	<i>GM2746-GM2689</i>	2.63	12.83	0.39
<i>QTL_{R5-LLS06}</i>	LLS-SI_Pooled mean	AhVIII	56.91	<i>GM2689-GM2690</i>	3.97	14.14	-0.32
<i>QTL_{R5-LLS07}</i>	LLS-SI_Pooled mean	AhVIII	62.61	<i>GM2690-TC9F10</i>	4.07	16.13	-0.34
<i>QTL_{R5-LLS08}</i>	LLS-SI_2009	AhI	65.51	<i>GM1090-GM1771</i>	2.90	12.31	0.37
<i>QTL_{R5-LLS09}</i>	LLS-SI_2007	AhXIV	146.41	<i>pPGSseq16C06-GM1734</i>	2.74	9.60	0.39
<i>QTL_{R5-LLS10}</i>	LLS-SI_2008	AhXIII	39.61	<i>GM1911-TC1D02</i>	2.54	8.85	0.16
<i>QTL_{R5-LLS11}</i>	LLS-SII_2008	AhXIII	146.01	<i>GM2407-TC3E02</i>	2.54	4.32	0.37
<i>QTL_{R5-LLS12}</i>	LLS-SII_2008	AhX	0.01	<i>IPAHM407c-GM2444</i>	2.52	3.27	-0.40
<i>QTL_{R5-LLS13}</i>	LLS-SII_2008	AhX	47.71	<i>PM137-IPAHM165</i>	4.13	5.42	0.52
<i>QTL_{R5-LLS14}</i>	LLS-SII_2007	AhVII	42.51	<i>GM1311-GM1867</i>	2.83	7.52	0.21
<i>QTL_{R5-LLS15}</i>	LLS-SII_2008	AhIIIc	3.51	<i>GM2215-pPGSseq13E06</i>	3.02	4.28	0.38

LLS-SI: Stage I =70DAS, LLS-SII: Stage II =90DAS, DAS: Days after sowing

R² (%) =% Phenotypic variance explained, a⁰ =Additive effect, '-' positive alleles come from TG 26, '+' positive alleles come from GPBD 4.



*Indicates the linkage groups having common markers and LGs assigned according to tetraploid reference map (Varshney *et al.*, 2009a)
QTL positions for LLS and rust are shown in colored bars according to legend key.

Fig 5: QTL map for LLS and rust based on TAG 24 × GPBD 4 (RIL-4) population

Table 25: Major and minor QTLs identified for rust in TAG 24 × GPBD 4 (RIL-4) population

QTLs	Stage and season	Linkage Group	Position	Marker	LOD	R ² (%)	a ⁰
QTL_{R4-Rust01}	Rust-SI_2006	AhXV	6.01	GM2009-GM1536	50.20	79.10	1.37
	Rust-SII_2006	AhXV	6.01	GM2009-GM1536	32.08	70.87	1.47
	Rust-SI_2007E1	AhXV	6.01	GM2009-GM1536	30.66	58.36	0.58
	Rust-SI_2007E2	AhXV	6.01	GM2009-GM1536	59.22	76.27	1.97
	Rust-SII_2007E2	AhXV	6.01	GM2009-GM1536	56.32	75.50	1.87
	Rust-SI_2008	AhXV	6.01	GM2009-GM1536	38.54	63.05	0.81
	Rust-SII_2008	AhXV	6.01	GM2009-GM1536	69.75	82.27	1.94
	Rust-SI_2009	AhXV	6.01	GM2009-GM1536	27.69	67.39	0.95
	Rust-SII_2009	AhXV	6.01	GM2009-GM1536	13.49	33.05	0.84
	Rust-SII_2010	AhXV	6.01	GM2009-GM1536	18.23	39.09	0.79
	Rust-SI_Pooled Mean	AhXV	6.01	GM2009-GM1536	47.79	68.73	0.68
	Rust-SII_Pooled Mean	AhXV	6.01	GM2009-GM1536	43.39	66.98	1.02
QTL_{R4-Rust02}	Rust-SI_2006	AhXV	12.51	GM1536-GM2301/GM2079	38.74	50.61	1.08
	Rust-SII_2006	AhXV	12.51	GM1536-GM2301/GM2079	10.44	12.62	0.87
	Rust-SI_2007E1	AhXV	12.51	GM1536-GM2301/GM2079	34.36	45.93	0.51
	Rust-SI_2007E2	AhXV	12.51	GM1536-GM2301/GM2079	51.56	58.98	1.69
	Rust-SII_2007E2	AhXV	12.51	GM1536-GM2301/GM2079	53.61	62.35	1.68
	Rust-SI_2008	AhXV	12.51	GM1536-GM2301/GM2079	35.78	45.90	0.69
	Rust-SII_2008	AhXV	12.51	GM1536-GM2301/GM2079	48.15	59.54	1.63
	Rust-SI_2009	AhXV	12.51	GM1536-GM2301/GM2079	25.07	36.99	0.69
	Rust-SII_2009	AhXV	12.51	GM1536-GM2301/GM2079	13.76	21.77	0.68
	Rust-SI_2010	AhXV	12.51	GM1536-GM2301/GM2079	12.99	19.86	0.28
	Rust-SII_2010	AhXV	12.51	GM1536-GM2301/GM2079	19.51	29.37	0.69
	Rust-SI_Pooled Mean	AhXV	12.51	GM1536-GM2301/GM2079	45.08	54.64	0.61
	Rust-SII_Pooled Mean	AhXV	12.51	GM1536-GM2301/GM2079	41.82	51.98	0.89

Rust-SI: Stage I =80DAS, Rust-SII: Stage II =90DAS, E1: Environment 1, E2: Environment 2, DAS=Days after sowing

R² (%) =% Phenotypic variance explained, a⁰ =Additive effect, '-' positive alleles come from TAG 24, '+' positive alleles come from GPBD 4.

Cont...

QTLs	Stage and season	Linkage Group	Position	Marker	LOD	R ² (%)	a ⁰
QTL_{R4-Rust}03	Rust-SI_2006	AhXV	20.51	<i>IPAHM103-GM1954</i>	48.99	79.37	1.37
	Rust-SII_2006	AhXV	20.51	<i>IPAHM103-GM1954</i>	14.25	46.83	1.42
	Rust-SI_2007E1	AhXV	18.51	<i>IPAHM103-GM1954</i>	36.65	57.55	0.57
	Rust-SII_2007E1	AhXV	16.51	<i>IPAHM103-GM1954</i>	38.62	53.40	0.60
	Rust-SI_2007E2	AhXV	18.51	<i>IPAHM103-GM1954</i>	64.24	75.80	1.95
	Rust-SII_2007E2	AhXV	18.51	<i>IPAHM103-GM1954</i>	61.37	75.10	1.87
	Rust-SI_2008	AhXV	18.51	<i>IPAHM103-GM1954</i>	42.80	68.10	0.85
	Rust-SII_2008	AhXV	18.51	<i>IPAHM103-GM1954</i>	78.41	82.96	1.96
	Rust-SI_2009	AhXV	20.51	<i>IPAHM103-GM1954</i>	32.18	69.96	0.97
	Rust-SI_2010	AhXV	18.51	<i>IPAHM103-GM1954</i>	11.92	23.12	0.30
	Rust-SII_2010	AhXV	22.51	<i>IPAHM103-GM1954</i>	18.31	62.58	1.00
	Rust-SI_Pooled Mean	AhXV	18.51	<i>IPAHM103-GM1954</i>	48.74	68.02	0.68
	Rust-SII_Pooled Mean	AhXV	18.51	<i>IPAHM103-GM1954</i>	42.56	66.77	1.01
QTL_{R4-Rust}04	Rust-SI_Pooled Mean	AhXVI	44.71	<i>GM2669-GM1392</i>	2.76	3.01	-0.14
	Rust-SII_Pooled Mean	AhXVI	44.71	<i>GM2669-GM1392</i>	2.67	2.96	-0.21
QTL_{R4-Rust}05	Rust-SI_2006	AhII	69.01	<i>TC1B02-pPGSseq18E07</i>	2.53	2.54	-0.24
	Rust-SI_2008	AhII	71.01	<i>TC1B02-pPGSseq18E07</i>	2.98	3.08	-0.17
	Rust-SII_Pooled Mean	AhII	71.01	<i>TC1B02-pPGSseq18E07</i>	3.28	3.29	-0.22
QTL_{R4-Rust}06	Rust-SII_2007E1	AhI	87.11	<i>GM2724a-GM1971a</i>	2.53	3.51	-0.15
QTL_{R4-Rust}07	Rust-SII_2007E2	AhXVIII	48.71	<i>pPGPseq5D05-GM2067</i>	3.27	2.83	0.36
QTL_{R4-Rust}08	Rust-SI_2007E1	AhXVIII	32.81	<i>TC4G10-GM624</i>	4.23	3.89	0.15

Rust-SI: Stage I =80DAS, Rust-SII: Stage II =90DAS, E1: Environment 1, E2: Environment 2, DAS=Days after sowing

R² (%) =% Phenotypic variance explained, a⁰ =Additive effect, '-' positive alleles come from TAG 24, '+' positive alleles come from GPBD 4.

Table 26: Major and minor QTLs identified for rust in TG 26 × GPBD 4 (RIL-5) population

QTLs	Stage and season	Linkage Group	Position	Marker	LOD	R ² (%)	a ⁰
QTL_{R5-Rust01}	RUST-SI_2004	AhXV	0.01	GM2009-GM1536	14.31	30.61	0.32
	Rust-SII_2004	AhXV	0.01	GM2009-GM1536	19.27	39.59	0.48
	Rust-SI_2005	AhXV	0.01	GM2009-GM1536	19.33	40.37	0.53
	Rust-SII_2005	AhXV	0.01	GM2009-GM1536	15.54	35.21	0.84
	Rust-SI_2007E1	AhXV	0.01	GM2009-GM1536	14.31	30.61	0.32
	Rust-SII_2007E1	AhXV	0.01	GM2009-GM1536	22.34	45.94	0.62
	Rust-SI_2007E2	AhXV	0.01	GM2009-GM1536	35.00	66.05	1.64
	Rust-SII_2007E2	AhXV	0.01	GM2009-GM1536	26.15	49.75	1.50
	Rust-SI_2008	AhXV	0.01	GM2009-GM1536	22.38	44.97	0.68
	Rust-SII_2008	AhXV	0.01	GM2009-GM1536	34.01	59.58	1.55
	Rust-SI_2009	AhXV	0.01	GM2009-GM1536	24.27	50.14	0.83
	Rust-SII_2009	AhXV	0.01	GM2009-GM1536	23.54	47.17	1.01
	Rust-SI_2010	AhXV	0.01	GM2009-GM1536	7.12	17.57	0.21
	Rust-SII_2010	AhXV	0.01	GM2009-GM1536	11.15	24.86	0.66
	Rust-SI_Pooled mean	AhXV	0.01	GM2009-GM1536	35.13	59.74	0.64
	Rust-SII_Pooled mean	AhXV	0.01	GM2009-GM1536	36.45	58.15	0.95
QTL_{R5-Rust02}	Rust-SI_2004	AhXV	7.41	IPAHM103-GM1954	7.90	21.17	0.27
	Rust-SII_2004	AhXV	5.41	IPAHM103-GM1954	12.78	28.45	0.42
	Rust-SI_2007E1	AhXV	7.41	IPAHM103-GM1954	7.90	21.17	0.27
	Rust-SII_2007E1	AhXV	5.41	IPAHM103-GM1954	16.24	36.25	0.55
	Rust-SI_2007E2	AhXV	9.41	IPAHM103-GM1954	25.31	77.25	1.75
	Rust-SII_2007E2	AhXV	7.41	IPAHM103-GM1954	20.04	52.94	1.59
	Rust-SI_2008	AhXV	7.41	IPAHM103-GM1954	15.47	41.70	0.67
	Rust-SII_2008	AhXV	9.41	IPAHM103-GM1954	30.78	78.96	1.89
	Rust-SI_2009	AhXV	7.41	IPAHM103-GM1954	16.79	46.52	0.79
	Rust-SII_2009	AhXV	13.41	IPAHM103-GM1954	6.620	23.54	0.72
	Rust-SI_2010	AhXV	9.41	IPAHM103-GM1954	5.665	17.41	0.21
	Rust-SII_2010	AhXV	7.41	IPAHM103-GM1954	10.33	27.71	0.71

Rust-SI: Stage I =80DAS, Rust-SII: Stage II =90DAS, E1: Environment 1, E2: Environment 2, DAS=Days after sowing

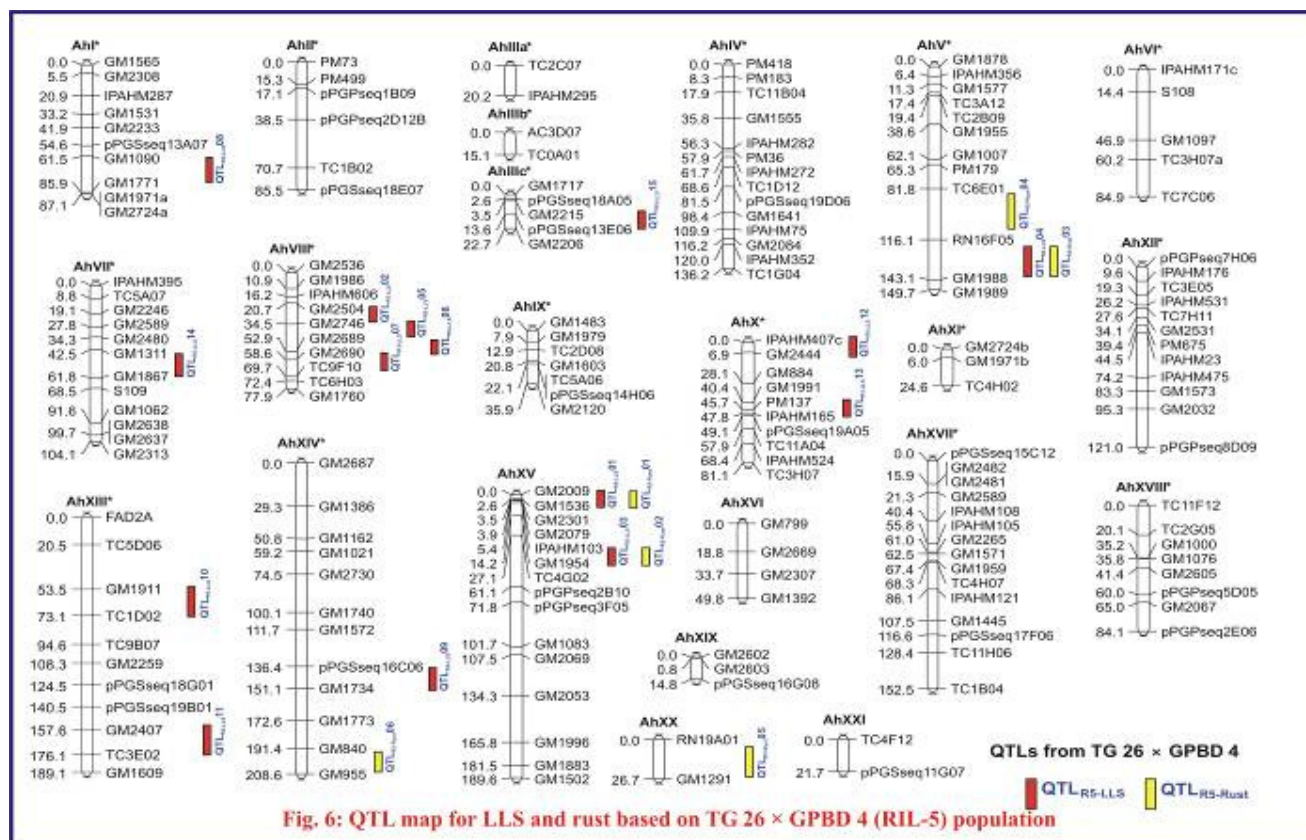
R² (%) =% Phenotypic variance explained, a⁰ =Additive effect, '-' positive alleles come from TG 26, '+' positive alleles come from GPBD 4.

Cont....

QTLs	Stage and season	Linkage Group	Position	Marker	LOD	R² (%)	a⁰
<i>QTL_{R5-Rust02}</i>	Rust-SI_Pooled mean	AhXV	7.41	<i>IPAHM103-GM1954</i>	23.36	60.94	0.66
	Rust-SII_Pooled mean	AhXV	7.41	<i>IPAHM103-GM1954</i>	27.46	62.88	1.00
<i>QTL_{R5-Rust03}</i>	Rust-SI_2005	AhV	124.11	<i>RN16F05-GM1988</i>	5.01	15.07	0.34
	Rust-SI_2010	AhV	124.11	<i>RN16F05-GM1988</i>	6.19	29.02	0.30
<i>QTL_{R5-Rust04}</i>	Rust-SII_2007E1	AhXX	22.01	<i>RN19A01-GM1291</i>	2.52	5.58	-0.20
<i>QTL_{R5-Rust05}</i>	Rust-SI_2010	AhV	107.81	<i>TC6E01-RN16F05</i>	5.84	30.16	0.31
<i>QTL_{R5-Rust06}</i>	Rust-SII_2008	AhXIV	191.41	<i>GM840-GM955</i>	2.56	2.89	0.34

Rust-SI: Stage I =80DAS, Rust-SII: Stage II =90DAS, E1: Environment 1, E2: Environment 2, DAS=Days after sowing

R² (%) =% Phenotypic variance explained, **a⁰** =Additive effect, '-' positive alleles come from TG 26, '+' positive alleles come from GPBD 4.



*Indicates the linkage groups having common markers and LGs assigned according to tetraploid reference map (Varshney *et al.*, 2009a)

QTL positions for LLS and rust are shown in colored bars according to legend key.

Fig 6: QTL map for LLS and rust based on TG 26 × GPBD 4 (RIL-5) population

Among the 14 QTLs identified in RIL-4 population none of them were found to be major QTL except the QTL_{R4-Plht01} and QTL_{R4-Plht02} which explained 11.47 and 13.55 % PV during Kharif 2010 (Table 27). However, in terms of consistency only two QTLs (QTL_{R4-Plht01} and QTL_{R4-Plht02}) were identified during all the three years of evaluation with a PVE ranging from 4.39-11.47% for QTL_{R4-Plht01} and 5.77-13.55% for QTL_{R4-Plht02}. Apart from these two QTLs a total of 4 QTLs (QTL_{R4-Plht03}, QTL_{R4-Plht04}, QTL_{R4-Plht05} and QTL_{R4-Plht06}) were identified during Kharif 2007, 3 QTLs (QTL_{R4-Plht07}, QTL_{R4-Plht08} and QTL_{R4-Plht09}) were detected during Kharif 2008 and 5 QTLs (QTL_{R4-Plht10}, QTL_{R4-Plht11}, QTL_{R4-Plht12}, QTL_{R4-Plht13} and QTL_{R4-Plht14}) during Kharif 2010 (Table 27).

Among the 5 QTLs identified in RIL-5 only four QTLs were found to be major in a particular season of evaluation viz., QTL_{R5-Plht01}, explained a phenotypic variance (PV) of 14.15% in Kharif 2007, 7.13% in Kharif 2008 and 11.61% in Kharif 2010. QTL_{R5-Plht02} explained a PV of 18.60% (Kharif 2007), 6.80% (Kharif 2008) and 13.30% during Kharif 2010. However, QTL_{R5-Plht04} was identified during Kharif 2007 only with 11.89% PVE and QTL_{R5-Plht05} was recognized during Kharif 2008 and 2010 with 9.77 and 10.51% PVE respectively (Table 28). Of the five QTLs, QTL_{R5-Plht01} and QTL_{R5-Plht02} were found consistently across all the three seasons of evaluation (Kharif 2007, 2008 and 2010). However, QTL_{R5-Plht05} was found across two environments/seasons (Kharif 2008 and 2010) and was not identified during Kharif 2007. Remaining 2 QTL (QTL_{R5-Plht03} and QTL_{R5-Plht04}) were identified only during Kharif 2007 (Table 28).

4.3.2.2 Number of primary branches:

QTL analysis for primary branches resulted in a total of 4 QTL viz., QTL_{R4-PBR01}, QTL_{R4-PBR02}, QTL_{R4-PBR03} and QTL_{R4-PBR04} distributed on three LGs (AhVIII*, AhI* and AhXVIII*) in RIL-4 population (Table 27; Fig. 7) and one QTL namely QTL_{R5-PBR01} located on AhVII* (Table 28; Fig. 8) in RIL-5 population. In either of the population major QTL for primary branches was not identified and the PVE ranged from 5.47-9.15% in RIL-4 and in RIL-5 population QTL_{R5-PBR01} explained 7.97% PV during Kharif 2007 (Table 28). Among the four QTL in RIL-4 population two were identified during Kharif 2007 and remaining two during Kharif 2008, none of the QTLs were identified during Kharif 2010 (Table 27).

4.3.2.3 Leaf length (mm):

QTL analysis for leaf length revealed a total of 4 QTLs viz QTL_{R4-LLN01}, QTL_{R4-LLN02}, QTL_{R4-LLN03} and QTL_{R4-LLN04} distributed on two LGs (Table 27; Fig. 7) in RIL-4 population (AhVI* and AhX*). In RIL-5 population three QTLs namely QTL_{R5-LLN01}, QTL_{R5-LLN02} and QTL_{R5-LLN03} (Table 28; Fig. 8) were identified and distributed on two LGs (AhIV* and AhV*). The PVE varied from 4.68-8.65% in RIL-4 and 7.97-12.67% in RIL-5 population (Table 27 and 28 respectively). The QTLs identified in RIL-4 population explained <10% PV and found to be minor QTLs, however QTL_{R4-LLN01} was found consistently across all three years of evaluation. On contrary in RIL-5 population none of the QTLs were consistent but revealed two major QTLs (QTL_{R5-LLN02} and QTL_{R5-LLN03}) during Kharif 2008 and remaining one QTL (QTL_{R5-LLN01}) explained 7.97% PV during Kharif 2007.

4.3.2.4 Leaf width (mm):

Only during Kharif 2007 four QTLs were identified in RIL-4 population. All these four QTLs viz., QTL_{R4-LWD01}, QTL_{R4-LWD02}, QTL_{R4-LWD03} and QTL_{R4-LWD04} were distributed on four LGs AhIV*, AhVI*, AhX* and AhV* respectively (Table 27; Fig. 7). All the QTLs identified were minor QTL as they explained <10% PV and were not consistent across the environments/seasons. The PVE ranged from 3.97 (QTL_{R4-LWD02}) to 7.12 (QTL_{R4-LWD01}) in RIL-4 population. However, in RIL-5 population only one major QTL was identified with a PV ranging from 10.89-15.64% and was detected during Kharif 2008 and 2010 (Table 28; Fig. 8).

4.3.3 QTLs for yield attributing traits

4.3.3.1 Number of pods per plant (NOP/P)

QTL analysis on three years of data available on both the populations revealed a total of 2 and 3 QTLs contributing for NOP/P in RIL-4 (Table 29; Fig. 7) and RIL-5 (Table 30; Fig. 8) population respectively. Among the identified two QTLs in RIL-4 population only one QTL (QTL_{R4-NOP/P02}) located on LG AhXIV* was a major QTL explaining 13.21% PV during Kharif 2010. Remaining one QTL identified during Kharif 2008 was a minor QTL with 9.64% PV and was located on LG AhXVIII*. In RIL-4 population PVE by the QTLs for NOP/P ranged from 9.64-13.21% (Table 29).

Table 27: QTLs identified for morphological traits in TAG 24 × GPBD 4 (RIL-4) population

QTL	Linkage Group	Marker Interval	Position range	LOD range	Kharif 2007 R ² (%)	a ⁰	Kharif 2008 R ² (%)	a ⁰	Kharif 2010 R ² (%)	a ⁰
Plant height (cm)										
<i>QTL_{R4-PIht}01</i>	AhV*	GM1955-GM1007	63.21-65.21	3.02-6.99	5.77	-0.96	6.18	-0.89	13.55	-1.17
<i>QTL_{R4-PIht}02</i>	AhV*	GM633-PM179	71.51-73.51	2.92-8.84	4.39	-0.84	7.58	-0.99	11.47	-1.21
<i>QTL_{R4-PIht}03</i>	AhV*	GM1577-TC3A12	43.21	3.54	7.03	-1.06				
<i>QTL_{R4-PIht}04</i>	AhXII*	GM1009-pPGPseq8D09	93.81	2.85	8.25	-1.15				
<i>QTL_{R4-PIht}05</i>	AhXVIII*	TC2G05-TC4G10	27.31	3.24	6.80	-1.05				
<i>QTL_{R4-PIht}06</i>	AhXVIII*	GM624-TC9H09	34.31	4.16	6.04	-0.98				
<i>QTL_{R4-PIht}07</i>	AhVII*	TC5A07-GM2246	9.61	3.38			4.50	0.77		
<i>QTL_{R4-PIht}08</i>	AhX*	PM137-GM670	66.81	4.71			6.28	-0.90		
<i>QTL_{R4-PIht}09</i>	AhXII*	IPAHM176-pPGPseq3E10	7.91	4.48			6.95	-0.95		
<i>QTL_{R4-PIht}10</i>	AhVII*	GM2637-GM2313	65.81	3.80					9.06	1.08
<i>QTL_{R4-PIht}11</i>	AhVII*	GM1062-GM2638	58.11	3.73					7.81	1.00
<i>QTL_{R4-PIht}12</i>	AhX*	pPGSseq19A05-TC11A04	74.11	3.84					4.81	-0.80
<i>QTL_{R4-PIht}13</i>	AhXII*	pPGPseq7H06-IPAHM176	0.01	5.89					7.87	-1.01
<i>QTL_{R4-PIht}14</i>	AhXII*	pPGPseq3E10-TC3E05	13.81	3.80					6.20	-0.90
Number of primary branches										
<i>QTL_{R4-PBR}01</i>	AhVIII*	GM2504-GM2746	33.01	3.82	5.81	-0.09				
<i>QTL_{R4-PBR}02</i>	AhVIII*	GM660-Ah-4-101	33.31	3.62	5.47	-0.09				
<i>QTL_{R4-PBR}03</i>	AhI*	GM2724a-GM1971a	89.11	2.58			5.92	0.23		
<i>QTL_{R4-PBR}04</i>	AhXVIII*	TC11F12-TC2G05	8.01	3.30			9.15	-0.28		
Leaf length (mm)										
<i>QTL_{R4-LLN}01</i>	AhVI*	S108-GM1097	10.71-14.71	3.06-4.98	6.13	-1.16	8.65	-1.65	6.97	-0.41
<i>QTL_{R4-LLN}02</i>	AhX*	GM670-pPGSseq19A05	70.91	2.8	4.68	1.02				
<i>QTL_{R4-LLN}03</i>	AhIX*	TC5A06-pPGSseq14H06	64.01	3.7	6.31	1.18				
<i>QTL_{R4-LLN}04</i>	AhX*	GM1991-PM137	64.01	2.9					5.02	0.28
Leaf width (mm)										
<i>QTL_{R4-LWD}01</i>	AhIV*	GM1555-IPAHM272	51.91	3.33	7.12	-0.56				
<i>QTL_{R4-LWD}02</i>	AhVI*	S108-GM1097	9.61	2.51	3.97	-0.42				
<i>QTL_{R4-LWD}03</i>	AhX*	GM1991-PM137	64.01	3.3	5.98	0.51				
<i>QTL_{R4-LWD}04</i>	AhV*	GM1577-TC3A12	51.21	2.73	6.77	0.55				

R² (%) = % Phenotypic variance explained, a⁰ = Additive effect, '-' positive alleles come from TAG 24, '+' positive alleles come from GPBD 4.

Table 28: QTLs identified for morphological traits in TG 26 × GPBD 4 (RIL-5) population

QTL	Linkage Group	Marker Interval	Position range	LOD range	Kharif 2007		Kharif 2008		Kharif 2010	
					R ² (%)	a ⁰	R ² (%)	a ⁰	R ² (%)	a ⁰
Plant height (cm)										
QTL_{R5-Plht}01	AhV*	GM1955-GM1007	58.61-60.61	2.57-4.65	14.15	-1.63	7.13	-1.02	11.61	-1.11
QTL_{R5-Plht}02	AhV*	PM179-TC6E01	69.31-71.31	2.76-5.09	18.60	-1.93	6.80	-0.99	13.30	-1.19
QTL_{R5-Plht}03	AhX*	GM884-GM1991	38.11	3.17	8.99	-1.38				
QTL_{R5-Plht}04	AhII*	pPGPseq2D12B-TC1B02	44.51	2.89	11.89	-1.59				
QTL_{R5-Plht}05	AhX*	GM2444-GM884	6.91-8.91	3.38-4.40			9.77	-1.21	10.51	-1.07
Number of primary branches										
QTL_{R5-PBR}01	AhVII*	GM1062-GM2638	99.61	2.52					6.63	0.12
Leaf length (mm)										
QTL_{R5-LLN}01	AhIV*	PM418-PM183	2.01	2.68	7.97	1.83				
QTL_{R5-LLN}02	AhV*	GM1955-GM1007	44.61	2.95			12.67	-1.79		
QTL_{R5-LLN}03	AhV*	TC2B09-GM1955	33.41	2.94			11.65	-1.72		
Leaf width (mm)										
QTL_{R5-LWD}01	AhIIIb*	AC3D07-TC0A01	8.01-10.01	3.05-4.29			10.89	0.81	15.64	0.97

R² (%) = % Phenotypic variance explained, a⁰ = Additive effect, '-' positive alleles come from TG 26, '+' positive alleles come from GPBD 4.

Table 29: QTLs identified for yield attributing traits in TAG 24 × GPBD 4 (RIL-4) population

QTL	Linkage Group	Marker Interval	Position range	LOD range	Kharif 2007 R ² (%)	a ⁰	Kharif 2008 R ² (%)	a ⁰	Kharif 2010 R ² (%)	a ⁰
Number of pods per plant										
<i>QTL_{R4-NOP/P01}</i>	AhXVIII*	TC11F12-TC2G05	4.01	3.24			9.64	-1.68		
<i>QTL_{R4-NOP/P02}</i>	AhXIV*	GM1386-GM1162	23.11	3.13					13.21	1.71
Pod yield per plant (g)										
<i>QTL_{R4-PY/P01}</i>	AhXV	IPAHM103-GM1954	18.51	7.08	15.37	-1.5				
<i>QTL_{R4-PY/P02}</i>	AhXV	GM1536-GM2301	12.51	6.71	10.74	-1.24				
<i>QTL_{R4-PY/P03}</i>	AhXV	GM2009-GM1536	6.01	5.63	14	-1.39				
<i>QTL_{R4-PY/P04}</i>	AhXII*	GM1009-pPGPseq8D09	87.81	5.49	12.51	-1.32				
<i>QTL_{R4-PY/P05}</i>	AhXV	GM2079-IPAHM103	12.81	5.47	8.57	-1.09				
<i>QTL_{R4-PY/P06}</i>	AhIII*	TC7E04-GM2691	0.01	2.9			6.9	1.49		
<i>QTL_{R4-PY/P07}</i>	AhVII*	GM2638-GM2637	60.11	3.84			8.4	-1.62		
<i>QTL_{R4-PY/P08}</i>	AhXIV*	GM1386-GM1162	15.11-21.11	3.04-7.17			15.16	-2.16	12.43	1.80
Shelling per cent (%)										
<i>QTL_{R4-SH%01}</i>	AhXV	GM2009-GM1536	10.01	8.25	15.2	-1.86				
<i>QTL_{R4-SH%02}</i>	AhXV	IPAHM103-GM1954	18.51	6.34	13.34	-1.74				
Hundred seed weight (g)										
<i>QTL_{R4-HSW01}</i>	AhXV	GM2009-GM1536	2.01	2.73	6.17	-1.24				
<i>QTL_{R4-HSW02}</i>	AhV*	GM633-PM179	71.51	3.75			5.83	1.13		
<i>QTL_{R4-HSW03}</i>	AhV*	TC3A12-GM1955	59.71	3.18			7.36	1.26		

R² (%) = % Phenotypic variance explained, a⁰ = Additive effect, '-' positive alleles come from TAG 24, '+' positive alleles come from GPBD 4.

Table 30: QTLs identified for yield attributing traits in TG 26 × GPBD 4 (RIL-5) population

QTL	Linkage Group	Marker Interval	Position range	LOD range	Kharif 2007 R ² (%) a ^u		Kharif 2008 R ² (%) a ^u		Kharif 2010 R ² (%) a ^u	
Number of pods per plant										
<i>QTL_{R5-NOP/p01}</i>	AhXV	GM2009-GM1536	0.01	3.98	9.41	-1.36				
<i>QTL_{R5-NOP/p02}</i>	AhXIV*	GM1740-GM1572	108.11	3.93	12.41	-1.56				
<i>QTL_{R5-NOP/p03}</i>	AhV*	PM179-TC6E01	65.31	3.43	8.28	-1.40				
Pod yield per plant (g)										
<i>QTL_{R5-PY/p01}</i>	AhXV	GM2009-GM1536	0.01	6.21	16.60	-1.54				
<i>QTL_{R5-PY/p02}</i>	AhXV	IPAHM103-GM1954	7.41	3.72	11.64	-1.28				
<i>QTL_{R5-PY/p03}</i>	AhXV	GM2301-GM2079	3.51	2.71	7.64	-1.04				
<i>QTL_{R5-PY/p04}</i>	AhXVII*	TC11H06-TC1B04	148.41	2.63			9.96	-2.07		
<i>QTL_{R5-PY/p05}</i>	AhIV*	TC1D12-pPGSseq19D06	68.61	2.90					7.40	1.47
<i>QTL_{R5-PY/p06}</i>	AhXIV*	GM1572-pPGSseq16C06	39.11	2.73					17.68	-2.23
Shelling per cent (%)										
<i>QTL_{R5-SH%01}</i>	AhXIX	GM2603-pPGSseq16G08	2.81	3.86	12.67	1.13				
<i>QTL_{R5-SH%02}</i>	AhI*	GM1090-GM1771	79.51	2.69					11.59	-1.17
Hundred seed weight (g)										
<i>QTL_{R5-HSW01}</i>	AhVI*	GM1097-TC3H07a	48.91	3.74			11.81	-1.64		
<i>QTL_{R5-HSW02}</i>	AhI*	pPGSseq13A07-GM1090	56.61	2.62			7.51	1.29		
<i>QTL_{R5-HSW03}</i>	AhXVII*	TC11H06-TC1B04	148.41	2.61			9.87	-1.50		

R² (%) = % Phenotypic variance explained, a⁰ = Additive effect, '-' positive alleles come from TG 26, '+' positive alleles come from GPBD 4.

Similarly in case of RIL-5 population three QTLs were located on LG AhXV, Ah XIV* and AhV*. The PVE by the QTLs ranged from 8.28-12.41%, only one QTL (QTL_{R5-NOP/P02}) was found to be a major QTL explaining 12.41% PV from among the three QTL identified. The remaining two QTLs were found to be minor QTLs (Table 30). However none of the QTLs identified from both (RIL-4 and RIL-5) the populations were not consistent across environments/seasons and were specific to a particular season of phenotyping.

4.3.3.2 Pod yield per plant (g)

For pod yield per plant, a total of eight and six QTLs were identified for pod yield in RIL-4 (Table 29; Fig. 7) and RIL-5 (Table 30; Fig 8) population respectively. The phenotypic variance explained ranged from 6.9-15.37% in RIL-4 and 7.40-17.68% in RIL-5 population. Among the eight QTLs identified in RIL-4 population five were found to be major QTLs (QTL_{R4-PY/P01}, QTL_{R4-PY/P02}, QTL_{R4-PY/P03}, QTL_{R4-PY/P04} and QTL_{R4-PY/P08}) explaining >10% PV (Table 29) and remaining three are minor QTLs (QTL_{R4-PY/P05}, QTL_{R4-PY/P06} and QTL_{R4-PY/P07}). All these QTLs were distributed on 5 LGs (AhXV, AhXII*, AhIII*, AhVII* and AhXIV*) in RIL-4 population. Among these only one QTL (QTL_{R4-PY/P08}) was found consistently during Kharif 2008 and 2010 with a PVE ranging from 12.46-15.16% and remaining 7 QTLs were specific to a particular season/environment (Table 29).

Likewise in RIL-5 population among the six QTL identified (Table 30) three were (QTL_{R5-PY/P01}, QTL_{R5-PY/P02} and QTL_{R5-PY/P06}) major QTLs with a phenotypic variance ranging from 11.64 (QTL_{R5-PY/P02}) -17.68% (QTL_{R5-PY/P06}) and remaining three QTLs (QTL_{R5-PY/P03}, QTL_{R5-PY/P04} and QTL_{R5-PY/P05}) were minor QTLs (Table 30). QTLs in RIL-5 population were distributed across four LGs (AhXV, AhXVII*, AhIV* and AhXIV*). However, none of the QTL could be consistently detected across the three seasons for pod yield per plant in RIL-5 population.

4.3.3.3 Shelling Percent (%)

For shelling percent a total of two QTLs each were identified in both the population [Table 29 (Fig. 7) and 30 (Fig. 8)]. The PVE ranged from 13.34 (QTL_{R4-SH%01}) to 15.20% (QTL_{R4-SH%02}) in RIL-4 population and 11.59 (QTL_{R5-SH%01}) to 12.67% (QTL_{R5-SH%02}) in RIL-5 population. In RIL-4 population both the QTLs were detected on LG AhXV and in case of RIL-5 population two QTLs (QTL_{R5-SH%01} and QTL_{R5-SH%02}) were distributed on two LGs AhXIX and AhI* respectively. QTLs identified for shelling percent in both the populations (RIL-4 and RIL-5) were found to be major QTLs [Table 29 (RIL-4) and 30 (RIL-5)] but were not consistent across environments.

4.3.3.4 Hundred seed weight (g)

In both the population 3 QTLs each were identified for hundred seed weight with a PV ranging from 5.83-6.17% in RIL-4 population (Table 29; Fig. 7) and 7.51-11.81% in RIL-5 population (Table 30; Fig 8). QTLs identified for hundred seed weight were distributed on two LGs (AhXV and AhV*) in RIL-4 and three LGs (AhVI*, AhI* and AhVII*) in case of RIL-5 population. None of the QTL (QTL_{R4-HSW01}, QTL_{R4-HSW02} and QTL_{R4-HSW03}) in RIL-4 population was a major QTL as they explained <10% PV for hundred seed weight (Table 29). However, only one QTL (QTL_{R5-HSW01}) in case of RIL-5 population was a major QTL with 11.81% PVE and remaining two QTLs (QTL_{R5-HSW02} and QTL_{R5-HSW03}) were minor QTLs with a PV <10%.

4.3.4 QTLs for quality traits:

4.3.4.1 Protein content:

A total of 6 QTL (QTL_{R4-Protein01}, QTL_{R4-Protein02}, QTL_{R4-Protein03}, QTL_{R4-Protein04}, QTL_{R4-Protein05} and QTL_{R4-Protein06}) and 3 QTL (QTL_{R5-Protein01}, QTL_{R5-Protein02} and QTL_{R5-Protein03}) were identified for protein content in case of RIL-4 (Table 31; Fig. 9) and RIL-5 (Table 32; Fig.10) population respectively. All the six QTLs in RIL-4 population were distributed on six LGs viz., AhVI*(QTL_{R4-Protein01}), AhVII*(QTL_{R4-Protein02}), AhXI*(QTL_{R4-Protein03}), AhVIII*(QTL_{R4-Protein04}), AhXII*(QTL_{R4-Protein05}) and AhII*(QTL_{R4-Protein06}). Similarly in RIL-5 population three QTLs were distributed on 3 LGs AhVIII*(QTL_{R5-Protein01}), AhXV (QTL_{R5-Protein02}) and AhIII*a (QTL_{R5-Protein03}). The PVE ranged from 3.88-20.35% in RIL-4 (Table 31) and 7.13-3.76% in RIL-5 (Table 32) population. Among the 6 QTLs identified in RIL-4 population for protein content only one QTL (QTL_{R4-Protein02}) was found to be major with a PV ranging from 18.85-20.35% and remaining 5 were minor QTLs explaining <10% PV. Of the 3 QTLs identified in RIL-5 population two were minor QTLs (QTL_{R5-Protein02} and QTL_{R5-Protein03}) and the QTL_{R5-Protein01} explained a PV of 13.76% and was a major QTL in summer 2007.

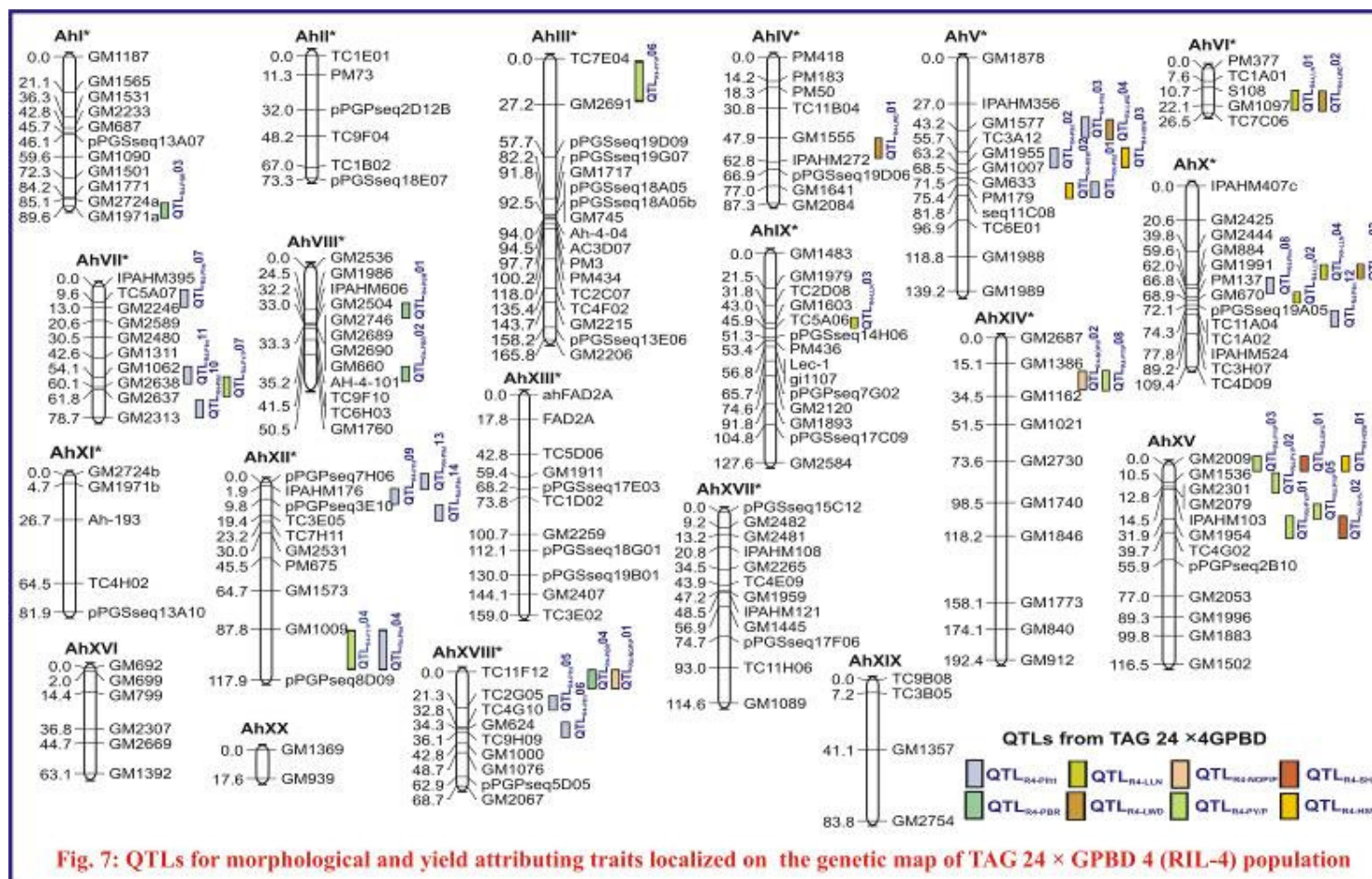


Fig 7: QTLs for morphological and yield attributing traits localized on the genetic map of TAG 24 × GPBD 4 (RIL-4) population

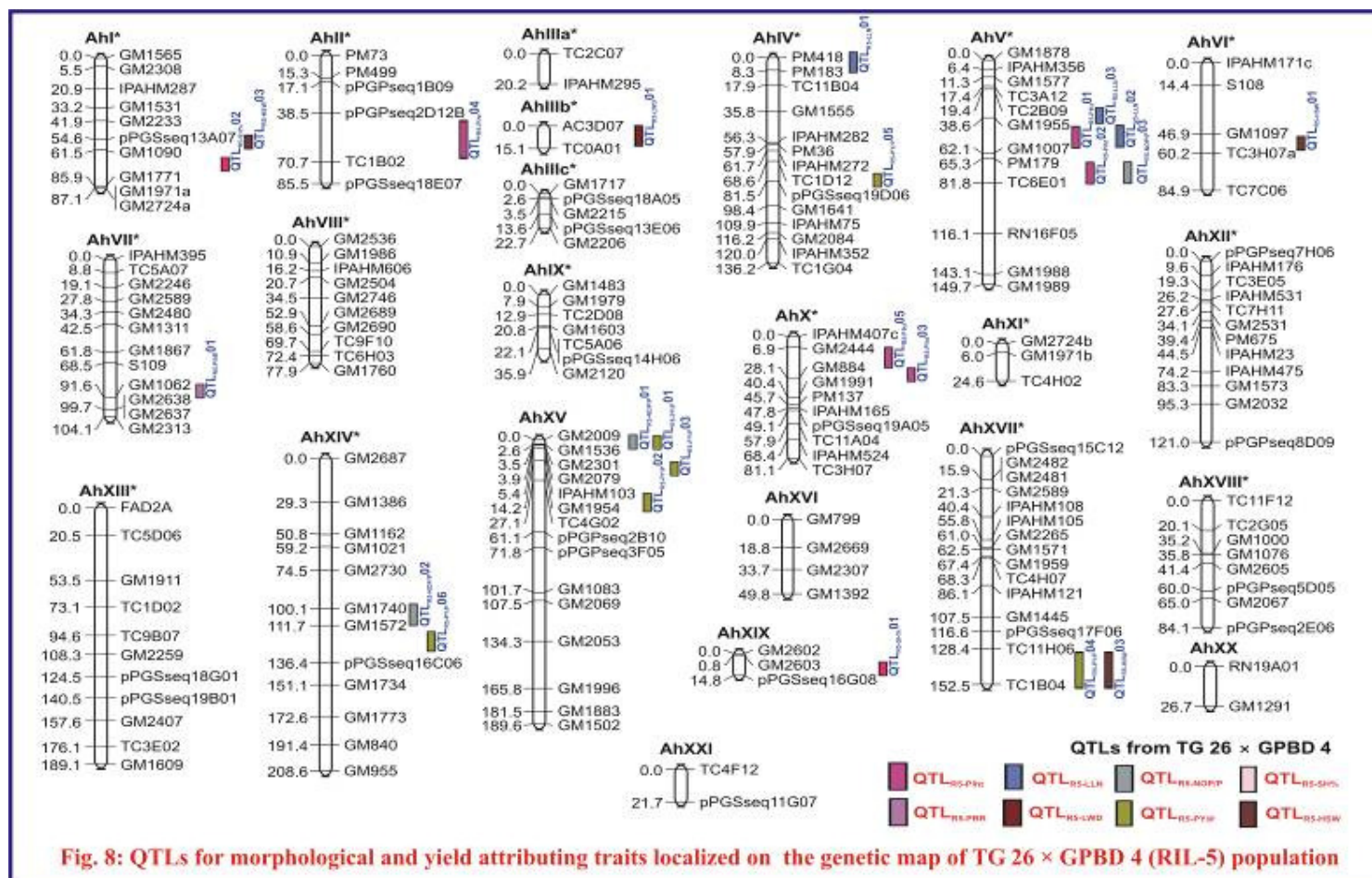


Fig 8: QTLs for morphological and yield attributing traits localized on the genetic map of TG 26 × GPBD 4 (RIL-5) population

Table 31: Major and minor QTLs identified for protein and oil content in TAG 24 × GPBD 4 (RIL-4) population

QTL	Linkage Group	Marker Interval	Position range	LOD range	Kharif 2008		Summer 2009	
					R ² (%)	a ⁰	R ² (%)	a ⁰
Protein content (%)								
<i>QTL_{R4-Protein}01</i>	AhVI*	<i>S108-GM1097</i>	18.71-21.11	4.21-4.97	9.27	0.62	8.74	0.50
<i>QTL_{R4-Protein}02</i>	AhVII*	<i>GM1311-GM1062</i>	46.61	7.58-8.16	18.85	-0.89	20.35	-0.77
<i>QTL_{R4-Protein}03</i>	AhXI*	<i>GM1971b-Ah-193</i>	22.71-24.61	3.16-3.78	9.44	-0.63	10.25	-0.55
<i>QTL_{R4-Protein}04</i>	AhVIII*	<i>Ah-4-101-TC9F10</i>	39.21	2.61	3.88	0.40		
<i>QTL_{R4-Protein}05</i>	AhXII*	<i>GM2531-PM675</i>	30.01	3.55	5.36	-0.48		
<i>QTL_{R4-Protein}06</i>	AhII*	<i>pPGPseq2D12B-TC9F04</i>	40.01	3.17			6.51	-0.44
Oil content (%)								
<i>QTL_{R4-Oil}01</i>	AhVI*	<i>S108-GM1097</i>	16.71-21.11	3.65-4.42	8.81	0.58	5.86	0.48
<i>QTL_{R4-Oil}02</i>	AhVII*	<i>GM1311-GM1062</i>	42.61-52.61	5.03-5.40	9.46	-0.60	10.40	-0.65
<i>QTL_{R4-Oil}03</i>	AhXV	<i>pPGPseq2B10-GM2053</i>	71.91-75.01	2.96-3.58	7.04	-0.52	8.72	-0.60
<i>QTL_{R4-Oil}04</i>	AhVIII*	<i>TC6H03-GM1760</i>	43.51	4.15	7.05	0.52		
<i>QTL_{R4-Oil}05</i>	AhVIII*	<i>Ah-4-101-TC9F10</i>	39.21	3.78	6.72	0.50		
<i>QTL_{R4-Oil}06</i>	AhX*	<i>IPAHM407c-GM2425</i>	8.01	2.93	6.65	-0.50		
<i>QTL_{R4-Oil}07</i>	AhVI*	<i>PM377-TC1A01</i>	6.01	3.26	5.35	0.45		
<i>QTL_{R4-Oil}08</i>	AhXIII*	<i>ahFAD2A(CAPS)-FAD2A</i>	10.01	3.22			6.61	0.51

R² (%) = % Phenotypic variance explained, a⁰ = Additive effect, '-' positive alleles come from TAG 24, '+' positive alleles come from GPBD 4.

Table 32: Major and minor QTLs identified for protein and oil content in TG 26 × GPBD 4 (RIL-5) population

QTL	Linkage Group	Marker Interval	Position range	LOD range	Summer 2007		Kharif 2007	
					R ² (%)	a ⁰	R ² (%)	a ⁰
Protein content (%)								
<i>QTL_{R5-Protein01}</i>	AhVIII*	<i>TC6H03-GM1760</i>	76.41	5.14	13.76	1.48		
<i>QTL_{R5-Protein02}</i>	AhXV	<i>GM2069-GM2053</i>	107.51	2.81	7.13	-1.06		
<i>QTL_{R5-Protein03}</i>	AhIIla*	<i>TC2C07-IPAHM295</i>	0.01	3.26			9.09	0.92
Oil content (%)								
<i>QTL_{R5-Oil01}</i>	AhV*	<i>PM179-TC6E01</i>	75.31	3.83			13.84	0.50
<i>QTL_{R5-Oil02}</i>	AhV*	<i>GM1988-GM1989</i>	147.11	2.65			8.13	0.38
<i>QTL_{R5-Oil03}</i>	AhXV	<i>TC4G02-pPGPseq2B10</i>	35.11	2.52			13.58	-0.51

R² (%) = % Phenotypic variance explained, a⁰ = Additive effect, '-' positive alleles come from TG 26, '+' positive alleles come from GPBD 4.

In terms of consistency of the QTL to be detected repeatedly across the environments only three QTLs (QTL_{R4-Protein}01, QTL_{R4-Protein}02 and QTL_{R4-Protein}03) were consistent across both seasons (Kharif 2008 and summer 2009) in RIL-4 population (Table 31). However it was not the same in case of RIL-5 population where none of the identified QTLs were consistent across environments (Table 32).

4.3.4.2 Oil content

QTL analysis on the data obtained on oil content from two seasons revealed a total of 8 QTLs (QTL_{R4-Oil}01, QTL_{R4-Oil}02, QTL_{R4-Oil}03, QTL_{R4-Oil}04, QTL_{R4-Oil}05, QTL_{R4-Oil}06, QTL_{R4-Oil}07 and QTL_{R4-Oil}08) in RIL-4 population (Table 31; Fig. 9) and 3 QTLs (QTL_{R5-Oil}01, QTL_{R5-Oil}02 and QTL_{R5-Oil}03) in RIL-5 population (Table 32; Fig. 10). The eight QTLs in RIL-4 population were distributed on 6 LGs viz., AhVI*(QTL_{R4-Oil}01 and QTL_{R4-Oil}07), AhVII*(QTL_{R4-Oil}02), AhXV (QTL_{R4-Oil}03), AhVIII*(QTL_{R4-Oil}04 and QTL_{R4-Oil}05), Ah X*(QTL_{R4-Oil}06) and AhXIII*(QTL_{R4-Oil}08). In case of RIL-5 the QTLs were distributed on two LGs viz., AhV*(QTL_{R5-Oil}01 and QTL_{R5-Oil}02) and AhXV (QTL_{R5-Oil}03). The PVE ranged from 5.35-10.40% in RIL-4 and 8.13-13.84% in RIL-5 population. Of the 8 QTLs identified in RIL-4 population none of them were found to be major as they explained <10% of phenotypic variance for oil content. However, 3 QTLs (QTL_{R4-Oil}01, QTL_{R4-Oil}02 and QTL_{R4-Oil}03) were consistently identified across both the environments in RIL-4 population (Table 31). Similarly QTL analysis in RIL-5 population, revealed 2 major QTLs [QTL_{R5-Oil}01 (13.84%) and QTL_{R5-Oil}02 (13.58%)] and one minor QTL [QTL_{R5-Oil}03 (8.13)]. However, none of the QTLs were consistent across the season/environment (Table 32).

4.3.4.3 Oleic acid content:

QTLs for oleic acid content were distributed on four LG (AhXIII*, AhVIII*, AhVI* and AhI*) harboring 7 QTLs (QTL_{R4-Oleic acid}01, QTL_{R4-Oleic acid}02, QTL_{R4-Oleic acid}03, QTL_{R4-Oleic acid}04, QTL_{R4-Oleic acid}05, QTL_{R4-Oleic acid}06 and QTL_{R4-Oleic acid}07) in case of RIL-4 population (Table 33; Fig. 9), similarly two QTLs were identified in RIL-5 population distributed on 2 LG (AhVIII* and AhVII*) (Table 34; Fig. 10). In RIL-4 population PV ranged from 3.77-23.79% and it was 11.45-14.08% in RIL-5 population. Among the seven QTLs identified in RIL-4 population only one major QTL (QTL_{R4-Oleic acid}01) contributing a phenotypic variance ranging from 14.34-23.79% was flanked by CAPS marker *ahFAD2A* and *FAD2A* (Allele specific marker) for *FAD2A* 'allele'. Remaining 6 QTLs were found to be minor QTLs with a PV ranging from 3.77-6.88% (Table 33). Likewise in RIL-5 Population two major QTLs [QTL_{R5-Oleic acid}01 (14.08%) and QTL_{R5-Oleic acid}02 (11.48%)] were identified and were specific to a particular environment (Table 34).

Considering the consistency of the QTL to be identified across environments a total of 5 QTLs were consistent across two seasons in RIL-4 population for oleic acid (Table 33). However none of the identified QTLs were consistent in case of RIL-5 Population (Table 34).

4.3.4.4 Linoleic acid:

QTL analysis for linoleic acid revealed a total of two (QTL_{R4-Linoleic acid}01 and QTL_{R4-Linoleic acid}01) and three (QTL_{R5-Linoleic acid}01, QTL_{R5-Linoleic acid}02 and QTL_{R5-Linoleic acid}03) QTLs in case of RIL-4 (Table 33; Fig. 9) and RIL-5 (Table 34; Fig. 10) population respectively. The PVE ranged from 8.34 (QTL_{R4-Linoleic acid}02) to 25.40% (QTL_{R4-Linoleic acid}01) in RIL-4 population (Table 33), similarly PV ranged from 7.26 (QTL_{R5-Linoleic acid}03) to 11.92% (QTL_{R5-Linoleic acid}01) in RIL-5 population (Table 34). QTLs identified in RIL-4 and RIL-5 populations were distributed on two LGs (AhXIII* and AhI*) and three LGs (AhXIII*, AhVII* and AhIV*) respectively. Only one QTL was found consistent across both the seasons of phenotyping of RIL-4 population. However, none of the QTL was consistent in RIL-5 population which was environment/season specific (Table 34).

4.3.4.5 O/L ratio:

QTL analysis for O/L ratio identified a total of 5 QTLs (QTL_{R4-O/L Ratio}01, QTL_{R4-O/L Ratio}02, QTL_{R4-O/L Ratio}03, QTL_{R4-O/L Ratio}04 and QTL_{R4-O/L Ratio}05) in RIL-4 (Table 33; Fig. 9) and 4 QTLs (QTL_{R5-O/L Ratio}01, QTL_{R5-O/L Ratio}02, QTL_{R5-O/L Ratio}03 and QTL_{R5-O/L Ratio}04) in case of RIL-5 population (Table 34; Fig. 10). The QTLs identified in RIL-4 population were distributed on 3 LGs (AhXIII*, AhI* and AhVI*) with a PVE ranging from 3.89-25.71%. Similarly the 4 QTLs identified in case of RIL-5 population had a PVE ranging from 7.76-14.00% and were distributed on AhVIII*, AhXIII* and AhVII* (Table 34).

Among the 5 QTLs identified in RIL-4 population only one QTL (QTL_{R4-O/L Ratio}01) was major and was consistently identified across two environments. In case of RIL-5 population of the 4 QTLs,

Table 33: Major and minor QTLs identified for oleic acid, linoleic acid and O/L ratio in TAG 24 × GPBD 4 (RIL-4) population

QTL	Linkage Group	Marker Interval	Position range	LOD range	Kharif 2008 R ² (%)	a ⁰	Summer 2009 R ² (%)	a ⁰
Oleic acid								
QTL_{R4-Oleic acid}01	AhXIII*	<i>ahFAD2A(CAPS)-FAD2A</i>	2.01	9.09-15.42	23.79	-1.42	14.34	-1.10
QTL_{R4-Oleic acid}02	AhVIII*	<i>Ah-4-101-TC9F10</i>	39.21-41.21	3.12-3.73	4.24	-0.59	4.89	-0.65
QTL_{R4-Oleic acid}03	AhVI*	<i>PM377-TC1A01</i>	0.01-6.01	3.79-4.50	6.06	-0.71	4.71	-0.64
QTL_{R4-Oleic acid}04	AhVIII*	<i>TC6H03-GM1760</i>	43.51	3.02-3.79	4.07	-0.57	5.50	-0.69
QTL_{R4-Oleic acid}05	AhVI*	<i>S108-GM1097</i>	9.61-10.71	2.68-5.28	6.88	-0.75	3.77	-0.57
QTL_{R4-Oleic acid}06	AhXIII*	<i>TC1D02-GM2259</i>	75.81	3.10	5.33	0.67		
QTL_{R4-Oleic acid}07	AhI*	<i>GM1531-GM2233</i>	36.31	2.93			4.41	-0.64
Linoleic acid								
QTL_{R4-Linoleic acid}01	AhXIII*	<i>ahFAD2A(CAPS)-FAD2A</i>	2.01	10.81-15.41	25.40	1.19	17.31	0.97
QTL_{R4-Linoleic acid}02	AhI*	<i>GM1531-GM2233</i>	36.31	5.07			8.34	0.80
O/L Ratio								
QTL_{R4-O/L Ratio}01	AhXIII*	<i>ahFAD2A(CAPS)-FAD2A</i>	2.01	12.51-15.93	25.71	-0.10	19.50	-0.09
QTL_{R4-O/L Ratio}02	AhI*	<i>GM1531-GM2233</i>	36.31	6.25			9.85	-0.07
QTL_{R4-O/L Ratio}03	AhI*	<i>GM1187-GM1565</i>	18.01	2.99			6.91	0.06
QTL_{R4-O/L Ratio}04	AhVI*	<i>PM377-TC1A01</i>	2.01	3.32			4.81	-0.04
QTL_{R4-O/L Ratio}05	AhVI*	<i>S108-GM1097</i>	9.61	2.79			3.89	-0.04

R² (%) = % Phenotypic variance explained, a⁰ = Additive effect, '-' positive alleles come from TAG 24, '+' positive alleles come from GPBD 4.

Table 34: Major and minor QTLs identified for oleic acid, linoleic acid and O/L ratio in TG 26 × GPBD 4 (RIL-5) population

QTL	Linkage Group	Marker Interval	Position range	LOD range	Summer 2007 R ² (%)	a ⁰	Kharif 2007 R ² (%)	a ⁰
Oleic acid								
QTL_{R5-Oleic acid}01	AhVIII*	GM2690-TC9F10	54.91	4.34	14.08	-3.18		
QTL_{R5-Oleic acid}02	AhVII*	GM2246-GM2589	19.11	4.33			11.48	-1.38
Linoleic acid								
QTL_{R5-Linoleic acid}01	AhXIII*	pPGSseq19B01-GM2407	144.61	4.59	11.92	2.02		
QTL_{R5-Linoleic acid}02	AhVII*	GM2246-GM2589	16.81	3.60			10.94	1.13
QTL_{R5-Linoleic acid}03	AhIV*	IPAHM282-PM36	56.31	2.98			7.26	1.05
O/L Ratio								
QTL_{R5-O/L Ratio}01	AhVIII*	GM2536-GM1986	6.01	3.69	14.00	0.22		
QTL_{R5-O/L Ratio}02	AhVIII*	GM2690-TC9F10	54.91	4.34	12.28	-0.19		
QTL_{R5-O/L Ratio}03	AhXIII*	pPGSseq19B01-GM2407	144.61	3.22	12.50	-0.19		
QTL_{R5-O/L Ratio}04	AhVII*	GM2246-GM2589	19.11	2.99			7.76	-0.10

R² (%) = % Phenotypic variance explained, a⁰ = Additive effect, '-' positive alleles come from TG 26, '+' positive alleles come from GPBD 4.

two QTLs were major (QTL_{R5-O/L Ratio}01 and QTL_{R5-O/L Ratio}02) and remaining two were minor QTLs. None of the QTLs from among the four were consistent for O/L ratio across environments.

4.4 Consensus QTL map for diseases, morphological, yield attributing and quality traits

As the consensus map has been developed based on RIL-4 and RIL-5 populations segregating for resistance to foliar diseases (LLS and rust), quality, morphological and yield attributing traits, the QTLs identified in RIL-4 and RIL-5 were placed on the respective position on the consensus map with the maximum number of common markers between the populations has significantly facilitated the comparison of QTLs across these two populations the details of the number of QTLs identified in each populations were reported and discussed earlier. However the Table 35 explains about the number of QTLs localized on each LG from both the populations and also about the total number of the QTLs localized on the consensus map. Further, linkage groups in the consensus map based on RIL-4 and RIL-5 was compared with recently published dense genetic consensus genetic map (Shirasawa *et al.*, 2013) in order to specify the genome specific LGs as compared to A and B genome (Table 35).

On the individual genetic map based on RIL-4 and RIL-5 population a total of 90 and 60 QTLs were localized on individual genetic map. All QTLs except QTL_{R5-SH}01 and QTL_{R5-Rust}04 could be placed at their respective positions on 20 LGs of the consensus map. However upon placing all these QTLs in their respective positions on the consensus map a total of 148 QTLs were localized on 20 LGs of the consensus map (Fig.12). From among the 148 QTLs identified a total of 54 QTLs were major (13 for LLS, 7 for rust, 6 for morphological traits, 13 for yield related traits and 14 for quality traits) and 95 QTLs localized were minor QTLs (15 for LLS, 6 for rust, 30 for morphological traits, 14 for yield related traits and 29 for quality traits). As listed above the present study reports a total of 28 QTLs for late leaf spot, 13 QTLs for rust, 36 QTLs for morphological characters, 28 QTLs for yield attributing traits and 43 QTLs for quality traits. The number of the QTLs placed on individual LGs ranged from one QTL (AhXI and AhXVI) to 24 QTLs (AhXV). However the LGs AhI, AhV, AhVI, AhVII, AhVIII, AhX, AhXII, AhXIII, AhXIV, AhXV and AhXVIII harbored maximum number of QTLs contributing across all the traits (Fig 12). Upon comparison it could be noticed that both the A and B genomes are important for groundnut crop improvement.

4.5 Validation of the linked markers to rust resistance

With an objective of validating the markers linked to rust resistance a germplasm comprising of 64 lines was phenotyped for rust at both the stages (SI-80 Days after sowing and SII-90 days after sowing) during Kharif 2007, 2008, 2009 and 2010. The germplasm exhibited wide range of disease incidence and genotypes were significantly differing for disease (Table 36). Based on the comparison of genotypic and phenotypic data a good congruence was observed between the pattern of the allele and their respective disease reaction (Table 36). However, there were some exceptions where in there was a difference in the allelic pattern exhibited by the genotypes with respect to GM 1536, GM 2301, GM 2079 and IPAHM 103. Though the genotypes namely D39d (Purple and small), VL2, VL1, ICGV 87264, ICGV 88256 and Dh 73 were resistant they exhibited the susceptible allelic pattern with respect to the markers GM 1536, GM 2301 and GM 2079 (Table 36) as compared to IPAHM 103 which had a resistant allelic pattern. Conversely all the four linked markers had a susceptible allelic form with respect to the genotypes NcAc 17090, Girnar mosaic, Girnar, MN 1-28 and ICGV 91173 which are resistant based on their phenotypic reaction across the years (Table 36). On the other hand resistant allelic form was observed for all these markers with the susceptible genotypes ICGV 86156 and JG thin shell which had a higher mean disease scores at both the stage. Another exception was observed with respect to IPAHM 103 which exhibited resistant allelic pattern with respect to VR 5 and ICGV 87921 which are susceptible to rust.

Further, with all these comparisons the genotyping and phenotyping data on the germplasm was subjected to single marker analysis (SMA). The analysis revealed a significant association with resistance as explained by the phenotypic variance presented in the Table 37. The highest PV was explained by IPAHM 103 ranging from 38.40-55.70 %. Similarly all other markers explained the same PV as they had the same allelic pattern and the PV ranged from 21.90 to 39.20 % (Table 37).

Nevertheless, in majority of the cases these markers differentiated successfully the resistant and susceptible genotypes/accessions indicating their potential to be used in marker assisted breeding program for rust resistance in groundnut.

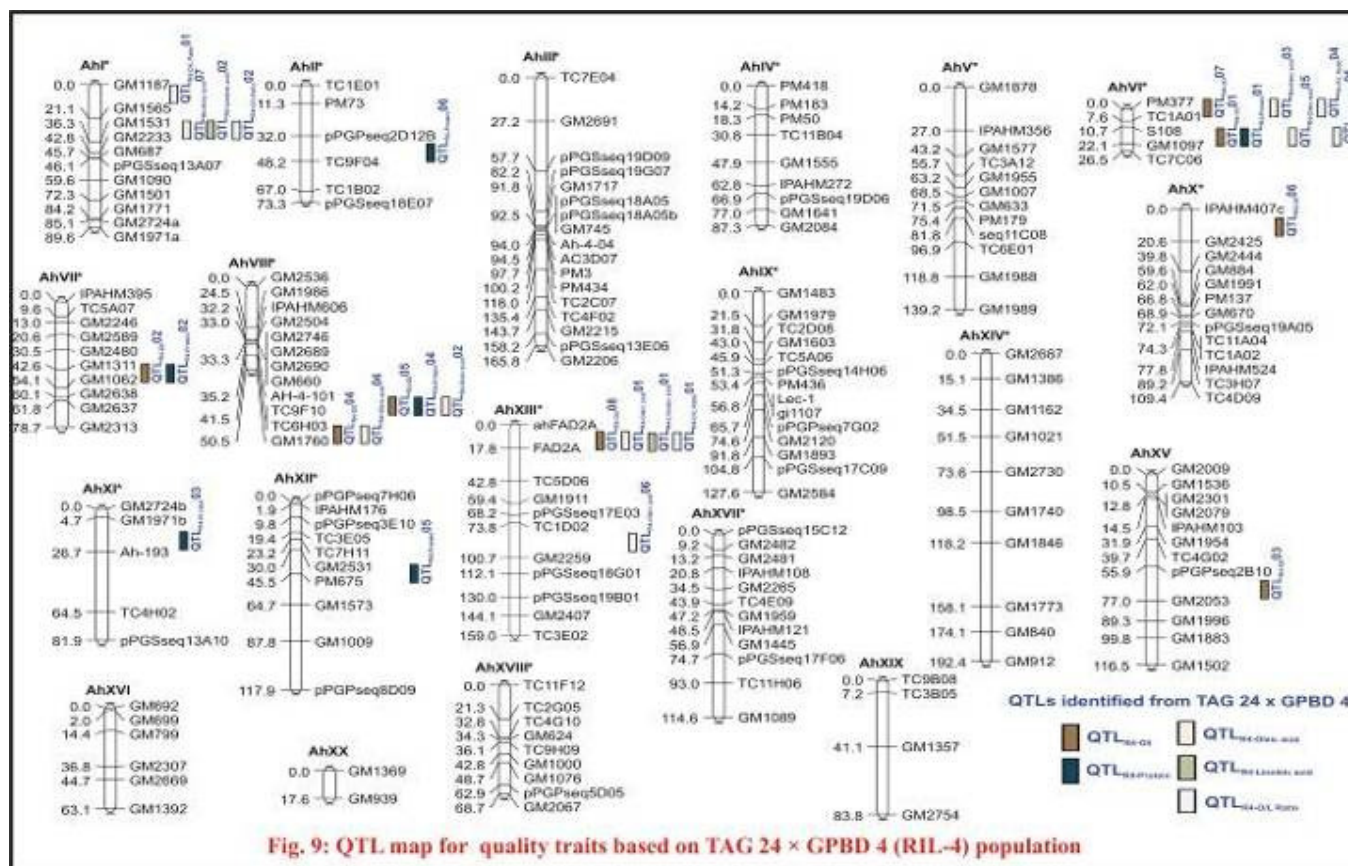


Fig 9: QTL map for quality traits based on TAG 24 × GPBD 4 (RIL-4) population

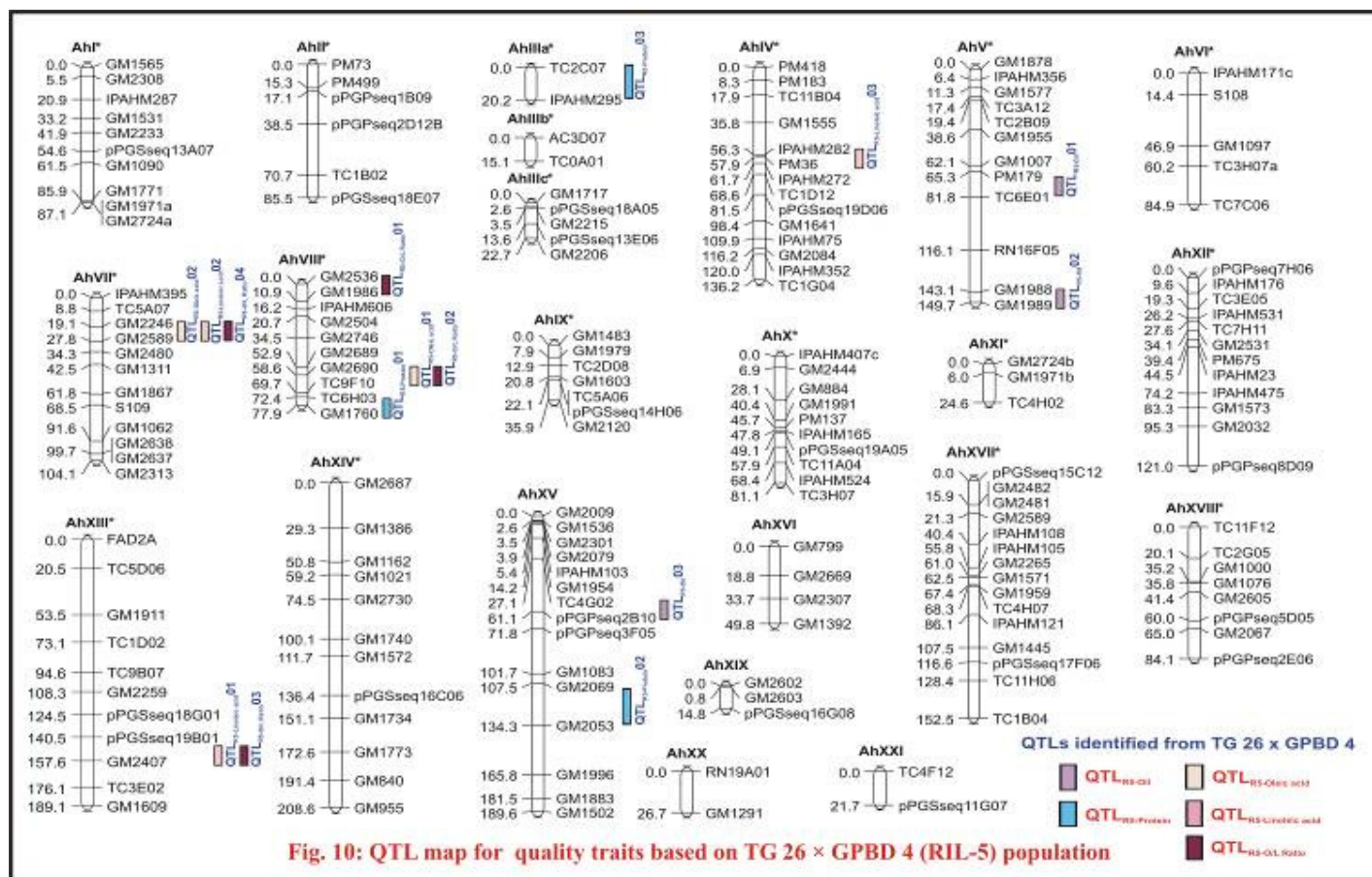


Fig 10: QTL map for quality traits based on TG 26 x GPBD 4 (RIL-5) population

Table 35: Details of consensus QTL map from RIL-4 and RIL-5 population placed on to consensus map and its comparison with the linkage groups of high density consensus map

Individual genetic maps		Consensus map based on RIL-4 and RIL-5 population	No of QTLs on individual LGs			Corresponding linkage group specifying A and B genome	Traits for which QTLs were identified	
RIL-4	RIL-5		RIL-4	RIL-5	Consensus map	(Shirasawa <i>et al.</i> , 2013)	RIL-4	RIL-5
AhXI*(5)	AhXI*(3)	AhXI*(5)	1	0	1	A01	Protein	-
AhI*(11)	AhI*(10)	AhI*(13)	6	3	9	B01	Rust, PBR, oleic acid, linoleic acid and O/Lratio	HSW, SH % and LLS
-	AhXXI(2)	-	0	0	0	A02	-	-
AhII*(6)	AhII*(6)	AhII*(8)	2	1	3	B02	Rust and protein	Plht
AhIII*(17)	AhIIIa,b,c*(9)	AhIII*(17)	1	3	4	A03	LWD	LLN, PY/P and linoleic acid
AhXV(12)/ AhXIV*(10)	AhXV(15)/ AhXIV*(12)	AhXV(15)/ AhXIV*(14)	16	14	30	B03	LLS, rust, PY/P, SH%, HSW and oil content	LLS, rust, PY/P, NOP/P, protein and oil content
AhVII*(10)	AhVII*(12)	AhVII*(12)	8	5	13	A04	LLS, Plht, PY/P, oil and protein content	LLS, PBR, oleic acid, linoleic acid and O/Lratio
AhXVII*(12)	AhXVII*(15)	AhXVII*(17)	0	2	2	B04	-	PY/P and HSW
AhV*(12)	AhV*(12)	AhV*(14)	9	9	18	A05	LLS, Plht, HSW and LWD	LLS, rust, LLN, Plht, NOP/P and oil content
AhIV*(9)	AhIV*(14)	AhIV*(15)	1	3	4	B05	LWD	LLN, PY/P and linoleic acid

* Linkage group assigned according to tetraploid reference map (Varshney *et al.* 2009a), Number in the parenthesis indicates the number of markers mapped in individual LGs

Contd...

Individual genetic maps		Consensus map based on RIL-4 and RIL-5 population	No of QTLs on individual LGs			Corresponding linkage group specifying A and B genome	Traits for which QTLs were identified	
RIL-4	RIL-5		RIL-4	RIL-5	Consensus map	(Shirasawa <i>et al.</i> , 2013)	RIL-4	RIL-5
AhVI*(5)	AhVI*(5)	AhVI*(7)	10	1	11	A06	LLS, LLN, LWD, oleic acid, O/L ratio, oil and protein content	HSW
AhX*(13)	AhX*(10)	AhX*(14)	6	3	9	B06	Plht, LLN and oil content	LLS and Plht
-	-	-	-	-	-	A07	-	-
AhXVIII*(9)	AhXVIII*(8)	AhXVIII*(11)	7	0	7	B07	LLS, rust, Plht, PBR and NOP/P	-
AhXIX(4)	-	AhXIX(4)	-	-	-	A08	-	-
AhVIII*(12)	AhVIII*(10)	AhVIII*(12)	8	7	15	B08	LLS, PBR, oleic acid, oil and protein content	LLS, oleic acid, O/L ratio and protein content
AhXIII*(11)	AhXIII*(10)	AhXIII*(13)	6	4	10	A09	LLS, oleic acid, linoleic acid, O/L ratio and oil content	LLS, O/L ratio and linoleic acid
AhIX*(14)	AhIX*(7)	AhIX*(14)	2	0	2	B09	LLS and LLN	-
AhXVI(6)	AhXVI(4)	AhXVI(6)	1	0	1	A10	Rust	-
AhXII*(10)	AhXII*(12)	AhXII*(14)	7	0	7	B10	LLS, Plht, PY/P and protein content	-
Total			90	59	149			

* Linkage group assigned according to tetraploid reference map (Varshney *et al.* 2009a), Number in the parenthesis indicates the number of markers mapped in individual LGs

Table 36: Mean disease scores across 4 environments and genotyping data for the rust resistance linked markers in the germplasm

Sl.No	Genotypes	Linked Markers				Mean Disease score over 4 environments	
		GM 1536	GM 2301	GM 2079	IPAHM 103	Rust-SI	Rust-SII
1	ICGV 86155	A	A	A	A	6	8
2	ICGV 86590	A	A	A	A	4	5
3	JLVR-1	A	A	A	A	5	7
4	JLVR 2	A	A	A	A	5	7
5	Dh 43	A	A	A	A	6	7
6	CHITRA	A	A	A	A	6	8
7	VB 9	A	A	A	A	6	7
8	NC Ac 343	A	A	A	A	6	8
9	KRG 1	A	A	A	A	7	8
10	M 28-2	A	A	A	A	6	7
11	R 9227	A	A	A	A	4	6
12	ICGV 86031	A	A	A	A	6	7
13	R 8808	A	A	A	A	6	8
14	R 9214	A	A	A	A	6	8
15	Dh 40 (Red seed)	A	A	A	A	6	8
16	Dh 40 (Tan)	A	A	A	A	7	8
17	ICGV 91180	A	A	A	A	5	6
18	K 134	A	A	A	A	6	8
19	Mutant 110	A	A	A	A	6	7
20	Mutant 45	A	A	A	A	6	7
21	TMV 2	A	A	A	A	7	8
22	JL 24	A	A	A	A	7	8
23	Dh 8	A	A	A	A	6	8
24	TAG 24	A	A	A	A	6	7
25	R 9251	A	A	A	A	7	8
26	ICGV 99004	A	A	A	A	5	7
27	ICGV 86158	A	A	A	A	7	8
28	ICGV 87378	A	A	A	A	6	8
29	TG 41	A	A	A	A	7	9
30	VR 5	A	A	A	B	5	7
31	ICGV 87921	A	A	A	B	5	7
32	NC Ac 17090	A	A	A	*	3	5
33	S 230	A	A	A	*	5	7
34	GIRNAR MOSAIC	A	A	A	A	3	3
35	GIRNAR	A	A	A	A	3	4
36	MN 1-28	A	A	A	A	4	5
37	ICGV 91173	A	A	A	*	3	3

‘A’ represent Susceptible reaction

‘B’ represent Resistant reaction

* Not amplified

Cont...

Sl. No	Genotypes	Linked Markers				Mean Disease score over 4 environments	
		GM 1536	GM 2301	GM 2079	IPAHM 103	Rust-SI	Rust-SII
38	ICGV 93008	B	B	B	B	3	4
39	D 39d(Purple & large)	B	B	B	B	3	4
40	B 37c	B	B	B	B	3	4
41	ICGV 90266	B	B	B	B	2	3
42	R 8972	B	B	B	B	3	4
43	ICGV 92188	B	B	B	B	3	3
44	ICGV 93023	B	B	B	B	3	3
45	A 30b	B	B	B	B	3	4
46	ICGV 91177	B	B	B	B	2	3
47	ICGV 86156	B	B	B	B	7	8
48	MG 8	B	B	B	B	4	5
49	IFDRV 11	B	B	B	B	3	4
50	GBFDS 272	B	B	B	B	3	3
51	JG Thin shell	B	B	B	B	4	6
52	ICG 99005	B	B	B	B	3	3
53	ICGV 87165	B	B	B	*	2	3
54	D 39d(GPBD 4)	B	B	B	*	3	4
55	ICGV 86699	B	B	B	*	2	3
56	VB 8	B	B	B	*	4	4
57	ICGV 99003	B	B	B	*	3	4
58	D 39d (Purple & small)	A	A	A	B	3	4
59	VL 2	A	A	A	B	3	4
60	VL 1	A	A	A	B	3	4
61	ICGV 87807	A	A	A	B	3	4
62	ICGV 87264	A	A	A	B	3	4
63	ICGV 88256	A	A	A	B	3	4
64	Dh 73	A	A	A	B	3	4

‘A’ represent Susceptible reaction

‘B’ represent Resistant reaction

* Not amplified

Table 37: Single marker analysis for rust at different stages in germplasm comprising resistant and susceptible accessions

Sl.No.	Linked markers /Stages	Band size		Status	Phenotypic variance explained (R^2_{adj}) by single marker analysis							
		A pattern	B pattern		Kharif 2007		Kharif 2008		Kharif 2009		Kharif 2010	
					Rust-SI	Rust-SII	Rust-SI	Rust-SII	Rust-SI	Rust-SII	Rust-SI	Rust-SII
1	IPAHM 103	140 bp	150 bp	Linked	54.90	54.90	38.40	45.40	47.20	55.70	46.20	46.70
2	GM 1536	486 bp	480 bp	Linked	28.60	28.90	21.90	23.80	32.60	39.20	37.60	34.70
3	GM 2301	136 bp	127 bp	Linked	28.60	28.90	21.90	23.80	32.60	39.20	37.60	34.70
4	GM 2079	409 bp	403 bp	Linked	28.60	28.90	21.90	23.80	32.60	39.20	37.60	34.70

A - Represent susceptible reaction

B - Represent resistant reaction

DISCUSSION

The prerequisite to initiate any molecular breeding program is the availability of sufficient genomic resources in terms of appropriate molecular markers, dense genetic maps, tightly linked markers or significant QTLs contributing for the trait of interest. With this view, in groundnut several studies have been reported earlier in establishing the genetic relationship between the cultivars/accessions using RFLPs, RAPDs, AFLPs and SSRs (Kochert *et al.*, 1991, Halward *et al.*, 1992, Paik-Ro *et al.*, 1992, and Stalker *et al.*, 1994). Nevertheless, with the concerted effort made by several research groups worldwide working on groundnut has enabled development of large set of SSR markers (Hopkins *et al.*, 1999; He *et al.*, 2003; Ferguson *et al.*, 2004; Moretzsohn *et al.*, 2005; Mace *et al.*, 2007; Cuc *et al.*, 2008; Liang *et al.*, 2009c; Yuan *et al.*, 2010) which are now available in the public domain. As a result, linkage mapping and QTL detection had been possible in cultivated groundnut (Varshney *et al.*, 2009a; Khedikar *et al.*, 2010; Hong *et al.*, 2010; Ravi *et al.*, 2011; Sarvamangala *et al.*, 2011) and using synthetic amphidiploids genetic mapping was done in BC₁F₁ population (Foncéka *et al.*, 2009). Though, genetic maps and QTL studies have been initiated in groundnut, they are still in infancy due to narrow genetic base, lack of sufficient polymorphic markers and have forced to develop partial genetic maps or maps with low marker density (Khedikar *et al.*, 2010; Sarvamangala *et al.*, 2011; Hong *et al.*, 2010). However, QTL mapping studies has been reported very recently with the development of partial linkage maps based on RIL-4 and RIL-5 population for foliar diseases (Khedikar *et al.*, 2010) and quality traits (Sarvamangala *et al.*, 2011) respectively. Therefore the present study was conducted to saturate these partial genetic maps with the new set of SSR markers available. Further, to determine the location and effects of QTLs for LLS, rust, yield and quality traits along with a consensus map which will assist groundnut molecular breeding.

5.1 Development of genetic maps

5.1.1 Marker polymorphism and segregation distortion

Though a variety of marker systems had been utilized in estimating DNA polymorphism, groundnut being an allopolyploid crop species exhibited low level of polymorphism. And this is a biggest impediment in the construction of a good skeletal / dense framework map in cultivated groundnut (Moretzsohn *et al.*, 2004, 2005; Ferguson *et al.*, 2004). Moreover, the low polymorphism in cultivated groundnut is attributed to its recent origin by a single event of polyploidization and further isolation from wild relatives (Halward *et al.*, 1991). However SSR markers due to their multi-allelic nature showed a relatively enhanced polymorphism in cultivated groundnut (He *et al.*, 2003; Mace *et al.*, 2006; Hong *et al.*, 2010; Varshney *et al.*, 2009a).

In the present study using the available resources, a total of 3,101 and 3,282 markers were screened on parental genotypes of RIL-4 and RIL-5 populations respectively. Along with the SSR markers two CAPS and two allele specific markers were also included in the Panel of markers to differentiate *FAD2A* and *FAD2B* alleles. A very low level of polymorphism was observed (RIL-4: 6.71% and RIL-5: 6.27%) between the parents (TAG 24, TG 26 and GPBD 4) of respective mapping population. As expected low level of polymorphism is observed in groundnut because of its narrow genetic base and majority of SSRs screened in this study were from ESTs which are from highly conserved genic regions (Varshney *et al.*, 2005). Similar results of low level of polymorphisms were also reported in earlier mapping studies (Moretzsohn *et al.*, 2004, 2005; Ferguson *et al.*, 2004; Varshney *et al.*, 2009a).

Of the 208 and 206 markers for which genotyping data were obtained, 44 (20%) and 53 (28%) markers showed segregation distortion in RIL-4 and RIL-5 population, respectively. Percent of distorted markers in the present study was relatively less as compared to earlier mapping studies by Burow *et al.*, 2001 (68%), Moretzsohn *et al.*, 2005 (51%) and Varshney *et al.*, 2009a (35%), Qin *et al.*, 2012 (26% and 28% in two populations) and Shirasawa *et al.*, 2012b (28 and 8%), 2013 (65%, 31% and 19%). Generally, mapping populations developed from highly diverse genotypes with less genome similarity (*e.g.*, cultivated × wild/synthetic genotypes) will reveal higher segregation distortion. In addition, varying degree of segregation distortion may be due to biological factors like chromosomal rearrangement, chromosome loss (Kasha *et al.*, 1970), genetic isolation mechanism (Zamir and Tadmor, 1986), and non biological factors like scoring error and sampling errors (Devey *et al.*, 1994; Echt *et al.*, 1997).

It is a known fact that distorted markers should not be considered for construction of framework/reference map as they may affect the estimation of map distances and the order of marker loci. However, these markers may be given their most possible location and order in the map. In the present study, since the number of the polymorphic markers was limited, the distorted markers were also used for construction of genetic maps and these markers were placed in most appropriate location and order using advanced function of mapping programs (Appendix I and II).

As observed in the earlier studies (Varshney *et al.*, 2009a, Ravi *et al.*, 2011) for amplification of more than two loci for a marker. And in the present study, amplification of more than two loci was observed for three markers (GM1971, GM2724 and pGSseq18A05) in case of RIL-4 and four markers (GM1971, GM2724, GM2589 and TC3H07) in RIL-5 population. The multi allelic loci could be attributed to amplification of duplicated locus or a different locus due to the allotetraploid/polyploidy nature of cultivated groundnut genome. Thus, the study clearly indicates that it is important and enviable to achieve higher number of marker polymorphism by making use of suitable marker system and selecting genetically diverse parents for developing mapping population.

5.1.2 Development of improved genetic maps

The primary rationale of developing genetic maps, in any of the crop species is for targeting the candidate genomic regions contributing towards the trait of interest and to critically define the genomic regions for its appropriate use in breeding programs. And the present study presents two genetic maps based on RIL-4 [1963.40 cM ; 190 loci mapped on 20 LGs; Table 20; Fig. 2] and RIL-5 [1982.90 cM; 182 loci mapped on 21 LGs; Table 20; Fig. 3] population. As expected, the map coverage was much higher than the diploid maps developed by Moretzsohn *et al.*, 2005 (1,230.89 cM, AA genome) and Moretzsohn *et al.*, 2009 (1,294.0 cM, BB genome). As compared to the partial genetic maps developed earlier using RIL-4 population by Khedikar *et al.*, 2010 (462.24 cM; 56 loci mapped on 14 LGs) and RIL-5 population by Sarvamangala *et al.*, 2011 (657.90 cM; 45 loci mapped on 8 LGs) the new genetic maps developed were dense with 190 and 182 marker loci, respectively. Nevertheless the tetraploid genetic maps are of direct use for the genetic improvement of cultivated groundnut and the present maps from this study with higher marker density was comparable to the earlier maps developed by Varshney *et al.*, 2009a (1,270.5 cM; 135 loci mapped on 22 LGs); Ravi *et al.*, 2011 (1785.4 cM; 191 loci mapped on 22 LGs) and also as good as with the recently developed genetic maps based on recombinant inbred lines (Table 38). Further, it was also comparable with genetic map developed based on backcross population using a synthetic amphidiploid by Foncéca *et al.*, 2009 (1,843.7 cM; 298 loci mapped on 21 LGs).

5.1.3 Construction of consensus map

The core objective of constructing the consensus map with several individual genetic linkage maps available is to place as many as informative (SSRs) markers onto a single map with a universal marker order that could be used as a reference for more-precise genetic studies for disease resistance and other traits (Khlestkina *et al.*, 2009; Marcel *et al.*, 2007; Danan *et al.*, 2011). Overall, the consensus map facilitates, the selection of markers along the length of the chromosome which can be used for detecting recombinant individuals, fixing loci, recovering the recurrent parent genetic background, assembling complex genotypes in complex crosses (Somers *et al.*, 2004; Mace *et al.*, 2009; Hong *et al.*, 2010 and Danan *et al.*, 2011). Consensus map could provide an excellent platform for further QTL mapping and its application in the marker assisted breeding programs for disease resistance especially for LLS and rust. A total of 144 markers were common between the two populations. Based on these common markers between the LGs of RIL-4 and RIL-5 genetic maps, an attempt was made to develop a consensus map using RIL-4 as a framework map. Unique loci of the genetic map developed based on RIL-5 population were added onto a fixed backbone of RIL-4 population following the “neighbors” map approach described by Cone *et al.* (2002). The consensus map had 1198.76 cM genome coverage with 227 SSR loci mapped onto 20 LGs of which 18 LGs were derived from the integration of LGs from the two (RIL-4 and RIL-5) genetic maps (Table 20 and 21; Fig. 4). Similarly, the aberrant status of genetic mapping prevailing in groundnut has made the researchers to develop consensus genetic map and now few consensus maps are available in groundnut (Hong *et al.*, 2010, Qin *et al.*, 2012 and Gautami *et al.*, 2012a).

Consensus map developed based on RIL-4 and RIL-5 population was comparable with earlier genetic maps developed (Varshney *et al.*, 2009a; 1,270.5 cM; 165 loci mapped on 22 LGs and Ravi *et al.*, 2011; 1785.4 cM; 191 loci mapped on 22 LGs) in terms of marker density and genome coverage. It was also equivalent with the recently developed integrated maps either based on two (Qin *et al.*, 2012) or three (Hong *et al.* 2010; Gautami *et al.* 2012a) mapping populations (Table 38). In turn dense

genetic maps based on trait specific mapping populations will precisely define the genomic regions contributing for the traits concerned. Furthermore, generalized use of increasingly larger sets of transferable markers and consensus mapping information will allow faster and more detailed investigations of QTL syntenies among species, validation of QTLs across variable genetic backgrounds and positioning of a large number of candidate genes to be tested in mapping experiments.

Further, along with other genetic maps reported earlier the genetic maps developed from the present study have been employed in developing dense consensus genetic maps by Gautami *et al.* (2012b) and Shirasawa *et al.* (2013). For the consensus map developed by Gautami *et al.* (2012b) a total of 176 and 168 marker loci were considered from RIL-4 and RIL-5 population, respectively. Similarly genotyping data for a set of 171 and 162 marker loci were considered from RIL-4 and RIL-5 populations for the consensus map with 3693 loci reported by Shirasawa *et al.* (2013). A detailed comparison was made in assigning the LGs corresponding to A and B genome while developing the dense genetic maps [Gautami *et al.* (2012b) and Shirasawa *et al.* (2013)]. Consecutively the comparison of two individual genetic maps and a consensus map developed from this present study had a good analogy with 18 LGs of dense genetic maps. And the LGs can specify whether a genomic region is contributed from A or B genome for a specific trait in cultivated groundnut (Table 39).

As an comparison for the position and order of markers on the genetic map developed in the present study, a good congruence for majority of the mapped loci was observed between the maps as well as with the reference genetic map based on TAG 24 × ICGV 86031 (Varshney *et al.*, 2009a; Ravi *et al.*, 2011). However, exceptions for few mapped loci were also found with respect to the position and order of the markers, which are commonly observed in polyploids (Somers *et al.*, 2004; Danan *et al.*, 2011). In general, discrepancy in the order and distance between the markers within linkage maps may be due to genome complexity of the crop, genotyping errors and missing data on the populations (Hackett, 2002). The small linkage groups observed in this study could be artificial and additional genetic markers can improve the linkage analysis for increasing the density of markers in the future. It is possible that missing or poor quality data and different algorithms being applied for developing the individual and consensus genetic maps lead to huge recombination gaps at terminal regions resulting in weak linkage in LGs of genetic maps (Gustafson *et al.*, 2009). Still extensive efforts were made worldwide for the development of genomic resources and for the past five years it has enabled an access to ~ 7,000 SSRs. With the available genomic resources development of dense genetic maps based on single mapping population are still in progress (Pandey *et al.*, 2012).

5.2 QTL analysis

5.2.1 Phenotyping

Accurate phenotyping of traits is one of the principal factors in QTL detection, since resistance to LLS and rust is complex with several components of resistance (Nevill 1982; Green and Wynne 1986; Dwivedi *et al.*, 2002). In addition to the phenotyping done earlier (During 2004, 2005, 2006, 2007) three more years of phenotyping was undertaken on diseases during Kharif season of 2008, 2009 and 2010. For morphological and yield attributing traits phenotyping was carried out during Kharif 2007, 2008 and 2010. With this enormous data generated on both the populations, critical analysis for components of variability has been done and was used in order to identify microsatellite markers/QTLs associated with foliar disease resistance and productivity traits. However, data on nutritional quality was obtained from the earlier studies by Sarvamangala *et al.* (2009) and Mukri (2011) on RIL-5 and RIL-4 populations respectively for QTL detection.

5.2.1.1 Phenotypic evaluation for LLS and rust

To improve the accuracy of QTL mapping by reducing background noise, utmost importance has to be given for the accuracy of phenotypic evaluation (Haley and Andersson, 1997). Then only reliable candidate QTLs can be identified from a reliable phenotypic data. However this will further help in potential use of the candidate QTLs and tightly linked markers for marker-assisted selection (MAS). Therefore, through the present study an attempt was made for extensive phenotyping of both the populations for LLS and rust. Analysis of variance revealed significant variation (Table 3 and 4) among the RILs, seasons and also significant G × E interaction for all the traits suggesting the need to screen in multiple seasons/locations (Table 7 and 8). Both the susceptible parents (TAG 24 and TG 26) exhibited higher incidence of diseases in all the environments as compared to the resistant parent GPBD 4. Mean values of RILs for LLS and rust in both the population was slightly inclined towards the susceptible parents the range of RILs were within the limit of mean disease scores exhibited by the

parents. However in some season/environments the disease scores were beyond the range as compared to resistant and susceptible parents for the diseases. The genetic estimates based on both the mapping populations exhibited considerable variability for resistance to LLS and rust. The magnitude of variability was moderate to high as revealed by PCV and GCV accompanied with moderate to high heritability in broad sense (Table 11, 12 and Fig. 11 to 14). Highest estimates of PCV and GCV values for these results are also reported by John *et al.* (2006), Khote *et al.* (2009) for LLS and rust disease score at 70 DAS. Shoba *et al.* (2009) reported moderate PCV and GCV values for LLS disease score at 90 and 102 DAS and similar findings were also reported by Sawargaonkar *et al.* (2010). High GAM coupled with moderate to high heritability indicated a better scope for selection while breeding for resistance to LLS and rust.

5.2.1.2 Phenotypic evaluation for morphological and yield related traits

Analysis of variance revealed significant variability (Table 5 and 6) among the RILs, seasons and also significant $G \times E$ interaction for all the traits suggesting the need to screen in multiple seasons/locations. Similar results were also obtained by Khedikar (2008), Sarvamangala (2009) and Azharudheen (2010) in segregating generations of groundnut population.

The magnitude of variability in both the populations as revealed by PCV was moderate to high for all the traits except for shelling percent. But lower heritability and GAM indicated lower heritable variation for most of these traits except plant height, pod yield per plant and 100-seed weight, which recorded moderate to high values (Table 13, 14 and Fig. 15 to 18). Similar results were obtained by John *et al.* (2008), Ladole *et al.* (2009) and Shinde *et al.* (2010). In the present investigation, low magnitude of GCV and PCV indicates the limited scope of selection for these traits. As compared to season wise estimates, pooled analysis resulted in lower heritable variability thus revealing the predominance of $G \times E$ interaction. Hence, improvement through conventional breeding will be more complex and molecular markers are likely to improve the effectiveness of selection.

Correlation coefficients measures the mutual relationship between various characters, which help in devising efficient strategies for indirect selection using component character and simultaneous selection of multiple traits. Correlations among the yield attributing traits in both the populations were found to be positively associated and morphological traits were also positively associated with yield related traits. Thus, indicating the importance of component traits for enhancing the productivity in the populations (Table 17 and 18). Based on the earlier reports, pod yield possessed significant positive association with kernel yield, number of pods per plant and test weight at both genotypic and phenotypic levels (Sah *et al.*, 2000, Laksmidevamma *et al.*, 2004; Upadhyaya and Nigam, 1998). However significant negative association was found for all the traits with LLS and rust in both the populations (Table 17 and 18), indicating the impact of disease on yield and quality of the produce.

5.2.2 Identification of QTLs

5.2.2.1 QTLs for Late leaf spot

There have been tremendous advances in the mapping of genes controlling quantitative traits through analysis of genomic data and quantitative trait loci (QTL) mapping. Such, attempts in dissecting the complex traits should lead to the identification of candidate genetic factors accountable for the heritable variation of the traits under study. Furthermore, once these genetic factors (QTLs) are mapped, they can be potentially used in the improvement of the traits using molecular markers. As a consequence, the accumulation of favorable alleles will provide genetic material for the development of new improved varieties. Though several genetic maps have been constructed in groundnut, there has been only one study based on RIL population (Khedikar *et al.*, 2010) to identify QTLs governing resistance to biotic stresses and reported a major QTL for rust and few minor QTLs for LLS based on the RIL-4 population. In this study, with the addition of more markers and QTL analysis based on multiple season phenotypic data for LLS on both RIL-4 and RIL-5 population, revealed a total of 13 and 15 QTLs for LLS respectively (Table 23 and 24; Fig. 5 and 6). Among the 13 QTLs, five were found to be major QTLs (QTL_{R4-LLS}01, QTL_{R4-LLS}02, QTL_{R4-LLS}03, QTL_{R4-LLS}04 and QTL_{R4-LLS}05) contributing for LLS with PVE ranging from 10.27-67.98% in RIL-4 population. Similarly in RIL-5 population, eight major QTLs were reported with a range of 7.58-63.13% PVE for LLS resistance. Through the present investigation, the addition of more SSR markers and development of dense genetic maps has facilitated in defining the proper genomic regions for disease resistance (Leal-Bertioli *et al.*, 2009) and to identify major QTLs for the first time in cultivated groundnut contributing for LLS.

Table 38: Comparison of genetic maps based on recombinant inbred lines in groundnut

Sl. No.	Population	Number of linkage groups	Number of mapped loci	Genome Coverage (cM)	References
Individual genetic maps					
1	TAG 24 × ICGV 86031	22	191	1785.4	Varshney <i>et al.</i> , 2009a; Ravi <i>et al.</i> , 2011
2	Yueyou 13 × Zhen Zhuhei	19	132	684.9	Hong <i>et al.</i> , 2010
3	Yueyou 13 × FU 95-5	21	109	540.69	Hong <i>et al.</i> , 2010
4	Yueyou 13 × J 11	13	46	401.7	Hong <i>et al.</i> , 2010
5	TAG 24 × GPBD4 (RIL-4)	14	56	462.24	Khedikar <i>et al.</i> , 2010
6	TG 26 × GPBD 4 (RIL-5)	8	45	657.90	Sarvamangala <i>et al.</i> , 2011
7	SunOleic 97R × NC 94022	22	172	920.7	Qin <i>et al.</i> , 2012
8	Tifrunner × GT-C20	26	239	1213.4	Qin <i>et al.</i> , 2012
9	ICGS 44 × ICGS 76	15	82	831.4	Gautami <i>et al.</i> , 2012a
10	ICGS 76 × CSMG 84-1	20	119	2208.2	Gautami <i>et al.</i> , 2012a
	Improved genetic maps based on (RIL-4)	20	190	1963.4	Genetic map from this study (Sujay <i>et al.</i> , 2012)
	Improved genetic maps based on (RIL-5)	21	182	1982.9	Genetic map from this study (Sujay <i>et al.</i> , 2012)
Consensus map					
1	Three RIL populations	20	175	885.4	Hong <i>et al.</i> , 2010
2	Two RIL populations	21	324	1352.1	Qin <i>et al.</i> , 2012
3	Three RIL populations	20	293	2840.8	Gautami <i>et al.</i> , 2012a
	Consensus map based on RIL-4 and RIL-5 from present study	20	227	1198.76	Genetic map from this study (Sujay <i>et al.</i> , 2012)

Table 39: Comparison of individual genetic maps and consensus maps with reference genetic maps of groundnut

TAG 24 × GPBD 4 (RIL-4)	TG 26 × GPBD 4 (RIL-5)	Consensus map based on RIL-4 and RIL-5 population	Reference genetic map (Varshney <i>et al.</i> , 2009a; Ravi <i>et al.</i> , 2012)	Corresponding linkage group of A and B genome based on consensus maps	
				Consensus map (Gautami <i>et al.</i> , 2012b)	Consensus map (Shirasawa <i>et al.</i> , 2013)
AhXI*(5)	AhXI*(3)	AhXI* (5)	LG_AhXI/LG_AhXV	a01	A01
AhI* (11)	AhI* (10)	AhI* (13)	LG_AhI	b01	B01
-	AhXXI (2)	-	LG_AhXX	a02	A02
AhII* (6)	AhII* (6)	AhII* (8)	LG_AhII	b02	B02
AhIII* (17)	AhIIIa,b,c* (9)	AhIII* (17)	LG_AhIII	a03	A03
AhXV (12)/ AhXIV* (10)	AhXV (15)/ AhXIV* (12)	AhXV (15)/ AhXIV* (14)	LG_AhXIV	b03	B03
AhVII* (10)	AhVII* (12)	AhVII* (12)	LG_AhVII	a04	A04
AhXVII* (12)	AhXVII* (15)	AhXVII* (17)	-	b04	B04
AhV* (12)	AhV* (12)	AhV* (14)	LG_AhV	a05	A05
AhIV* (9)	AhIV* (14)	AhIV* (15)	LG_AhIV	b05	B05
AhVI* (5)	AhVI* (5)	AhVI* (7)	LG_AhVI	a06	A06
AhX* (13)	AhX* (10)	AhX* (14)	LG_AhX	b06	B06
-	-	-	LG_AhXVI	a07	A07
AhXVIII* (9)	AhXVIII* (8)	AhXVIII* (11)	LG_AhXVIII	b07	B07
AhXIX (4)	-	AhXIX (4)	-	a08	A08
AhVIII* (12)	AhVIII* (10)	AhVIII* (12)	LG_AhVIII	b08	B08
AhXIII* (11)	AhXIII* (10)	AhXIII* (13)	LG_AhXIII	a09	A09
AhIX* (14)	AhIX* (7)	AhIX* (14)	LG_AhIX	b09	B09
AhXVI (6)	AhXVI (4)	AhXVI (6)	LG_AhXII	a10	A10
AhXII* (10)	AhXII* (12)	AhXII* (14)	-	b10	B10

* Linkage group assigned according to tetraploid reference map (Varshney *et al.* 2009a), Number in the parenthesis indicates the number of markers mapped in individual LGs

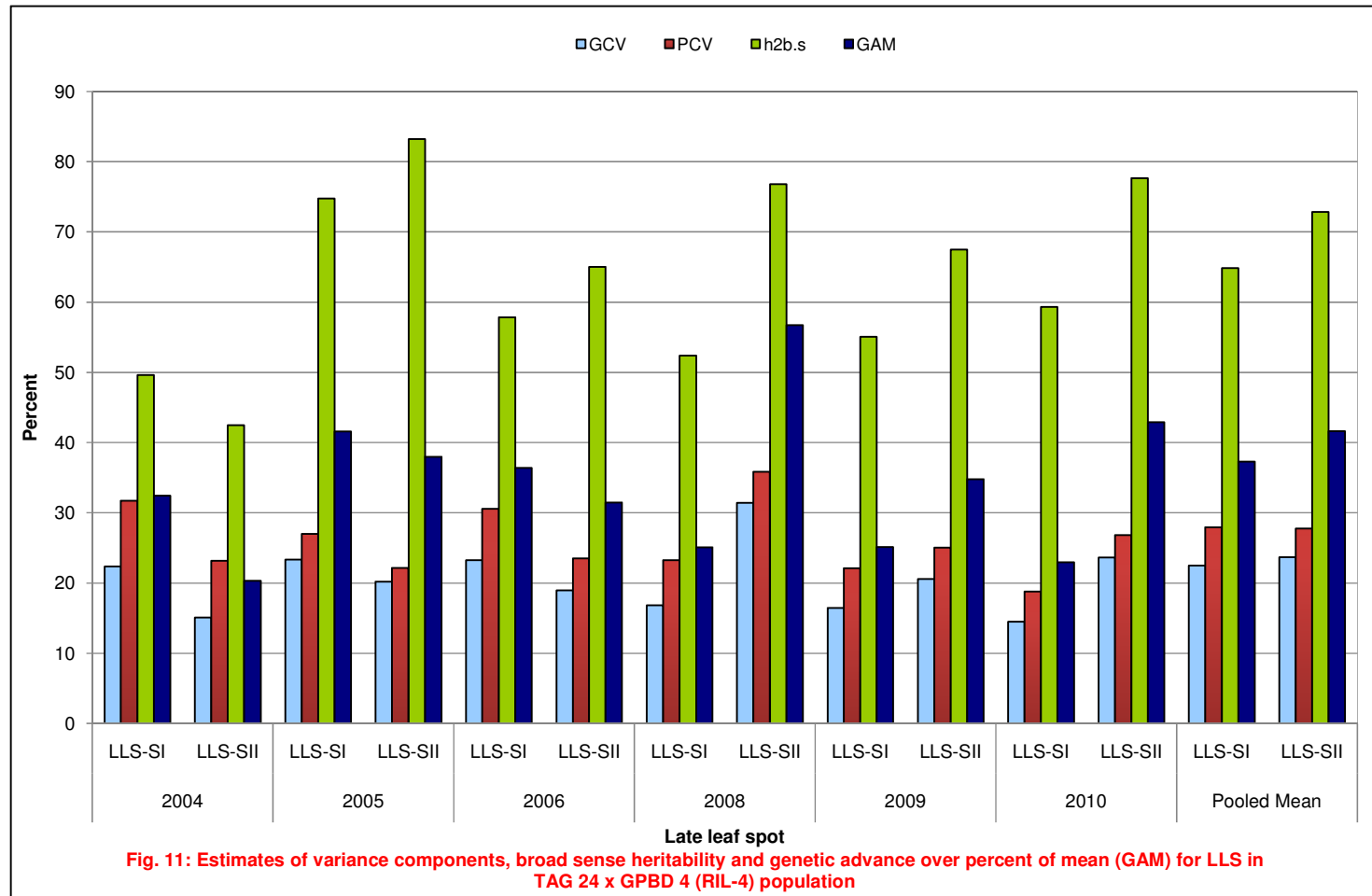


Fig 11: Estimates of variance components, broad sense heritability and genetic advance over percent of mean (GAM) for LLS in TAG 24 x GPBD 4 (RIL-4) population

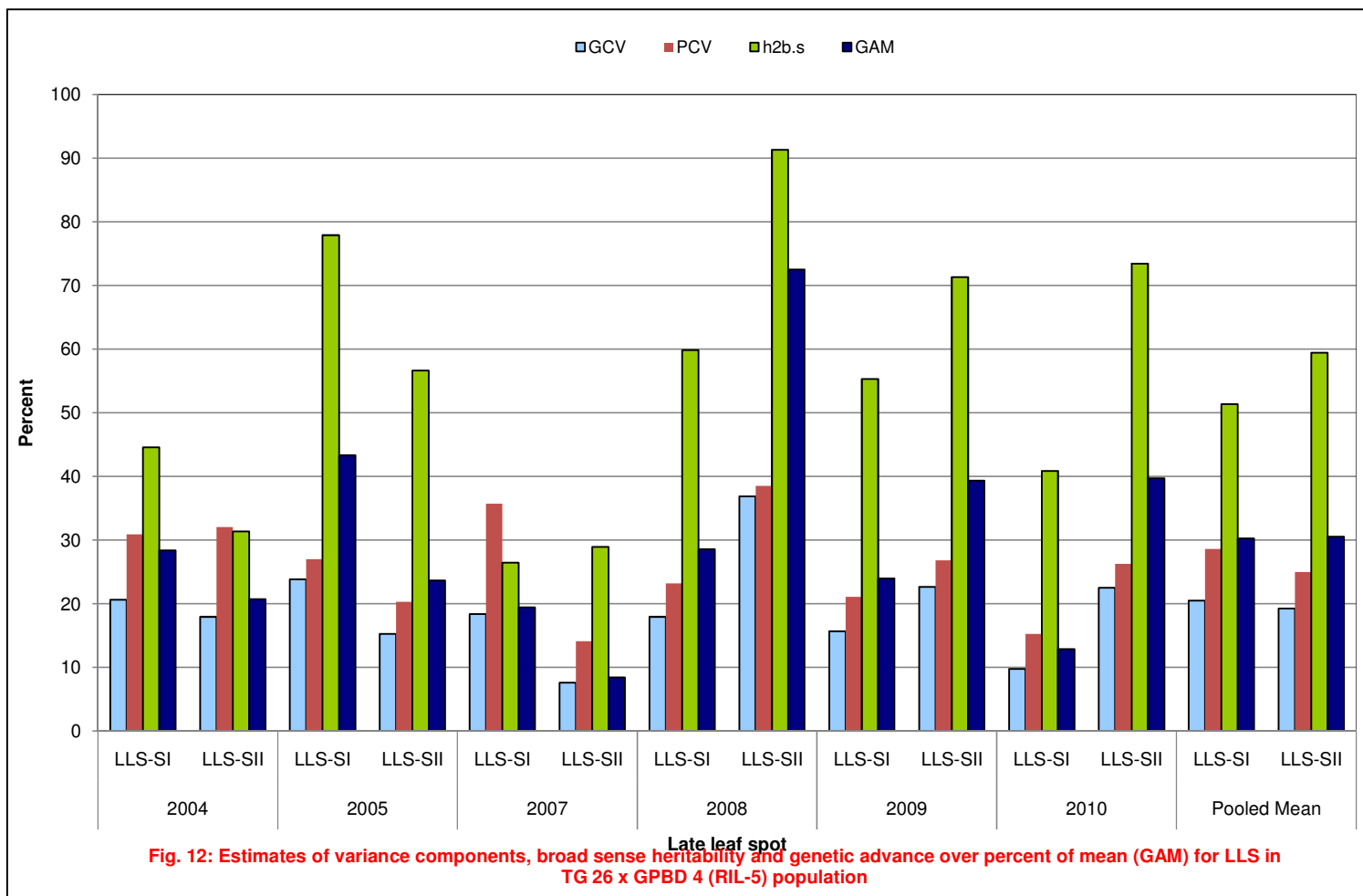


Fig 12: Estimates of variance components, broad sense heritability and genetic advance over percent of mean (GAM) for LLS in TG 26 x GPBD 4 (RIL-5) population

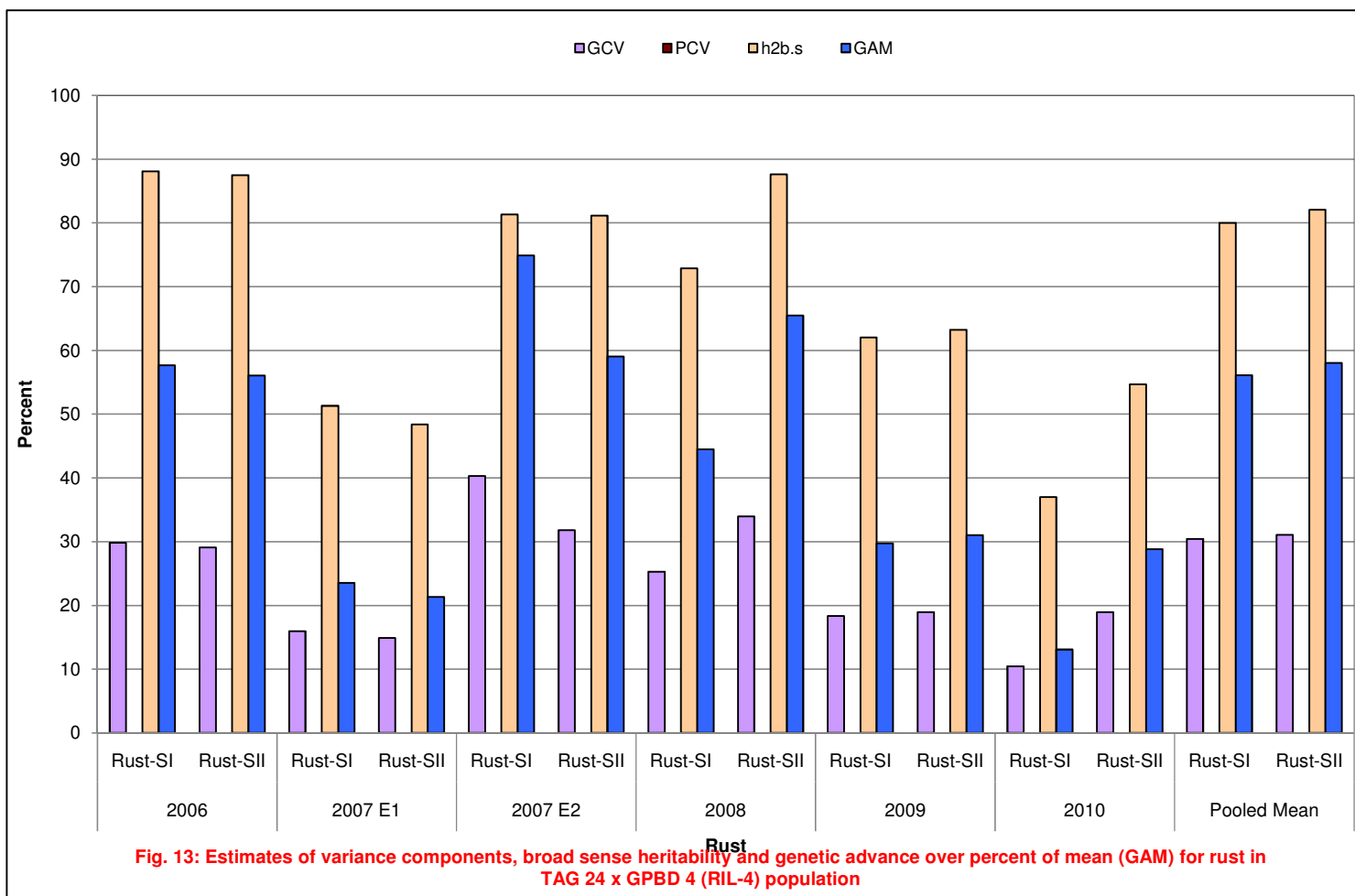


Fig 13: Estimates of variance components, broad sense heritability and genetic advance over percent of mean (GAM) for rust in TAG 24 x GPBD 4 (RIL-4) population

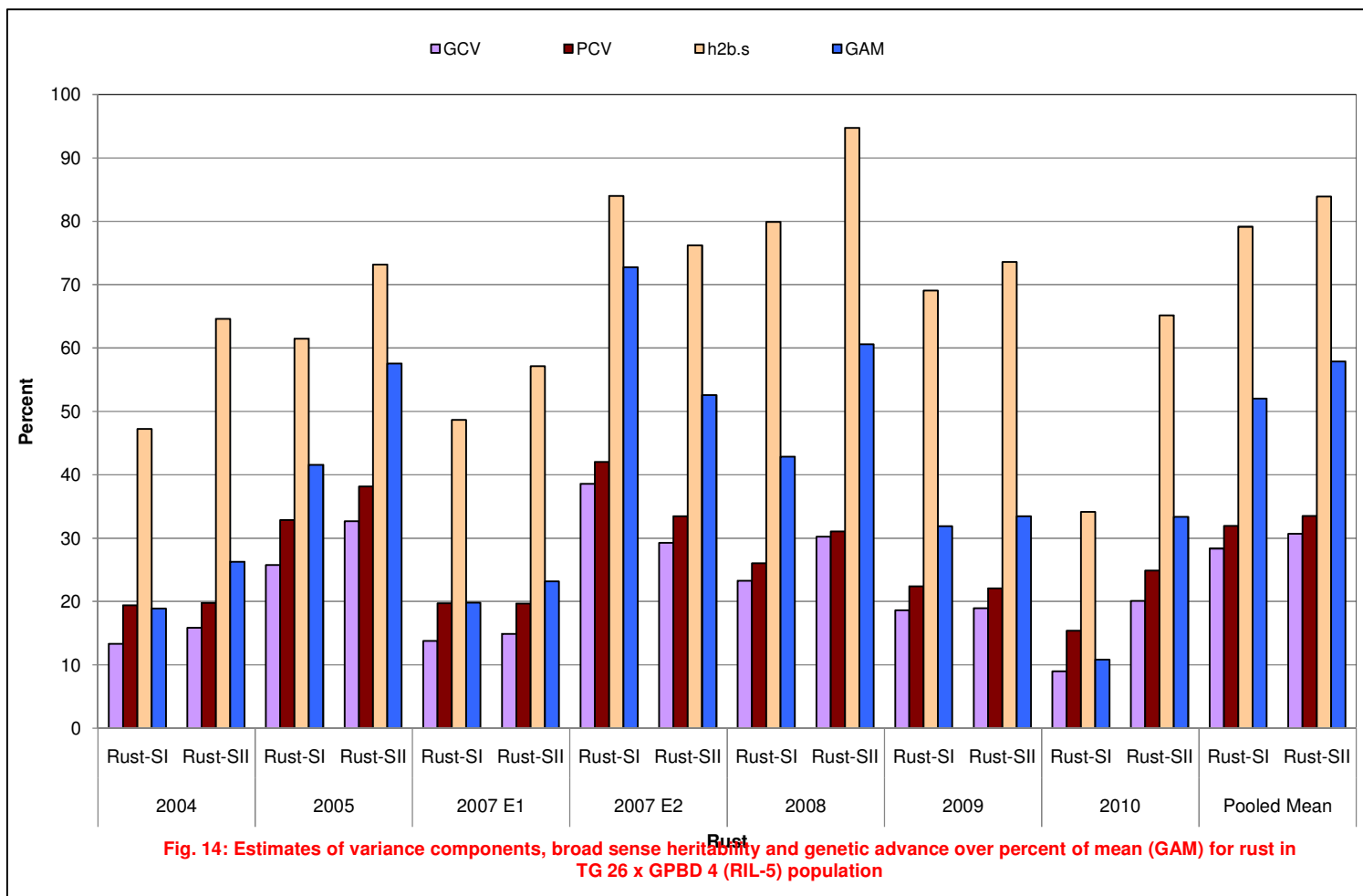


Fig 14: Estimates of variance components, broad sense heritability and genetic advance over percent of mean (GAM) for rust in TG 26 x GPBD 4 (RIL-5) population

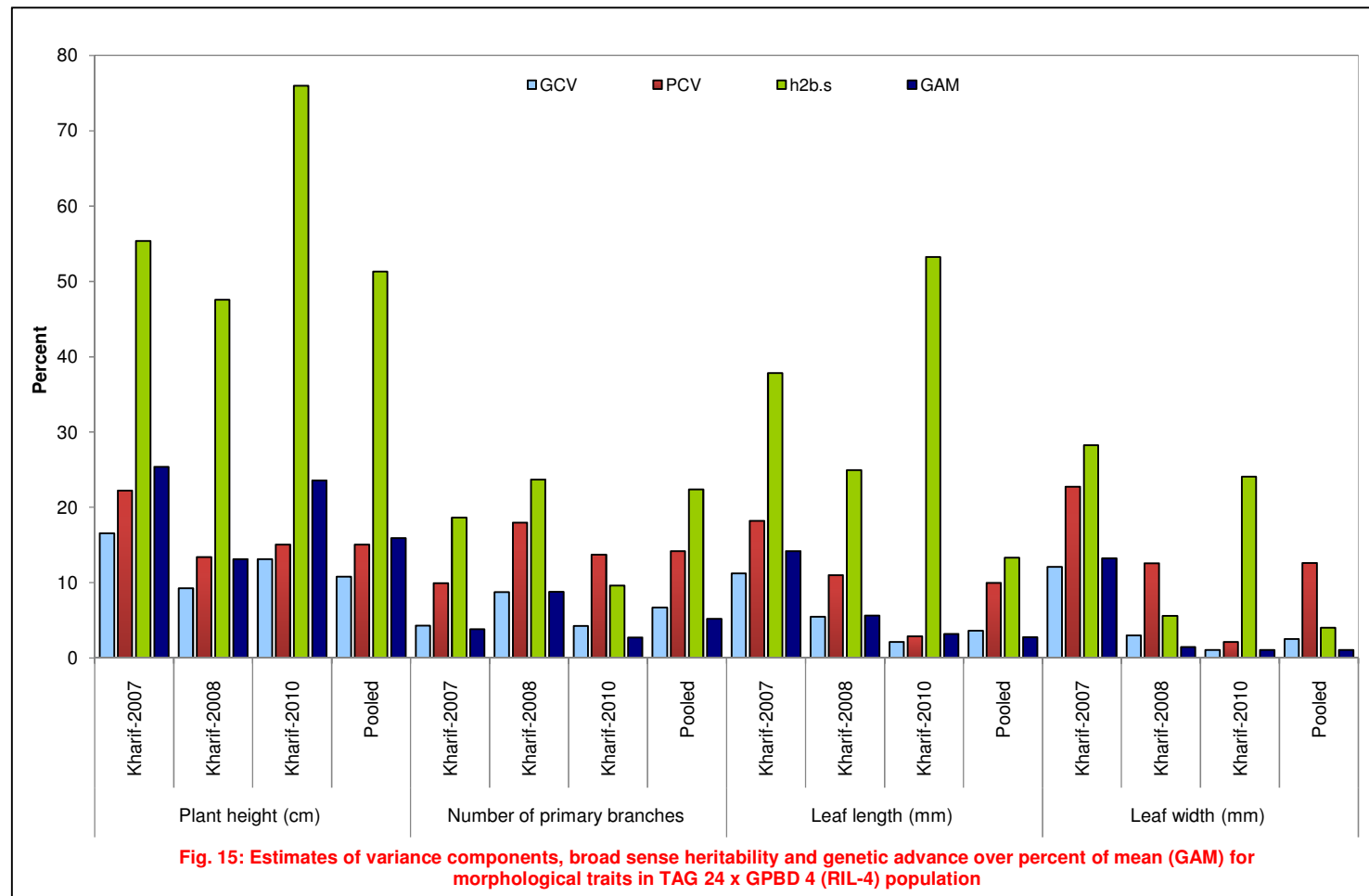


Fig 15: Estimates of variance components, broad sense heritability and genetic advance over percent of mean (GAM) for morphological traits in TAG 24 x GPBD 4 (RIL-4) population

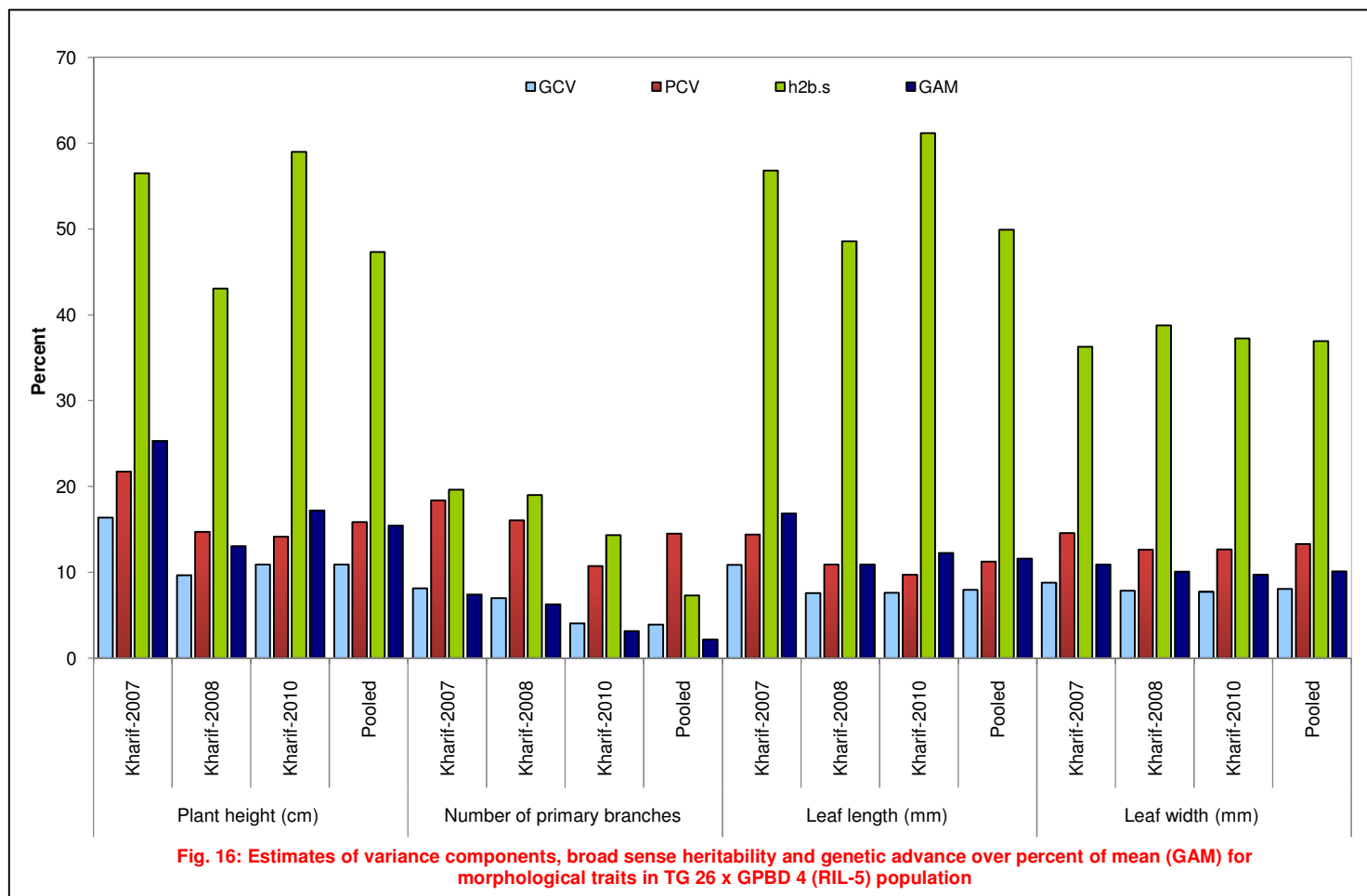


Fig 16: Estimates of variance components, broad sense heritability and genetic advance over percent of mean (GAM) for morphological traits in TG 26 x GPBD 4 (RIL-5) population

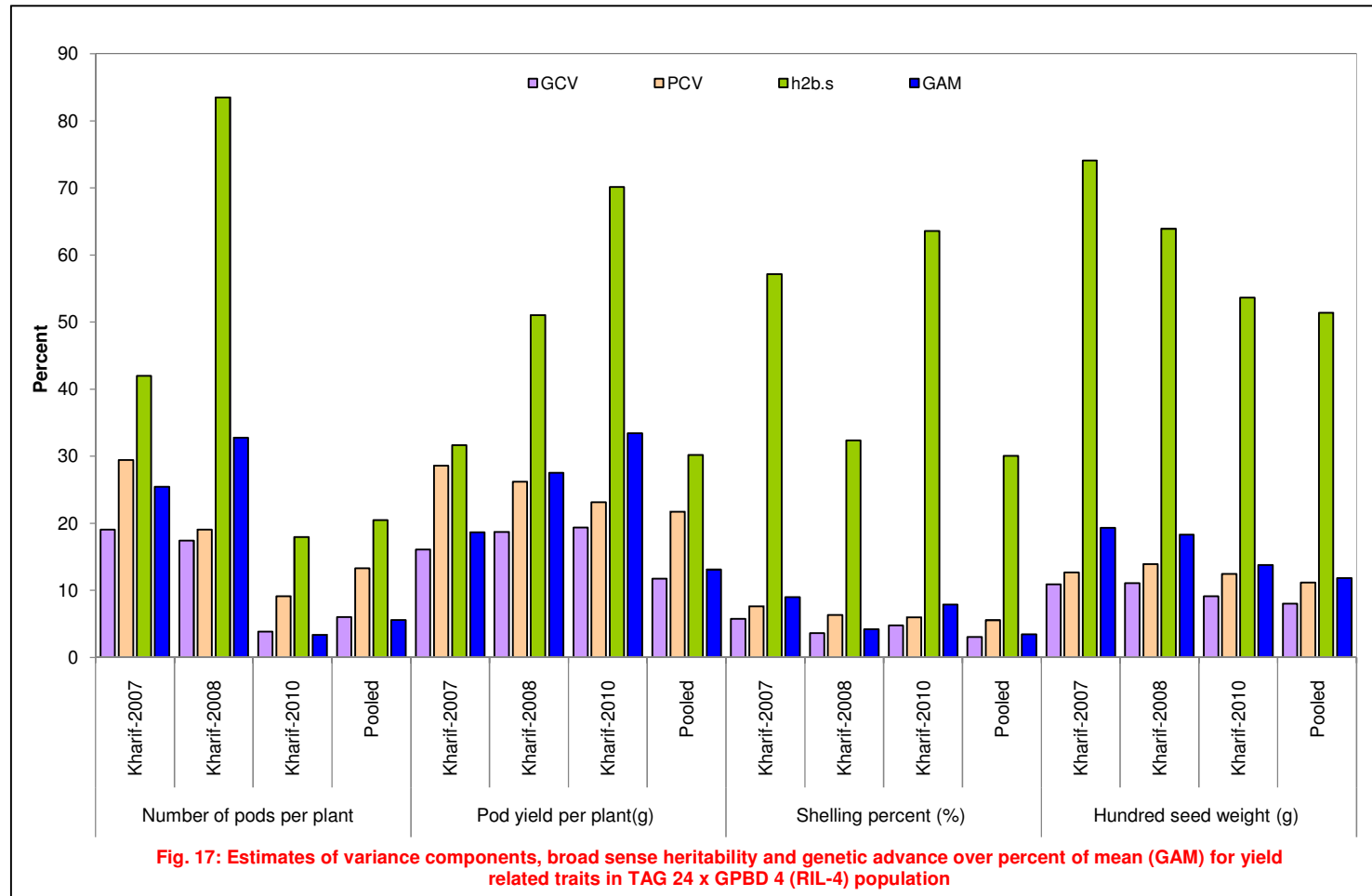


Fig 17: Estimates of variance components, broad sense heritability and genetic advance over percent of mean (GAM) for yield related traits in TAG 24 x GPBD 4 (RIL-4) population

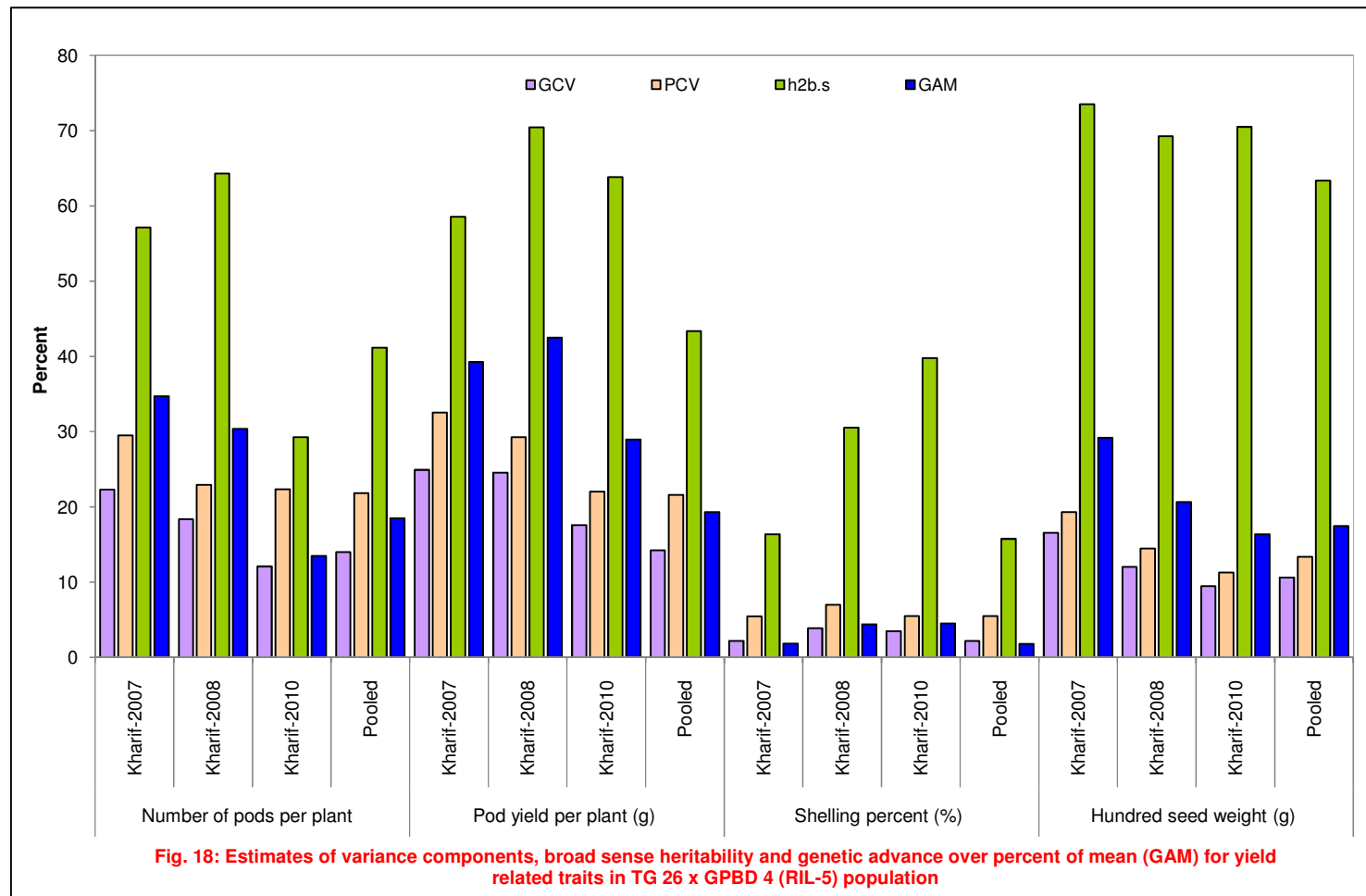


Fig 18: Estimates of variance components, broad sense heritability and genetic advance over percent of mean (GAM) for yield related traits in TG 26 x GPBD 4 (RIL-5) population

In terms of stability of the identified QTLs in RIL-4 population, only QTL_{R4-LLS01} (10.27-62.34% PVE), was found consistently in all six season and at both stages of disease scoring, followed by QTL_{R4-LLS05} (10.81-15.34% PVE in four seasons) and QTL_{R4-LLS09} (3.39-8.5% PVE in three seasons). However, the other 10 QTLs identified were detected in less than three seasons and in either of the two stages or in both the stages with a range of 3.38-67.98% PVE. In case of RIL-5 population, of the 15 QTLs identified none of the QTLs were present in more than three seasons. But two QTLs *i.e.*, QTL_{R5-LLS01} (7.58-49.64% PVE) and QTL_{R5-LLS02} (9.79-22.46% PVE) were present at least in two seasons of disease scoring. Though, other QTLs had high phenotypic variance explained they were not consistent in their appearance in more than three environments. A comparison among the QTLs for their specificity at a particular stage (Either LLS-SI or LLS-SII) of disease scoring revealed three QTLs (QTL_{R4-LLS03}, QTL_{R4-LLS05} and QTL_{R4-LLS09}) exclusively contributing only for LLS-SII. However, exception was observed for QTL_{R4-LLS03} and QTL_{R4-LLS05} for their appearance in LLS-SII, instead they also appeared in LLS-SI of Kharif 2009. Such, reports of inconsistent QTLs which are either season and/or stage specific were also reported earlier (Ender and Kelly 2005; Leal-Bertioli *et al.*, 2009; Khedikar *et al.*, 2010).

With the comparison of the QTLs identified for LLS between the populations revealed three common genomic regions contributing for LLS *viz.*, QTL_{R4-LLS02} of RIL-4 and QTL_{R5-LLS01} of RIL-5, QTL_{R4-LLS04} of RIL-4 and QTL_{R5-LLS03} of RIL-5. Similarly, the QTL_{R4-LLS06} (RIL-4) and QTL_{R5-LLS10} (RIL-5) had a common left flanking marker (GM1911) which defines the common QTL region (Table 23 and 24). However, the major QTL, QTL_{R4-LLS01} (10.27-62.34% PVE) identified in RIL-4 population could not be validated in the other population since the tightly linked marker for LLS (GM1009) was found to be monomorphic between TG 26 and GPBD 4. Further when considered the disease reaction of TG 26 for LLS, it was moderately resistant and it could be due to the different source of resistance genes or a different mechanism of resistance. And validation of the major QTL QTL_{R4-LLS01} is a must for employing in marker-assisted breeding programs.

As compared to the earlier study based on RIL-4 population with 56 marker loci (Khedikar *et al.*, 2010), further saturation with additional marker loci through the present study made it possible to identify a major QTLs for LLS for the first time in cultivated groundnut. However, phenotyping in additional environments identified the QTLs which were specific to the additional environments of disease screening. For instance the three QTLs, QTL_{R4-LLS02}, QTL_{R4-LLS03} and QTL_{R4-LLS04} were identified particularly in additional seasons (*i.e.* 2008 and 2009) of disease screening, but not in the earlier seasons (2004, 2005 and 2006). So there is a need to focus on the precision of phenotyping for LLS and also to study the components of resistance in detail. However, increasing the marker density further by SNPs and other marker system will assist in defining the proper genomic regions contributing for LLS.

5.2.2.2 QTLs identified for rust

Similarly detailed QTL analysis for rust resistance with respect to both the populations was done with additional SSRs integrated onto the map and multiple seasons of disease scoring with two stages. The analysis revealed a total of eight (RIL-4) and six (RIL-5) QTLs (Table 25 and 26; Fig. 5 and 6) for rust resistance from both the populations with the PVE ranging from 2.54-82.96% in RIL-4 and 2.89-78.96% in RIL-5 population. However, the number of major QTLs identified was three (10.68-82.96% PVE) in RIL-4 and four (15.04-78.96% PVE) in RIL-5 population, remaining QTLs were minor as they explained <10% PV (Collard *et al.*, 2005).

Among the major QTLs identified for rust in RIL-4 population, three QTLs (QTL_{R4-Rust01}, QTL_{R4-Rust02} and QTL_{R4-Rust03}) were found consistently in all the six seasons of disease scoring, except for few stages in specific seasons like QTL_{R4-Rust01} was absent in Rust-SII_2007E2 and Rust-SI_2010. Similarly QTL_{R4-Rust02} and QTL_{R4-Rust03} were absent in either of the two stages of disease scoring or absent in a particular season of disease screening. In the case of RIL-5 mapping population only two QTLs (QTL_{R5-Rust01} and QTL_{R5-Rust02}) were present in all the seven seasons of disease scoring except for QTL_{R5-Rust02} which was absent in the year 2005 at both the stages of disease scoring (Table 26). The remaining two minor QTLs explained <10% PV (Collard *et al.*, 2005). However, stage specific QTLs for rust were also observed (Table 25 and 26) in both the populations, which were not consistent across the seasons of experimental studies (Ender and Kelly 2005; Leal-Bertioli *et al.*, 2009; Khedikar *et al.*, 2010).

Though the genomic region contributing for rust was defined with the same set of flanking markers (GM2009, GM1536, GM2301, GM2079, IPAHM103 and GM1954), the number of QTLs defined for rust on LG AhXV varied (three QTLs in RIL-4 and two QTLs in RIL-5 population) between

the populations. However, the variation could be attributed to the difference in the inter-marker distance between markers flanking the QTL region in both the populations (Fig. 5 and 6) where it was less than 5-10 cM. However, it is difficult to distinguish two QTLs that are having less inter-marker distance (<10-20 cM) between the markers. Hence two or more QTLs within this interval may be misinterpreted as one (Lebreton *et al.*, 1998). And this may also result in a large ghost QTL being located between the two true QTL if they are linked in coupling phase and possibly no QTL being identified if they are linked in repulsion phase (Martinez and Curnow, 1993). However, in the genetic maps, disagreement observed for the order of closely linked markers within linkage groups of different maps may be attributed to recombination heterogeneity between populations (Gustafson *et al.*, 2009).

Saturation of both partial linkage maps developed earlier with additional markers reconfirms the presence of the major QTL for rust resistance in both the population. Further, it has enabled to identify new closely linked markers (GM2009, GM1536, GM2301, GM2079 and GM 1954) with IPAHM103 and to define proper QTL positions with less inter marker distance. Whereas in the earlier report (Khedikar *et al.*, 2010) the QTL region was flanked by IPAHM103-pPGSseq19D06 with an interval of 49.9cM between markers and it was due to the less number of markers used in the earlier studies which might have defined a putative QTL region. With a comparison of the QTLs identified earlier with that of the present study we could reconfirm the presence of only four QTLs (PM436-Lec-1, PM179-seq11C08, IPAHM103-GM1954 and TC5A07-GM2246) for LLS and three QTLs (IPAHM103-GM1954, TC1B02-pPGSseq18E07 and TC4G10-GM624) for rust. However, with the saturation of the genetic map of RIL-4 population with 190 loci mapped the remaining QTLs reported earlier for LLS and rust in Khedikar *et al.* (2010) could not be identified in the present study. Nevertheless, in both the maps there were no other linkage groups which harbored consistent major QTLs for rust resistance except the LG AhXV. It has a well defined candidate genomic region consistently contributing for rust resistance across the environments and also the contribution of the QTL has been increased with the saturation of the QTL region. Further to the best of our knowledge we report for the first time, identification of major QTLs for LLS in cultivated groundnut. However, similar kind of genetic mapping with additional markers (specifically RGAs) facilitated in identifying the candidate genomic regions for disease resistance (Leal-Bertioli *et al.*, 2009). QTL analysis for both the foliar disease under study revealed the appearance of majority of inconsistent minor QTLs. This could be due to varying environmental conditions across seasons, size of the mapping populations, incomplete genotyping data and errors in the genotyping/phenotyping data (Miklas *et al.*, 2001). Such inconsistencies have also been found in other studies, which report identification of small effect QTLs, specific to the environments and treatments in the experimental studies (Ender and Kelly 2005; Leal-Bertioli *et al.*, 2009; Khedikar *et al.*, 2010; Ravi *et al.*, 2011). Such minor QTLs with small effects found across the environments could be validated and can be used in marker assisted breeding programmes (Ravi *et al.*, 2011 and Ribaut *et al.*, 2010).

In summary, major QTLs for LLS has been identified for the first time in cultivated groundnut and further reconfirmed the presence of rust QTL with closely linked new SSR markers in addition to IPAHM 103. Further it is needed to validate the tightly linked markers for LLS and rust resistance for the future use in marker assisted breeding programs and to identify candidate genes for disease resistance.

5.2.2.3 Common QTLs conferring resistance to LLS and rust

QTL analysis revealed three common QTLs for both the disease from both the populations. In case of RIL-4 the QTL regions contributing for LLS and rust were GM2009-GM1536, GM1536-GM2301/GM2079 and IPAHM103-GM1954. Similarly in the RIL-5 population common QTL regions for both the diseases were defined with the following QTL regions GM2009-GM1536, IPAHM103-GM1954 and RN16F05-GM1988 (Fig. 19). However, it is quite possible to observe the increased effect of QTLs with the saturation of map with more number of markers. And QTL analysis in the present study with few major QTLs and several minor QTLs for disease resistance (Leal-Bertioli *et al.*, 2009; Khedikar *et al.*, 2010) was in comparison to that of classical genetic studies conducted in the past on LLS and rust, indicating multiple recessive genes governing resistance in LLS (Nevill 1982) and as few recessive genes conferring rust resistance (Kalekar *et al.*, 1984; Paramasivam *et al.*, 1990). Further the present study reconfirms that resistance to rust is controlled by both major and minor QTLs with complex inheritance pattern (Dwivedi *et al.*, 2002) and also occurrence of major QTL and minor QTLs is a pervasive fact in disease resistance studies (Welter *et al.*, 2007; Leal-Bertioli *et al.*, 2009).

5.2.2.4 Distribution of QTLs for LLS and rust on the consensus map

In the present study an attempt was made to develop a consensus map of 227 mapped loci spanning around 1198.76 cM of map distance was, which in turn provided an opportunity to place the QTLs identified in the respective populations for both LLS and rust. A total 42 QTLs (20 major and 22 minor QTLs for both LLS and rust) were mapped in their respective positions from both the RIL-4 and RIL-5 population (Fig. 19) were placed on their respective positions. In the consensus map LG AhXII with 30.0 cM genomic region had one major QTL (QTL_{R4-LLS01}) contributing consistently across all the six environments with PVE from 10.57-67.98 % and another genomic region with 38.7 cM on LG AhVIII had five QTLs for LLS. Similarly for rust the LG AhXV had the candidate genomic region of 20 cM harboring a total of three and two QTLs from RIL-4 and RIL-5 population respectively. However, the consensus QTL map enabled to identify common QTLs contributing for both LLS and rust as observed in the QTL region flanked by GM1878-RN16F05 in LG AhV and three QTLs flanked by GM2009-GM1536, GM1536-GM2301/GM2079 and IPAHM103-GM1954 in LG AhXV, which were contributing for both the diseases (Fig. 19). Based on the co-localization of these QTLs, it can be inferred that genomic (QTL) regions flanked by GM2009-GM1536, GM1536-GM2301/GM2079 and IPAHM103-GM1954 contribute to the resistance for both the diseases (Fig. 19). Though, it was not possible to find maximum number of QTL clusters in the consensus map and may explain the possibility of less number of genomic regions governing both the foliar diseases. Further, implying to saturate with more number of polymorphic markers, for better understanding of the disease resistance mechanism for these two (LLS and rust) foliar diseases. Nonetheless, upon validation of QTLs/tightly linked marker the marker assisted breeding programs can be initiated for enhancing resistance to LLS and rust in cultivated groundnut.

5.2.3 Identification of QTLs for morphological traits

QTL analysis across three seasons of phenotyping revealed a total of fourteen QTLs in RIL-4 and five QTLs in RIL-5 population for plant height; it was three QTLs for number of primary branches in RIL-4 and one QTL in RIL-5 population. Similarly for both leaf length and leaf width four QTLs for each of the trait were identified in RIL-4 population. However it was only three and one QTL for leaf length and leaf width respectively in case of RIL-5 population. From among the QTLs identified for morphological traits in RIL-4 population none of them were found to be major QTLs except QTL_{R4-Plht01} (GM 1955-GM 1007) and QTL_{R4-Plht02} (GM 633-PM 179) during Kharif 2010. However, these two QTLs explained <10% PV (Collard *et al.*, 2005) during Kharif 2007 and 2008 (Table 27 and Fig. 7). Similarly, in case of RIL-5 population during Kharif 2007 and 2010 two QTLs (QTL_{R5-Plht01} and QTL_{R5-Plht02}) were identified as major QTLs. However, during Kharif 2008 these two QTLs explained <10% PV (Table 28 and Fig. 8) and were found to be minor QTLs (Collard *et al.*, 2005). Similar, inconsistencies in detection of QTLs were also observed for leaf length, leaf width and number of primary branches in the present study. Such discrepancies were also reported by earlier studies involving RIL-4 and RIL-5 populations by Khedikar, 2008 and Sarvamangala, 2009, respectively. Such, reports of inconsistent QTLs which are either season and/or stage specific with respect to diseases were also reported earlier (Ender and Kelly 2005; Leal-Bertioli *et al.*, 2009; Khedikar *et al.*, 2010).

However comparison of the two populations for the QTLs identified to be common and based on the consistency of a QTL to be identified across the seasons. Two QTLs were found to be common and stable between the populations for plant height the QTLs were flanked with the same set of flanking markers for QTL_{R4-Plht01} and QTL_{R5-Plht01} viz., GM.1955 and GM 1007. However, for the QTL_{R4-Plht02} and QTL_{R5-Plht02} only one marker (PM 179) was found to be common and based on this it was considered to be a common QTL between populations. Previously, QTL flanked with GM633-PM179 was reported earlier for plant height by khedikar (2008) and the same QTL was repeatedly detected (Table 27) in the present analysis over three years. Similarly QTL_{R4-LWD01} flanked by S 108 and GM 1097 marker loci was consistently identified for leaf length in all the three seasons (Kharif 2007, 2008 and 2010) with a PV < 10% in case of RIL-4 population. Though these QTLs explained less PV but are very important when genomic assisted breeding program are postulated in the future. Based on the additive effects of the QTLs (Table 27 and 28), it was observed that the contribution of favorable alleles is accomplished from both the parents for morphological traits. Though, favorable alleles were contributed from both the parents, the contributions from female parents (TAG 24 and TG 26) were found to be prominent for all the morphological traits in both the populations. Earlier similar conclusions were drawn for these traits in both the populations by Khedikar (2008) and Sarvamangala (2009).

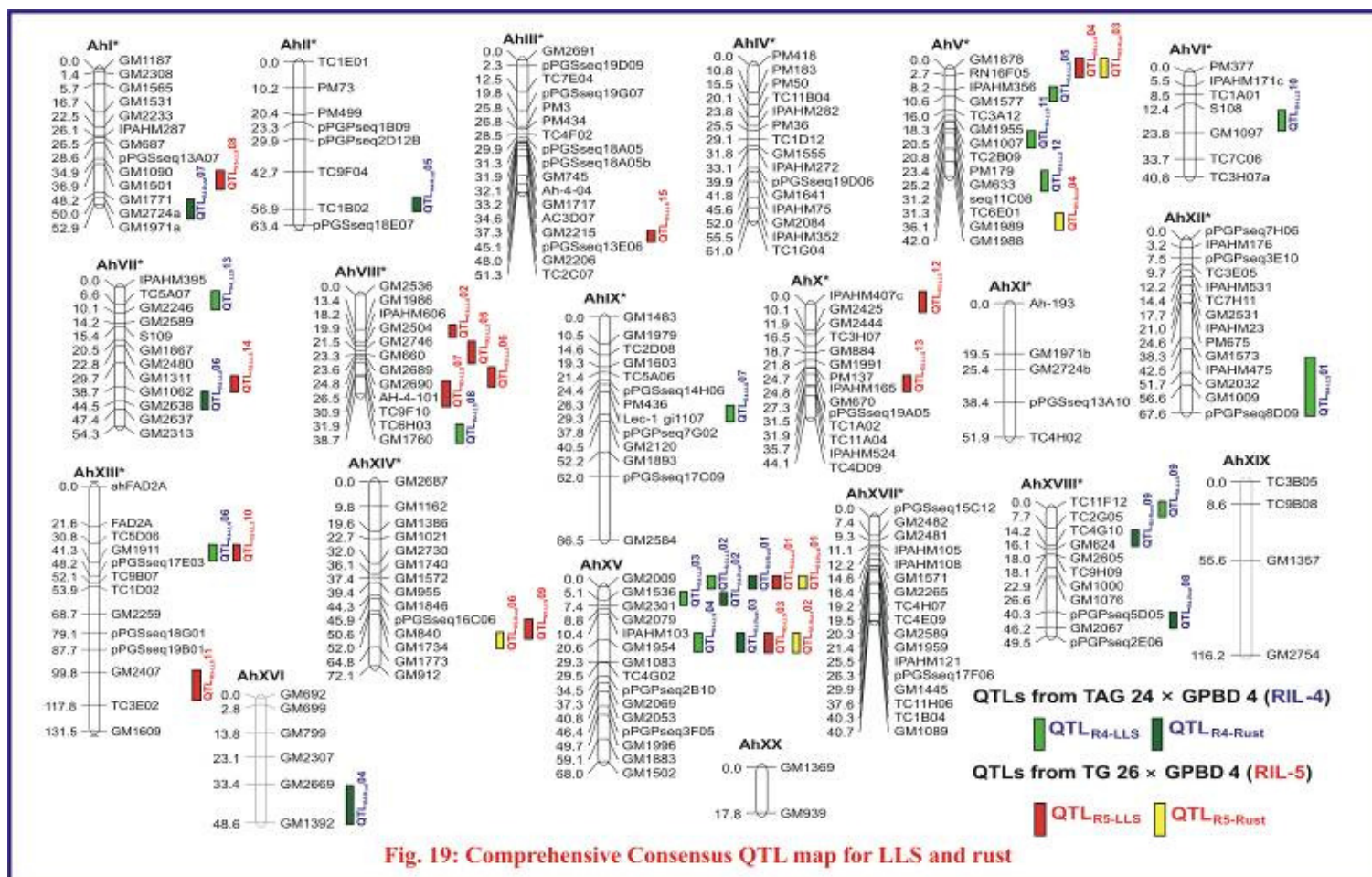


Fig 19: Comprehensive Consensus QTL map for LLS and rust

5.2.4 QTL identification for yield attributing traits

QTL detection on the data obtained across three years of phenotyping revealed total of 2 (RIL-4) and 3 (RIL-5) QTLs for number of pods per plant, 8 QTLs in case of RIL-4 and 6 QTLs in RIL-5 population for pod yield per plant (Table 29, 30 and Fig. 7-8). Two QTLs each for shelling percent and three QTLs each for hundred seed weight were identified in both RIL-4 and RIL-5 population. As compared to the QTLs identified for morphological traits in terms of consistency none of the QTLs were consistent across three seasons (Table 29 and 30). However, the genomic region flanked between the markers GM 2009 and GM 1954 with a genomic region of 18.71 cM in case of RIL-4 and 7.41 cM in case of RIL-5 contributed for pod yield per plant during Kharif 2007. Though QTLs within this genomic region were not consistent across the seasons, but their common contribution for a particular trait between the populations can be considered for further validation and their utilization in the marker assisted breeding programs. However, contribution of favorable alleles from the female parents remained prominent for yield attributing traits.

5.2.5 QTL identification for quality traits

Earlier QTL analysis for quality traits has been reported by Sarvamangala *et al.*, 2011 in RIL-5 population followed by Mukri (2011) in RIL-4 population. However, the data on *AhFAD2A* allele based on CAPS marker was available only on RIL-4 population. With the improved genetic maps along with phenotyping data obtained from the earlier studies by Sarvamangala (2009) and Mukri (2011) was subjected to QTL analysis.

QTL analysis revealed a total of 6 and 3 QTLs for protein content in RIL-4 and RIL-5 respectively. Similarly for oil content it was 8 QTLs in RIL-4 and 3 QTLs in RIL-5 population. QTL analysis for oil and protein content revealed both major and minor QTLs contributing for the trait (Table 31, 32 and Fig. 9-10). However in terms of consistency of the QTL to be identified across two environments was seen only in the case of RIL-4 population where in a total of three QTLs viz., QTL_{R4-Protein}01, QTL_{R4-Protein}02 and QTL_{R4-Protein}03 were consistent for protein content and similarly for oil content three QTLs (QTL_{R4-Oil}01, QTL_{R4-Oil}02 and QTL_{R4-Oil}03) were consistently identified across the environments. In general protein and oil content are negatively correlated as seen in the earlier reports by Sarvamangala (2009) and Mukri (2011). However, two QTLs were found to be common for protein and oil content. The QTL flanked with S108-GM1097 was common for QTL_{R4-Protein}01 and QTL_{R4-Oil}01 and similarly QTL flanked with GM1311-GM1062 was common for QTL_{R4-Protein}02 and QTL_{R4-Oil}02 (Table 31). Based on the earlier reports (Sarvamangala, 2009; Mukri, 2011) it is concluded that GPBD 4 is good for both protein and oil content and simultaneously it is reported that TAG 24 constitutes a total 29 percent protein and 45 percent oil content. Since both the parents of RIL-4 population are comparatively good for protein and oil content it could be possible that both parents are contributing for protein and oil content. Further it needs to be understood in terms of biochemical pathway how common genomic regions could contribute simultaneously for protein and oil content.

QTL analysis for protein and oil content in RIL-5 population did not reveal any significant and consistent QTLs. However QTL_{R5-Protein}01 (TC6H03-GM1760) was found to be major for protein and two QTLs viz., QTL_{R5-Oil}01 (PM179-TC6E01) and QTL_{R5-Oil}03 (TC4G02-pPGPseq2B10) for oil content. QTL_{R5-Protein}01 (TC6H03-GM1760) was identified earlier by Sarvamangala (2009) and another QTL flanked with IPAHM295 was also reported earlier and in the present study also. However the QTL flanked by TC2E05-TC3E02 identified earlier could not be identified, which could be due to the order/position of the marker loci in the present improved genetic map.

QTL analysis for other quality traits viz oleic acid, linoleic acid and O/L ratio revealed a total of 7 QTLs in RIL-4 and 2 QTLs for oleic acid in RIL-5 population. For linoleic acid 2 QTLs were identified in RIL-4 and 3 QTLs in case of RIL-5 and for O/L ratio it was 5 and 4 QTLs in RIL-4 and RIL-5 population respectively (Table 33, 34 and Fig. 9-10).

Further, with a view of mapping *FAD2* alleles *FAD2A* and *FAD2B* parental polymorphism was assessed for allele specific markers. Polymorphism was only observed for *FAD2A* allele between the parents of RIL-4 and RIL-5 population and was monomorphic for the *FAD2B* allele indicating the absence of B allele in the population. The marker data was generated on both the populations for *FAD2A* allele (Plate 3) using the allele specific PCR based markers (Chen *et al.*, 2010). *FAD2A* allele was then mapped on to LG AhXIII* in both the populations. As expected to identify a QTL with the marker data of allele specific CAPS marker and PCR based assay for *FAD2* marker for the *FAD2A* allele of 'A' genome. We could identify the marker trait association and consistent QTL has been

identified for oleic acid, linoleic acid and O/L ratio. However, it was not the same for with RIL-5 population. Where in the marker segregated in the expected ratio of 1:1 and fitted with χ^2 chisquare values. However the possible reasons could be ascribed to the content of linoleic acid with respect to the female parent TG26 which is high for linoleic acid and probably would have contributed for the aberrant variation for oleic acid, linoleic acid and O/L ratio which in turn did not have proper marker trait association this could also be due to the population size handled to explain the variation in RIL-5 population. The gene specific markers has been validated for its contribution for *FAD2A* allele governing the fatty acid composition and ratio by Mukri *et al.* (2011) in the minicore collection and also with mapping studies with earlier partial genetic map using CAPS marker. However, in the present study allele specific PCR based marker for *FAD2A* allele has been validated in both the population and was mapped on the genetic map on AhXIII.

Along with *AhFAD2A*, *FAD2A* few more QTLs were found consistent across environment for oleic acid in RIL-4 population. Additional QTLs were minor and alleles were contributed from the female parent TAG 24. These QTLs can be validated and utilized in marker assisted breeding. Though marker trait association was not observed for allele specific marker, the genomic region flanked with GM 2690-TC9F10 and GM 2246-GM 2589 was identified for oleic acid, linoleic acid and O/L ratio (Table 34). Nevertheless it was not consistent across the environments. However based on the earlier reports and the present study it can be concluded that improving nutritional quality in groundnut can be successfully achieved by marker assisted breeding programme.

5.3 Genomic regions contributing across different traits

5.3.1 Common QTLs contributing across different traits

An attempt was made to compare the contribution of the QTLs for different traits and is presented in the Table 40. The present study we could identify a total of 19 genomic regions comparatively contributing across the traits. The contribution from a QTL ranged from two to six traits as mentioned in the Table 40. For instance in case of the genomic region flanked by S108 and GM 1097 located on LG AhVI contributed for a total of 6 traits and was corresponding to A genome and represents A06 linkage group of groundnut. Similarly candidate QTLs for rust resistance flanked by GM 2009-GM 1536, IPAHM 103-GM1954 and GM1536-GM2301 were also significantly contributing for LLS, pod yield per plant, shelling percent and hundred seed weight indicating its possibility of controlling the disease and enhancing the productivity. As compared with the high density consensus map it was considered that the whole candidate genomic region contributing for rust resistance was contributed from the B genome (B03) in groundnut. However the present study could also validate the presence of the *FAD2A* allele and its contribution for improving quality. Wherein, it was contributing for oleic acid, linoleic acid, O/L ratio and oil content indicating the major contribution in controlling the desaturase activity (Chen *et al.*, 2010). Likewise several other traits were also controlled in the same fashion by common genomic region and the details are presented in Table 40.

Similar comparisons were made in case of RIL-5 population which revealed a total of 9 genomic regions flanked with the marker intervals as presented in the Table 41, the number of traits controlled by a genomic region ranged from 2 to 4 traits per QTL. Genomic regions contributing for diseases also contributed for the yield attributing traits as seen earlier in case of RIL-4 population. Similar observations were made earlier by Khedikar (2008) and Sarvamangala (2009) for the QTLs governing disease resistance. In general these genomic regions are of prime importance and could be targeted for further saturation and dissecting the QTLs in a precise manner for the identification of candidate gene/genomic regions in the future.

5.3.2 Common genomic regions between populations

Mere comparison of the genomic regions across traits may not give a proper idea or clarity regarding the genomic regions responsible for the trait of interest. It could also be checked across different genetic back ground in a different mapping population in the present study the availability of the two genetic maps has made it possible to compare whether a genomic region is either contributing for the same trait or even for other traits or for an entirely different trait. The comparison concluded all the three possibilities as mentioned in Table 42, where in genomic regions common between populations are contributing for the same trait across populations as explained by the disease resistance QTLs flanked by GM2009 and GM1536, IPA103 and GM 1954 in both RIL-4 and RIL-5 population. Similarly, two QTLs contributing for plant height was flanked by the flanking markers for QTL_{R4-PIht}01 and QTL_{R5-PIht}01 viz., GM.1955 and GM 1007 in RIL-4 and RIL-5 population.

Table 40: Common genomic regions /QTLs contributing across different traits in RIL-4 population

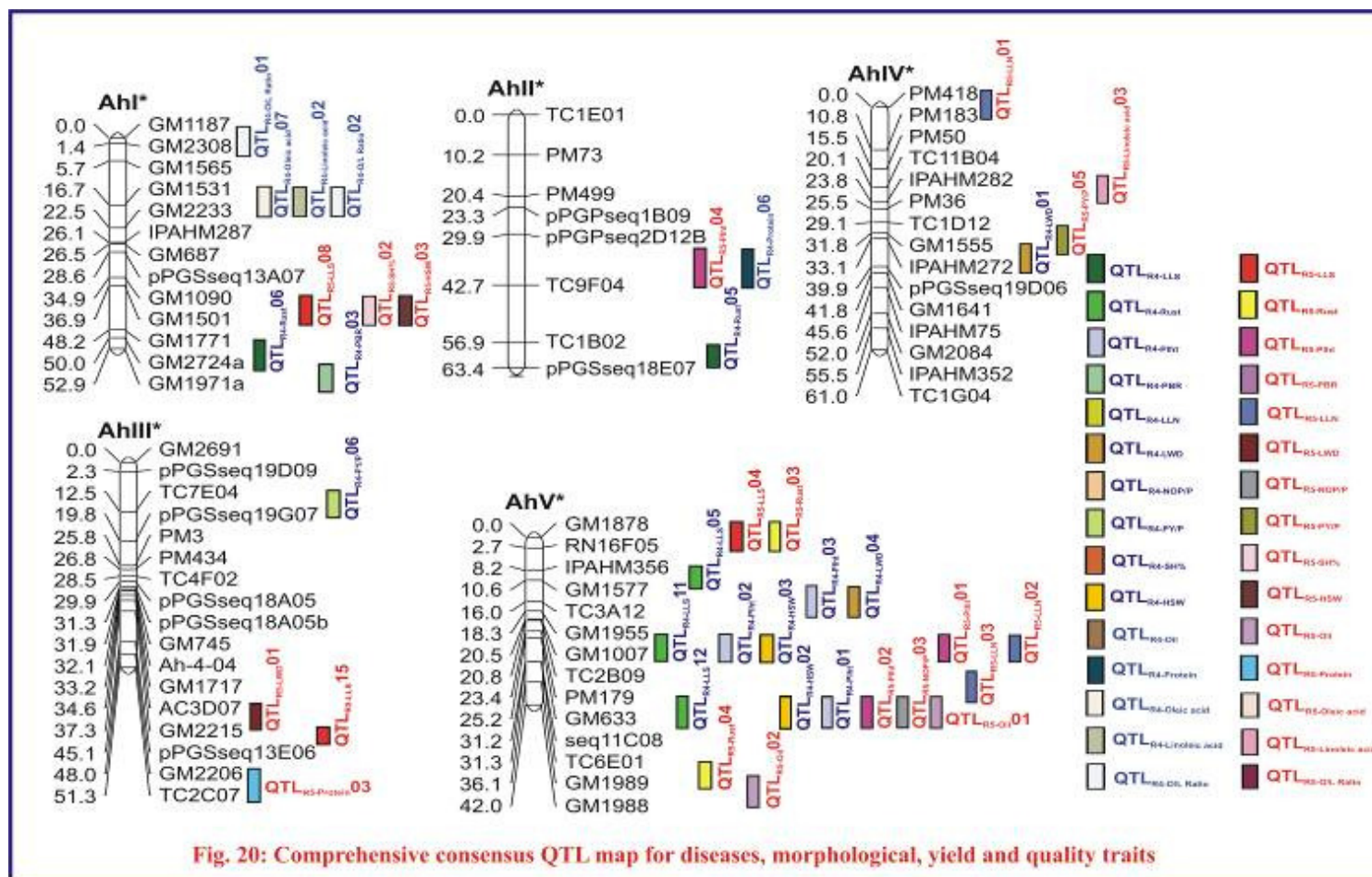
Sl.No.	Genomic regions contributing across traits in RIL-4 population	Contribution of genomic regions for the following traits	Total number of traits	R^2_p Range
1	GM1573-pPGPseq8D09	LLS (10.27-62.34), Plht (8.25) and PY/P (12.50)	3	8.25-62.34
2	GM2009-GM1536	LLS (12.49-67.98), rust (33.05-82.27), PY/P (14.00), SH% (15.20) and HSW (6.17)	5	6.17-82.27
3	IPAHM103-GM1954	LLS (16.14-42.66), rust (23.12-82.96), PY/P (15.37) and SH% (13.30)	4	13.30-82.96
4	GM1536-GM2301	LLS (4.83-10.13), rust (12.62-62.35) and PY/P (10.74)	3	4.83-62.35
5	S108-GM1097	LLS (4.72), LLN (6.13-8.65), oleic acid (3.77-6.88), O/L ratio (3.89), Protein (8.74-9.27) and oil content (5.86-8.81)	6	3.77-9.27
6	GM1955-GM1007	LLS (4.46) and Plht (5.77-13.55)	2	4.46-13.55
7	TC5A07-GM2246	LLS (4.01) and Plht (4.50)	2	4.01-4.50
8	TC11F12-TC2G05	LLS (3.39-8.50), PBR (9.15) and NOP/P (9.64)	3	3.39-9.64
9	TC6H03-GM1760	LLS (3.38-3.76), oleic acid (4.07-5.50) and oil content (7.05)	3	3.38-7.05
10	GM2724a-GM1971a	Rust (3.51) and PBR (5.92)	2	3.51-5.92
11	GM1386-GM1162	NOP/P (13.21) and PY/P (12.43)	2	12.43-13.21
12	GM1577-TC3A12	Plht (7.03) and LWD(6.77)	2	6.77-7.03
13	GM1991-PM137	LLN (5.02) and LWD (5.98)	2	5.02-5.98
14	GM633-PM179	Plht (4.39-11.47) and HSW(5.83)	2	4.39-11.47
15	ahFAD2A(CAPS)-FAD2A	oleic acid (14.34-23.79), linoleic acid (17.31-25.40), O/L ratio (19.50-25.71), and oil content (6.61)	4	6.61-25.71
16	Ah-4-101-TC9F10	oleic acid (4.24-4.89), Protein (3.88) and oil content (6.72)	3	3.88-6.72
17	GM1531-GM2233	oleic acid (4.41), linoleic acid (8.34) and O/L ratio (9.85)	3	4.41-9.85
18	PM377-TC1A01	oleic acid (4.71-6.06), O/L ratio (4.81), and oil content (5.35)	3	4.71-6.06
19	GM1311-GM1062	Protein (18.85-20.35) and oil content (9.46-10.40)	2	9.46-20.35

Table 41: Common genomic regions /QTLs contributing across different traits in RIL-5 population

Sl.No.	Genomic regions contributing across traits RIL-5 population	Contribution of genomic regions for the following traits	Total number of traits	R^2_p Range
1	GM2009-GM1536	LLS (7.59-49.64), rust (17.57-66.05), NOP/P (9.41) and PY/P (16.6)	4	7.59-66.05
2	IPAHM103-GM1954	LLS (63.17), rust (17.41-78.96) and PY/P (11.64)	3	11.64-78.96
3	RN16F05-GM1988	LLS (16.17) and rust (15.07-29.02)	2	15.07-29.02
4	PM179-TC6E01	Plht (6.80-18.60), NOP/P (8.28) and oil content (13.84)	3	6.80-18.60
5	GM1955-GM1007	Plht (7.13-14.15) and LLN (12.67)	2	7.13-14.15
6	TC11H06-TC1B04	PY/P (9.96) and HSW (9.87)	2	9.87-9.96
7	GM2246-GM2589	Oleic acid (11.48), linoleic acid (10.94) and O/L ratio (7.76)	3	7.76-11.48
8	GM2690-TC9F10	Oleic acid (14.08) and O/L ratio (12.28)	3	12.28-14.08
9	pPGSseq19B01-GM2407	Linoleic acid (11.92) and O/L ratio (12.50)	2	11.92-12.50

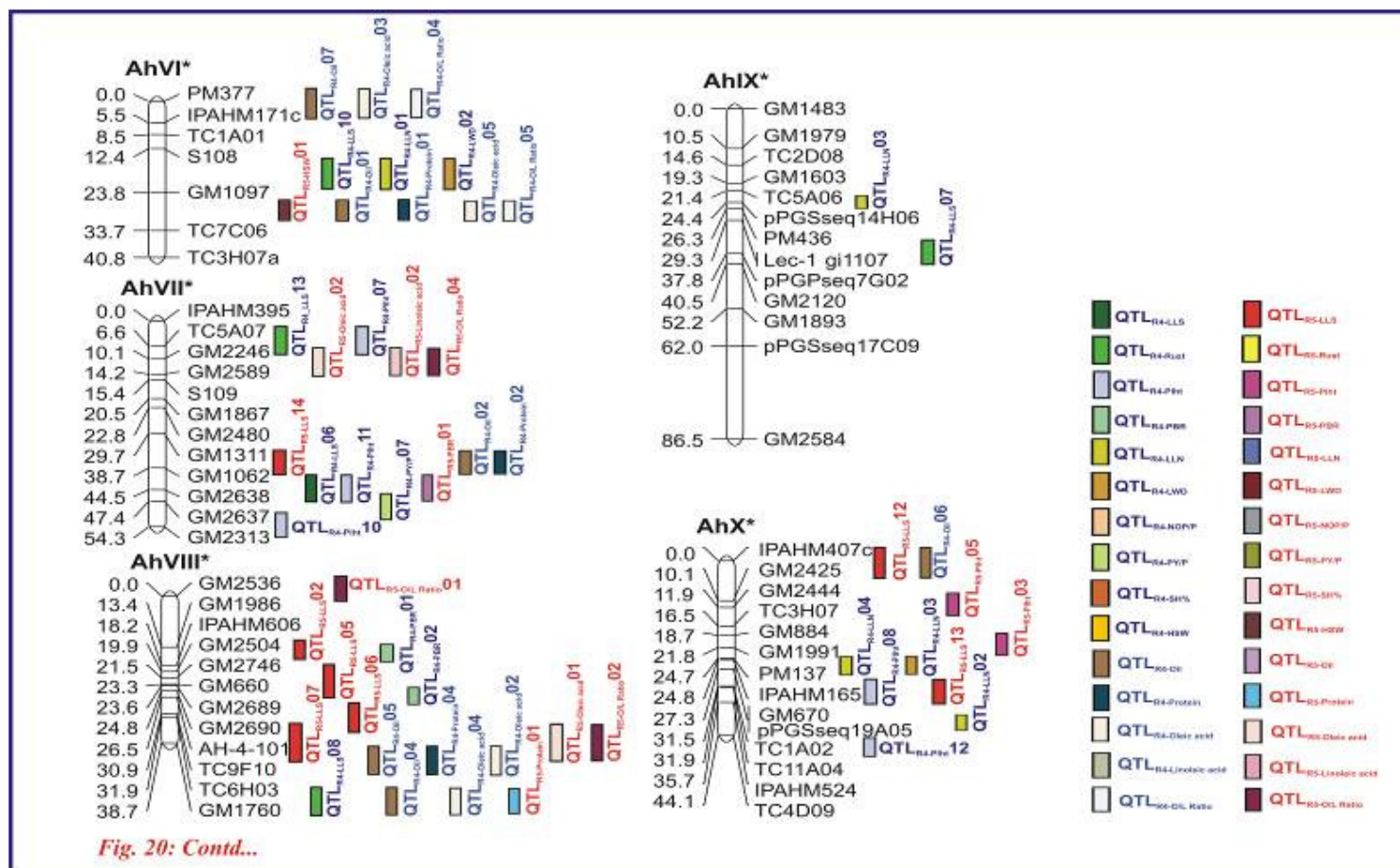
Table 42: Common genomic regions /QTLs between populations contributing across different traits in RIL-4 and RIL-5 population

Sl.No	Common genomic regions between RIL-4 and RIL-5 population	Population	Total number of traits	Traits governed by the common genomic regions
1	GM2009-GM1536	RIL-4	5	LLS, rust, pod yield per plant, shelling percent and hundred seed weight
	GM2009-GM1536	RIL-5	4	LLS, rust, number of pods per plant and pod yield per plant
2	IPAHM103-GM1954	RIL-4	4	LLS, rust, pod yield per plant and shelling percent
	IPAHM103-GM1954	RIL-5	3	LLS, rust and pod yield per plant
3	GM633-PM179	RIL-4	2	Plant height and hundred seed weight
	PM179-seq11C08	RIL-4	1	LLS
	PM179-TC6E01	RIL-5	3	Plant height, number of pods per plant and oil content
4	GM1955-GM1007	RIL-4	2	LLS and plant height
	GM1955-GM1007	RIL-5	2	Plant height and leaf length
5	TC6H03-GM1760	RIL-4	3	LLS, oleic acid and oil content
	TC6H03-GM1760	RIL-5	1	Protein
6	GM1062-GM2638	RIL-4	2	Rust and Plant height
	GM1062-GM2638	RIL-5	1	Number of primary branches
7	GM1311-GM1062	RIL-4	2	Protein and oil content
	GM1311-GM1867	RIL-5	1	LLS
8	GM1991-PM137	RIL-4	2	Leaf length and leaf width
	PM137-GM670	RIL-4	1	Plant height
	PM137-IPAHM165	RIL-5	1	LLS
9	GM1911-pPGSseq17E03	RIL-4	1	LLS
	GM1911-TC1D02	RIL-5	1	LLS
10	GM2079-IPAHM103	RIL-4	1	Number of plant per plant
	GM2301-GM2079	RIL-5	1	Pod yield per plant
11	GM2504-GM2746	RIL-4	1	Number of primary branches
	GM2504-GM2746	RIL-5	1	LLS
12	IPAHM407c-GM2425	RIL-4	1	Oil content
	IPAHM407c-GM2444	RIL-5	1	LLS



*Indicates the linkage groups having common markers and LGs assigned according to tetraploid reference map (Varshney *et al.*, 2009a)
 QTL positions for all the traits are shown in colored bars according to legend key.

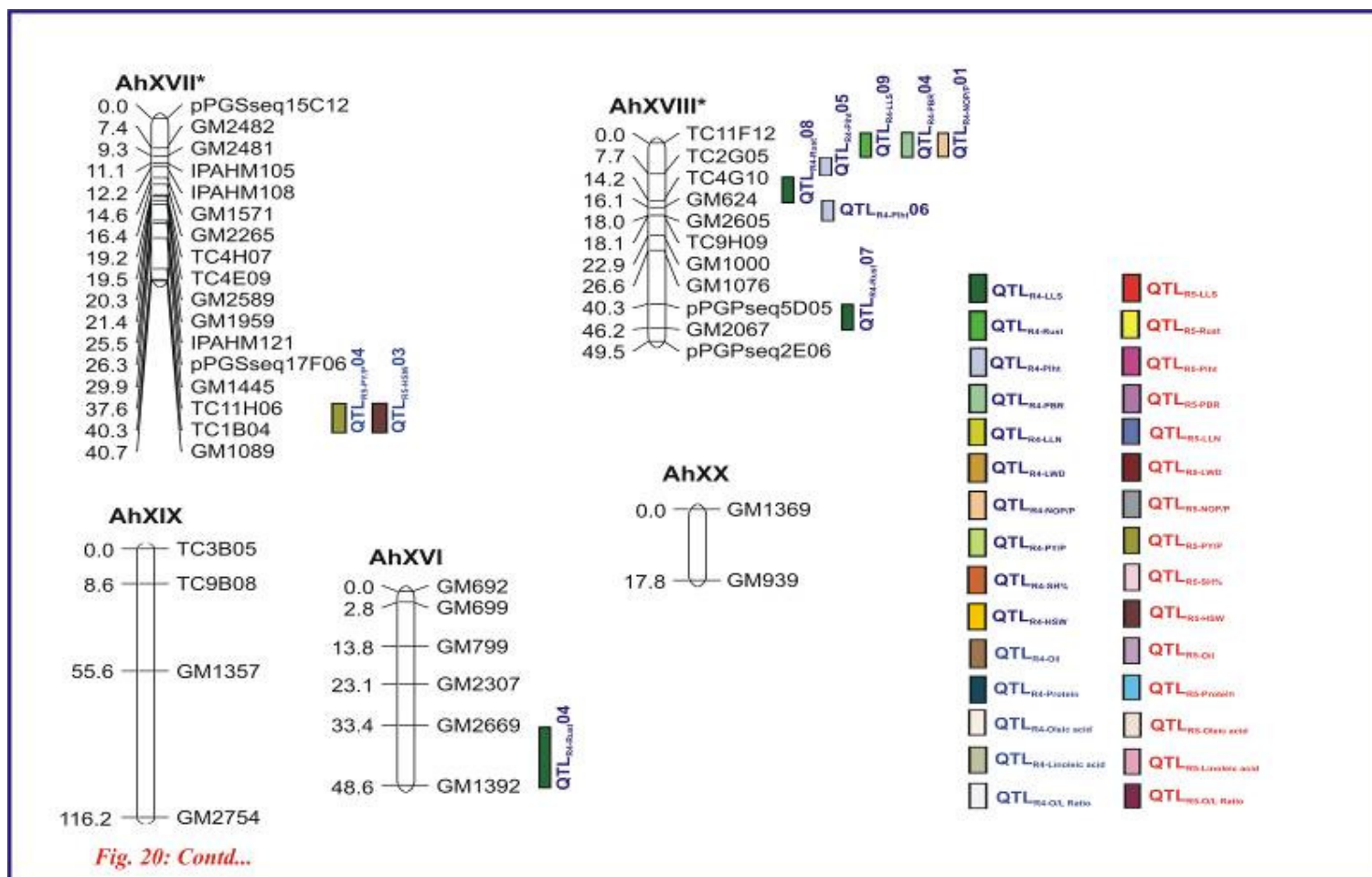
Fig 20 Comprehensive consensus QTL map for diseases, morphological, yield and quality traits



*Indicates the linkage groups having common markers and LGs assigned according to tetraploid reference map (Varshney *et al.*, 2009a)

QTL positions for all the traits are shown in colored bars according to legend key.

Fig 20: Contd...



*Indicates the linkage groups having common markers and LGs assigned according to tetraploid reference map (Varshney *et al.*, 2009a)

QTL positions for all the traits are shown in colored bars according to legend key.

Fig 20: Contd...

However, for the QTL_{R4-Plht}02 (GM 633-PM 179) and QTL_{R5-Plht}02 (PM 179- TC6E01) only one marker (PM 179) was found to be common and based on this it was considered to be a common QTL between populations. These two QTLs consistently contributed for plant height across three seasons in both the populations. Likewise for plant height and leaf length and LLS followed by TC6H03 -1760 in RIL-4 contributed for LLS, oleic acid and oil content on contrary contributed for protein content in RIL-5 population. For the genomic region flanked by GM 1311 and GM 1062 in RIL-4 controlled protein and oil however the same genomic region was not identified for protein and oil content in RIL-5 population, instead it is contributing entirely in a different way towards late leaf spot disease. Similar trends were also observed for the other common genomic regions in both RIL-4 and RIL-5 populations (Table 42). In general it is important to validate the markers before employing them for molecular breeding.

5.4 Consensus QTL map for disease, quality, morphological and yield attributing traits

As the consensus map was developed based on RIL-4 and RIL-5 populations segregating for resistance to foliar diseases (LLS and rust), quality, morphological and yield attributing traits, the QTLs identified separately using RIL-4 and RIL-5 were placed on the respective position on the consensus map. Comparison of QTLs across these two populations the details of the number of QTLs identified in each population were reported and discussed earlier.

A total of 90 and 60 QTLs were localized on the genetic map developed for RIL-4 and RIL-5 population (Table. 35), respectively. All QTLs except QTL_{R5-SH%}01 and QTL_{R5-Rust}04 could be placed at their respective positions on 20 LGs of the consensus map. The number of the QTLs placed on individual LGs ranged from one QTL (AhXI and AhXVI) to 24 QTLs (AhXV) with an average of 8 QTLs per LG. Of the 148 QTLs were placed on the consensus map (Fig. 20), 54 (13 for LLS, 7 for rust, 6 for morphological traits, 14 for yield related traits and 14 for quality traits) were major and 94 (15 for LLS, 6 for rust, 30 for morphological traits, 14 for yield related traits and 29 for quality traits) were minor QTLs. In total, 28 QTLs for late leaf spot, 13 for rust, 36 for morphological characters, 28 for yield attributing traits and 43 QTLs for quality traits were identified in this study.

Consensus QTL map developed in this study was compared with the high density consensus map developed using only tetraploid (Gautami *et al.*, 2012b) and tetraploid/diploids (Shirasawa *et al.*, 2013). Genomic regions, GM 1573-GM 1009/ pPGPseq8D09 (on AhXII) governing resistance to LLS and GM 2009-GM 1954 (on AhXV) governing resistance to rust were contributed by B10 and B03 linkage group of B genome. The QTL for high O/L localized on AhXIII was contributed by A09 linkage group of A genome. Likewise, it was evident that both A and B genomes contributed for other important agronomic traits. Hence, the breeding programs for improving tetraploid groundnut with respect to disease resistance, agronomic and quality traits should depend on recombining selected genomic regions of A and B genomes.

5.5 Validation of rust linked markers

Molecular markers are especially advantageous for traits where conventional phenotypic selection is difficult, expensive and lacks accuracy or precision of phenotyping. Molecular mapping and identification of molecular markers associated with genes and QTLs for traits are prerequisites for the MAS. In this view QTL analysis for rust resistance in RIL-4 and RIL-5 population has enabled the identification potential QTLs for rust resistance. In addition to the earlier identified tightly linked marker IPAHM 103, the present study has significantly contributed for identifying new major QTLs contributing for rust resistance with other new tightly linked markers viz., GM 1536, GM 2301 and GM 2079 contributing up to 82.96 % of phenotypic variance explained. Further for their utilization in marker assisted breeding programs, they need to be validated in determining the target phenotype in independent populations and different genetic backgrounds, which is referred to as 'marker validation' (Cakir *et al.*, 2003; Collins *et al.*, 2003; Jung *et al.*, 1999; Langridge *et al.*, 2001; Li *et al.*, 2001; Sharp *et al.*, 2001).

With an objective of validating the markers linked to rust resistance a germplasm comprising of 64 accessions with wide genetic background was utilized. The germplasm consisted of susceptible and resistant accessions derived from diverse breeding strategies. The germplasm exhibited wide range of disease incidence and genotypes were significantly differing for disease (Table 36). Based on the comparison of genotypic and phenotypic data a good association was observed between the pattern of the allele and their respective phenotype (Table 36 and 37). Single marker analysis (SMA)

was carried out and analysis revealed a significant association with resistance as explained by the phenotypic variance presented in the Table 36. The highest PV was explained by IPAHM 103 ranging from 38.40-55.70%. Similarly all the other markers explained the same PV as they had the same allelic pattern and the PV range was 21.90 to 39.20% (Table 37). However, there were some exceptions with all the markers. IPAHM 103 revealed a disagreement with the genotypes namely Girnar Mosaic, ICGV 91173, Girnar, MN 1-28 and ICGV 86590 which had a resistance reaction against rust but they were identified as susceptible based on susceptible ('A') allelic pattern by IPAHM 103. Conversely the genotypes VR 5, ICGV 87921 and ICGV 86156 though susceptible were identified as resistant lines by IPAHM 103. Similarly the other markers also exhibited exception with the resistant genotypes viz., VL2, VL1, ICGV 87264, ICGV 88256 and Dh 73 but identified as the susceptible genotypes based on susceptible ('A') allelic pattern as compared to IPAHM 103 which exhibited resistant allelic pattern. Genotypes VR 5 and ICGV 87921 susceptible to rust were identified as resistant accessions by IPAHM 103, but in comparison with other new markers linked to resistance these genotypes were identified as susceptible accessions exactly in relation to their disease scores (Table 36). However such a sort of exceptions were observed when a trait is controlled by few number of genes, which will not guarantee that whether DNA markers identified in one population will be useful in different populations, especially when the populations originate from distantly related germplasm (Yu *et al.*, 2000). These exceptions can be resolved when the candidate genes will be identified for rust resistance in the future. In majority of the cases the exceptions were observed in case of accessions derived from mutations and cross derivatives from landraces, however, in case of interspecific derivatives the markers differentiated the resistant and susceptible accessions. Nevertheless, in majority of the cases these markers differentiated successfully the resistant and susceptible genotypes/accessions, indicating their potential to be used in marker assisted breeding program for rust resistance in groundnut.

Future line of work

1. Marker assisted back cross breeding programs can be initiated for rust resistance using the tightly linked markers in the genetic background of elite cultivars,
2. Marker assisted selection in combination with *FAD2A* and *FAD2B* allele can be initiated for simultaneous improvement of rust resistance and quality in groundnut,
3. Identification of "candidate gene" for rust resistance can be initiated to dissect the mechanism of rust resistance,
4. Validation of the QTL linked to late leaf spot can be initiated for further use in molecular breeding
5. Saturation of the genetic maps with high throughput genotyping platforms like SNP's and by sequencing can put new insights in identifying the candidate genomic regions contributing for morphological and yield related traits.

SUMMARY AND CONCLUSIONS

There has been tremendous advances in the mapping of genes controlling quantitative traits through analysis of genomic data and quantitative trait loci (QTL) mapping. Such, attempts in dissecting the complex traits should lead to the identification of candidate genetic factors accountable for the heritable variation of the traits under study. Furthermore, once these genetic factors (QTLs) are mapped, they can be potentially used in the improvement of the traits using molecular markers. As a consequence, the accumulation of favorable alleles will provide genetic material for the development of new improved varieties. With a rationale of identifying the genomic regions contributing for LLS, rust, yield and quality traits two mapping populations based on Recombinant Inbred Lines (RILs) of $F_{6:7}$ generations were developed at the University of Agricultural Sciences, Dharwad, India from the cross TAG 24 $\times$ GPBD 4 (RIL-4; 266 RILs) and TG 26 $\times$ GPBD 4 (RIL-5, 146 RILs) using single-seed descent (SSD) method. Based on these studies two partial genetic maps were developed earlier based on RIL-4 (Khedikar *et al.*, 2010) and RIL-5 (Sarvamangala *et al.*, 2011). With the efforts made earlier only 56 and 45 SSR loci were mapped onto 14 and 8 linkage groups in RIL-4 and RIL-5 population respectively. Keeping in view of the status of genetic mapping on RIL-4 and RIL-5 population, the present aimed at saturating the partial genetic maps and comprehensive QTL analysis for LLS, rust, quality, morphological and yield attributing traits in groundnut.

In addition to the available data on both the populations (Khedikar, 2008, Sarvamangala, 2009 and Mukri, 2011), phenotyping on diseases was undertaken for three more years during Kharif season of 2008, 2009 and 2010. Phenotyping for morphological and yield attributing traits was carried out during Kharif 2007, 2008 and 2010. With the data obtained on both the populations, comprehensive analysis for components of variability has been conducted. Further, the data was also used for QTL analysis to identify associated microsatellite markers/QTLs for foliar disease resistance, nutritional quality and productivity traits.

Analysis of variance showed significant variation among the genotypes, environments and $G \times E$ interaction for all the traits suggesting the need for extensive phenotyping across seasons and/or locations. Both the susceptible parents (TAG 24 and TG 26) exhibited higher incidence of diseases in all the environments as compare to the resistant parent GPBD 4.

Variance components estimated in all the environments for LLS and rust on both the populations indicated ample variability for improving disease resistance. Heritability estimates in broad sense were estimated for all the environments at both the stages of disease scoring for LLS and rust in RIL-4 and RIL-5 population. Heritability for both LLS and rust in RIL-4 population was moderate to high. Genetic advance over percent of mean (GAM) for LLS was high (20.29-56.74) and it was moderate (13.10) to high (74.91) GAM for rust. In case of RIL-5 population GAM was low to high for LLS (8.41-72.50) and rust (10.8-72.76). Heritability for LLS was low to high (26.42-91.31) and moderate to high for rust (34.16-84.01).

For morphological and yield attributing traits, the magnitude of variation in both the population as revealed by PCV was moderate to high for all the traits except for shelling percent. But lower heritability and GAM indicated lower heritable variation for most of these traits except plant height, pod yield per plant and 100-seed weight, which recorded moderate to high values. As compared to season wise estimates, pooled analysis resulted in lower heritable variation thus indicating the predominance of $G \times E$ interaction. Correlations among the yield attributing traits in both the populations were found to be positively associated. Positive association was observed between yield and morphological indicating the role of morphological traits for enhancing the productivity.

In summary, a total of 3,101 SSR markers were screened on parents of RIL-4 population. Similarly a total of 3,284 SSR markers were screened on parental genotypes of RIL-5 population. Segregation data on RILs were obtained for 211 and 210 marker loci in RIL-4 and RIL-5 population.

As the earlier linkage maps developed based on the two populations under study had very less mapped loci, the linkage analysis was done on entire marker dataset in each of RIL population. As a result, an improved genetic map with 190 SSR loci mapped onto 20 linkage groups (LGs) spanning 1963.40 cM was developed based on RIL-4 population. Similarly another genetic map comprising of 182 loci on 21 LGs covering 1982.90 cM distance was developed based on RIL-5 population. As developed genetic maps are based on the populations (RIL-4 and RIL-5) that segregate for LLS and rust, a consensus map was developed based on the 144 common markers. As a result, the consensus map developed in the present study has 227 SSR loci mapped onto 20

LGs of which 18 LGs were integrated from both genetic maps using common markers and remaining two LGs were adopted as such from framework map.

Eventually in the present study with the addition of more markers and QTL analysis based on multiple season phenotypic data for LLS on both RIL-4 and RIL-5 population, identified a total of 13 and 15 QTLs respectively. Among the 13 QTLs five were found to be major QTLs (QTL_{R4-LLS}01, QTL_{R4-LLS}02, QTL_{R4-LLS}03, QTL_{R4-LLS}04 and QTL_{R4-LLS}05) contributing for LLS with PVE ranging from 10.27-67.98% in RIL-4 population. Similarly in RIL-5 population, eight major QTLs were reported with a range of 7.58-63.13% PVE for resistance to LLS. The addition of more SSR markers and development of dense genetic maps has facilitated in defining the proper genomic regions for LLS resistance and for the first time major QTLs contributing for LLS has been identified in cultivated groundnut.

In terms of stability of the identified QTLs in RIL-4 population, only QTL_{R4-LLS}01 (10.27-62.34% PVE), was found consistently in all six season and at both stages of disease scoring, followed by QTL_{R4-LLS}05 (10.81-15.34% PVE in four seasons) and QTL_{R4-LLS}09 (3.39-8.5% PVE in three seasons). However, the other 10 QTLs identified were detected in less than three seasons and/or in either of the two stages or in both the stages with a range of 3.38-67.98% PVE. In case of RIL-5 population, of the 15 QTLs identified none of the QTLs were present in more than three seasons. But two QTLs *i.e.*, QTL_{R5-LLS}01 (7.58-49.64% PVE) and QTL_{R5-LLS}02 (9.79-22.46% PVE) were present at least in two seasons of disease scoring.

Among the major QTLs identified for rust in RIL-4 population, three QTLs (QTL_{R4-Rust}01, QTL_{R4-Rust}02 and QTL_{R4-Rust}03) were found consistently in all the six seasons of disease scoring, except for few stages in specific seasons. Similarly in RIL-5 population only two major QTLs (QTL_{R5-Rust}01 and QTL_{R5-Rust}02) were present in all the seven seasons of disease scoring except for QTL_{R5-Rust}02 which was absent in the year 2005 at both the stages of disease scoring.

Saturation of partial linkage maps developed earlier with additional markers reconfirms the presence of the major QTL for rust resistance in both the population. Further, it has enabled to identify new closely linked markers (GM2009, GM1536, GM2301, GM2079 and GM 1954) with IPAHM103.

In summary as compared to partial genetic maps of RIL-4 and RIL-5 population developed earlier, the present genetic maps are dense in terms of marker density and genome coverage. It has enabled to identify major QTLs for LLS and also new QTLs for rust with new SSR markers.

QTL analysis for morphological traits across three seasons of phenotyping revealed a total of fourteen QTLs in RIL-4 and five QTLs in RIL-5 population for plant height, for number of primary branches it was three QTLs in RIL-4 and one QTL in RIL-5 population. Similarly for both leaf length and leaf width four QTLs for each of the trait were identified in RIL-4 population. However it was only three QTLs for leaf length and one QTL for leaf width in RIL-5 population.

From among the QTLs identified for morphological traits, two QTLs (QTL_{R4-Plht}01 and QTL_{R5-Plht}01; QTL_{R4-Plht}02 and QTL_{R5-Plht}02) were found to be common and stable between the populations for plant height. Similarly, a consistent QTL QTL_{R4-LLN}01 was identified for leaf length in all the three seasons (Kharif 2007, 2008 and 2010) in case of RIL-4 population. Though these QTLs explained less PV but are very important when genomic assisted breeding program are postulated in the future.

QTL detection on the data obtained for yield attributing traits across three years revealed a total of 2 (RIL-4) and 3 (RIL-5) QTLs for number of pods per plant, 8 QTLs in case of RIL-4 and 6 QTLs in RIL-5 population for pod yield per plant. Two QTLs each for shelling percent and three QTLs each for hundred seed weight were identified in both the population (RIL-4 and RIL-5). As compared to the QTLs identified for morphological traits in terms of consistency none of the QTLs were consistent across three seasons. Based on the present study, a total of 90 and 60 QTLs were localized on individual genetic map based on RIL-4 and RIL-5 population respectively. As listed above the present study reports a total of 28 QTLs for late leaf spot, 14 QTLs for rust, 36 QTLs for morphological characters, 29 QTLs for yield attributing traits and 43 QTLs for quality traits.

An attempt was made to compare the contribution of the QTLs for different traits. We could identify a total of 19 genomic regions comparatively contributing across the traits in RIL-4 population. Similarly, 9 genomic regions in RIL-5 population were contributing for more than one trait.

Upon placing all these QTLs in their respective positions on the consensus map a total of 148 QTLs were localized on 20 LGs of the consensus map one QTL from RIL-5 population could not be localised on consensus map. From among the 149 QTLs identified a total of 54 QTLs were major (13

for LLS, 7 for rust, 6 for morphological traits, 14 for yield related traits and 14 for quality traits) and 94 QTLs localized were minor QTLs (15 for LLS, 6 for rust, 30 for morphological traits, 14 for yield related traits and 29 for quality traits). The number of the QTLs placed on individual LGs ranged from one QTL (AhXI and AhXVI) to 24 QTLs (AhXV). However the LGs AhI, AhV, AhVI, AhVII, AhVIII, AhX, AhXII, AhXIII, AhXIV, AhXV and AhXVIII harbored maximum number of QTLs contributing across all the traits. Further upon comparing the QTLs identified individually from both the populations a total of 12 genomic regions were found to be common between populations which are contributing for several traits across populations.

Comparison of the consensus QTL map with high density reference maps developed recently has revealed the contribution of both A and B genome for all the traits under study. However the genomic region responsible for rust resistance is contributed from the B03 linkage group of B genome (AhXV in RIL-4 and RIL-5) and for late leaf spot the QTL flanked with GM 1573-GM 1009/pPGPseq8D09 was contributed from B10 linkage group of B genome (AhXII in RIL-4). Similarly it was observed that the QTL identified for fatty acids linked with allele specific marker of *FAD2A* was harbored on AhXIII in RIL-4 (A09 linkage group of A genome). In general both the genomes are important for the crop improvement programs in tetraploid groundnut.

Identification of new major QTLs contributing for rust resistance with other new linked markers viz., GM 1536, GM 2301 and GM 2079 contributing upto 82.96 % of phenotypic variance explained is a significant contribution from the present study. These markers have been validated in the germplasm of diverse genetic background and can be potentially used in marker assisted breeding program for rust resistance in groundnut.

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*Original articles not seen _____

Appendix I: Chi square values for polymorphic maker loci in two mapping populations

TAG 24 × GPBD 4 Mapping population							
SINo	Locus	a	b	-	χ^2	Signif.	
1	GM1187	130	50	86	35.56	*****	
2	GM1565	98	81	87	1.61	-	
3	GM1531	120	55	91	24.14	*****	
4	GM2233	73	58	135	1.72	-	
5	GM687	135	107	24	3.24	*	
6	pPGSseq13A07	93	75	98	1.93	-	
7	GM1090	92	84	90	0.36	-	
8	GM1501	97	81	88	1.44	-	
9	GM1771	125	54	87	28.16	*****	
10	GM2724a	126	53	87	29.77	*****	
11	GM1971a	119	61	86	18.69	*****	
12	TC1E01	87	93	86	0.2	-	
13	PM73	84	93	89	0.46	-	
14	pPGPseq2D12B	131	117	18	0.79	-	
15	TC9F04	134	122	10	0.56	-	
16	TC1B02	129	111	26	1.35	-	
17	pPGSseq18E07	80	93	93	0.98	-	
18	TC7E04	57	117	92	20.69	*****	
19	GM2691	91	76	99	1.35	-	
20	pPGSseq19D09	113	114	39	0	-	
21	pPGSseq19G07	136	116	14	1.59	-	
22	GM1717	90	90	86	0	-	
23	pPGSseq18A05	96	84	86	0.8	-	
24	pPGSseq18A05b	129	123	14	0.14	-	
25	GM745	127	123	16	0.06	-	
26	Ah-4-04	121	112	33	0.35	-	
27	AC3D07	125	127	14	0.02	-	
28	PM3	126	117	23	0.33	-	
29	PM434	132	110	24	2	-	
30	TC2C07	129	123	14	0.14	-	
31	TC4F02	147	111	8	5.02	**	
32	GM2215	97	81	88	1.44	-	
33	pPGSseq13E06	131	126	9	0.1	-	
34	GM2206	98	80	88	1.82	-	
35	PM418	72	107	87	6.84	***	
36	PM183	139	118	9	1.72	-	
37	PM50	132	119	15	0.67	-	
38	TC11B04	122	58	86	22.76	*****	
39	GM1555	83	97	86	1.09	-	
40	IPAHM272	135	112	19	2.14	-	
41	pPGSseq19D06	124	112	30	0.61	-	
42	GM1641	92	85	89	0.28	-	
43	GM2084	88	87	91	0.01	-	
44	GM1878	81	94	91	0.97	-	
45	IPAHM356	99	80	87	2.02	-	
46	GM1577	89	91	86	0.02	-	
47	TC3A12	88	92	86	0.09	-	
48	GM1955	96	84	86	0.8	-	

Contd...

TAG 24 × GPBD 4 Mapping population							
SINo	Locus	a	b	-	χ^2	Signif.	
49	GM1007	83	95	88	0.81	-	
50	GM633	119	141	6	1.86	-	
51	PM179	133	126	7	0.19	-	
52	seq11C08	117	130	19	0.68	-	
53	TC6E01	96	84	86	0.8	-	
54	GM1988	92	87	87	0.14	-	
55	GM1989	99	80	87	2.02	-	
56	PM377	144	113	9	3.74	*	
57	TC1A01	125	139	2	0.74	-	
58	S108	93	87	86	0.2	-	
59	GM1097	85	88	93	0.05	-	
60	TC7C06	83	95	88	0.81	-	
61	IPAHM395	112	127	27	0.94	-	
62	TC5A07	130	116	20	0.8	-	
63	GM2246	98	78	90	2.27	-	
64	GM2589	93	84	89	0.46	-	
65	GM2480	101	79	86	2.69	-	
66	GM1311	90	89	87	0.01	-	
67	GM1062	81	99	86	1.8	-	
68	GM2638	87	93	86	0.2	-	
69	GM2637	79	101	86	2.69	-	
70	GM2313	80	98	88	1.82	-	
71	GM2536	97	80	89	1.63	-	
72	GM1986	79	99	88	2.25	-	
73	IPAHM606	92	88	86	0.09	-	
74	GM2504	85	91	90	0.2	-	
75	GM2746	84	94	88	0.56	-	
76	GM2689	82	97	87	1.26	-	
77	GM2690	83	97	86	1.09	-	
78	GM660	133	126	7	0.19	-	
79	AH-4-101	132	119	15	0.67	-	
80	TC9F10	132	132	2	0	-	
81	TC6H03	132	132	2	0	-	
82	GM1760	88	90	88	0.02	-	
83	GM1483	92	83	91	0.46	-	
84	GM1979	94	86	86	0.36	-	
85	TC2D08	104	76	86	4.36	**	
86	GM1603	93	84	89	0.46	-	
87	TC5A06	125	113	28	0.61	-	
88	pPGSseq14H06	98	82	86	1.42	-	
89	PM436	123	104	39	1.59	-	
90	Lec-1	132	106	28	2.84	*	
91	gi1107	112	100	54	0.68	-	
92	pPGPseq7G02	134	124	8	0.39	-	
93	GM2120	83	91	92	0.37	-	
94	GM1893	87	90	89	0.05	-	
95	pPGSseq17C09	131	123	12	0.25	-	
96	GM2584	93	84	89	0.46	-	
97	IPAHM407c	137	122	7	0.87	-	
98	GM2425	99	81	86	1.8	-	
99	GM2444	98	82	86	1.42	-	

Contd...

TAG 24 x GPBD 4 Mapping population							
SINo	Locus	a	b	-	χ^2	Signif.	
100	GM884	97	82	87	1.26	-	
101	GM1991	96	84	86	0.8	-	
102	PM137	136	123	7	0.65	-	
103	GM670	106	129	31	2.25	-	
104	pPGSseq19A05	71	64	131	0.36	-	
105	TC11A04	115	147	4	3.91	**	
106	TC1A02	113	140	13	2.88	*	
107	IPAHM524	97	101	68	0.08	-	
108	TC3H07	90	89	87	0.01	-	
109	TC4D09	114	130	22	1.05	-	
110	GM2724b	82	97	87	1.26	-	
111	GM1971b	86	86	94	0	-	
112	Ah-193	74	103	89	4.75	**	
113	TC4H02	88	88	90	0	-	
114	pPGSseq13A10	70	84	112	1.27	-	
115	pPGPseq7H06	92	87	87	0.14	-	
116	IPAHM176	142	117	7	2.41	-	
117	pPGPseq3E10	89	85	92	0.09	-	
118	TC3E05	145	108	13	5.41	**	
119	TC7H11	151	103	12	9.07	****	
120	GM2531	106	74	86	5.69	**	
121	PM675	95	84	87	0.68	-	
122	GM1573	82	95	89	0.95	-	
123	GM1009	91	87	88	0.09	-	
124	pPGPseq8D09	145	101	20	7.87	***	
125	TC5D06	91	88	87	0.05	-	
126	GM1911	94	84	88	0.56	-	
127	pPGSseq17E03	120	76	70	9.88	****	
128	TC1D02	80	100	86	2.22	-	
129	GM2259	81	99	86	1.8	-	
130	pPGSseq18G01	122	133	11	0.47	-	
131	pPGSseq19B01	67	109	90	10.02	****	
132	GM2407	87	92	87	0.14	-	
133	TC3E02	69	105	92	7.45	***	
134	GM2687	98	81	87	1.61	-	
135	GM1386	113	67	86	11.76	*****	
136	GM1162	86	94	86	0.36	-	
137	GM1021	98	81	87	1.61	-	
138	GM2730	97	82	87	1.26	-	
139	GM1740	87	89	90	0.02	-	
140	GM1846	81	96	89	1.27	-	
141	GM1773	86	94	86	0.36	-	
142	GM840	74	98	94	3.35	*	
143	GM912	55	123	88	25.98	*****	
144	GM2009	89	91	86	0.02	-	
145	GM1536	85	93	88	0.36	-	
146	GM2301	83	97	86	1.09	-	
147	GM2079	84	94	88	0.56	-	
148	IPAHM103	126	134	6	0.25	-	
149	GM1954	84	96	86	0.8	-	
150	TC4G02	82	97	87	1.26	-	

Contd...

TAG 24 × GPBD 4 Mapping population						
SiNo	Locus	a	b	-	χ^2	Signif.
151	pPGPseq2B10	122	127	17	0.1	-
152	GM2053	96	81	89	1.27	-
153	GM1996	95	82	89	0.95	-
154	GM1883	87	91	88	0.09	-
155	GM1502	92	80	94	0.84	-
156	GM692	107	143	16	5.18	**
157	GM699	115	131	20	1.04	-
158	GM799	94	81	91	0.97	-
159	GM2307	95	84	87	0.68	-
160	GM2669	88	91	87	0.05	-
161	GM1392	89	90	87	0.01	-
162	pPGSseq15C12	110	68	88	9.91	****
163	GM2482	109	70	87	8.5	****
164	GM2481	98	81	87	1.61	-
165	IPAHM108	135	110	21	2.55	-
166	GM2265	85	95	86	0.56	-
167	TC4E09	150	107	9	7.19	***
168	GM1959	95	82	89	0.95	-
169	IPAHM121	143	111	12	4.03	**
170	GM1445	95	85	86	0.56	-
171	pPGSseq17F06	96	84	86	0.8	-
172	TC11H06	96	84	86	0.8	-
173	GM1089	82	94	90	0.82	-
174	TC11F12	94	86	86	0.36	-
175	TC2G05	109	129	28	1.68	-
176	TC4G10	114	146	6	3.94	**
177	GM624	115	142	9	2.84	*
178	TC9H09	113	137	16	2.3	-
179	GM1000	92	84	90	0.36	-
180	GM1076	92	87	87	0.14	-
181	pPGPseq5D05	137	127	2	0.38	-
182	GM2067	96	84	86	0.8	-
183	TC9B08	120	134	12	0.77	-
184	TC3B05	103	105	58	0.02	-
185	GM1357	68	112	86	10.76	****
186	GM2754	92	88	86	0.09	-
187	GM1369	42	136	88	49.64	*****
188	GM939	66	114	86	12.8	*****
189	GM822	64	115	87	14.53	*****
190	GM1391	117	63	86	16.2	*****
191	GM1073	72	108	86	7.2	***
192	GM1418	82	98	86	1.42	-
193	GM1477	97	78	91	2.06	-
194	GM1745	69	110	87	9.39	****
195	GM1752	87	89	90	0.02	-
196	GM1821	69	111	86	9.8	****
197	GM1902	114	61	91	16.05	*****
198	GM1949	124	55	87	26.6	*****
199	GM2671	78	99	89	2.49	-
200	TC3H02	135	127	4	0.24	-
201	GM1395	91	84	91	0.28	-

Contd...

TAG 24 × GPBD 4 Mapping population							
SINo	Locus	a	b	-	X ²	Signif.	
202	GM2747	82	98	86	1.42	-	
203	pPGPseq2E06	127	109	30	1.37	-	
204	GM2250	95	83	88	0.81	-	
205	pPGSseq9E08	91	141	34	10.78	****	
206	GM1809	102	78	86	3.2	*	
207	GM1494	92	88	86	0.09	-	
208	GM1839	95	85	86	0.56	-	
209	TC4F12	122	139	5	1.11	-	

Contd...

TG 26 x GPBD 4 Mapping population						
SINo	Locus	a	b	-	χ^2	Signif.
1	GM1565	60	77	9	2.11	-
2	GM2308	64	72	10	0.47	-
3	IPAHM287	71	66	9	0.18	-
4	GM1531	69	69	8	0	-
5	GM2233	64	72	10	0.47	-
6	pPGSseq13A07	57	65	24	0.52	-
7	GM1090	70	64	12	0.27	-
8	GM1771	78	60	8	2.35	-
9	GM1971a	78	60	8	2.35	-
10	GM2724a	75	59	12	1.91	-
11	PM73	65	70	11	0.19	-
12	PM499	63	75	8	1.04	-
13	pPGPseq1B09	65	73	8	0.46	-
14	pPGPseq2D12B	67	63	16	0.12	-
15	TC1B02	59	70	17	0.94	-
16	pPGSseq18E07	67	70	9	0.07	-
17	TC2C07	30	107	9	43.28	*****
18	IPAHM295	26	120	0	60.52	*****
19	AC3D07	71	72	3	0.01	-
20	TC0A01	63	81	2	2.25	-
21	GM1717	65	73	8	0.46	-
22	pPGSseq18A05	65	72	9	0.36	-
23	GM2215	72	66	8	0.26	-
24	pPGSseq13E06	70	68	8	0.03	-
25	GM2206	75	63	8	1.04	-
26	PM418	68	70	8	0.03	-
27	PM183	80	51	15	6.42	**
28	TC11B04	84	54	8	6.52	**
29	GM1555	82	55	9	5.32	**
30	IPAHM282	84	54	8	6.52	**
31	PM36	78	53	15	4.77	**
32	IPAHM272	87	48	11	11.27	*****
33	TC1D12	88	51	7	9.85	****
34	pPGSseq19D06	86	43	17	14.33	*****
35	GM1641	85	56	5	5.96	**
36	IPAHM75	80	62	4	2.28	-
37	GM2084	70	61	15	0.62	-
38	IPAHM352	77	67	2	0.69	-
39	TC1G04	81	57	8	4.17	**
40	GM1878	61	67	18	0.28	-
41	IPAHM356	62	76	8	1.42	-
42	GM1577	53	85	8	7.42	***
43	TC3A12	64	74	8	0.72	-
44	TC2B09	58	72	16	1.51	-
45	GM1955	63	74	9	0.88	-
46	GM1007	62	73	11	0.9	-
47	PM179	65	73	8	0.46	-
48	TC6E01	63	71	12	0.48	-

Contd...

TG 26 x GPBD 4 Mapping population							
SINo	Locus	a	b	-	χ^2	Signif.	
49	RN16F05	49	81	16	7.88	***	
50	GM1988	58	71	17	1.31	-	
51	GM1989	76	62	8	1.42	-	
52	IPAHM171c	72	65	9	0.36	-	
53	S108	70	68	8	0.03	-	
54	GM1097	68	69	9	0.01	-	
55	TC3H07a	78	60	8	2.35	-	
56	TC7C06	86	52	8	8.38	****	
57	IPAHM395	72	66	8	0.26	-	
58	TC5A07	72	65	9	0.36	-	
59	GM2246	73	63	10	0.74	-	
60	GM2589a	92	44	10	16.94	*****	
61	GM2480	82	56	8	4.9	**	
62	GM1311	74	60	12	1.46	-	
63	GM1867	82	56	8	4.9	**	
64	S109	79	59	8	2.9	*	
65	GM1062	60	77	9	2.11	-	
66	GM2638	60	78	8	2.35	-	
67	GM2637	61	74	11	1.25	-	
68	GM2313	65	73	8	0.46	-	
69	GM2536	75	62	9	1.23	-	
70	GM1986	68	70	8	0.03	-	
71	IPAHM606	65	73	8	0.46	-	
72	GM2504	76	62	8	1.42	-	
73	GM2746	82	56	8	4.9	**	
74	GM2689	63	75	8	1.04	-	
75	GM2690	68	68	10	0	-	
76	TC9F10	70	68	8	0.03	-	
77	TC6H03	78	64	4	1.38	-	
78	GM1760	81	53	12	5.85	**	
79	GM1483	73	65	8	0.46	-	
80	GM1979	76	62	8	1.42	-	
81	TC2D08	73	65	8	0.46	-	
82	GM1603	77	60	9	2.11	-	
83	TC5A06	75	63	8	1.04	-	
84	pPGSseq14H06	74	63	9	0.88	-	
85	GM2120	62	76	8	1.42	-	
86	IPAHM407c	69	65	12	0.12	-	
87	GM2444	65	73	8	0.46	-	
88	GM884	80	58	8	3.51	*	
89	GM1991	85	46	15	11.61	*****	
90	PM137	70	70	6	0	-	
91	IPAHM165	72	66	8	0.26	-	
92	pPGSseq19A05	54	61	31	0.43	-	
93	TC11A04	78	61	7	2.08	-	
94	IPAHM524	68	69	9	0.01	-	
95	TC3H07	63	75	8	1.04	-	
96	GM2724b	55	83	8	5.68	**	
97	GM1971b	69	65	12	0.12	-	
98	TC4H02	72	71	3	0.01	-	
99	pPGPseq7H06	59	78	9	2.64	-	

Contd...

TG 26 × GPBD 4 Mapping population						
SINo	Locus	a	b	-	χ^2	Signif.
100	IPAHM176	64	79	3	1.57	-
101	TC3E05	62	77	7	1.62	-
102	IPAHM531	51	79	16	6.03	**
103	TC7H11	55	84	7	6.05	**
104	GM2531	61	77	8	1.86	-
105	PM675	62	76	8	1.42	-
106	IPAHM23	55	90	1	8.45	****
107	IPAHM475	69	75	2	0.25	-
108	GM1573	65	73	8	0.46	-
109	GM2032	64	69	13	0.19	-
110	pPGPseq8D09	61	77	8	1.86	-
111	TC5D06	58	80	8	3.51	*
112	GM1911	82	56	8	4.9	**
113	TC1D02	64	74	8	0.72	-
114	TC9B07	68	61	17	0.38	-
115	GM2259	78	60	8	2.35	-
116	pPGSseq18G01	77	65	4	1.01	-
117	pPGSseq19B01	73	65	8	0.46	-
118	GM2407	71	66	9	0.18	-
119	TC3E02	73	67	6	0.26	-
120	GM1609	73	63	10	0.74	-
121	GM2687	77	61	8	1.86	-
122	GM1386	63	75	8	1.04	-
123	GM1162	63	75	8	1.04	-
124	GM1021	44	94	8	18.12	*****
125	GM2730	64	74	8	0.72	-
126	GM1740	68	70	8	0.03	-
127	GM1572	62	76	8	1.42	-
128	pPGSseq16C06	68	70	8	0.03	-
129	GM1734	73	65	8	0.46	-
130	GM1773	66	72	8	0.26	-
131	GM840	56	82	8	4.9	**
132	GM955	63	74	9	0.88	-
133	GM2009	76	61	9	1.64	-
134	GM1536	75	62	9	1.23	-
135	GM2301	74	60	12	1.46	-
136	GM2079	74	60	12	1.46	-
137	IPAHM103	75	64	7	0.87	-
138	GM1954	72	60	14	1.09	-
139	TC4G02	94	44	8	18.12	*****
140	pPGPseq2B10	71	67	8	0.12	-
141	pPGPseq3F05	52	82	12	6.72	***
142	GM1083	73	60	13	1.27	-
143	GM2069	64	74	8	0.72	-
144	GM2053	60	78	8	2.35	-
145	GM1996	56	78	12	3.61	*
146	GM1883	56	75	15	2.76	*
147	GM1502	51	86	9	8.94	****
148	GM799	68	70	8	0.03	-
149	GM2669	63	75	8	1.04	-
150	GM2307	68	70	8	0.03	-

Contd...

TG 26 x GPBD 4 Mapping population							
SINo	Locus	a	b	-	χ^2	Signif.	
151	GM1392	68	70	8	0.03	-	
152	pPGSseq15C12	82	54	10	5.76	**	
153	GM2482	79	57	10	3.56	*	
154	GM2481	76	58	12	2.42	-	
155	GM2589b	72	58	16	1.51	-	
156	IPAHM108	75	66	5	0.57	-	
157	IPAHM105	80	64	2	1.78	-	
158	GM2265	75	61	10	1.44	-	
159	GM1571	75	61	10	1.44	-	
160	GM1959	79	59	8	2.9	*	
161	TC4H07	69	62	15	0.37	-	
162	IPAHM121	80	48	18	8	****	
163	GM1445	73	65	8	0.46	-	
164	pPGSseq17F06	69	64	13	0.19	-	
165	TC11H06	73	65	8	0.46	-	
166	TC1B04	75	64	7	0.87	-	
167	TC11F12	73	64	9	0.59	-	
168	TC2G05	72	64	10	0.47	-	
169	GM1000	65	69	12	0.12	-	
170	GM1076	71	65	10	0.26	-	
171	GM2605	66	72	8	0.26	-	
172	pPGPseq5D05	69	69	8	0	-	
173	GM2067	65	67	14	0.03	-	
174	pPGPseq2E06	72	66	8	0.26	-	
175	GM2602	71	67	8	0.12	-	
176	GM2603	67	69	10	0.03	-	
177	pPGSseq16G08	72	66	8	0.26	-	
178	RN19A01	70	64	12	0.27	-	
179	GM1291	94	43	9	18.99	*****	
180	TC4F12	72	65	9	0.36	-	
181	pPGSseq11G07	79	61	6	2.31	-	
182	GM2250	66	70	10	0.12	-	
183	seq4E08	68	62	16	0.28	-	
184	TC4F10	56	73	17	2.24	-	
185	TC2E05	64	74	8	0.72	-	
186	PM433	79	49	18	7.03	***	
187	GM1958	85	50	11	9.07	****	
188	GM1995	95	43	8	19.59	*****	
189	GM2020	73	65	8	0.46	-	
190	GM1937	51	87	8	9.39	****	
191	GM1533	73	65	8	0.46	-	
192	GM1540	66	66	14	0	-	
193	GM1562	73	64	9	0.59	-	
194	GM1356	67	71	8	0.12	-	
195	TC9B08	70	71	5	0.01	-	
196	GM822	56	82	8	4.9	**	
197	GM1369	58	80	8	3.51	*	
198	pPGSseq19D09	84	54	8	6.52	**	
199	GM2754	68	70	8	0.03	-	
200	GM1418	67	71	8	0.12	-	
201	GM1391	61	77	8	1.86	-	
202	GM1357	100	38	8	27.86	*****	
203	GM1494	59	79	8	2.9	*	
204	GM1949	62	76	8	1.42	-	
205	GM1745	77	61	8	1.86	-	
206	GM1752	87	51	8	9.39	****	
207	GM1821	78	60	8	2.35	-	
208	GM1902	55	83	8	5.68	**	
209	GM2350	95	37	14	25.48	*****	

Appendix II: Details of individual and consensus maps with marker distances

Colored text indicates common mapped loci between populations					
TAG 24 × GPBD 4 Mapmaker 3.0		TG 26 × GPBD 4 Mapmaker 3.0		Consensus Map JoinMap 4.0 (Kosambi)	
RIL-4		RIL-5		Consensus Map	
Ahl*		Ahl*		Ahl*	
GM1187	0.00	GM1565	0.00	GM1187	0.00
GM1565	21.10	GM2308	5.50	GM2308	1.40
GM1531	36.30	IPAHM287	20.90	GM1565	5.67
GM2233	42.80	GM1531	33.20	GM1531	16.70
GM687	45.70	GM2233	41.90	GM2233	22.51
pPGSseq13A07	46.10	pPGSseq13A07	54.60	IPAHM287	26.13
GM1090	59.60	GM1090	61.50	GM687	26.55
GM1501	72.30	GM1771	85.90	pPGSseq13A07	28.61
GM1771	84.20	GM1971a	87.10	GM1090	34.86
GM2724a	85.10	GM2724a	87.10	GM1501	36.91
GM1971a	89.60			GM1771	48.17
				GM2724a	50.02
				GM1971a	52.91
AhII*		AhII*		AhII*	
TC1E01	0.00	PM73	0.00	TC1E01	0.00
PM73	11.30	PM499	15.30	PM73	10.22
pPGPseq2D12B	32.00	pPGPseq1B09	17.10	PM499	20.44
TC9F04	48.20	pPGPseq2D12B	38.50	pPGPseq1B09	23.27
TC1B02	67.00	TC1B02	70.70	pPGPseq2D12B	29.91
pPGSseq18E07	73.30	pPGSseq18E07	85.50	TC9F04	42.74
				TC1B02	56.92
				pPGSseq18E07	63.37
AhIII*		AhIIIa*		AhIII*	
TC7E04	0.00	TC2C07	0.00	GM2691	0.00
GM2691	27.20	IPAHM295	20.20	pPGSseq19D09	2.25
pPGSseq19D09	57.70			TC7E04	12.52
pPGSseq19G07	82.20	AhIIIb*		pPGSseq19G07	19.81
GM1717	91.80	AC3D07	0.00	PM3	25.82
pPGSseq18A05	92.50	TC0A01	15.10	PM434	26.82
pPGSseq18A05b	92.50			TC4F02	28.50
GM745	92.50	AhIIIc*		pPGSseq18A05	29.93
Ah-4-04	94.00	GM1717	0.00	pPGSseq18A05b	31.31
AC3D07	94.50	pPGSseq18A05	2.60	GM745	31.87
PM3	97.70	GM2215	3.50	Ah-4-04	32.11
PM434	100.20	pPGSseq13E06	13.60	GM1717	33.20
TC2C07	118.00	GM2206	22.70	AC3D07	34.62
TC4F02	135.40			GM2215	37.27
GM2215	143.70			pPGSseq13E06	45.11
pPGSseq13E06	158.20			GM2206	47.99
GM2206	165.80			TC2C07	51.31

Contd...

RIL-4		RIL-5		Consensus Map	
AhIV*		AhIV*		AhIV*	
PM418	0.00	PM418	0.00	PM418	0.00
PM183	14.20	PM183	8.30	PM183	10.79
PM50	18.30	TC11B04	17.90	PM50	15.54
TC11B04	30.80	GM1555	35.80	TC11B04	20.07
GM1555	47.90	IPAHM282	56.30	IPAHM282	23.84
IPAHM272	62.80	PM36	57.90	PM36	25.52
pPGSseq19D06	66.90	IPAHM272	61.70	TC1D12	29.11
GM1641	77.00	TC1D12	68.60	GM1555	31.83
GM2084	87.30	pPGSseq19D06	81.50	IPAHM272	33.06
		GM1641	98.40	pPGSseq19D06	39.86
		IPAHM75	109.90	GM1641	41.80
		GM2084	116.20	IPAHM75	45.61
		IPAHM352	120.00	GM2084	52.03
		TC1G04	136.20	IPAHM352	55.46
				TC1G04	60.97
AhV*		AhV*		AhV*	
GM1878	0.00	GM1878	0.00	GM1878	0.00
IPAHM356	27.00	IPAHM356	6.40	RN16F05	2.68
GM1577	43.20	GM1577	11.30	IPAHM356	8.24
TC3A12	55.70	TC3A12	17.40	GM1577	10.59
GM1955	63.20	TC2B09	19.40	TC3A12	16.05
GM1007	68.50	GM1955	38.60	GM1955	18.30
GM633	71.50	GM1007	62.10	GM1007	20.48
PM179	75.40	PM179	65.30	TC2B09	20.83
seq11C08	81.80	TC6E01	81.80	PM179	23.40
TC6E01	96.90	RN16F05	116.10	GM633	25.23
GM1988	118.80	GM1988	143.10	seq11C08	31.20
GM1989	139.20	GM1989	149.70	TC6E01	31.34
				GM1989	36.08
				GM1988	41.97
AhVI*		AhVI*		AhVI*	
PM377	0.00	IPAHM171c	0.00	PM377	0.00
TC1A01	7.60	S108	14.40	IPAHM171c	5.52
S108	10.70	GM1097	46.90	TC1A01	8.51
GM1097	22.10	TC3H07a	60.20	S108	12.40
TC7C06	26.50	TC7C06	84.90	GM1097	23.78
				TC7C06	33.67
				TC3H07a	40.77

Contd...

RIL-4		RIL-5		Consensus Map	
AhVII*		AhVII*		AhVII*	
IPAHM395	0.00	IPAHM395	0.00	IPAHM395	0.00
TC5A07	9.60	TC5A07	8.80	TC5A07	6.59
GM2246	13.00	GM2246	19.10	GM2246	10.11
GM2589	20.60	GM2589	27.80	GM2589	14.22
GM2480	30.50	GM2480	34.30	S109	15.38
GM1311	42.60	GM1311	42.50	GM1867	20.51
GM1062	54.10	GM1867	61.80	GM2480	22.78
GM2638	60.10	S109	68.50	GM1311	29.69
GM2637	61.80	GM1062	91.60	GM1062	38.69
GM2313	78.70	GM2638	99.70	GM2638	44.51
		GM2637	99.70	GM2637	47.37
		GM2313	104.10	GM2313	54.30
AhVIII*		AhVIII*		AhVIII*	
GM2536	0.00	GM2536	0.00	GM2536	0.00
GM1986	24.50	GM1986	10.90	GM1986	13.44
IPAHM606	32.20	IPAHM606	16.20	IPAHM606	18.24
GM2504	33.00	GM2504	20.70	GM2504	19.94
GM2746	33.30	GM2746	34.50	GM2746	21.47
GM2689	33.30	GM2689	52.90	GM660	23.32
GM2690	33.30	GM2690	58.60	GM2689	23.55
GM660	33.30	TC9F10	69.70	GM2690	24.76
AH-4-101	35.20	TC6H03	72.40	AH-4-101	26.53
TC9F10	41.50	GM1760	77.90	TC9F10	30.86
TC6H03	41.50			TC6H03	31.85
GM1760	50.50			GM1760	38.67
AhIX*		AhIX*		AhIX*	
GM1483	0.00	GM1483	0.00	GM1483	0.00
GM1979	21.50	GM1979	7.90	GM1979	10.48
TC2D08	31.80	TC2D08	12.90	TC2D08	14.62
GM1603	43.00	GM1603	20.80	GM1603	19.30
TC5A06	45.90	TC5A06	22.10	TC5A06	21.42
pPGSseq14H06	51.30	pPGSseq14H06	22.10	pPGSseq14H06	24.36
PM436	53.40	GM2120	35.90	PM436	26.27
Lec-1	56.80			Lec-1	29.27
gi1107	56.80			gi1107	29.33
pPGPseq7G02	65.70			pPGPseq7G02	37.84
GM2120	74.60			GM2120	40.54
GM1893	91.80			GM1893	52.18
pPGSseq17C09	104.80			pPGSseq17C09	61.99
GM2584	127.60			GM2584	86.51

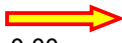
Contd...

RIL-4		RIL-5		Consensus Map	
AhX*		AhX*		AhX*	
IPAHM407c	0.00	IPAHM407c	0.00	IPAHM407c	0.00
GM2425	20.60	GM2444	6.90	GM2425	10.09
GM2444	39.80	GM884	28.10	GM2444	11.95
GM884	59.60	GM1991	40.40	TC3H07	16.46
GM1991	62.00	PM137	45.70	GM884	18.73
PM137	66.80	IPAHM165	47.80	GM1991	21.82
GM670	68.90	pPGSseq19A05	49.10	PM137	24.74
pPGSseq19A05	72.10	TC11A04	57.90	IPAHM165	24.76
TC11A04	74.30	IPAHM524	68.40	GM670	27.31
TC1A02	74.30	TC3H07	81.10	pPGSseq19A05	27.33
IPAHM524	77.80			TC1A02	31.54
TC3H07	89.20			TC11A04	31.85
TC4D09	109.40			IPAHM524	35.68
				TC4D09	44.14
AhXI*		AhXI*		AhXI*	
GM2724b	0.00	GM2724b	0.00	Ah-193	0.00
GM1971b	4.70	GM1971b	6.00	GM1971b	19.51
Ah-193	26.70	TC4H02	24.60	GM2724b	25.44
TC4H02	64.50			pPGSseq13A10	38.39
pPGSseq13A10	81.90			TC4H02	51.86
AhXII*		AhXII*		AhXII*	
pPGPseq7H06	0.00	pPGPseq7H06	0.00	pPGPseq7H06	0.00
IPAHM176	1.90	IPAHM176	9.60	IPAHM176	3.23
pPGPseq3E10	9.80	TC3E05	19.30	pPGPseq3E10	7.50
TC3E05	19.40	IPAHM531	26.20	TC3E05	9.72
TC7H11	23.20	TC7H11	27.60	IPAHM531	12.22
GM2531	30.00	GM2531	34.10	TC7H11	14.38
PM675	45.50	PM675	39.40	GM2531	17.75
GM1573	64.70	IPAHM23	44.50	IPAHM23	20.98
GM1009	87.80	IPAHM475	74.20	PM675	24.63
pPGPseq8D09	117.90	GM1573	83.30	GM1573	38.30
		GM2032	95.30	IPAHM475	42.50
		pPGPseq8D09	121.00	GM2032	51.74
				GM1009	56.60
				pPGPseq8D09	67.57
					Contd...

RIL-4		RIL-5		→	Consensus Map	
AhXIII*		AhXIII*			AhXIII*	
ahFAD2A	0.00				ahFAD2A	0.00
FAD2A	17.80	FAD2A	0.00		FAD2A	21.63
TC5D06	42.80	TC5D06	20.50		TC5D06	30.81
GM1911	59.40	GM1911	53.50		GM1911	41.32
pPGSseq17E03	68.20	TC1D02	73.10		pPGSseq17E03	48.18
TC1D02	73.80	TC9B07	94.60		TC9B07	52.12
GM2259	100.70	GM2259	108.30		TC1D02	53.92
pPGSseq18G01	112.10	pPGSseq18G01	124.50		GM2259	68.67
pPGSseq19B01	130.00	pPGSseq19B01	140.50		pPGSseq18G01	79.13
GM2407	144.10	GM2407	157.60		pPGSseq19B01	87.73
TC3E02	159.00	TC3E02	176.10		GM2407	99.76
		GM1609	189.10		TC3E02	117.80
					GM1609	131.53

AhXIV*		AhXIV*		→	AhXIV*	
GM2687	0.00	GM2687	0.00		GM2687	0.00
GM1386	15.10	GM1386	29.30		GM1162	9.84
GM1162	34.50	GM1162	50.80		GM1386	19.62
GM1021	51.50	GM1021	59.20		GM1021	22.70
GM2730	73.60	GM2730	74.50		GM2730	32.00
GM1740	98.50	GM1740	100.10		GM1740	36.06
GM1846	118.20	GM1572	111.70		GM1572	37.40
GM1773	158.10	pPGSseq16C06	136.40		GM955	39.42
GM840	174.10	GM1734	151.10		GM1846	44.34
GM912	192.40	GM1773	172.60		pPGSseq16C06	45.93
		GM840	191.40		GM840	50.58
		GM955	208.60		GM1734	52.04
					GM1773	64.83
					GM912	72.14

AhXV		AhXV		→	AhXV	
GM2009	0.00	GM2009	0.00		GM2009	0.00
GM1536	10.50	GM1536	2.60		GM1536	5.12
GM2301	12.80	GM2301	3.50		GM2301	7.39
GM2079	12.80	GM2079	3.90		GM2079	8.79
IPAHM103	14.50	IPAHM103	5.40		IPAHM103	10.38
GM1954	31.90	GM1954	14.20		GM1954	20.56
TC4G02	39.70	TC4G02	27.10		GM1083	29.27
pPGPseq2B10	55.90	pPGPseq2B10	61.10		TC4G02	29.48
GM2053	77.00	pPGPseq3F05	71.80		pPGPseq2B10	34.54
GM1996	89.30	GM1083	101.70		GM2069	37.34
GM1883	99.80	GM2069	107.50		GM2053	40.83
GM1502	116.50	GM2053	134.30		pPGPseq3F05	46.36
		GM1996	165.80		GM1996	49.69
		GM1883	181.50		GM1883	59.11
						67.96
		GM1502	189.60		GM1502	

RIL-4		RIL-5		Consensus Map	
AhXVI		AhXVI 		AhXVI	
GM692	0.00	GM799	0.00	GM692	0.00
GM699	2.00	GM2669	18.80	GM699	2.00
GM799	14.40	GM2307	33.70	GM799	13.76
GM2307	36.80	GM1392	49.80	GM2307	23.08
GM2669	44.70			GM2669	33.39
GM1392	63.10			GM1392	48.62
AhXVII*		AhXVII* 		AhXVII*	
pPGSseq15C12	0.00	pPGSseq15C12	0.00	pPGSseq15C12	0.00
GM2482	9.20	GM2482	15.90	GM2482	7.35
GM2481	13.20	GM2481	15.90	GM2481	9.25
IPAHM108	20.80	GM2589	21.30	IPAHM105	11.07
GM2265	34.50	IPAHM108	40.40	IPAHM108	12.17
TC4E09	43.90	IPAHM105	55.80	GM1571	14.63
GM1959	47.20	GM2265	61.00	GM2265	16.35
IPAHM121	48.50	GM1571	62.50	TC4H07	19.24
GM1445	56.90	GM1959	67.40	TC4E09	19.45
pPGSseq17F06	74.70	TC4H07	68.30	GM2589a	20.28
TC11H06	93.00	IPAHM121	86.10	GM1959	21.44
GM1089	114.60	GM1445	107.50	IPAHM121	25.46
		pPGSseq17F06	116.60	pPGSseq17F06	26.26
		TC11H06	128.40	GM1445	29.91
		TC1B04	152.50	TC11H06	37.62
				TC1B04	40.30
				GM1089	40.74
AhXVIII*		AhXVIII* 		AhXVIII*	
TC11F12	0.00	TC11F12	0.00	TC11F12	0.00
TC2G05	21.30	TC2G05	20.10	TC2G05	7.66
TC4G10	32.80	GM1000	35.20	TC4G10	14.18
GM624	34.30	GM1076	35.80	GM624	16.13
TC9H09	36.10	GM2605	41.40	GM2605	17.96
GM1000	42.80	pPGPseq5D05	60.00	TC9H09	18.05
GM1076	48.70	GM2067	65.00	GM1000	22.92
pPGPseq5D05	62.90	pPGPseq2E06	84.10	GM1076	26.63
GM2067	68.70			pPGPseq5D05	40.34
				GM2067	46.18
				pPGPseq2E06	49.50
AhXIX		AhXIX 		AhXIX	
TC9B08	0.00	-	-	TC3B05	0.00
TC3B05	7.20	-	-	TC9B08	8.61
GM1357	41.10	-	-	GM1357	55.56
GM2754	83.80	-	-	GM2754	116.16
AhXX		AhXX 		AhXX	
GM1369	0.00	-	-	GM1369	0.00
GM939	17.60	-	-	GM939	17.82

GENETIC MAPPING AND QTL ANALYSIS FOR DISEASE RESISTANCE, YIELD AND QUALITY IN GROUNDNUT (*Arachis hypogaea* L.)

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2013

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ABSTRACT

Recombinant Inbred Line (RIL) populations of TAG 24 × GPBD 4 (RIL-4) and TG 26 × GPBD 4 (RIL-5) were phenotypically evaluated and genotyped with SSR markers to construct the linkage map and identify the QTL governing resistance to late leaf spot (LLS) and rust, yield and quality traits in groundnut. Ample variability was observed for both the diseases (LLS and rust) however it was low to moderate for morphological and yield related traits in both the populations.

A total of 3,101 and 3,284 SSR markers were screened on parents of mapping populations, of which 211 and 210 markers were considered for linkage map construction. A map of 1982.90 cM with 20 linkage groups (LGs) was obtained for RIL-4 with 190 markers, while RIL-5 had a map of 1963.40 cM with 21 LGs with 182 marker loci. Consensus map was developed with 227 SSR loci of which 144 were common between two maps. It had 20 LGs with genome coverage of 1198.76 cM.

QTL analysis with composite interval mapping identified a total of 90 (RIL-4) and 60 (RIL-5) QTLs localized on individual maps. From both the populations, a total of 28 QTLs for late leaf spot, 14 QTLs for rust, 36 QTLs for morphological traits, 29 QTLs for yield and 43 QTLs for quality were identified. Among the 150 QTLs identified, 55 were major QTLs and 95 were minor QTLs.

Major QTLs for LLS were identified for the first time in cultivated groundnut and they need to be validated. Presence of major QTL for rust was reconfirmed, and in addition to IPAHM103, a few new markers (GM1536, GM2301 and GM2079) linked to rust resistance were identified and validated in diverse germplasm. Further, *FAD2A* allele specific marker has been validated and mapped on the genetic map. Tightly linked markers for rust and *FAD2A* allele for oil quality can be deployed simultaneously in marker assisted breeding in cultivated groundnut.