CONSTRUCTION OF GENETIC LINKAGE MAP AND QTL ANALYSIS FOR FOLIAR DISEASE RESISTANCE, NUTRITIONAL QUALITY AND PRODUCTIVITY TRAITS IN GROUNDNUT (*Arachis hypogaea* L)

SARVAMANGALA S. CHOLIN

DEPARTMENT OF GENETICS AND PLANT BREEDING

COLLEGE OF AGRICULTURE, DHARWAD
UNIVERSITY OF AGRICULTURAL SCIENCES,

DHARWAD – 580 005

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SARVAMANGALA S. CHOLIN

DEPARTMENT OF GENETICS AND PLANT BREEDING

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DEPARTMENT OF GENETICS AND PLANT BREEDING
COLLEGE OF AGRICULTURE, DHARWAD
UNIVERSITY OF AGRICULTURAL SCIENCES, DHARWAD

CERTIFICATE

This is to certify that the thesis entitled " **CONSTRUCTION OF GENETIC LINKAGE MAP AND QTL ANALYSIS FOR FOLIAR DISEASE RESISTANCE, NUTRITIONAL QUALITY AND PRODUCTIVITY TRAITS IN GROUNDNUT (*Arachis hypogaea* L)**" *submitted by* **Ms. SARVAMANGALA S. CHOLIN.**, *for the degree of* **DOCTOR OF PHILOSOPHY** in **GENTICS AND PLANT BREEDING**, *to the University of Agricultural Sciences, Dharwad is a record of research work done by her during the period of her study in this university under my guidance and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.*

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4. _____
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Affectionately Dedicated

to

My husband
Sunil

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DHARWAD

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ABBREVIATIONS

QTL	: Quantitative Trait Loci
LOD	: Log of odd ratio
MAS	: Marker-assisted selection
cM	: CentiMorgan
%	: Per cent
% S	: Per cent of saturated fatty acid
g	: Grams
cm	: Centi meter
µl	: Microliter
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
E1	: *Rainy* 2005
E2	: *Rainy* 2006
E3	: *Rainy* 2007
E4	: Post *rainy* 2007
E5	: Post *rainy* 2008
S1	: 70 days after sowing
S2	: 90 days after sowing
S3	: 110 days after sowing

1. INTRODUCTION

Tailoring the genetic architecture of crop plants to suit the growing needs of human beings in terms of increased yield and improved quality has paid significant dividend in case of cereals like rice and wheat. However, plant breeding efforts are yet to make such an impact in case of groundnut (*Arachis hypogaea* L.), an important oil seed crop, globally grown in an area of 25.20 m ha with the production of 35.90 m t (FAO, 2006). It is native to South America and grown in six continents but mainly concentrated in Asia, America and Africa in over 100 countries. China, India and USA are the major producers of groundnut. Cultivated groundnut is a segmental amphidiploid (2n=4x=40) which is believed to be originated from a single hybridization event between *A. duranensis* and *A. ipaensis* not too distant in the past (Kochert *et al.*, 1996).

Among oilseeds, groundnut is unique in that it can be consumed directly as an item of food and also utilized in diverse ways. In the developed countries like USA, bulk of the produce is processed for consumption as peanut butter, salted peanuts and confectionary while in India, the bulk is crushed for expulsion of oil (Carley and Stainly, 1995). With about 26 per cent protein, 48 per cent oil and 3 per cent fiber and high in calcium, thiamine and niacine contents, it has all the potential to be used as a highly economical food supplement to fight malnutrition that occurs due to deficiencies of these nutrients in the cereal grains like wheat and rice. Thus groundnut is a nature's gift to man in general and to children, pregnant and nursing women and the poor in particular.

Until 1976, India contributed substantially to the world trade of edible groundnut. With the liberalization process under the WTO regime, the availability of cheaper oil from non-conventional sources like rice bran, palm oil, cottonseed and soybean oil has increased. In India groundnut share in oil fell from 54 per cent in 1974-75 to 14 per cent in 1999-2000 (Hegde, 2005), the lost share being captured by soybean and palm oil. With a wide fluctuation of price, because of stiff competition from cheaper source of edible oil, groundnut is no longer considered as an economical source of edible oil in India and it is losing its ground as a main source of oilseed. But there is a greater interest to promote the use of groundnut for food in India and also to

revive the trade, as there has been growing demand for good quality groundnut for human consumption in several Asian countries.

Development of cultivars in groundnut varies with the purpose for which it is put to use (Bandyopadhyay and Desai, 2000). For instance, high protein, high oil content and high O/L ratio are important in developing cultivars for edible oil and cultivars with high O/L ratio, low oil/fat and high protein are suitable for confectionary purpose. Although, larger genetic variation is available for these traits in the groundnut germplasm, selection for the seed quality is practiced only in advanced breeding lines, as biochemical estimation of these quality traits in segregating population is too costly, cumbersome, time consuming, high resource requiring and biochemical analysis of most of these traits is postmortem. Therefore, it is beyond the capacity of the breeders to undertake large scale breeding program for quality improvement through conventional breeding techniques.

Higher productivity is the ultimate objective in any crop. Groundnut yields are restricted in most of the areas of the world by diseases. The two important diseases *viz*., late leaf spot (LLS) and rust are the worst among the foliar diseases in groundnut, together cause an yield loss up to 50-70 per cent (Subramanyam *et al*., 1984). Besides, adversely affecting the productivity, they affect the quality of the seeds and fodder, making it unsuitable for consumption. Though, chemical control is possible, development of resistant cultivars is considered to be the best strategy to surmount the additional cost of production and hazardous effect of fungicides on the soil and environment. In addition, the seeds harvested form unsprayed plots will have significantly better quality than those treated with chlorothalonil, a fungicide used to control rust and LLS in groundnuts (Hammonds *et al*., 1976). But because of their co-occurrence and defoliating nature of late leaf spot, it is difficult to differentiate resistant and susceptible cultivars for both the diseases in field conditions by conventional screening techniques. Even identified also, the resistant sources often suffer from undesirable traits like low productivity, long duration and poor adaptability besides poor yield and seed traits. Hence, conventional resistance breeding would not alone give the expected results.

Because of its complex nature and polygenic inheritance, breeding for high yield is one of the great challenges in groundnut. Although, an abundance of morphological

variation within *A. hypogaea* is known, but, most of the agronomically important traits in groundnut are quantitatively inherited (Wynne and Coffelt, 1982), significant genotype and environment interaction exists in them and most of the yields contributing traits have low heritable variation. Hence, phenotypic selection based on conventional breeding techniques alone will have very limited practical utility in the breeding program for yield improvement.

The above problems of conventional breeding for crop improvement can be solved by employing new biotechnological tools such as, use of DNA markers for mapping and tagging of the markers with desirable traits. Constructing a molecular linkage maps is now routine to trace the valuable alleles in a segregating population. Once the framework maps are generated, a large number of markers derived from various techniques (RFLP, AFLP, SSR etc) are used to saturate the maps. Hence, DNA marker based genetic linkage map would enable breeders to effectively pyramid genes for better seed quality, resistance to biotic and abiotic stresses in to an agronomically enhanced breeding population in a much shorter time than would be possible by conventional techniques.

Earlier studies using RAPD and RFLP approaches have found limited DNA variation among genotypes in groundnut which is mainly attributed to its origin by single event of hybridization followed by polyploidization (Kochert *et al*., 1991, Halward *et al.,* 1992 and Paik-Ro *et al*., 1992*)*. Similarly isozyme and seed protein studies have revealed limited variation among peanut cultivars (Stalker *et al*., 1994). However, He and Prakash (1997) detected polymorphism in peanut using DAF, and AFLP markers but the percentage of polymorphism relative to the total number of primers screened was low. Simple sequence repeats (microsatellites) are considered to be most efficient and breeder friendly DNA markers because they are ubiquitous through out the genome, multi-allelic, co-dominant and transferable between the populations (Gupta and Varshney, 2000). In groundnut, several hundreds of SSRs are currently available (Hopkins, *et al*., 1999; He *et al*., 2003; Ferguson *et al*., 2004; Moretzohn *et al*., 2004 and 2005; Mace *et al*., 2007; Cuc *et al*., 2008; Bertioli *et al*., unpublished and Knapp *et al*., unpublished).

Mapping population plays a crucial role in linkage map construction. Care must be taken while selecting parents for developing a mapping population which should be

as diverse as possible to generate complete linkage map with large number of molecular markers. Recombinant Inbred lines obtained by hybridization of diverse parents followed by single seed descent method from F_2 onwards are considered to be the best among the mapping populations to use them in molecular mapping and tagging. As RILs consist of a series of homozygous lines, each containing a unique combination of chromosomal segments from the original parents, and they are immortal; hence, multi-location, and multi-environment data can be generated. It can also be transferable between different laboratories for further linkage analysis by addition of markers to the existing linkage maps. The time required for development of RILs is the major disadvantage as it takes six to eight generations to achieve the highest homozygosity.

The selection of the parents for developing a mapping population plays very important role for phenotyping followed by identification of quantitative trait loci (QTLs) for the desired traits. The mapping population developed from TG 26 x GPBD 4 at University of Agricultural Sciences, Dharwad is one of the best among the RILs in groundnut as it is segregating for disease resistance, nutritional qualities such as protein content, oil content, major fatty acids like oleic and linoleic acid besides productivity traits. Hence, the population consisting of 146 RILs at F9 generation obtained from the cross TG 26 x GPBD 4 was used to fulfill the objectives.

In the light of the above facts, present study on "**Construction of Genetic linkage map and QTL analysis for foliar disease resistance, nutritional quality and productivity traits in groundnut (*Arachis hypogaea* L)"** was undertaken with the following specific objectives

1. Phenotyping for rust and LLS, nutritional quality, agronomic and productivity traits in parents (GPBD 4 and TG 26) and mapping population.
2. Screening for parental polymorphism (TG 26 and GPBD 4) and genotyping of mapping population.
3. Construction of genetic linkage map using polymorphic microsatellite markers for TG 26 x GPBD 4 mapping population.
4. Identification of QTLs associated with foliar disease resistance, nutritional quality, agronomic and productivity traits in TG 26 x GPBD 4 mapping population.

2. REVIEW OF LITERATURE

The review of literature of the present study has been divided into following sub-headings

2.1 Late leaf spot and Rust

2.2 Nutritional quality traits

2.3 Agronomic and productivity traits

2.4 Molecular marker studies

2.5 Genetic linkage map studies

2.6 Marker trait association/ QTL studies

2.1 Late leaf spot and rust

Late leaf spot and rust are the most destructive, widely distributed and economically important foliar diseases of the groundnut causing severe damage to the crop (McDonald *et al*., 1985; Kokalis-Burette *et al*., 1997). They are commonly present wherever groundnut is grown but their incidence and severity vary between localities and seasons. Each disease alone is capable of causing substantial yield loss but when they occur together losses are further increased. For instance, rust and late leaf spot (LLS) together can cause up to 70 per cent yield loss in India (Subramanyam *et al*., 1984).

These foliar diseases besides reducing the yield, also have an adverse effect on seed quality and grade characteristics, deteriorate the quality of plant biomass and thus render the foliage unsuitable as animal feed. Further, control of these diseases through the application of plant protection measures will not only increase the cost of cultivation but also lead to environmental and health hazards. Use of disease resistant cultivars is one of the best means of reducing crop losses from these diseases.

Identification of resistance sources, knowledge of components, mechanism of resistance and the number of loci contributing to resistance are the pre-requisite for the success of disease resistance breeding programme (Dwivedi *et al*., 2002).

Different sources of resistance to LLS and Rust have been reported in *A. hypogaea* (Waliyar *et al*., 1993a; Anderson *et al*., 1993; Mehan *et al*., 1996; Singh *et al*., 1997). Majority of the resistant sources belong to subspecies *fastigiata* var *fastigiata* and are landraces from South America (Subramanyam *et al*., 1989).

There are only moderate levels of LLS resistance available in the cultivated groundnut gene pool. In contrast, several wild *Arachis* species possess very high levels of rust resistance.

There has been limited success in transferring LLS resistance from wild *Arachis* to cultivated groundnut, mainly because of inter-specific compatibility barriers, resistance being linked with many undesirable pod/seed characteristics and longer periods required for developing stable tetraploid interspecific derivatives. In spite of these obstacles, a few interspecific derivatives (ICGV87165, GPNCW1, GPNCW2, GPNCW3, GPNCW4, ICGV86699 and ICGV87167) possessing high levels of resistance to foliar diseases have been developed in India and USA (Nigam *et al*., 1992; Stalker and Beaute, 1993; Reddy *et al*., 1996). But due to the agronomically undesirable traits like late maturity, inferior pod and seed characteristics in comparison to commercially grown cultivars, they have not been released for cultivation. Although, most of the released cultivars are susceptible to late leaf spot and rust diseases, a few cultivars with moderate resistance to these diseases have been released in India namely ICGV87160 and ICGV86590 (Reddy *et al*., 1992).

Progress in resistance breeding is limited because of absence of high levels of resistance in cultivated peanut and the linkage of resistance with long duration, lower partitioning with undesirable pod and seed characteristics (Singh *et al*., 1997).

An inter-specific derivative GPBD-4 released at U.A.S. Dharwad combined early maturity, high yield potential and high shelling outturn with minimum yield reduction due to high level of resistance to rust and late leaf spot, pod growth rate, partitioning coefficient and harvest index (Gowda *et al*., 2001).

2.1.1 Components of resistance

There are only few studies dealing with components of resistance to rust and LLS and their association among themselves in groundnut.

Several authors reported complex nature of resistance to leaf spot (Anderson *et al*., 1986 and 1993; Green and Wynne, 1987; Iroume and Knauft 1987; Jogloy *et al*., 1987) and several components attribute to resistance including infection, lesion size, sporulation and defoliation (Green and Wynne, 1986; Chiteka *et al.,* 1988, Anderson *et al*., 1993; Waliyar *et al*., 1993b).

Resistance to LLS is partial in nature. Sporulation rate, lesion size, lesion number and latent period are important components that contribute to a desired field score for LLS (Chiteka *et al*., 1988; Anderson *et al*., 1990). Resistant genotypes have longer incubation period, fewer lesions and lower sporulation rates than susceptible genotypes (Nevill, 1981).

Resistance to LLS in *A. hypogaea* L. is due to longer latent period, reduced sporulation of pathogen and less defoliation on host (Nevill, 1981). Sporulation, lesion size and latent period are important components of resistance to LLS and are highly correlated with each other and with per cent of leaf necrotic area (Chiteka *et al*., 1988). Lesion diameter, defoliation and sporulation from glass house study are correlated with field disease scores (Subramanyam *et al*., 1982). Motagi (2001) reported that incubation period, lesion size and lesions on main stem are the important components of resistance having strong association with field disease scores.

Resistance to rust in *A. hypogaea* L. was reported to be associated with longer incubation period, less number of pustules, smaller pustules, less ruptured pustules and less leaf area damage (Subramanyam *et al*., 1983; Reddy and Khare, 1988; Mehan *et al*., 1994). Infection frequency, pustule diameter, per cent ruptured pustules and leaf area damage are correlated with each other and with mean field rust scores.

Rust resistant lines have an increased pathogen incubation periods, decreased infection frequencies and reduced pustule size, spore production and spore germinability (Subramanyam *et al*., 1983; Mehan *et al*., 1994). In contrast, the characterized sources of resistance in wild *Arachis* species and their interspecific derivatives have more dramatic effects on pathogen such as uredosori on the accessions are observed to be very small (containing very few uredospores), slightly depressed and do not rupture to release their uredospores (Subramanyam *et al*., 1983).

The incubation period is negatively correlated with other components. Most of the wild *Arachis* species in sections Erectoides, Triseminale, Extranervosae and Rhizonatosae show immunity to rust with no recognizable symptoms of the disease even after incubation period of 40 days (Subramanyam *et al*., 1983).

2.1.2 Genetics of Resistance:

Although resistance to LLS is reported as partial type similar to slow rusting, different sources of resistance to LLS have been reported as digenic recessive basis (Tiwari *et al*., 1989) or being conferred by a five gene model (Nevill, 1981). Other studies report predominantly additive genetic variance for most of the components of resistance to LLS (Kornegay *et a*., 1980; Hamid *et al*., 1981; Anderson *et al*., 1986; Jogloy *et al*., 1987).

Motagi (2001) reported duplicate recessive genes controlling resistance to LLS and the favourable resistant alleles coming from interspecific source like CS_16 (ICGV 86855). Resistance to LLS has been reported to be conferred by five loci recessive genes in the crosses involving cultivated groundnut and wild *Arachis* species (Sharief *et al*., 1978). Most of the components of resistance to LLS are controlled by additive genetic variance (Kornegay *et al*., 1980; Hamid *et al*., 1981; Anderson *et al*., 1986; Jogloy *et al* 1987).

Resistance to rust in *Arachis hypogaea* L. is conferred either by a few recessive genes (Kalekar *et al*., 1984; Tiwari *et al*., 1984; Knauft, 1987; Paramasivam *et al*., 1990) or predominantly controlled by additive, dominance and additive x additive and additive x dominance genetic effects (Reddy *et al*., 1987; Varman *et al*., 1991). In addition, partial dominance is reported in some diploid species (Singh *et al*., 1984).

Singh *et al* (1984) concluded that rust resistance in diploid species is partially dominant as compared to the recessive in *Arachis hypogaea* L. While, Motagi (2001) reported that resistance to rust is conferred by duplicate complementary genes (9:7).

2.2 Nutritional quality traits

Groundnut is an ideal food crop to reduce malnutrition due to its rich nutritional properties. The quality of groundnut can vary with the purpose for which it is put to

(Bandyopadhyay and Desai, 2000). Chemical and nutritional factors include oil and protein content, carbohydrates, amino and fatty acid composition, mineral and vitamins. The seed has several uses as a whole seed or processed to make groundnut butter, oil and other products.

2.2.1 Protein content

Groundnut protein is increasingly becoming popular as food and feed sources, especially in developing countries where protein from animal sources is not within the means of majority of the population.

The groundnut kernels contain high quality protein than meat, eggs and most of other vegetables. Hence, it is important for children, women and people eating more meatless meals (Misra, *et al.,* 2000). In absence of adequate carbohydrate and fat in diets, dietary proteins are broken down to provide energy (4Kcal/g).

Groundnut protein comprises almost entirely of two globulins *viz.,* arachin (63 per cent) and conarachin (33 per cent). As both arachin and conarachin contain 18.3 per cent nitrogen, hence, the nitrogen protein conversion factor for groundnut is 5.46. However, there is possibility of variation in the value of nitrogen to protein conversion factor due to differences in genotypes and geographical locations (Misra, *et al.,* 2000).

The development of nutritionally balanced foods to feed the growing population of dietary deficiencies of proteins is receiving increasing attention of the food scientist and nutritionists, oilseeds are used to manufacture and market high protein foods at reasonably low prices (Bookwaltes *et al.,* 1979),

Reddy *et al.,* (1987) indicated that the seeds of groundnut contain 25 to 32 per cent protein and the cake, the residual matter after oil extraction contains 46 to 60 per cent protein. Where as, Gupta and coworkers (1982) reported the protein content in the range of 24.05 to 33.25 per cent among the twenty five genotypes of groundnut grown at Hissar. A range of 16.00 to 34.00 per cent protein was observed in 8000 germplasm accessions analyzed at ICRISAT (Dwivedi *et al*., 1993)

Pancholy *et al*. (1978) reported crude protein content of whole seed groundnut range between 22 to 30 per cent showing large variation which is generally influenced by genotype and environmental conditions.

The drought at the end of season results in increased protein content of kernels (Dwivedi *et al*., 1996) and the kernels obtained from the rainy season generally contain higher protein than that of summer season.

Protein content varies much between accessions of botanical varieties but between accessions of botanical varieties it ranges from 16.10 to 34.00 per cent (Singh *et al*., 1998).

Association studies revealed significant inverse correlation between protein and oil content (Holley and Hammons, 1968; Tai and Young, 1975; Dwivedi *et al*., 1990). For chemical traits like low oil, high protein and sugar, the genotypes DCG-24 (TG49 x R 9227, 8), DCH 26 (TG 49 x R9227, 19) and TKG 19A were reported to be superior (Yashoda, 2005 and 2007)

2.2.2 Oil content

The most important quality requirement of groundnut as a source of oil are high oil content in seed and high oleic acid resulting in high oleic/linoleic acid ratio for longer stability. About 80 per cent of total groundnut production in India is crushed for the extraction of oil. Hence, improvement in oil yield and quality is of interest to plant breeders and millers.

Compared to refined oil, raw groundnut oil is fairly stable because of its iodine number, saponification number, acetyl number and free fatty acids do not change during heat treatments and hence, groundnut oil is highly reusable. Besides, groundnut oil can be stored at room temperature for 18 months without significant deterioration in quality. Hence groundnut oil is considered as an excellent cooking medium (Misra, *et al.,* 2000).

The oil content of kernels shows significant genotypic variations. The crop season, habit group, geographical location, soil fertility, moisture availability, maturity of crop at harvest, seed mass have a bearing on the oil content (Misra, 2004).

Tai and young (1975) reported that oil content is quantitatively inherited, while Martin (1967) estimated that only two pairs of major genes control oil content in peanut seeds. Martin (1967) and Patil (1972) obtained high heritability estimates of oil content.

Estimates of the correlation between seed size and oil content were generally negative (Holley and Hammons, 1968, Patil, 1972) but Mital and Mehta (1954) found a positive relationship for these traits among spreading type peanuts.

Cherry (1977) determined the quantity of oil from 37 selected wild species and 21 cultivars and found that oil content in seeds was 46.50 to 63.10 per cent for wild species and 43.6 to 55.50 per cent for the cultivars. The oil content of groundnut has been reported to range from 35.80 to 54.20 per cent and average near 45 per cent (Jambunathan *et al*., 1985; Dwivedi *et al*., 1990).

2.2.3 Oil quality/fatty acid composition

Nutritional quality of oil is determined by its fatty acid composition. Groundnut oil comprised glycosides of about 14 types of fatty acids of which about 80 per cent are unsaturated and 20 per cent are saturated fatty acids. Nutritionally however, only eight fatty acids *viz*., Palmitic, Stearic, Oleic, linoleic, Arachidic, Eicosenoic, Behenic and Lignoseric are considered important. Oleic acid a monounsaturated fatty acid and linoleic a polyunsaturated fatty acid account for 75 to 80 per cent of the total fatty acids in peanut oil. Among the saturated fatty acids, which comprise the remaining 20 per cent of the total fatty acids, palmitic acid (10%) has the largest proportion.

Palmitic acid is considered to be a major contributor to increased levels of total blood cholesterol, especially LDL (Worthington and Hammons, 1977; Groff *et al*., 1996; Lukange *et al*., 2007). Consumption of oils with reduced palmitic acid content is desirable to reduce the health risks of coronary diseases and breast, colon and prostrate cancer properties associated with this fatty acid (Henderson, 1991). Stearic acid, (18:0) the other main saturate present in plant oils does not raise LDL-cholesterol like other saturates (Dougherty *et al*., 1995) and generally considered to be neutral with respect to risk of cardiovascular disease. Long chain fatty acids such as arachidic, lignoseric acids have been implicated in the elevated artherogenic effect (Kritchevsky *et al*., 1971; Worthington and Hammons, 1977; Slack and Browse, 1984).

Saturated fatty acids are hypercholesterolemic and polyunsaturated fatty acids are hypocholesteromic and it was assumed that monounsaturated fatty acids are neutral in this regard (Groff *et al*., 1996; Grande and Denke, 1990). Oleic acid, a monounsaturated fat in most edible vegetable oils, lowers LDL as effectively as linoleic

acid, but does not affect HDL levels (O' Bryne *et al*., 1997; Kris-Eterton *et al*., 2001). A large body of evidences consistently showed that consumption of tree nut and groundnuts is associated with a reduced risk of coronary heart disease (CHD). In addition, results from Adventist Health Study demonstrated that the consumption of nuts 5 times per week reduced the risk of death from CHD by 39 % (Fraser *et al*., 1997).

Oils with higher proportion of unsaturated fatty acids can be heated to high temperatures without smoking, leading to faster cooking time and absorption of less oil (Miller *et al*., 1987).

The *Virginia* types had higher mean oleic acid and a lower mean linoleic acid content than Spanish and Valencia types (Dwivedi *et al*., 1993).

The O/L ratio among 200 germplasm lines of different botanical groups ranged from 0.48 to 1.36 in the Spanish and *Valencia* types and from 1.0 to 2.2 in the *Virginia* types (Norden *et al*., 1987).

Two breeding lines originating from natural mutation in Florida (USA) are reported to have high O/L ratio. The high oleate trait found in Florida collections have shown to be controlled by two recessive genes, one of which occurs commonly in groundnut germplasm (Norden *et al*., 1987).

Seven hundred and thirty two groundnut plant introductions evaluated for fatty acid composition showed range of 8.20 to 15 per cent for palmitate, 1.1 to 7.2 per cent for stearate, 31.5 to 60.2 per cent for oleate, 19.9 to 45.4 per cent for linoleate, 0.8 to 3.2 per cent for arachidate, 0.6 per cent for ecosinoate, 1.8 to 5.4 per cent for behenate and 0.5 to 2.5 per cent for lignoserate (Hammond *et al*., 1997).

Khan *et al*. (1974) reported a wide range of genetic variability for iodine value in the F_2 populations and opined that iodine values are under the control of few additive genes and highly heritable.

Inheritance of high oleic and low linoleic acid is shown to be under simple genetic control (Moore and Knauft, 1989) and duplicate recessive alleles *viz*., Ol_1 and Ol_2 are responsible for this character.

Two major recessive genes have been identified in peanut, which increases the oleic acid content to as high as 80 per cent and reduce the linoleic acid content to around 2 per cent (Moore *et al*., 1989).

Jung *et al.* (2000) reported that high oleate groundnut resulted form reduction in the activity or transcript level of microsomal oleoyl-PC desaturase. They isolated two non-allelic but homeologous genes, ahFAD2A and ahFAD2B from the developing peanut seed with normal oleate seeds. Reduction in ahFAD2B transcript levels in the developing seeds is correlated with high oleate trait.

Oleic acid content is also reported to be influenced by additive and additive x additive genetic effects (Layrisse *et al*., 1980; Moore and Knauft, 1989; Mercer *et al*., 1990).

Groundnut seeds with high O/L ratios have long product stability and shelf-life (Branch *et al*., 1990). Oil content and O/L ratios are influenced by G x E interaction (Dwivedi *et al*., 1993)

GPBD 4 recorded higher oleic acid (46-48 %) and high O/L ratio (1.50-1.70) with high unsaturated fatty acid content (78.80 %) among the foliar disease resistant genotypes screened and which was significantly superior over currently cultivated Spanish bunch genotypes for oil quality (Motagi *et al*., 2005). Ajay *et al*., (2008) reported that, GPBD 4 had highest protein content (30.33 %) and high O/L ratio (1.68-2.01) with low saturated fatty acid content among the 17 groundnut varieties.

Strong negative correlation between oleate with linoleate and palmitate has been reported (Hammond *et al*., 1997; Anderson *et al*., 1998; Kavera., 2008).

Sekhon *et al.* (1980) reported that oil content has no correlation with any of the major fatty acids. However, a negative correlation between oil content and oleic acid concentration has been reported (Emeroglu and Mortuary. 1984; Kavera., 2008).

Kavera (2008) studied on oil quality improvement through induced mutagenesis, where in, GPBD 4 and TPG 41 were used as parents for developing mutant population. The improvement in oleic and corresponding decrease in linoleic ranged for 14.66 per cent from gamma rays treatment to 6.00% from EMS in GPBD 4 mutant population and

8.00 per cent from gamma rays treatment to 3.00 per cent from EMS in TPG 41 mutant population.

2.3 Agronomic and productivity traits

Among the various oil seed crops, groundnut is a unique commercial crop and has been aptly described as "natures" 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 x 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.

Many agronomically important traits in groundnut are quantitatively inherited (Murthy and Reddy, 1993). Additive, non-additive and epistatic genetic effects are reported for pod yield, pods and seeds per plant, pod length and shelling outturn (Parker *et al*., 1970; Layrisse *et al*., 1980; Dwivedi *et al*., 1989; Upadhyaya and Nigam, 1998).

Jaswal and Gupta (1967) suggested that branches contributed to the yield in Spanish types. Increase in the branch number and node number resulted in the increased peg number and yield in the case of Spanish mutants (Prasad *et al*., 1984).

Highest pod yield per plant was reported in *hypogaea* group with the range of 6.68 d to 11.49 g) rather than *fastigiata* group ranged from 3.69 to 8.76 g (Mallikarjuna Swamy *et al*., 2003). They also reported that pod yield per plant had a significant negative correlation with oil content and positive correlation with test weight.

Upadhyaya (2003) reported significant variation for shelling per cent between *hypogaea* and *fastigiata* and *fastigiata* showed higher shelling per cent than *hypogaea* type in two seasons studied from an evaluation of core collection consisting of 1704 accessions of which 910 belonged to sub spp. *fastigiata* and 794 to sub spp *hypogaea.*

Association studies revealed positive correlation for pod yield per plant with number of pods per plant, 100-seed weight, oil yield and it was negatively correlated with shelling percentage (Sah *et al*., 2000)

Pod yield possessed significant positive association with kernel yield, test weight and oil yield at both genotypic and phenotypic levels (Laksmidevamma *et al*., 2004).

The size of kernel is one of the important factors for export. Normally varieties with hundred seed mass of 60 g or more are considered as large seeded groundnut and are preferred for confectionary purpose.

100-seed mass is qualitatively inherited trait controlled by additive, dominance and epistatic effects (Garet, 1976; Sandhu and Khera, 1976; Layrisse *et al*., 1980; Arunachalam *et al*., 1984; Upadhyaya and Nigam, 1998).

Large seeds of groundnut have a greater consumer preference and fetch higher prices in domestic and international markets. Birsa 1 was one of the first confectionary varieties released for cultivation in India (Rahaman *et al*., 1995) in pod/seed yield and 15 % in 100-seed mass.

Genetic variability components revealed high heritability for shelling per cent, 100-seed weight and low for pod yield per plant (Upadhyaya *et al.,* 2005 and Vasanthi *et al.,* 1998)

2.4 Molecular marker studies in groundnut

2.4.1 Problems in conventional breeding and need for MAS in groundnut improvement

Groundnut is predominantly an inbreeding crop so the most commonly used breeding methods are pedigree selection, bulk pedigree selection and single seed descent. Back cross breeding has not been extensively utilized because most of the economically important traits in the crop have complex inheritance pattern (Wynne and Gregory, 1981; Knauft and Wynne, 1995).

Conventional plant breeding have had limited success in enhancing genetic resistance against LLS and rust due to lack of genetic information, complexicity of the genome and most of the time the two diseases occur together and interfere with each other, hence, it is difficult to identify resistant lines to these diseases in the field condition.

Selection for most of the quality traits such as protein content, oil quality (fatty acid composition) is practiced only in advanced breeding lines as analysis of these traits in segregating population is too costly, cumbersome, time consuming, high resource requiring. And most of these biochemical analyses are post mortem, as analysis will be done after the harvest of the crop. Therefore, it is beyond the capacity of the researchers to undertake large scale quality breeding programme to improve these traits through conventional breeding strategy.

Conventional breeding methods can investigate the genetic control of quantitative traits such as yield and yield contributing traits in segregating population. Although, valuable but it is insufficient to provide information on chromosomal regions regulating the variations of each trait, the simultaneous effects of each chromosome regions on other traits and genetic basis of such associated traits (Hallauer and Miranda, 1988). Transfer of most of the desirable traits like disease resistance, high oil content, O/L ratio *etc* from land races and wild relatives to cultivated background is difficult due to linkage drag such as undesirable traits like thick shell, low yield; poor adaptability and long duration *etc* are associated with these desirable traits.

With the advent of molecular markers it is now common to trace valuable alleles in a segregating populations and mapping them. Once the frame work of maps is generated, a large number of markers derived from various techniques are used to saturate the maps as much as possible. Once mapped, these markers are efficiently employed in tagging the desirable traits and using them in Marker Assisted Selection (MAS). This will not only eliminate the need of chemical analysis, screening for individual traits phenotypically in the early generation breeding programme, but also minimize the time required to develop new genotype with desirable genotype in the seedling stage itself, instead of waiting till harvest.

2.4.2 Advantages of marker-assisted selected (MAS)

Molecular markers help in easy identification and transfer of recessive genes and to monitor alien gene introgression and also in eliminating undesirable traits in much shorter time frame than those expected through conventional breeding programme.

MAS may be able to break linkage drag to deleterious traits, increase the speed and efficiency of creating acceptable inter-specific derivatives and facilitate the

pyramiding of different sources of resistance from the cultivated and wild gene pools in order to develop varieties with all the desirable traits (Knauft and Wynne, 1995).

MAS could also reduce the need for phenotypic selection that may be inappropriate in identifying genotypic differences and in selection of rare recombinants between tightly linked resistance genes. It also facilitates map based cloning of disease resistant genes and aids in faster recovery of recurrent parent genome in the backcross breeding programme (Tanskley *et al*., 1989).

2.4.3 Requirements of marker-assisted selection (MAS)

Molecular markers offer great scope for improving the efficiency of conventional plant breeding. The essential requirements for developing MAS system are i) availability of germplasm with substantially contrasting genotypes for the traits of interest ii) highly accurate and precise screening techniques for phenotyping of mapping population for the trait of interest iii) identification of flanking markers closely associated with the loci of interest and the flanking region on either side and iv) simple robust DNA marker technology to facilitate rapid and cost effective screening of large population (Paterson *et al*, 2004).

The application of biotechnology to the improvement of allotetraploid (2n=4x=40) peanut has been hampered by an inability to visualize genetic variation in germplasm lines. Studies on isozymes (Grieshammer and Wynne 1990) and seed proteins (Tombs, 1963; Bianchi-Hall *et al*., 1991) and total proteins (Savoy, 1976) identified very low level of polymorphism in groundnut.

The cultivated groundnut has been analyzed by several marker systems including RFLPs, RAPDs (DAF and SCAR), AFLPs and microsatellites. Studies on each of these markers in *Arachis* are explained below.

2.4.4 Restriction fragment length polymorphism (RFLP)

RFLP is the most widely used hybridization based molecular marker. RFLPs are produced by digesting genomic DNA with restriction endonucleases that recognize specific sites on a DNA sequence and then cleave the DNA strand in or near the recognition sites of the sequence. Fragments thus produced can be separated by size on a gel electrophoresis. For complex genomes a probe is made from cloned DNA that is

homologous to a specific DNA sequence in the species being investigated (Botstein, *et al*., 1980). Radioactivity is used to label probes and bands are visualized when the un-hybridized radioactivity is washed away and then an autoradiograph is produced. The major strength of RFLP markers are high reproducibility, co-dominant inheritance, good transferability between laboratories, provide locus specific markers that allow synergy studies, no sequence information required and relatively easy to score due to large size difference between fragments but it requires high quality and quantity of DNA, time consuming, laborious, expensive and requires radioactive labeled probes.

In *Arachis,* as early as 1991 Halward *et al*., reported high polymorphism among wild *Arachis* species but very little among cultivated groundnut from a study involving 25 unadapted germplasm, 2 U.S cultivars and wild allotetraploid progenitors of cultivated peanut.

Kochert *et al*. (1991) reported very low levels of RFLP variability among the allotetraploids which included the U.S cultivars and *Arachis monticola*, a wild tetraploid species.

Paik-Ro *et al*. (1992) observed significant amount of variation among the *Arachis* section (*A. hypogaea, A. monticola, A. batizocoi, A. cardenasi, A.duranensis and A. glandutifera*) using RFLP markers and found that *A. monticola* was more closely related to *A. hypogaea* subspecies *hypogaea* than to subspecies *fastigiata.*

Stalker *et al*. (1995) used RFLP to study genetic diversity among eighteen accessions of *A. duranensis* and found large amount of variation in the species.

Kochert *et al*. (1996) observed no variation between *Arachis hypogaea* and *Arachis monticola* using RFLP technique and concluded that the cultivated groundnut resulted from the cross between *Arachis duranensis* and *Arachis ipaensis* and based on the chloroplast analysis they concluded that *Arachis duranensis* as the female progenitor of cultivated groundnut.

Gimens *et al*. (2002) studied genomic relationship between AA genome, BB genomes and AABB genome species using RFLP technique. The lowest genetic variation was detected within accessions of *Arachis duranensis* (17 accessions)

followed by *Arachis batizocoi* (4 accessions) and *Arachis cardenasii* (9 plants of accession GKP 10017).

2.4.5 Random amplified polymorphic DNA (RAPD)

The assay developed by Williams *et al.* (1990) uses a single arbitrary oligonucleotide primer to amplify template DNA without prior knowledge of the target sequence. RAPDs are quick, simple, and inexpensive. Multiple loci from a single primer is possible and small amount of DNA is required to carryout this assay but is less popular due to problems such as dominance inheritance pattern, poor reproducibility and transferability, faint or fuzzy products and difficulty in scoring bands, which lead to inappropriate inferences.

Lanham *et al* (1992) detected significant amount of variation (81.66%) between *Arachis hypogaea* and synthetic amphidiploids using RAPD.

Hilu and Stalker (1995) observed maximum variation among accessions of *Arachis cardensii* and *Arachis glandulifera* where as, the least amount of variation was observed in *Arachis hypogaea* and *Arachis monticola*. Based on RAPD assay it was proposed that *Arachis duranensis* was most closely related to the domesticated groundnut and is believed to be the donor of *Arachis genome*.

Bhagwat *et al.* (1997) observed 5.5 per cent of polymorphism and detected variation among the different plant height mutants and pod size mutants by using RAPD assays in peanut.

Subramaniam *et al.* (2000) studied 70 genotypes representing variability for several morphological, physiological and other characteristics using 48 RAPD primers and 7 primers yielded polymorphism. The total number of bands from the 7 primers was 408 of which 27 were polymorphic.

Santos *et al.* (2001) analyzed 38 RAPD markers (80 RAPD bands from 10 polymorphic primers) to establish genetic relationships among the 48 accessions of five sections of the genes *Arachis*. They found wide variation among the accessions and low variation within the accessions and they demonstrated that RAPDs can be used to determine the genetic relationships of genes *Arachis*.

Dwivedi *et al.* (2001) assessed genetic diversity among 26 accessions using eight random primers and identified five accessions with diverse profiles for mapping and genetic enhancement studies.

2.4.6 Amplified fragment length polymorphism (AFLP)

AFLP technique combines the power of RFLP with the flexibility of PCR based technology by ligating primer recognition sequences (adaptors) to the restricted DNA. PCR amplification of restriction fragments is achieved by using the adapter and restriction site sequence as target sites for primer annealing. The selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying those fragments in which the primer extensions match the nucleotide flanking restriction sites (Vos *et al.*, 1995). AFLP technique is highly reliable and reproducible, does not require prior sequence information, multiple loci can be detected and it is possible to generate high level of polymorphism but it is tedious, require large amount of DNA and complicated methodology, requires both restriction endonuclease and adapters.

He and Prakash (1997) used DAF (DNA Amplified Fingerprinting) and AFLP technique to detect genetic variation in peanut. They found that AFLP approach was more efficient as 43 per cent of the primer combinations detected polymorphism in contrast to 3 per cent with the DAF approach.

He and Prakash (2001) studied genetic relationships among 44 accessions of cultivated peanut representing 6 botanical varieties of 2 subspecies along with 3 accessions of the wild relative *Arachis monticola* krapov et Rigoni using AFLP marker technology. They concluded that, by employing AFLP approach sufficient DNA variation can be detected in the cultivated peanut germplasm to conduct evolutionary studies.

Gimens *et al.* (2002) used AFLP to establish the genetic relationship among 20 species from 7 sections of genus *Arachis* and revealed that AFLPs are good markers for establishing genetic relationships among *Arachis* species and can also detect high level of polymorphism than RAPDs and RFLPs.

Herselman (2003) detected polymorphism between 21 closely related cultivated southern Africa peanut genotypes using AFLP technique. These 21 genotypes were

divided in to two main groups corresponding to the two subspecies of *Arachis hypogaea* namely *fastigiata* and *hypogaea*.

Milla *et al.* (2005) used AFLP technique to determine intra and inter specific relationships among and within 108 accessions of 26 species. Based on genetic distances and cluster analysis, "*A" genome* accessions KG 30029 (*A. helodes*) and KSSC 36009 (*A. simpsonii*) and B genome accession KGBSPSC 30076 (*A. ipaensis*) were most closely related to both *A. hypogaea* and *A. monticola*.

2.4.7 Microsatellites/simple sequence repeats (SSRs)

Microsatellites also known as simple sequence repeats are most preferred, widely used molecular markers and detects highest polymorphism in groundnut. The primer designed for amplification of DNA is crop specific and complementary to the flanking region of the repeat motifs. Hence, the polymorphism will be detected based on the number of tandem repeats (VNTRs) in a given repeat motif. Because of their multi-allelic nature, co-dominant inheritance, relative abundance, extensive genome coverage SSRs can be used for a variety of applications (Gupta and Varshney, 2000). This method is technically simple, robust, reliable and transferable between the laboratories and mapping populations. But requires large amount of time, cost and labor to generate primers and as it requires polyacrylamide gel electrophoresis hence, laborious and hazardous to environment and human health.

Hopkins *et al.* (1999) for the first time identified 6 polymorphic SSR markers from 26 primers among 22 accessions belonging to different subspecies of *Arachis hypogaea* and concluded that these markers detected more variation in cultivated peanut than other molecular marker studies *viz*., Isozymes, RFLPs, RAPDs and AFLPs.

He *et al.* (2003) designed 56 micro-satellites by using SSR enrichment procedure and observed 33.90 per cent polymorphism among the genotypes suggesting higher level of polymorphism by these markers than other DNA markers in cultivated groundnut.

Moretzsohn *et al.* (2004) screened 67 TTG SSR markers to study polymorphism of seven accessions and observed only 4.40 per cent polymorphism in cultivated groundnut.

Ferguson *et al.* (2004) identified and characterized 110 sequence tagged micro-satellite markers in a diverse array of 24 peanut landraces and found that 81 per cent of (ATT)n and 70.8 per cent of (GA)n showed polymorphism.

Luo *et al.* (2005) generated 44 EST derived SSR primers to detect polymorphism among the 24 genotypes of cultivated peanut and concluded that the rate of polymorphism among the peanut lines is higher on the basis of EST derived SSR markers than SSR derived from genomic sequences of peanut.

Krishna *et al.* (2004) studied molecular diversity in the cultivated *Valencia* groundnut subspecies *fastigiata* using micro satellites and indicated considerable genetic variation among the analyzed genotypes.

He *et al.* (2005) observed 29.23 per cent polymorphism from 130 SSR markers among 24 groundnut accessions. Eight markers found useful to classify botanical varieties. Mace *et al.* (2006) screened 23 SSR markers across 22 groundnut genotypes with varying levels of resistance to rust and late leaf spot and showed 52 per cent polymorphism with PIC value grater than 0.50.

Bravo *et al.* (2006) worked on transferability of micro-satellites primers and analyzed the genetic variability between and within the germplasm of same species of *Arachis* section and reported 78 per cent polymorphism and also showed that all loci had transferability to all the species analyzed.

Kottapalli *et al.* (2007) used 73 micro-satellite markers to genotype 72 accessions from the US groundnut mini-core and found moderate level of genetic variation and the genetic distances (D) values ranged from 0.88 to 0.254.

Tang *et al.*, (2007) assessed the genetic variation from the four sets of 24 accessions each from the four botanical varieties of the cultivated groundnut using 34 microsatellites and observed polymorphism for 10-16 micro-satellite primers.

Upadhyaya *et al.* (2007) studied genetic diversity in composite collection containing 916 accessions with 21 SSR markers and revealed considerable variation among the accessions (0.819 PIC values; 490 alleles). They identified 101, 50 and 11 group specific unique alleles in wild *Arachis fastigiata* and *Arachis hypogaea* respectively.

2.5 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. However, it is difficult to obtain useful linkage maps of cultivated groundnut as it presents extremely low levels of polymorphism due to single event hybridization followed by polyploidization. But recently explosion of robust molecular marker methods revealed significant amount of polymorphism in the crop (Table 1).

Halward *et al.* (1993) for the first time constructed RFLP based linkage map in groundnut aimed at improving the cultivated species (*Arachis hypogaea*). An F_2 (87 individual) population derived from the interspecies hybridization of two related diploid species in the section *Arachis* (*Arachis stenosperma*, *Arachis cardenasii*) were used to construct map. RFLP markers from both genomic and c-DNA clones of groundnut *Arachis hypogaea* CVGK7 were used. Out of these, 100 genomic and 300 c-DNA clones, 15 and 190 respectively revealed polymorphism among the parents but due to complex banding pattern, only 132 markers analyzed for segregation in the population and 117 could be mapped on 11 linkage groups. A total map distance of 1400 cM was covered with a 20 cM resolution. This map covers 80 per cent of the groundnut genome.

Garcia *et al.* (1995) constructed a genetic linkage map for the population 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.

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* K9484X *(Arachis cardenasii* GKP10017 X *Arachis digoi* GKP10602)^{4X}} was

used as a donor parent to generate a back cross (BC1) population of 78 progeny. 370 RFLP loci were mapped on to 23 linkage groups spanning 2210 cM which was slightly greater than twice the length of (1063 cM) the diploid map (Garcia *et al*., 1995).

Milla (2003) constructed a genetic linkage map for an F2 population of *A. kuhlmannii* X *A. digoi*. The map consisted of 102 AFLP markers grouped in to 12 linkage groups and spanning 1068.1 cM.

Herselman *et al*. (2004) used F2:3 population (60) developed from a cross using the aphid resistant parent ICG 12991 and the aphid susceptible male parent ICGVSM93541 to construct AFLP based linkage map. 308 AFLP primer combinations (20 ECoRI + 3/MSe I + 3144 M14 I+3/MSeI + 3 and 144 Pst I + 3/MSeI+3) were used to identify markers associated with the aphid resistance. Twenty putative markers were identified of which 12 mapped to five linkage groups covering a map distance of 139.4cM. A single recessive gene was mapped on linkage group1, 3.9cM from a marker originating from the susceptible parent that explained 76.1% of the phenotype variation for Aphid resistance. This study represents the first partial AFLP based genetic linkage map for cultivated peanut and this is the first report on identification of molecular markers linked to Aphid resistance to groundnut rosette disease (GRD).

Garcia *et al.* (2005) used a backcross population *Arachis stenosperma* X (*Arachis stenosperma* X *A. cardenasii*) and 39 shared RFLPs and 167 RAPD loci to locate on the RFLP map. The RAPDs covered a total genetic length of 800 cm and mapped on to 11 linkage groups.

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 271 new markers plus another 162 published for peanut were screened against both progenitors and 204 of these (47.1%) were polymorphic with 170 co-dominant markers and 34 dominant markers. The 80 co-dominant markers segregating 1:2:1 were initially used to establish the linkage groups. Distorted and dominant markers were subsequently included in the map. The resulting linkage map consists of 11 linkage groups covering 1,230.89 cm of total map distance with an average distance of 7.24 cM between markers.

Gobbi *et al.* (2006) constructed B genome map of groundnut. They used F2 population of 93 individuals obtained from the cross between *Arachis ipaensis* (KG 30076) and *Arachis magna* (KG 30097) both diploid species with B genome for map construction. Eleven linkage groups were obtained from 94 polymorphic micro-satellite markers covering a total distance of 754.8 cM. The size of linkage groups ranged from 5.6 to 130 cM.

Khedikar (2008) constructed a molecular genetic linkage map in cultivated groundnut from in a mapping population consisting of 268 recombinant inbred lines obtained from a cross TAG-24 x GPBD-4 using 67 microsatellite markers. A total of 59 markers mapped on 13 linkage groups spanning 909.4 cM with an average marker interval of 15.25 cM.

Varshney *et al.*, (2008) used 318 RILs obtained from a cross of TAG 24 x ICGV 86031 to construct the SSR based genetic linkage map in cultivated groundnut. 135 out of 150 SSR loci were mapped on 22 linkage groups with the total span of 1270.5 cM with an average intermarker distance of 9.4cM. QTL analysis identified 2-5 QTLs each for transpiration, transpiration efficiency, specific leaf area and SPAD chlorophyll meter reading with the phenotypic variance of 3.5 to 14.1 %.

2.6 Marker trait association studies in groundnut

2.6.1 Late leaf spot and rust

Varma *et al.* (2005) identified SSR markers associated with resistance to rust in groundnut. The parents and F_2 populations of the two crosses were evaluated for resistance to rust under green house conditions. None of the SSR primer pairs showed intra-accession variability among parents. Seven and eight primer pairs detected polymorphism between ICGV 9903 X TMV-2 and ICGV99005 X TMV-2 respectively. They either belonged to ATT or AG SSR repeat families. Based on Wilcoxin test of significance, rust resistance was associated with two SSR alleles (pPGP seq 3A1 271 and pPGP seq 3A1390) in ICGV 99003X TMV-2 and seven SSR alleles (pPGP seq 5D5270, pPGP seq 5D5295, pPGP seq 5D5 325, pPGP seq16F1325, pPGP seq 16F1424, pPGP seq17F6 128 and pPGP seq 13A7 292) in ICGV 99005 X TMV-2.

Mace *et al*. (2006) conducted an experiment to identify diverse disease resistant germplasm for the development of mapping population and their introduction in to breeding programs. 22 genotyped with differing levels of resistance to rust and LLS were screened by 23 SSRs. Overall, 135 alleles across 23 loci were observed in the 22 genotypes screened. 12 of the 23 SSRs (52%) showed a high level of polymorphism with PIC values more than 0.50. Locus by locus AMOVA and Kruskal-wallis one way ANOVA identified candidate SSR loci that may be valuable for mapping rust and LLS resistance.

Mondal *et al*. (2007) identified RAPD markers linked to rust resistance in *Arachis hypogaea* L. They developed F2 mapping population (117) from a cross between the rust resistant parent VG 9514 and rust susceptible parent TAG 24. They tagged RAPD marker J171300 by using modified Bulk Segregate Analysis (BSA), which was tightly linked to rust resistance gene at a distance of 18.50 cM. Out of 160 RAPD primers, 11 primers detected reproducible polymorphism between the parents. One primer (J7) out of eleven primers generated polymorphic DNA fragments, J71350 and J71300 between the resistant and susceptible bulks. Based on linkage analysis results confirmed that J1300 was in repulsion phase and J7 1350 in coupling phase. To test the magnitude of association with rust resistance, simple regression analysis was carried out. The results showed that J7 1300 and J7 1350 individually explained 9.4 per cent and 27.9 per cent of phenotypic variance, respectively.

Khedikar (2008) identified 12 minor QTLs for LLS with the phenotypic variance ranging from 1.40 to 6.20 % and two QTLs (TC2G05-TC9H09 and Seq5D5-TC2G05) were common across the environments. One major QTL (XIP103-Seq 19D06) and 4 minor QTLs were identified for rust in the mapping population (TAG 24 x GPBD 4) consisting of 268 RILs. Six QTLs were identified for nine agronomic traits with the phenotypic variance ranged from 3.20-11.30 per cent.

2.6.2 Other diseases

Stalker and Mozingo (2001) reported association of RAPD markers with a gene conferring resistance to *Cercosporium arachidicola* based on Sporulation, lesion

diameter, defoliation and overall rating. A marker was also associated with resistance to Southern corn rootworm damage. In addition, they associated markers with *Cylindrocladium* black rot resistance and sporulation of *C. arachidicola* in a cross between cultivar NC7 and PI 109839, which represent the first report of molecular markers being associated with resistance genes in an *Arachis hypogaea* x *A. hypogaea* cross.

Burrow *et al.* (1996) reported three RAPD markers linked to a single dominant *Meloidogyne arenaria* resistance gene that was derived from tetraploid plants of the hybrid Florunner with three wild peanut species.

Garcia *et al.* (1996) used RAPD and SCAR technology to map two dominant genes that conferred resistance to the root knot nematode, *Meloidogyne arenaria* (Neal) chit wood Race 1 in a segregating F2 population derived from a cross between wild *Arachis cardenasii krapov* and W. C. Gregory and cultivated *A. hypogaea* species.

Milla (2003) used AFLP markers to establish marker trait association for tomato spotted wilt virus (TSWV) resistance in groundnut. 179 individuals from F2 population of *A. kuhlmannii krapov* and W. C. Gregory X *A. digoi* with total of 13 ECoRI / MSeI primer combinations were used to tag markers associated with TSWV. The study identified five closely linked markers to TSWV resistance. All the five markers located on the same linkage group within a distance of 62.7 cm and among them, four markers originated from *Arachis digoi*.

Lei *et al.* (2006) from twenty genotypes reported SCAR markers AFs-412 converted from AFLP marker E45/M53-440 which was closely linked with resistance to *Aspergilus flavus* infection.

Mace *et al.* (2007) quantified the genetic diversity among 46 selected bacterial wilt resistant lines in comparison with the levels of variation observable within the cultivated *A. hypogaea* gene pool. 32 SSR markers were used to assess the degree of polymorphism. 107 alleles of which 101 (99.40 %) were polymorphic among the 46 genotyped. Analysis of molecular variance (AMOVA) performed to calculate the

contribution of each locus to the differentiation of resistant and susceptible groups within the germplasm tested. Six of the 107 alleles found to contribute significantly to the differentiation between the BW resistant and susceptible genotypes. Furthermore, markers pPGP Seq-1698 229 and pPGP Seq-12 F7 307 contributed 74.9% of the total genetic difference between the two disease response groups.

Varshney *et al.,* (2008) constructed a genetic linkage map using 135 SSR loci for mapping population (TAG 24 x ICGV 86031) consisting of 318RILs. QTL analysis for drought tolerant traits identified one to three QTLs each for T and TE, four to five QTLs for SLA at the start of drought, two to three QTLs for SLA at the time of harvest and eight QTLs for SCMR. The phenotypic variance for these QTLs ranged from 3.50 to 14.10 %.

Table 1: Present status of studies on construction of genetic linkage map in groundnut

S. No.	Mapping population	Features of Genetic map	Genome coverage	Genome	References
1	F2, interspecific; *A. stenosperma x A. cardenasii*	11 Linkage groups with 117 RFLP loci	1063 cM	AA	Halward *et al.*, 1993
2	Backcross population; *A. batizacoi* (*A. cardenasii x A. digoi*)	23 Linkage groups with 370 RFLP loci	2210cM	AABB	Burow *et al.*, 2001
3	F2 population; *A. kuhlmanni x A. digoi*	12 Linkage groups with 102 AFLP loci	1068.1 cM	AA	Milla, 2003
4	F2:3 population; ICG 12991 (Spanish) x ICGVSM 93541(land race)	5 Linkage groups with 12 AFLP loci	139.4 cM	AABB	Herselman *et al.*, 2004
5	F2 population; *A. duranensis x A. stenosperma*	11 Linkage groups with 204 microsatellite loci	1230.89 cM	AA	Moretzohn *et al.*, 2005
6	Back cross population; *A. stenosperma x (A. stenosperma x A. cardenassi*)	11 Linkage groups with 167 RAPD loci	800 cM	AA	Garcia *et al.*, 2005
7	F2 population ; *A. ipaensis x A. magna*	11 Linkage groups with 94 microsatellite loci	754.8 cM	BB	Gobbi *et al.*, 2006
8	Recombinant inbred lines; GPBD 4 (Spanish) x TAG 24 (Spanish)	13 Linkage groups with 59 microsatellite loci	900 cM	AABB	Khedikar, 2008
9	Recombinant inbred line, TAG 24 x ICGV86031	22 Linkage Groups with 135 microsatellite loci	1270.5cM	AABB	Varshney *et al.,* 2008

3. MATERIAL AND METHODS

The mapping population consisting of one hundred and forty six Recombinant Inbred Lines (RILs) segregating for late leaf spot and rust, protein, oil content, oil quality parameters, other agronomic and productivity traits *viz*., plant height, number of branches, number of pods/plant, pod yield/plant 100-seed weight, shelling %, were used for the study. The population was derived from the cross TG 26 x GPBD 4. Both the parents and RILs were subjected to phenotyping for the above traits and genotyping was carried out using 53 polymorphic SSR markers.

3.1 Salient features of parents and mapping population

The salient features of parents of mapping population are as follows

- **TG 26**: An improved Spanish bunch variety, derived form a cross of BARCG1 x TG 23 released in 1998. 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 and rust with high linoleic acid content but susceptible to LLS (Kale *et al*., 1997; Badigannavar *et al*., 2002).

- **GPBD 4**: An improved Spanish bunch groundnut variety derived from KRG1 x 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 (*Arachis hypogaea x A. cardenasii*, ICGV 86855) resistant to foliar diseases developed at ICRISAT, Patanacheru, India.

Mapping population consisting of 146 recombinant inbred lines was developed at the University of Agricultural Sciences, Dharwad from the cross TG 26 x GPBD 4. F_1 obtained from this cross was advanced to F_2 by selfing. One hundred and forty six individual plants selected in F_2 were advanced through single descent method till F_6

generation. Each of the RILs obtained from F_6 generation were maintained by random mating. The population was at F_9 generation when it was used for phenotyping (scoring for the diseases of Rust and late leaf spot, quality traits, agronomic and other productivity traits) and genotyping for the present study.

3.2 Experimental site

Field experiments of the present study were conducted at Botany Garden, Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, during *Rainy* 2005 (E1), *Rainy* 2006 (E2), *Rainy* 2007 (E3), post *Rainy* 2007 (E4) and post *Rainy* 2008 (E5). The soil type of the experimental block was vertisol with pH in the range of 7.0 to 7.5.

3.3 Climatic conditions

Dharwad is located in the transitional tract of Karnataka at $15^0$13' north latitude, 75°07' east longitude and at an altitude of 678 m above mean sea level with an average rainfall of about 800 mm. The rainfall is well distributed between June to October.

3.4 Phenotyping

Phenotyping was carried out for 146 RILs and the parents (TG 26 and GPBD 4) of the mapping population for different traits in five seasons *viz*., E1, E2, E3, E4 and E5 (Table 2).

3.4.1 Rust and late Leaf spot diseases

Disease scoring on rust was recorded in *Rainy* 2005 (E1), *Rainy* 2007 (E3, Experiment I and II) and post *Rainy* 2007 (E4) at 70 days (stage I), 90 days (stage II) and 110 days (stage III only in E3 and E4). Phenotyping on late leaf spot was carried out in E1 and E3 at 70 days (stage I) and 90 days (Stage II).

3.4.1.1 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

Table 2: Phenotypic observations recorded for various traits in different seasons

Rainy 2005 (E1)	*Rainy* 2006 (E2)	*Rainy* 2007 (E3)	Post *Rainy* 2007 (E4)	Post *Rainy* 2008 (E5)
Diseases	**Quality traits**	**Quality traits**	**Quality traits**	**Morphological Traits**
Rust	Protein content (%)	Protein content (%)	Protein content (%)	Plant height (cm)
Stage I (70 DAS)	Oil content (%)	Oil content (%)	Oil content (%)	Number of Branches
Stage II (90 DAS)	**Morphological Traits**	**Fatty acid composition**	**Fatty acid composition**	Pod weight per plant (gms)
Late Leaf spot	Plant height (cm)	Palmitic acid	Palmitic acid	Shelling percentage (%)
Stage I (70 DAS)	Number of Branches	Stearic acid	Stearic acid	100-seed weight (gms)
Stage II (90 DAS)	Number of pods per plant	Oleic acid	Oleic acid	
	Pod weight per plant (gms)	Linoleic acid	Linoleic acid	
	Shelling percentage (%)	Arachidic acid	Arachidic acid	
		Behenic acid	Behenic acid	
		Eicosenoic acid	Eicosenoic acid	
		Lignoseric acid	Lignoseric acid	
		Fatty acid derivatives	**Fatty acid derivatives**	
		O/L ratio	O/L ratio	
		Iodine Value	Iodine Value	
		U/S ratio	U/S ratio	
		% S	% S	
		Morphological Traits	**Morphological Traits**	
		Plant height (cm)	Plant height (cm)	
		Number of Branches	Number of Branches	
		Number of pods per plant	100-seed weight (gms)	
		Pod weight per plant (gms)	**Diseases**	
		Shelling percentage (%)	**Rust**	
		100-seed weight (gms)	Stage I (70DAS)	
		Diseases	Stage II (90 DAS)	
		Rust (Experiment I)	Stage III (110 DAS)	
		Stage I (70DAS)		
		Stage II (90 DAS)		
		Stage III (110 DAS)		
		Rust (Experiment II)		
		Stage I (70DAS)		
		Stage II (90 DAS)		
		Late Leaf Spot		
		Stage I (70 DAS)		
		Stage II (90 DAS)		

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 10^{th} 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 carbendizim (bavistin) 1g/litre and tridemorph (Calixin) 1ml/liter, respectively.

3.4.1.2 Disease scoring

Modified 9 point scale (1-9 score) was followed for both the diseases as per Subbarao *et al*., 1990 (Table 3a and 3b and Figure 1a and 1b). Disease scoring for rust was carried out at E1, E3 (Experiment I and II) and E4 and LLS at E1 and E3 at different stages.

3.4.2 Quality traits

Parents and RILs of the mapping population were subjected to phenotyping for the following quality traits.

3.4.2.1 Protein (%)

Phenotyping for protein content was done in three seasons *viz., Rainy* 2006 (E2) by Kjeldhahl method and *Rainy* 2007 (E3) and post *Rainy* 2007 (E4) were estimated by

Table 3a: Modified 9-point scale used for field screening groundnut genotypes for resistance to rust diseases

Disease score	Description	Disease severity (%)
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 of 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 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	61-80
9	Almost all leaves withered, bare stems seen	81-100

Table 3b: Modified 9-point scale used for field screening groundnut genotypes for resistance to late leaf spot disease

Disease score	Description	Disease severity (%)
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 leaflets 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 lesions 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 may remain, but show severe leaf spot	81-100

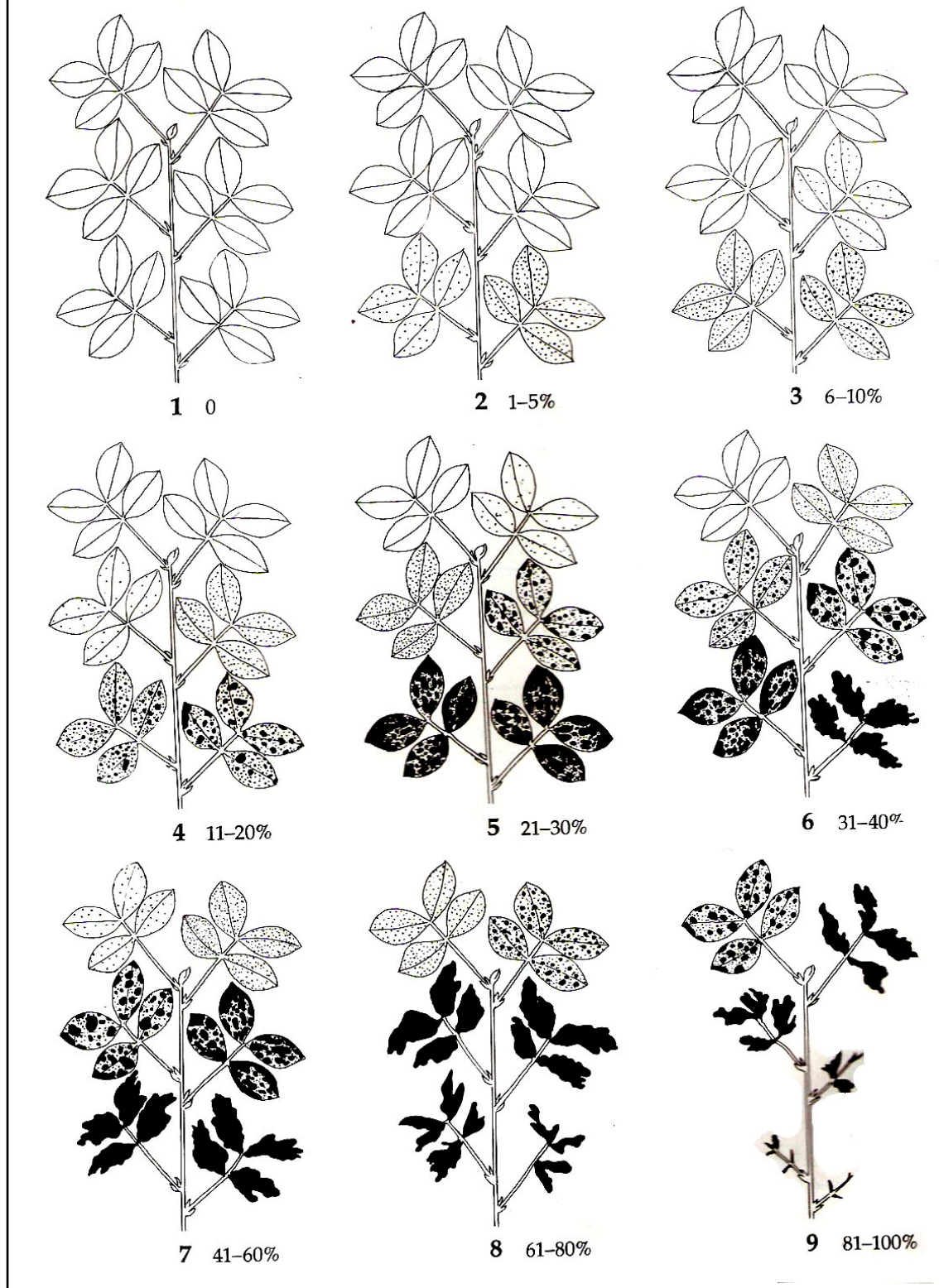


Fig. 1a: The modified 9-point scale for field evaluation of rust of groundnut

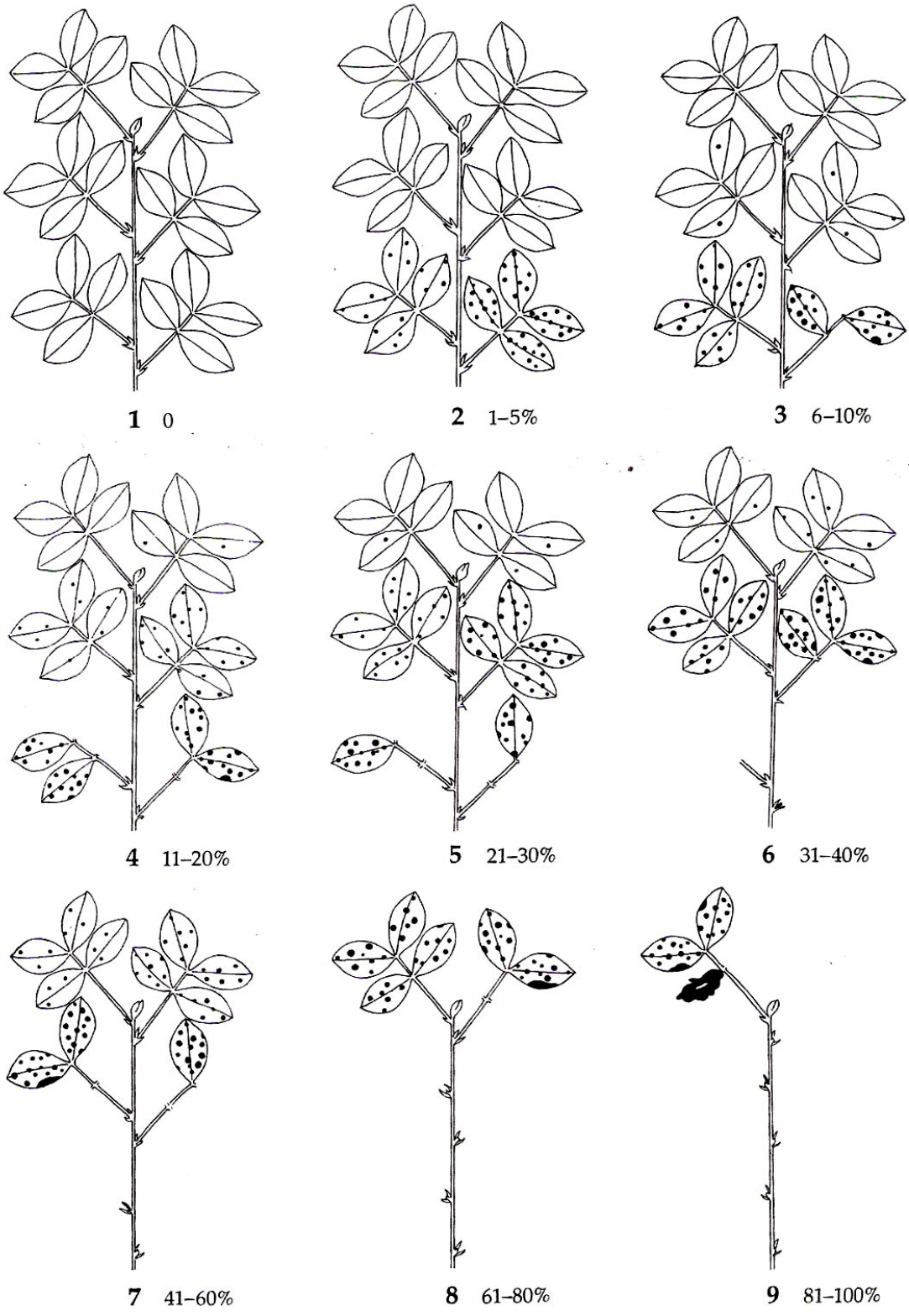


Fig. 1b: The modified 9-point scale for field evaluation of late leaf spot of groundnut

P1

X

P2

F_1

⊗

F_2

Followed single seed decent (SSD) method after F2 generation

LEGEND

X = Crossed with

⊗= Self pollination

Recombinant inbred lines segregating for foliar diseases, nutritional quality and productivity traits in F_9 generation

Plate 1: Development of TG 26 x GPBD4 mapping population

Rust

LLS

Plate 2a: Diseases symptoms of rust and LLS

Rust

LLS

Plate 2b: RILs segregating for rust and LLS

near Infrared spectroscopy (NIRS) at seed quality testing and research lab, U.A.S. Dharwad.

3.4.2.1.1 Kjeldhahl Method of crude protein estimation

One representative sample from each parent and RI line in each replication was taken for protein analysis. The total nitrogen was estimated by Kjeldhahl distillation method. The crude protein was computed by multiplying total nitrogen by the factor 5.46 to arrive at the protein content and is expressed in percentage.

$$\text{Nitrogen (\%)} = \frac{\text{TV x Normality of acid x } 0.014 \text{ x } V_1}{\text{Weight of sample x } V_2}$$

Where,

TV= Titre value

N= Normality of Acid (HCl)

V_1= Volume of digested sample

V_2= Volume taken for distillation.

Phenotyping in other two seasons *viz*., E3 and E4 was done using Near Infrared Spectroscopy (NIRS). The phenotypic data of protein content obtained in E2 (Kjeldhahl method) was used for NIRS calibration.

3.4.2.1.2 Near infrared spectroscopy (NIRS)

Near infrared spectroscopy either in reflectance (NIRS) or transmittance mode (NITS) is a multi-trait technique of large scale applications in the analysis of quality traits in food and agricultural commodities (Shenk and Westerhaus, 1995)

a. Principle

The detection and measurement of chemical composition of biological material in NIRS is based on the vibrational responses of chemical bonds to NIR radiation.

b. Spectral measurements of NIR

NIR diffused reflectance spectra were collected by a monochromator NIR spectrometer model 6500 (Foss NIR systems, France) with the range from 400 to 2500 nm, which consisted of a light source of tungsten halogen lamps of 50 w 12 volts. The spectrometer was equipped with silicon detector. For analysis, the seeds were placed in a special adapter of about 3 mm thick with the diameter of 37 mm and a central hole of 6mm. Scanning was done by inserting the adapter in a standard ring cup (IH-0325, Infrasoft International, LLC, France) and a sample was placed in a central hole. Before spectra acquisition, a reference spectrum was collected from a standard check cell (IH-0324A, Infrasoft Interantional, LLC, France). The instrument diagnostics was carried out to test the response of instrument, wavelength and NIR repeatability to avoid the effect of surrounding environment on the instrument performance. The absorbance spectra (log 1/R) from 400 to 2500 nm were recorded at 8 nm intervals. The method permitted the analysis of about 40 single seeds per hour. Mathematical procedures on the spectral information were carried out with WinISI II Project Manager software, version 1.50 (Infrasoft International, LLC).

c. Calibration

Before using NIR spectrophotometer for any quantitative analysis, it has to be calibrated first using chemical reference method with the application if multivariate regression models to interpret chemical information encoded in the spectral data. Original reflectance spectra were corrected prior to calibration by applying first and second derivative information, standard normal variate transformation and de-trend scatter correction, and four passes were used to eliminate outliers. Second derivative was calculated from log (1/R) spectra of 6 data points and a smoothing over segments of 4 data points (2, 6, 4 and 1). This combination was selected after having tested six additional math treatments (1,4,4,1;1,10,10,1;2,10,10,1;3,10,10,1;4,10,10,1 and 2,5,5,1) with and without spectral corrections and data pretreatments. The 2,6,4,1 treatment was either equal or superior in all cases based on standard error of cross validation and 1-VR

(1 minus the ratio of unexplained variance to total variance) statistics. The calibration equations were developed using principle component regression (PCR), partial least square and modified partial least square (mPLS) regression models. Wavelengths at interval of 8 nm across the entire visible-plus-near-infrared spectrum (visible:408-1092 nm;near infrared: 1108-2492 nm) were used for calibration. The standard error of calibration (SEC), standard error of cross-validation (SECV), correlation coefficient (r), and 1-VR statistics were used to select the best calibration equations.

d. Validation of calibration statistics

The performance of the calibration equations were monitored using the cross validation and external validation of set of samples (n=100). SECV, standard error of prediction (SEP) and r were used to determine the accuracy of prediction.

The best equation for determining the protein, oil and fatty acid composition were developed and used for the subsequent analysis of fatty acid profile 146 RILs of the mapping population.

3.4.2.2 Oil content

Phenotyping for oil content was estimated in three seasons *viz., Rainy* 2006 (E2) by Soxhlet method and *Rainy* 2007 (E3) and post *Rainy* 2007 (E4) by near Infrared spectroscopy (NIRS).

3.4.2.2.1 Soxhlet method for estimation of oil content

Oil content was estimated by Soxhlet method as given by Jambunathan *et al*., (1985) with some modifications. 5 gms of groundnut seeds from each RILs and parents in two replications from the mapping population were made into fine powder in a pestle and mortar. Groundnut meal was extracted with petroleum ether (60-80^{0}C bp) for 5 hrs approximately in a Soxhlet apparatus. Petroleum ether was evaporated and the oil content was estimated by difference in the weight between the two was expressed in percentage. The phenotypic data obtained for oil content by this method was used for calibrating for oil in NIRS. Phenotyping for oil in other two seasons (E3 and E4) were estimated by NIRS.

3.4.2.3 Oil quality/fatty acid profiles and their derivatives

Phenotyping for oil quality traits has been carried out during *Rainy* 2007 (E3) and post *Rainy* 2007 (E4) using NIRS. Preliminary evaluation was done to study the fatty acid profiles in the parents (TG 26 and GPBD 4) and RILs of the mapping population using Gas Chromatography (GC) by taking seed samples of parents and 30 representative RILs from the mapping population.

3.4.2.3.1 Preliminary evaluation for fatty acid profiles

Randomly chosen sound mature seeds of unshelled pods were obtained from each parent (TG 26 and GPBD 4) and thirty randomly selected RILs from population. A small portion of the distal end removed and middle portion of the seed was used for the analysis. According to Kartha (1963) and Zeile *et al.,* (1993), tissue samples from the middle sections of groundnut seed gave optimal representation for fatty acid composition and iodine value (IV). Preliminary evaluation of thirty RILs and two parents were subjected to fatty acid analysis using a modified method of Young and Waller (1972). A brief summary of this method is as follows.

a. Esterification procedure (Young and Waller, 1972)

Esterification procedure involved solvent extraction and esterification of the fatty acids to form fatty acid methyl esters (FAME).

1. The 200-300 mg of grounded samples were placed in test tube to which 2 ml of petroleum ether (HPLC grade, boiling range 35-60^0 C) was added, the tubes were sealed with Teflon-lined caps, vortexed and allowed to stand overnight at room temperature.

2. The following day, supernatant was transferred to another set of tubes and tubes were left open at room temperature to evaporate petroleum ether.

3. The 2 ml of 0.5 M NaOH in methanol was added; tubes were vortexed and heated at 100^{0}C in water bath for 5 minutes.

4. Tubes were cooled and 2 ml of boron triflouride in methanol (12%) (Sigma, Aldrich) was added; tubes were vortexed and heated at 100 ^{0}C in water bath for 5 minutes. Tubes were cooled and 2 ml of deionized water was added to stop the reaction and a pinch of $NaSO_4$ was added to absorb the water.

5. Two ml of petroleum ether was added to tubes. After a thorough mixing and phase separation, 1.5 ml of the upper phase (FAME) was removed by pipette into 2 ml screw cap glass auto sampler vials fitted with a septum and used for gas chromatography analysis.

b. Gas chromatography analysis

A gas chromatograph, model GC-2010 equipped with automatic sample injector AOC-20i, flame ionization detector (Shimadzu, Kyoto, Japan) and fitted with a narrow bore capillary column: Rtx- (film thickness-0.25µm; I.D-0.25mm; length-30m) was used to separate methyl esters. The initial column temperature was set at 170 ^{0}C and held for 3 minutes, then programmed at an increase of 10 ^{0}C per minute to a final temperature of 230 ^{0}C, at which it was held for 1 minute. Injector and detector temperature were both set at 250 ^{0}C. The flow rates for nitrogen (carrier gas), hydrogen and air were 45, 40 and 400 ml per minute, respectively. A split ratio of 10:1 was employed and 1µl of sample was injected using an auto sampler. The fatty acid methyl ester was identified by a comparison of retention time to standard methyl ester fatty acid mixtures (Sigma, Aldrich). Concentration of each fatty acid was recorded by normalization of peak areas as per cent of particular fatty acid (Fig. 2).

Phenotyping for fatty acid composition during E3 and E4 was analyzed using Near Infrared Spectroscopy (NIRS). Eight fatty acids *viz*., Palmitic acid (16:0), Stearic acid (18:0), Oleic acid (18:1), Linoleic acid (18:2), Arachidic acid (20:0), eicosenoic acid (20:1), behenic acid (22:0), and lignoseric acid (24:0). Among these, Palmitic, Stearic, Arachidic, Behenic and Lignoseric are unsaturated fatty acids with no double bonds in their fatty acid chain and oleic and eicosenoic acids are monounsaturated fatty acids with single double bond and the linoleic acid is the polyunsaturated fatty acid with two double bonds in their fatty acid chain. The values in the brackets indicate the number of carbon atoms and the number of double bonds in the fatty acid chain.

Oil stability indices *viz.,* Oleic/Linoleic acid (O/L) ratio, Iodine value (IV), Unsaturated/saturated (U/S) fatty acid ratio and % of saturated fatty acids (% S) were estimated as follows

- **Oleic/Linoleic acid (O/L) ratio:** % of Oleic acid (C18:1)/ % of Linoleic acid (C18:2)

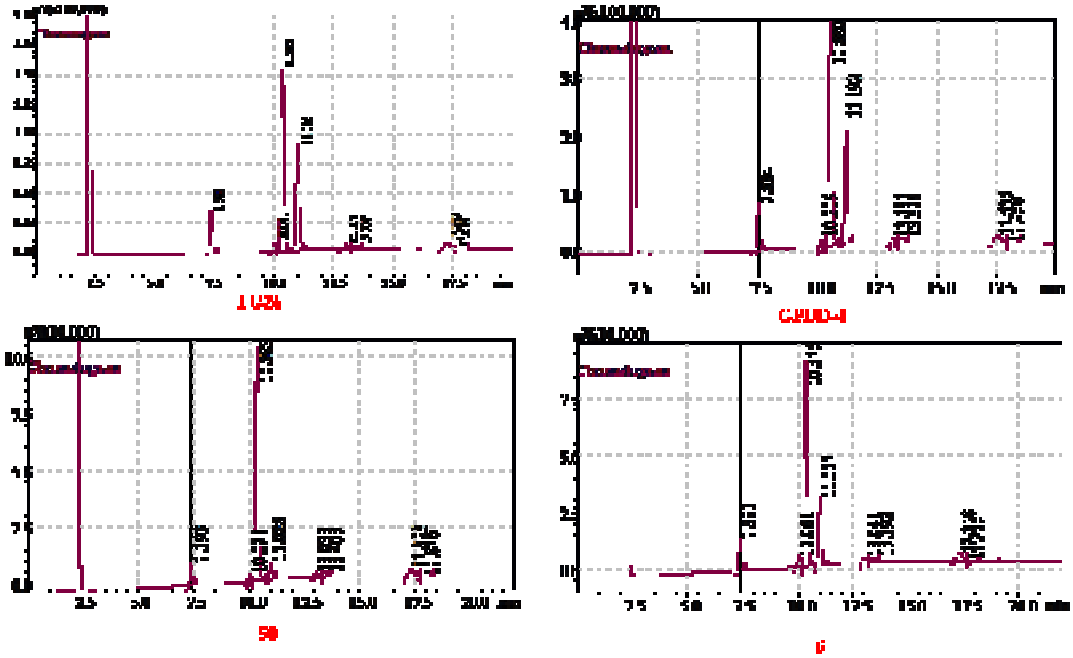


Fig 2: Chromatogram of parents and RILs of mapping population

- **Iodine value (IV):** (% Oleic x 0.8601)+(% Linoleic x 1.7321)+(% Eicosenoic x 0.7854) (Mozingo *et al.,* 1988)

- **Unsaturated/saturated fatty acid (U/S):** % (Oleic + Linoleic + Eicosenoic)/% (Palmitic + Stearic + Arachidic + Behenic + Lignoseric).

- **% of Saturated fatty acids (% S):** % (Palmitic + Stearic + Arachidic + Behenic + Lignoseric acid) (Mozingo *et al.,* 1988).

3.4.3 Agronomic and other productivity traits

Data on agronomic and productivity traits were recorded during *Rainy* 2006 (E2), *Rainy* 2007 (E3), post *Rainy* 2007(E4) and post *Rainy* 2008 (E5). Phenotyping was done from two random plants selected from each RILs and parents of the mapping population in two replications. The following observations were recorded.

1. **Plant height (cm):** Phenotyping on plant height was recorded during *Rainy* 2006 (E2), *Rainy* 2007 (E3), post *Rainy* 2007 (E4) and post *Rainy* 2008 (E5). 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.

2. **Number of primary branches per plant:** Number of primary branches in each RI line was recorded before the harvest during *Rainy* 2006 (E2), *Rainy* 2007 (E3), post *Rainy* 2007 (E4) and post *Rainy* 2008 (E5).

3. **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 during *Rainy* 2006 (E2), *Rainy* 2007 (E3) and post *Rainy* 2008 (E5).

4. **Pod yield per plant (gms):** Phenotyping on pod weight per plant was done during *Rainy* 2006 (E2), *Rainy* 2007 (E3). Pod weight per plant was recorded from two plants of each RIL in two replications.

5. **Shelling Percentage (%):** Shelling % was calculated as kernel weight /pod weight and expressed in percentage during *Rainy* 2006 (E2), *Rainy* 2007 (E3) and post *Rainy* 2008 (E5).

6. 100-Seed weight (gms): Weight of 100 seeds in grams was recorded after the harvest from two plants of each RIL in two replications during *Rainy* 2007 (E3), post *Rainy* 2007 (E4) and post *Rainy* 2008 (E5).

3.5 Genotyping of mapping population

3.5.1 DNA isolation of parents and RILs

Young leaves and tissues of parents and RILs were collected from two weeks old plants grown in green house and SIGMA Genelute plant genomic DNA extraction kit was used to isolate DNA as per the manufacturer's recommendations as follows.

1. The young leaves and tissues were ground in to a fine powder in liquid nitrogen using a mortar and pestle and transferred up to 100 mg of the powder to a microcentrifuge tube, and kept the sample on ice for immediate use or frozen at -70 ^{0}C until use.

2. 350 µl of lysis solution (A) and 50 µl of lysis solution (B) were added to the tube; mixed it thoroughly by vortexing and inverting. A white precipitate was formed upon the addition of lysis solution. The tube was incubated at 65^{0}C for 10 minutes with occasional inversion to dissolve the precipitate.

3. To this mixture, 130 µl of precipitation solution was added and mixed completely by inversion and placed the samples on ice for 5 minutes. The samples were centrifuged at maximum speed (12,000-16,000 rpm) for 5 minutes to pellet the cellular debris, proteins and polysaccharides.

4. The supernatant from step 3 was carefully pipetted on to a Genelute filtration column and centrifuged at maximum speed for 1minute. This removes any cellular debris not removed in step 3. Discarded the filtration column and retained the collection tube.

5. 700 µl of binding solution was added directly to the flow-through liquid from step 4. The solution was mixed thoroughly by inversion.

6. Inserted a Genelute miniprep binding column into a provided microcentrifuge tube. Five hundred microlitres of the column preparation solution was added to each

miniprep column and centrifuged at 12,000 rpm for 30 seconds to 1 minute. The flow through liquid was discarded.

7. 700 µl of the mixture from step 5 was transferred on to the column prepared in step 6 and centrifuged at maximum speed for 1 min. The flow through liquid was discarded and the collection tube was retained. The column was returned to the collection tube. The remaining lysate from step 5 was applied on to the column. The centrifugation was repeated and discarded the flow through liquid and collection tube.

8. Prior to first time use, ethanol was added to the wash solution concentrate. Binding column was placed into a fresh 2ml collection tube and applied 500 µl of the diluted wash solution to the column. The tube was centrifuged at maximum speed for 1 minute and discarded the flow through liquid and retained the collection tube.

9. Another 500 µl of diluted wash solution was added to the column and centrifuged at maximum speed for 3 minutes to dry the column. Care was taken not allow the flow-through liquid to contact the column; wiped off any fluid that adheres to the outside of the column.

10. In the next step, the binding column was transferred to a fresh 2ml collection tube and applied 100 µl of pre-warmed (65 ^{0}C) elution solution to the column and centrifuged at maximum speed for 1 minute. Elute was collected in the same collection tube. Alternatively, a second collection tube was used for the second elution to prevent dilution of the first elute.

3.5.2 Quantification of DNA

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

3.5.3 Genotyping using SSR primers

Initially the parents TG 26 and GPBD 4 were screened for polymorphism by using 1043 available SSR primers (Hopkins *et al*., 1999; He *et al*., 2003; Ferguson *et al*., 2004; Moretzohn *et al*., 2004 and 2005; Mace *et al*., 2007; Cuc *et al*., (unpublished); Bertoli *et al*., (Unpublished) and Knapp *et al*., (unpublished)). Out of these, 894 primers produced scorable bands and 53 markers found polymorphism between the parents TG 26 and GPBD 4 (Table 4). These 53 polymorphic primers were employed for genotyping the mapping population.

3.5.4 DNA amplification

Polymerase chain reaction (PCR) was employed by using a touch down PCR profile and an amplification protocol appropriate for each pair of primers (Table 4). DNA amplification was performed in 5 µl reaction mixture in Gene Amp® PCR system 9700 (Applied Biosystems). The recipes for PCR reaction mixture for all the labeled and unlabelled primers were common except the primer concentration and Taq polymerase. Reaction mixture consisted of 25 mM $MgCl_2$ (Bioline), 2 mM dNTPs, 10X PCR buffer (Bioline for unlabelled and Amplitaq Gold for labeled primers) and 5 U/µl Taq polymerase (1U Bioline Taq for unlabeled and 5U Amplitaq gold for labeled primers). Concentration of the primer for unlabeled and florescent labeled primers was 10 pm/µl (Forward and Reverse primers mixture) and for M-13 tailed primers, the primer concentration of 1pm/µl of forward+2pm/µl of reverse primers mixture was used (Table 6a, b and c).

3.5.5 Electrophoresis

PCR products were confirmed for amplification on 1.2 % agarose gel before loading them in the sequencing gel. For separation of amplified DNA fragments, non-denaturing polyacrylamide gel electrophoresis (PAGE) and capillary electrophoresis (ABI 3700) were employed.

3.5.6 Non-Denaturing gel electrophoresis

After PCR amplification, one µl of orange dye was added to 5 µl reaction mixture and mixed by short spinning. Then 2.5 µl of this reaction mixture was loaded in each lane of 96 track of 6% non-denaturing PAGE using multichannel pipette

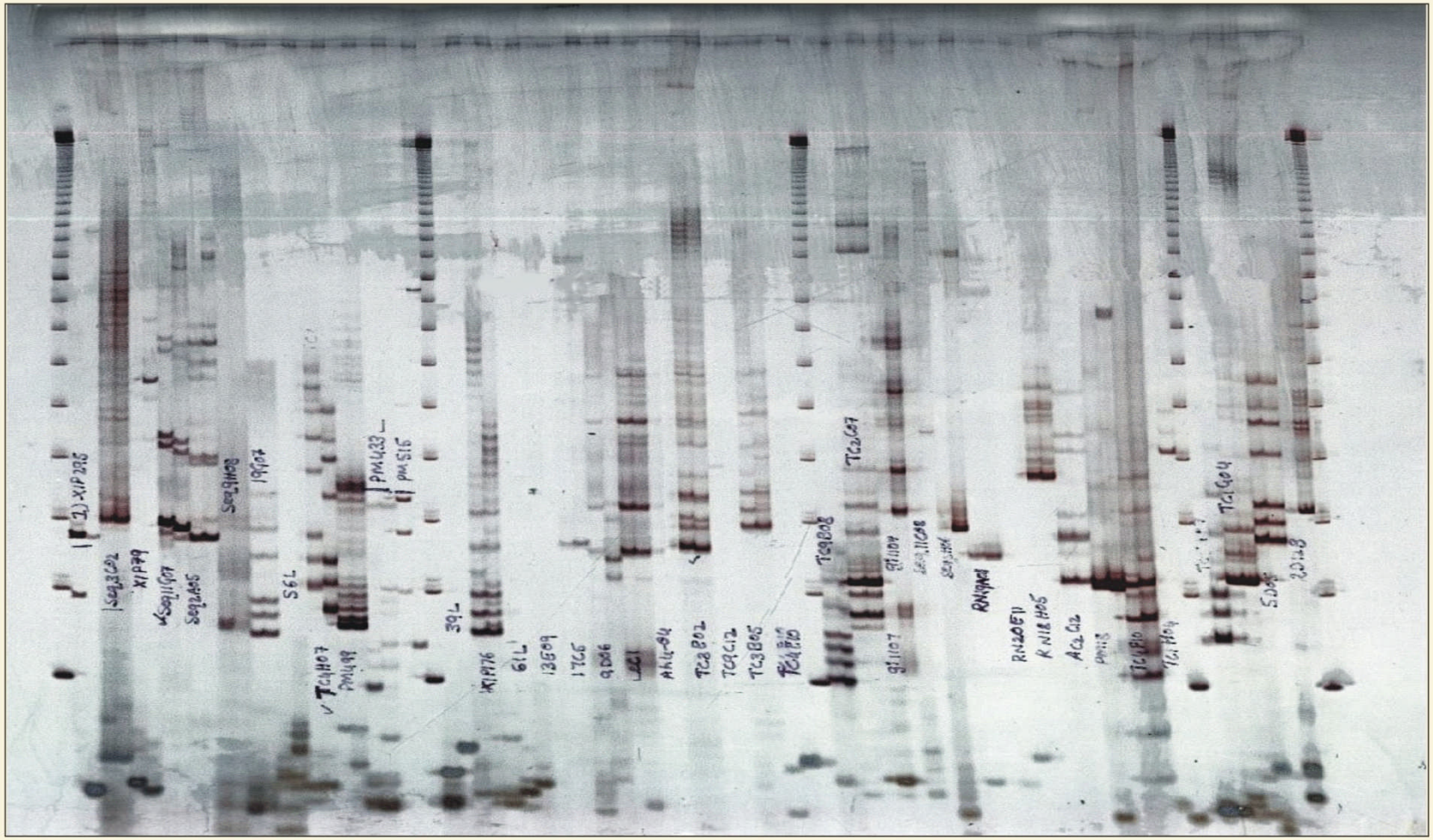


Plate 3a: Screening of parents using SSR markers on PAGE

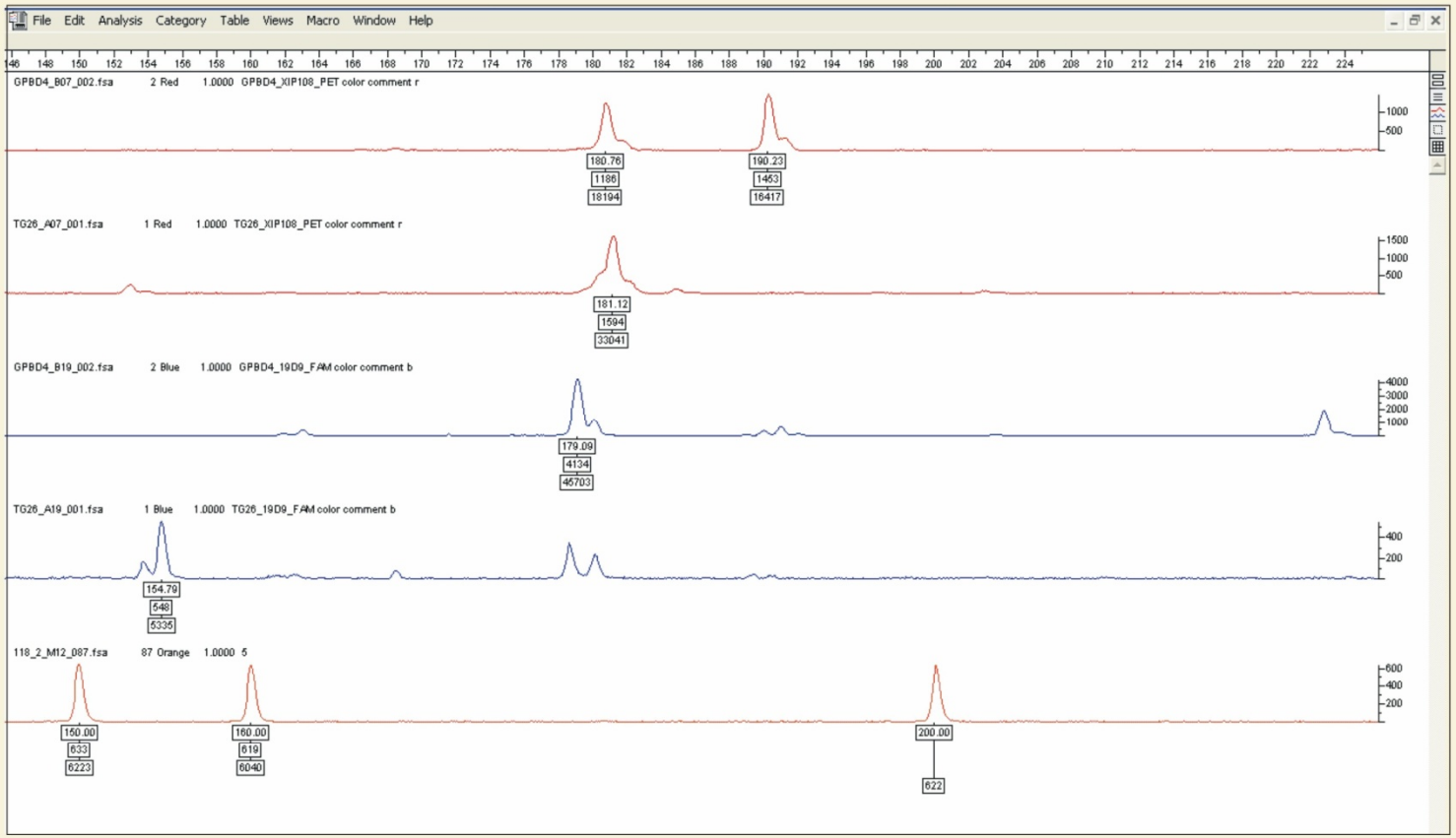
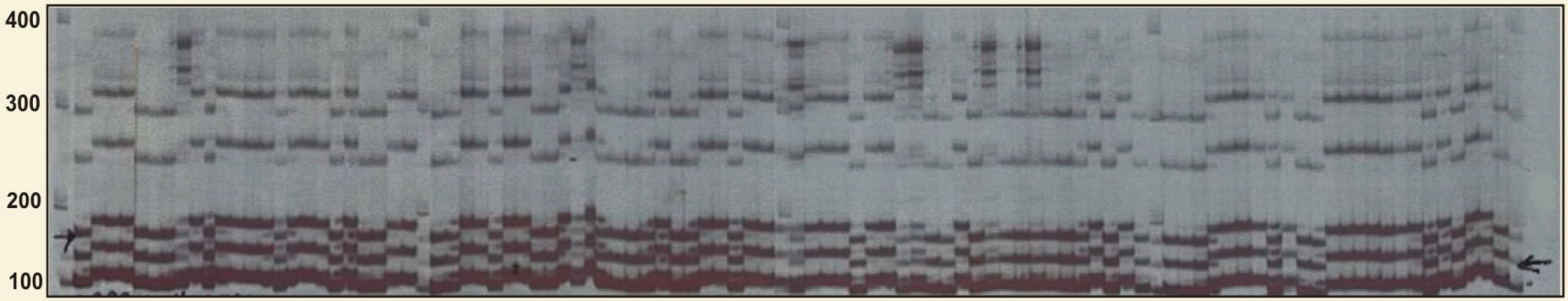
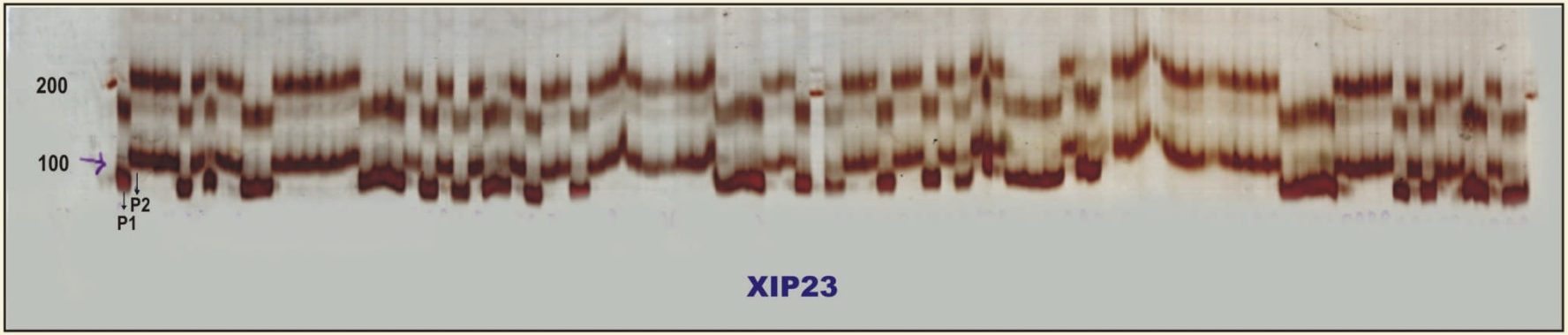


Plate 3b: Screening of parents using capillary electrophoresis (ABI 3700) genetic analyzer



TC3A12



XIP23

Plate 4a: Genotyping of RILs with SSR markers on PAGE

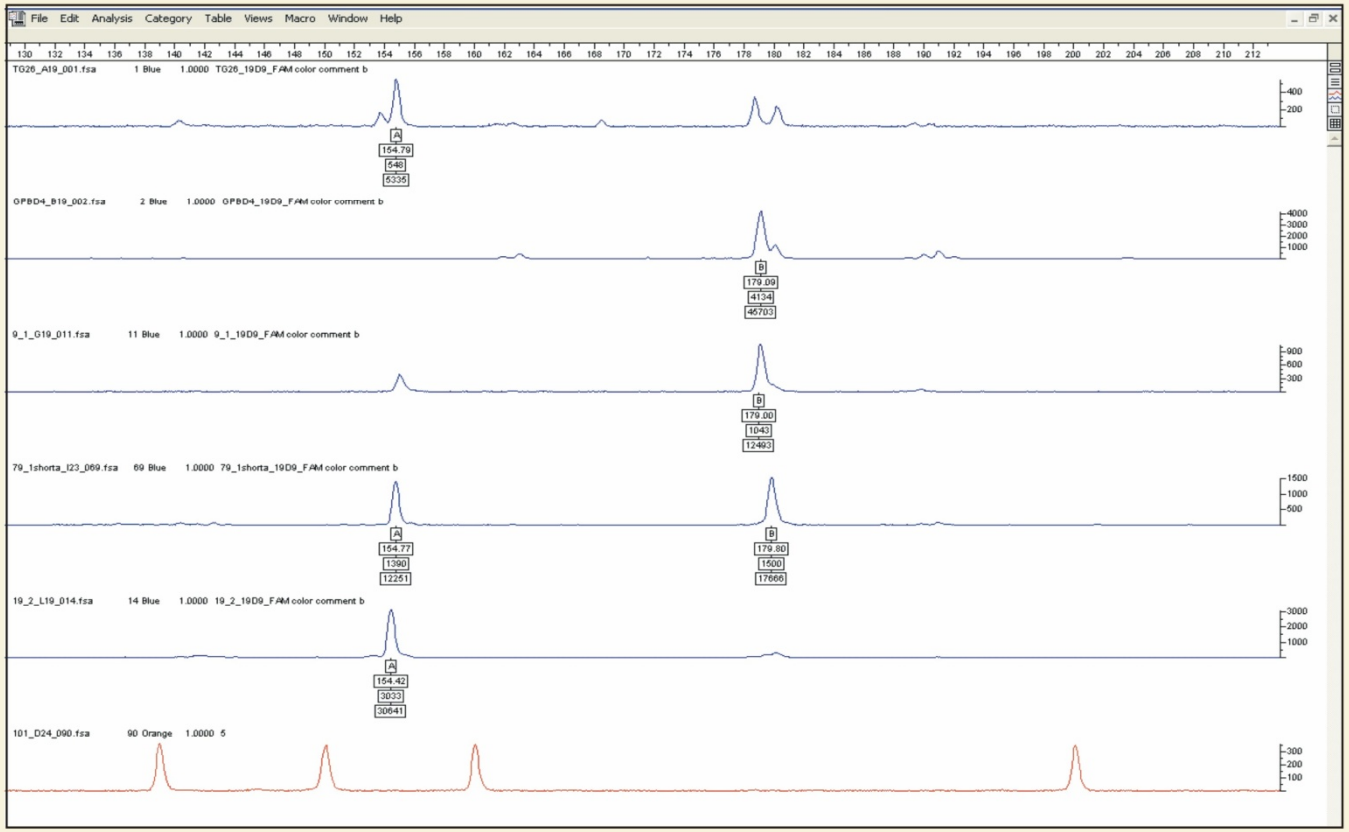


Plate 4b : Genotyping of RILs with SSR markers on capillary electrophoresis (ABI 3700) genetic analyzer

(Finipipette) and the 100 base pair ladder was loaded after every 24 samples. 75 ml of 6 % non denaturing polyacrylamide gel was sufficient for Biorad PAGE unit. The recipe for 75 ml of gel consisted of 7.5 ml of 10X TBE buffer, 15 ml of 29:1 (w/w) acrylamide/bisacrylamide, 53 ml distilled water, 450 µl of Ammonium per sulphate (APS) and 100 µl of TEMED. After polymerization, the gel plate was set for prerunning for 10 minutes at 750 volts to warm the plate. Then the samples were loaded Biorad sequencing gel unit. Electrophoresis was run at 800 volts for 2 hours 30 minutes or until the desired resolution has been reached (determined by the dye front) in 0.5 X TBE running buffer using.

Amplified products were then visualized by using silver staining protocol (Kolodny 1984). Initially the gel was rinsed in distilled water for 3-5 minutes, with gentle shaking followed by soaking the gel in 1.5 liters of 0.1 % CTAB for 12-15 minutes then kept in 1.5 liters of 0.3 % liquid ammonia for 15 minutes with gentle shaking. In the next step the gel was placed in silver nitrate solution (1.5 g silver nitrate, 6 ml of 1M NaOH, in 1.5 liters of water and then titrated with 6-8 ml of 25 % ammonia until the solution becomes clear) for 15 minutes with gentle shaking. Then the gel was cleaned with distilled water to stop staining further. In the last step, the gel was kept in developer solution (22.5 g of sodium carbonate + 400 µl of formaldehyde in 1.5 µl of distilled water) until the bands become visible.

The gel was kept in water for 5 minutes to remove the gel debris attached to another side of plate and to stop further staining further. After staining the gel, bands were scored as A, B, H and O. Where, A represents homozygosity for the allele from female parent (TG 26) and B indicates the homozygosity for the allele from male parent (GPBD 4) and H represents the heterozygotes *i.e* the presence of both A and B alleles and O represents offtypes (neither A nor B) and missing values (Appendix 1).

3.5.7 Capillary electrophoresis

Amplified products of M13-tailed primers and florescent labeled primers were separated by capillary electrophoresis (ABI 3700). After the amplification, ABI run plate, a total of 11 µl containing a mixture of 1.2 µl of PCR product, 2.8 µl of deionized water, 7.0 µl of Hi-di formamide, 0.2 µl of LIZ 500 size standard was prepared. Then

the plate containing this mixture was centrifuged and kept for denaturation at 95^0 C for 5 minutes followed by chilling on ice for 5 minutes. Then again the plate was centrifuged at 900 rpm for 1 minute and immediately the plate was wrapped with aluminium foil (to protect the mixture from exposing to light as LIZ is sensitive and losses its activity after exposing it to light). Then the plate was kept in ABI 3700 genetic analyzer. The "G5" dye set, "GeneScan Pope" run module and GS 500 LIZ analysis module were employed and the fragments were separated in 36 cm capillary array. After completion of the run, the A and B peaks were sized using Genescan software and scoring was done with the help of Genotyper software (Plate 3b and 4b).

3.6 Statistical Analysis

3.6.1 Phenotypic data analysis

3.6.1.1 Analysis of variance

The analysis of variance (ANOVA) for different characters was carried out by using the mean phenotypic data for all the seasons separately and pooled across the seasons in order to partition the variability due to different sources following the method given by Panse and Sukhatme (1964).

The structure of ANOVA is as follows

Source of variation	d.f.	MSS	Expected value of MSS	Cal F.
Replication	(r-1)	M_1	-	
Genotypes	(g-1)	M_2	$\sigma^2 e + r\sigma^2 g$	M_2/M_3
Error	(r-1) (g-1)	M_3	$\sigma^2 e$	
Total	(rg-1)	$M_1+M_2+M_3$		

3.6.1.2 Mean and range

The mean, range and variance of each character were calculated for each RI line at each season.

i) Mean $(\bar{X})$ $= \dfrac{\text{Sum of observations of all the plants}}{\text{Number of plants}}$

ii) Range = The minimum and maximum values for each trait

Where,

Xi = Individual value

X = Population mean

n = Number of observations

3.6.1.3 Frequency distribution:

Phenotypic data of LLS and rust diseases, quality traits and productivity traits and of one hundred and forty six RILs in comparison with the parents TG 26 and GPBD 4 were subjected for frequency distribution. SPSS software was used for analysis.

3.6.1.4aEstimation of genetic variability components

In order to assess and quantify the genetic variability among the RILs for the characters under study, the following parameters were estimated.

Phenotypic and genotypic variances were estimated using the following formula (Singh and Chaudhary, 1979).

$$\text{Genotypic variance } (\sigma_g^2) = \frac{\text{MSS (genotypes) - MSS (error)}}{\text{Number of replications}} = \frac{M_2 - M_3}{R}$$

$$\text{Phenotypic variance } (\sigma_p^2) = \sigma_g^2 + \text{MSS error} = \frac{M_2 - M_3}{r} + M_3$$

b) Coefficient of variability

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

i) Genotypic coefficient of variability (GCV)

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

ii) 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 as indicated by Siva Subramanian and Menon (1973). It is as follows

0-10% : Low

10-20% : Moderate

20% and above : High

c) Heritability (h^2)

Heritability 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.

$$\text{Heritability } (h^2) = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where,

σ_g^2 = Genotypic variance

σ_p^2 = 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

d) 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 of selection (Lush, 1949)

σ_p = Phenotypic standard deviation

e) Genetic advance as per cent of mean (GAM)

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

Where,

GA= Genetic advance

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

Genetic advance as per cent 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

3.6.1.5 Correlation analysis

The correlation coefficients were worked out to determine the degree of association for a group of characters (Diseases, quality and productivity traits). The correlations were calculated in all the five seasons *viz*., E1, E2, E3, E4 and E5 among the quality traits, disease resistance to LLS and rust and among morphological traits.

Phenotypic correlations were computed by using the formula given by Webber and Moorthy (1952).

$$r_p = \frac{\text{Cov } XY_p}{\sqrt{\sigma_p^2 x X\ \sigma_p^2 y}}$$

Where,

r_p = Phenotypic correlation

Cov XY_p = Phenotypic covariance between the characters 'x' and 'y'

$\sigma_p^2 x$ and $\sigma_p^2 y$ = Phenotypic variance of the characters 'x' and 'y' respectively

Phenotypic correlation coefficients were compared against table value at (n-2) degrees of freedom at the probability levels of 0.05 and 0.01 to test their significance (Fisher and Yates, 1963).

3.6.2 Genotypic data analysis

3.6.2.1 Linkage map construction

Fifty three marker data of one hundred and forty recombinant inbred lines of mapping population were subjected for linkage map construction. Chi square test was performed on the genotypic data to test the null hypothesis of expected 1:1 Mendelian segregation. Of these, fifteen markers showed Segregation Distortion (SD). Due to less number of markers the distorted markers were also used for linkage map construction.

The linkage analysis was performed using MAPMAKER/EXP 3.0 (Lander *et al*., 1987; Lincolin *et al*., 1992). A minimum LOD of 3.0 and maximum recombination fraction of 0.50 were set as threshold values for linkage groups determination. Linkage groups were defined with "Sequence All" command. The most likely order within each linkage group was estimated by using three point analyses ("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. The "try" and "build" commands were used to determine the exact position and linkage group of the new marker orders. 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 centi Morgans (cM) using Kosambi mapping function. The intermarker distances calculated from mapmaker were used to construct the Linkage map by using MAPCHART Version 2.2 (Voorrips, 2006). Out of 53 markers, 45 markers could land on 8 linkage groups, which spanned 657.9cM of the groundnut genome and eight markers remained ungrouped. The previously mapped markers were used to designate and orientate linkage groups.

3.6.3 Marker-trait association

3.6.3.1 Single marker analysis (SMA)

Single marker analysis was performed to tag potential SSR markers linked to the phenotypic data of rust and LLS disease resistance, quality traits, agronomic and productivity traits and genotypic data of 53 markers pertaining to the 146 RILs, which is based on simple linear regression method (Haley and Knott, 1992).

3.6.3.2 QTL analysis

Phenotypic data of quality traits, disease resistance to LLS and Rust and productivity traits in each individual seasons and across the seasons were combined

with genotypic data and linkage map in order to identify the QTLs associated with these traits using PLABQTL version 1.1w (Utz and Melchinger, 1996).

The replicated means of 146 RILs for quality traits, disease resistance and morphological traits were used for QTL mapping in each individual season. To determine the QTLs across the seasons, replicated means of across seasons means of 146 RILs were used. QTL analysis was performed using the method of composite interval mapping (CIM) (Zeng 1994, Jansen and Stam, 1994) as in PLABQTL version 1.1w.

Composite interval mapping combines the approaches of interval mapping (IM) and single marker analysis (SMA) in multiple regression frame works (Haley and knott, 1992). Cofactors are identified using stepwise regression with an F to enter and F to delete threshold value of 3.5 in PLAB QTL. Once the model containing cofactors is built, the entire genome is rescanned using interval mapping.

The presence of putative QTL in an interval was tested by using a critical value for LOD threshold as determined by PLABQTL 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 x 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

LOD=n/n (1+p8F/Df)*0.2171

Where,

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

F ratio=SSR(full)-SSR(red)/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)=SSE/DEF=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):

Additive effect= (Parent P2-Parent P1)/2

Table 4: Screening for parental polymorphism in TG 26 x GPBD 4 parents of mapping population

Source	No. of primers screened	Polymorphic primers	Percent polymorphism
Hopkins *et al*., 1999	26	0	0.00
He *et al*., 2003	158	4	2.53
Ferguson *et al*., 2004	226	10	4.42
Moretzshon *et al*., 2004, 2005	338	20	5.92
Mace *et al*., 2006	79	0	0.00
Cuc *et al*., (Unpublished)	170	16	9.41
Bertioli *et al*., (Unpublished)	46	3	6.52
Total	**1043**	**53**	**5.08**

Table 5: Touch down PCR for labeled and unlabelled primers used for Genotyping of mapping population of TG 26 x GPBD 4

S. No.	Steps	Unlabelled Primers			Labeled Primers			Cycles
		Temperature (^{0}C)		Time	Temperature (^{0}C)		Time	
		60-55 (56)	65-60 (59)		60-55 (56)	65-60 (59)		
1	Initial denaturation	95	95	3 min	95	95	15 min	5 Cycles
2	Denaturation	94	94	20 sec	94	94	20 sec	
3	Annealing	60	65	20 sec	60	65	20 sec	
4	Primer extension	72	72	30 sec	72	72	30 sec	
5	Denaturation	94	94	20 sec	94	94	20 sec	30 Cycles
6	Annealing	56	59	20 sec	56	59	20 sec	
7	Primer extension	72	72	30 sec	72	72	30 sec	
8	Final extension	72	72	20 min	72	72	20 min	
9	Store at	4	4		4	4		

Table 6a: PCR reactions for unlabelled primers

Components	Concentration^{-1}/µl	Unlabelled primers		
		Protocol 1	Protocol 2	Protocol 3
		PCR reaction (5 µl)	PCR reaction (5 µl)	PCR reaction (5 µl)
Primers (F+R)	10pM	0.30	0.50	0.30
Taq buffer (Bioline)	10 X	0.50	0.50	0.50
Mg 2+ (Bioline)	25mM	0.15	0.20	0.20
dNTP's	2 mm	0.50	0.50	0.50
Template	5ng/l	1.00	1.00	1.00
Deionised water		2.40	2.15	2.15
Taq polymerase (Bioline)	1 U	0.15	0.15	0.15

Protocol 1: Universal protocol
Protocol 2: Seq19D06, XIP 121
Protocol 3: Seq4E08, XIP407c and XIP295

Table 6b: PCR reactions for the M13-tailed primers

Components	Concentration^{-1}/µl	PCR reaction (5 µl)
Primers (F+R)	1pM F+2pM R	0.50
Taq buffer (Amplitaq Gold)	10 X	0.50
Mg 2+ (Amplitaq Gold)	25mM	0.50
dNTP's	2 mm	0.25
Template	5ng/l	1.00
Deionised water		1.73
Taq polymerase (Amplitaq Gold)	5 U	0.02
Dye (FAM, NED, PET, VIC)	2pM/l	0.50

Table 6c: PCR reactions for the Florescent labeled primers

Components	Concentration^{-1}/µl	PCR reaction (5 µl)
Primers (F+R)	10pM	0.3
Taq buffer (Amplitaq Gold)	10 X	0.5
Mg 2+ (Amplitaq Gold)	25mM	0.5
dNTP's	2 mm	0.25
Template	5ng/l	1
Deionised water		2.43
Taq polymerase (Amplitaq Gold)	5 U	0.02

4. EXPERIMENTAL RESULTS

Phenotyping was done for diseases (rust and LLS), nutritional quality (protein, oil twelve oil quality parameters), agronomic (plant height and number of branches) and productivity traits (number of pods/plant, pod yield/plant, shelling % and 100-seed weight) in five seasons *viz., Rainy* 2005 (E1), *Rainy* 2006 (E2), *Rainy* 2007 (E3), post rainy 2007 (E4) and post rainy 2008 (E5) at University of Agricultural Sciences, Dharwad. Genotypic data was generated using 53 polymorphic microsatellite markers at ICRISAT, Patanacheru, Andhra Pradesh. Mapping population consisting of 146 RILs obtained from a cross between TG 26 and GPBD 4 was used for the study. The population was segregating for all the above traits. The experimental results of the present investigation have been divided in to following sub headings.

4.1 Phenotypic data analysis

4.2 Genotypic data analysis

4.3 Marker-Trait Association

4.1 Phenotypic data analysis

4.1.1 Analysis of variance (ANOVA)

Analysis of variance pooled across the seasons for different traits is given in the Table 7. Analysis of variance across the seasons for disease scores of rust and LLS, nutritional quality traits and productivity traits revealed significant variation among the genotypes, seasons and Genotype x season interaction for all the traits. The coefficient of variation (CV) for nutritional quality traits was less compared to diseases and productivity traits.

4.1.2 Components of variation

The nature and magnitude of variation was assessed by phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2) and genetic advance as per cent mean (GAM) for all the traits studied are explained below.

4.1.2.1 Rust and late leaf spot

Table 7: Pooled ANOVA for disease resistance, nutritional quality and productivity traits in TG 26 x GPBD 4 mapping population

Traits/Source of variation	Mean sum of squares							
	Season	Replication	S x R	Genotypes	S X G	Error	CV	Sed
Diseases								
df	2	1	2	145	290	435		
Rust								
Stage I	189.89**	11.72	7.59	7.26**	1.50**	0.43	17.60	0.33
Stage II	316.58**	1.17	5.67	11.56**	1.95**	0.71	17.07	0.42
df	1	1	1	145	145	290		
Stage III	87.46**	6.16	1.16	6.06**	1.65**	1.09	18.66	0.74
Late leaf spot								
Stage I	263.12**	30.75	75.51	4.44**	2.42**	1.11	21.33	0.74
Stage II	234.40**	15.78	0.36	2.11**	1.27**	0.73	12.58	0.60
Nutritional quality traits								
df	2	1	2	145	290	435		
Protein (%)	65.31**	2.50	2.19	26.69**	13.66**	1.45	4.02	0.70
Oil (%)	38.87**	0.63	0.06	9.23**	5.96**	1.18	2.38	0.63
df	1	1	1	145	145	290		
Palmitic acid	333.55**	0.65	0.27	1.52**	0.64**	0.13	3.50	0.26
Stearic acid	0.14	0.31	0.13	0.72**	0.31**	0.15	11.08	0.27
Oleic acid	292.63**	0.38	0.63	82.84**	35.81**	2.13	3.16	1.03
Linoleic acid	1678.63**	0.06	0.06	59.68**	27.03**	1.72	4.08	0.93
Arachidic acid	29.74**	0.01	0.01	0.049**	0.029**	0.02	6.39	0.08
Eicosenoic acid	24.59**	0.01	0.01	0.03**	0.014**	0.01	7.58	0.04
Behenic acid	4.40**	0.10	0.01	0.26**	0.13**	0.03	4.60	0.13
Lignoseric acid	17.71**	0.02	0.00	0.05**	0.021**	0.01	7.80	0.08
O/L ratio	3.36**	0.00	0.01	0.01*	0.26**	0.01	1.20	0.81
Iodine value (IV)	2741.00**	2.00	1.50	31.4**	15.38**	1.32	1.20	0.81
U/S ratio	19.87**	0.005	0.01	0.17**	0.08**	0.01	3.11	0.08
%S	351.25**	0.031	0.19	2.99**	1.20**	0.25	2.08	0.35
Productivity traits								
df	3	1	3	145	435	580		
Plant height	1598.56**	449.88	39.48	42.15**	30.34**	15.02	16.05	1.94
No. of Branches	163.05**	0.016	3.81	1.91**	1.81**	1.59	21.58	0.63
df	1	1	1	145	145	290		
No. of pods/plant	0.03**	7.45	13.58	83.84**	5.94**	34.12	34.91	4.13
df	2	1	2	145	290	435		
Pod weight/plant	1.04**	3.94	194.58	55.49**	20.43**	22.67	36.53	2.75
Shelling %	5009.75**	810	60.25	61.82**	54.49**	48.99	9.44	4.04
100-Seed weight	4077.44**	112.62	139.88	139.40**	49.99**	28.74	14.62	3.09

df- Degrees of Freedom, SxR-Season x Replication, S x G- Season x Genotype, CV-Coefficient of variation, Sed-Standard error difference

The phenotypic and genotypic coefficients of variation for both rust and LLS revealed significant variation indicating moderate to high level of variability at all the stages in all the seasons except for LLS at stage II in E3. A similar trend was evident for heritability and genetic advance for both the diseases indicating highly heritable mature of the variation. The reduction in different components of variability under pooled analysis was more for LLS as compared to rust indicating preponderance of G x E interaction (Table 8).

4.1.2.2 Nutritional quality

4.1.2.2.1 Protein and oil content

Although moderate PCV and GCV were recoded for protein, but very high heritability followed by moderate to high GA revealed high heritable variation. In contrast, lower magnitude of variation (PCV and GCV) was observed for oil coupled with higher heritability but low GA. Across the seasons, there was reduction in values of components of variability for both the traits, but the reduction was more for oil compared to protein indicating preponderance of G x E interaction for oil as compared to protein (Table 9).

4.1.2.2.2 Oil quality parameters

All the oil quality parameters except O/L ratio had low to moderate PCV and GCV with high to very high heritability coupled with low to moderate GAM in both the seasons. O/L ratio recorded higher magnitude and heritable variation as evidenced by high PCV, GCV, heritability and GAM. A comparable trend was observed under pooled analysis with very slight reduction in the variability components indicating lesser role for G x E interaction for these traits (Table 10).

4.1.2.3 Agronomic and productivity traits

In general, the phenotypic coefficient of variation revealed higher magnitude of variation for all the traits except shelling %. But low to moderate heritability has resulted in very low genetic advance for number of branches, number of pods per plant and pod yield per plant, while, higher heritability had led to moderate genetic advance for plant height and 100-seed weight. When the data was analyzed across the seasons, there was significant reduction in all the components of variation indicating predominant role for G x E interaction for these traits (Table 11).

Table 8: Mean, range and genetic variability components for rust and late leaf spot in TG 26 x GPBD 4 mapping population

	Parents		RILs					
Traits	**TG 26**	**GPBD 4**	**Mean**	**Range**	**PCV**	**GCV**	**h^2**	**GAM**
***Rainy* 2005 (E1)**								
Rust (Stage I)	3.00	2.00	2.98	2.00-5.00	31.41	23.62	56.60	36.63
Rust (Stage II)	5.00	3.00	4.03	2.00-8.00	37.50	31.74	71.70	55.28
LLS (Stage I)	7.00	2.00	5.62	2.50-7.00	26.49	23.28	77.20	42.20
LLS (Stage II)	8.00	3.00	6.16	3.00-8.00	19.49	14.21	52.90	21.26
***Rainy* 2007 {E 3 (EXPI)}**								
Rust (Stage I)	4.00	2.50	3.57	2.00-5.00	18.70	12.15	42.20	16.24
Rust (Stage II)	5.00	3.00	4.51	3.00-6.00	18.53	14.29	59.40	22.62
Rust (Stage III)	6.00	3.00	5.23	3.00-7.00	19.30	14.25	54.50	21.61
***Rainy* 2007 {E3 (EXPII)}**								
Rust (Stage I)	5.00	2.00	4.88	2.00-8.00	41.83	38.28	83.70	7.13
Rust (Stage II)	7.50	3.00	6.43	3.00-9.00	33.27	28.96	75.70	51.94
LLS (Stage I)	4.00	2.50	4.27	2.00-9.00	35.74	18.30	26.20	19.19
LLS (Stage II)	7.50	4.00	7.43	5.00-9.00	13.25	5.85	19.50	5.38
Post *Rainy* 2007 (E4)								
Rust (Stage I)	4.50	2.00	3.52	2.00-6.00	31.91	26.13	67.00	12.48
Rust (Stage II)	6.00	2.00	4.83	2.00-8.00	33.21	27.45	68.30	9.71
Rust (Stage III)	6.50	3.50	6.00	2.00-9.00	33.03	24.69	55.90	6.33
Across seasons								
Rust (Stage I)	4.06	2.13	3.73	2.12-5.12	28.73	22.70	62.50	37.00
Rust (Stage II)	5.07	2.75	4.95	2.75-6.62	27.96	22.14	62.70	36.16
Rust (Stage III)	6.45	3.00	5.61	3.00-7.50	26.42	18.70	50.10	27.27
LLS (Stage I)	5.50	2.25	4.99	2.75-7.00	25.71	14.36	31.20	16.43
LLS (Stage II)	7.75	3.5	6.79	5.50-8.00	14.25	6.69	22.00	6.48

RILs-Recombinant Inbred Lines
PCV-Phenotypic coefficient of variation
GCV-Genotypic coefficient of variation
h^2- Heritability (%)
GAM- Genetic advance as percent mean

Table 9: Mean, Range and Genetic variability components for protein and oil in TG 26 x GPBD 4 mapping population

Traits	Parental means		RILs					
	TG 26	GPBD 4	Mean	Range	PCV	GCV	h^2	GAM
***Rainy* 2006 (E2)**								
Protein (%)	26.33	33.18	30.36	21.40-38.98	11.23	10.51	87.50	20.26
Oil (%)	43.65	50.28	45.50	36.36-52.67	6.56	5.59	72.70	9.823
***Rainy* 2007 (E3)**								
Protein (%)	26.03	34.79	30.12	24.02-36.64	8.76	8.23	88.30	15.93
Oil (%)	44.17	47.38	45.31	40.76-49.03	3.14	2.67	72.60	4.70
Post *Rainy* (E4)								
Protein (%)	23.96	32.57	29.45	21.12-37.51	11.05	9.90	80.30	10.2
Oil (%)	43.85	46.27	46.02	42.40-49.55	2.59	2.04	62.20	2.50
Across seasons								
Protein (%)	25.44	33.51	29.98	24.18-35.42	6.35	4.92	60.20	12.35
Oil (%)	43.88	47.98	45.61	41.77-49.22	2.88	1.62	31.70	2.10

E2-*Rainy* 2006, E3-*Rainy* 2007, E4-Post *Rainy* 2007

RILs-Recombinant Inbred Lines

PCV-Phenotypic coefficient of variation

GCV- Genotypic coefficient of variation

h^2- Heritability (%)

GAM- Genetic advance as percent mean

Table 10: Mean, range and Genetic variability components for oil quality parameters in TG 26 x GPBD 4 mapping population

Traits	Parental means		RILs					
	TG 26	GPBD 4	Mean	Range	PCV	GCV	h^2	GAM
***Rainy* 2007 (E3)**								
Palmitic acid	11.27	10.09	10.98	8.90-13.37	6.00	4.70	61.30	7.56
Stearic acid	2.81	2.32	3.49	2.24-4.98	13.59	9.50	48.90	13.77
Oleic acid	40.15	51.35	46.88	34.47-59.98	9.08	8.74	92.60	17.32
Linoleic acid	38.03	28.98	30.41	19.65-40.56	11.73	11.24	91.90	22.19
Arachidic acid	1.55	1.09	1.64	0.71-2.12	11.25	6.90	37.60	8.56
Eicosenoic acid	1.05	1.09	1.03	0.80-1.25	7.73	5.24	45.90	7.73
Behenic acid	3.94	3.67	4.08	3.04-5.12	9.05	7.81	74.50	13.97
Lignoseric acid	1.20	1.41	1.42	0.94-1.84	8.97	5.48	37.30	7.05
O/L ratio	1.06	1.77	1.58	0.85-3.05	22.05	21.28	93.20	42.38
Iodine value (IV)	101.22	95.22	93.81	85.91-100.97	2.77	2.59	87.30	4.98
U/S ratio	3.82	4.40	3.64	3.10-4.23	5.46	4.80	77.30	8.80
%S	23.75	21.53	24.60	22.11-27.35	3.77	3.30	76.90	5.98
Post *Rainy* 2007 (E4)								
Palmitic acid	10.01	8.87	9.47	6.32-11.51	9.30	8.72	87.90	1.77
Stearic acid	3.32	2.32	3.52	1.68-5.26	18.86	14.32	57.60	6.39
Oleic acid	33.99	51.94	45.46	27.89-65.90	14.48	13.99	93.20	0.61
Linoleic acid	44.76	28.83	33.80	16.11-49.11	16.83	16.19	92.60	0.95
Arachidic acid	2.51	2.08	2.09	1.56-2.46	6.64	5.26	62.60	4.13
Eicosenoic acid	0.31	0.81	0.62	0.21-1.18	21.68	18.86	75.70	53.86
Behenic acid	4.34	3.72	3.91	2.85-5.02	7.79	6.28	64.90	2.69
Lignoseric acid	0.71	1.34	1.07	0.55-1.56	15.04	12.97	74.40	21.83
O/L ratio	0.76	1.80	1.43	0.57-4.06	37.07	35.78	93.10	49.95
Iodine value (IV)	107.00	95.25	98.15	84.78-109.46	4.32	4.10	89.90	0.08
U/S ratio	3.79	4.45	4.00	3.20-5.15	7.73	6.91	79.90	3.18
%S	23.88	22.56	23.05	19.25-26.82	5.28	4.73	80.30	0.38
Across seasons								
Palmitic acid	10.64	9.48	10.22	6.32-13.37	5.80	4.59	62.70	7.53
Stearic acid	3.06	2.27	3.50	1.68-5.26	14.34	9.10	40.30	11.99
Oleic acid	37.07	51.65	46.17	27.89-65.90	8.07	7.43	84.60	14.08
Linoleic acid	41.39	28.90	32.11	16.11-49.11	9.79	8.90	82.60	16.66
Arachidic acid	2.03	1.58	1.86	0.71-2.46	7.44	3.81	26.20	3.76
Eicosenoic acid	0.68	0.95	0.83	0.21-1.25	10.37	7.07	46.60	9.65
Behenic acid	4.14	3.69	3.99	2.85-5.12	6.34	4.37	47.50	6.26
Lignoseric acid	0.95	1.38	1.24	0.55-1.84	9.79	6.45	43.40	8.84
O/L ratio	0.91	1.79	1.51	0.57-4.06	18.78	17.08	82.70	31.89
Iodine value (IV)	104.11	95.23	95.98	84.78-109.46	2.41	2.09	75.20	3.72
U/S ratio	3.81	4.42	3.82	3.10-5.15	5.02	3.94	61.60	6.28
%S	23.81	21.43	23.82	19.25-27.35	3.49	2.81	64.60	4.66

PCV-Phenotypic coefficient of variation, GCV-Genotypic coefficient of variation
h^2- Heritability (%), GAM- Genetic advance as percent mean, E3-*Rainy* 2007, E4-Post *Rainy* 2007

Table 11: Mean, range and genetic variability components for agronomic and productivity traits in TG 26 x GPBD 4 mapping population

Traits	Parents			RILs				
	TG 26	GPBD 4	MEAN	Range	PCV	GCV	h^2	GAM
***Rainy* 2006 (E2)**								
Plant height (cm)	18.03	30.95	27.18	8.00-43.00	21.68	11.77	29.40	13.13
Number of branches	5.00	6.00	5.02	3.50-8.50	24.19	9.59	15.70	7.76
No. of pods/plant	11.00	24.50	16.74	4.00-42.50	40.14	14.87	13.70	11.35
Yield/plant (g)	7.98	19.11	13.05	2.00-35.50	41.90	19.15	20.90	18.00
shelling %	76.23	73.44	76.47	57.50-90.91	8.85	2.49	7.90	1.44
***Rainy* 2007 (E3)**								
Plant height (cm)	15.90	29.45	23.08	8.00-33.30	21.94	16.03	53.30	1.04
Number of branches	4.75	4.50	6.09	3.00-13.50	22.52	0.52	0.10	0.00
No. of pods/plant	13.04	16.67	16.72	5.50-45.50	34.80	12.82	13.60	0.60
Pod Yield/plant (g)	7.02	10.21	12.97	2.75-37.25	37.51	17.84	22.60	1.39
shelling %	68.71	75.99	76.64	61.70-87.50	5.24	1.95	13.90	0.03
100-seed weight	32.65	50.76	34.21	17.36-58.74	21.69	14.77	46.40	20.64
Post *Rainy* 2007 (E4)								
Plant height (cm)	15.50	31.90	24.66	8.85-39.80	18.40	8.81	22.90	1.43
Number of branches	4.50	5.50	6.77	4.00-13.00	23.81	5.30	4.90	5.16
100-seed weight	26.26	45.00	40.97	21.88-62.20	16.60	12.76	59.10	11.20
Post *Rainy* 2007 (E5)								
Plant height (cm)	19.58	25.28	21.66	14.7-29.83	18.00	12.49	48.20	17.91
Number of branches	4.92	5.25	5.50	3.5-7.5	17.66	7.19	16.60	6.00
Pod Yield/plant (g)	10.23	20.15	12.99	3.44-24.13	40.87	12.24	9.00	7.62
shelling %	71.23	74.62	69.40	48.95-83.06	14.19	3.54	6.20	1.83
100-seed weight	31.25	49.52	34.79	16.75-54.52	22.51	13.98	38.60	17.91
Across seasons								
Plant height (cm)	17.25	29.4	24.15	16.56-31.54	16.82	5.03	8.90	3.11
Number of branches	4.79	5.31	5.85	4.50-7.06	21.66	1.86	7.00	0.34
No. of pods/plant	11.42	20.44	16.73	7.50-32.38	43.75	26.38	36.30	5.48
Pod Yield/plant (g)	7.5	14.66	13.04	6.49-20.76	40.96	18.54	20.50	2.25
shelling %	72.06	74.68	74.17	63.14-81.92	9.55	1.49	2.40	0.36
100-seed weight	30.05	48.43	36.67	22.78-46.23	18.02	10.53	34.10	4.65

RILs-Recombinant Inbred Lines
PCV-Phenotypic coefficient of variation
GCV-Genotypic coefficient of variation
h^2- Heritability (%)
GAM- Genetic advance as percent mean
E2-*Rainy* 2006, E3-*Rainy* 2007, E4-Post *Rainy* 2007, E5-Post *Rainy* 2008

4.1.3 Frequency distribution, mean and range

The frequency distribution among the RILs in comparison with parents is given in the figures (Fig. 7 to 14). X-axis represents traits which are divided into equal class intervals and Y-axis represents the genotype frequencies for respective traits. The parental mean, mean and range among the RILs for disease resistance to rust and LLS, nutritional quality and agronomic and productivity traits are presented in the Tables 8-11.

4.1.3.1 Rust and late leaf spot

The frequency distribution was bimodal (E3 (Exp II) and E4) to normal (E1, E3, Exp I) for rust. Exceptionally, the distribution was skewed towards the resistance in one season (E3, Exp I). Majority of the RILs fell within the range of parents however; few RILs showed transgressive segregation for susceptibility (Fig 3 (i) & (ii)).

For late leaf spot, the distribution was mostly normal but skewed towards susceptibility at stage I in E1. Majority of the RILs were within the range of parents in the first stage in both the seasons but many RILs exhibited higher susceptibility than the susceptible parent, TG 26 at later stage (Fig. 4).

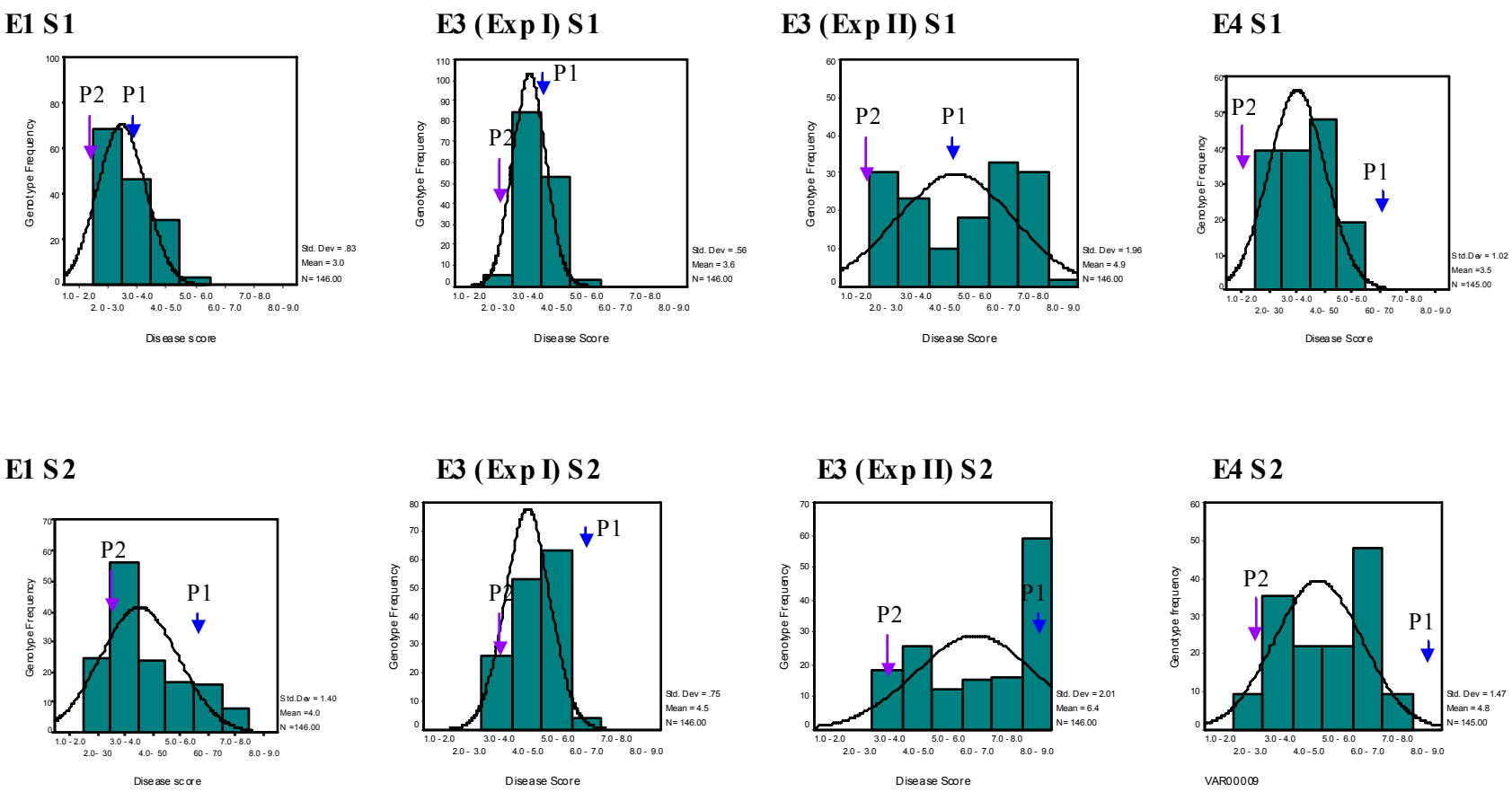
4.1.3.2 Nutritional quality traits

4.1.3.2.1 Protein and oil content

Distribution was normal for both oil and protein in all the three seasons with few RILs exhibiting transgressive segregation beyond both higher (GPBD 4) and lower value (TG 26) parents (Fig. 5).

4.1.3.2.2 Oil quality parameters

Frequency distribution was mostly normal for all the twelve oil quality parameters. Few RILs showed transgressive segregation in both the direction except for stearic acid where many RILs showed transgressive segregants beyond the higher stearic acid parent (TG 26). GPBD 4 was higher value parent for oleic acid, eicosenoic acid, lignoseric acid, O/L ratio and U/S ratio. Whereas, TG 26 was higher value parent for palmitic acid, stearic acid, linoleic acid, arachidic acid, behenic acid, Iodine value and %S (Fig.6 (i) to 6 (iv)).



E1 – Rainy 2005
S1 – Stage 1

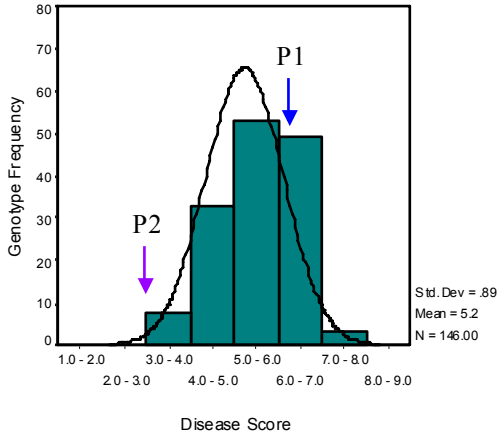
E3 – Rainy 2007
S2 – Stage 2

E4 – Post rainy 2007

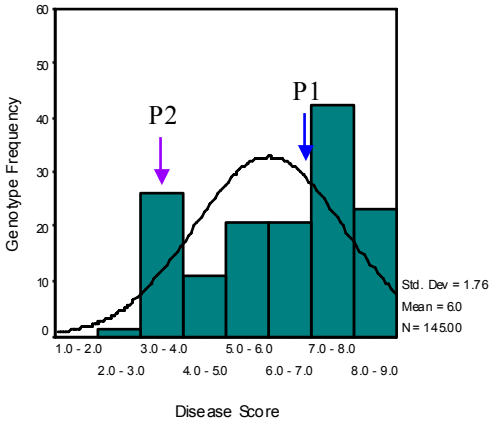
P1 – TG 26
P2 – GPBD 4

Fig. 3 (i): Frequency distribution of RILs for rust verses stage I and stage II in TG 26 x GPBD 4 mapping population

E3 (Exp II) S3



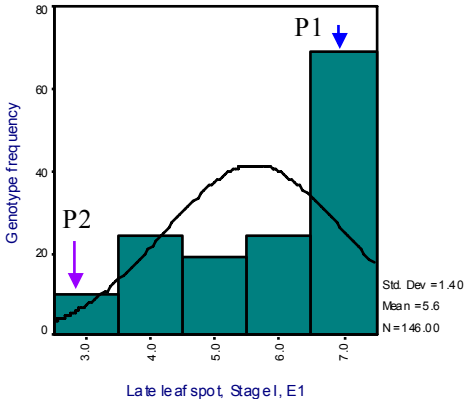
E4 S3



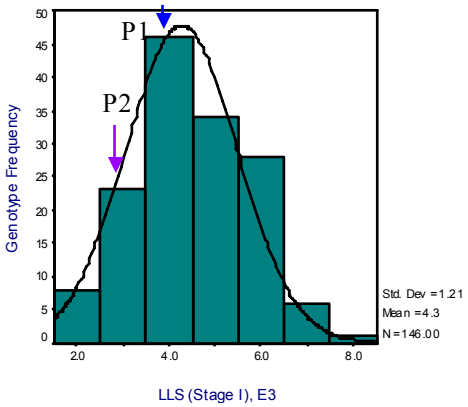
E3 – Rainy 2007 E4 – Post-rainy 2007 P1 – TG 26
S3 – Stage 3 P2 – GPBD 4

Fig. 3 (ii): Frequency distribution of RILs for rust in TG 26 x GPBD 4 mapping population

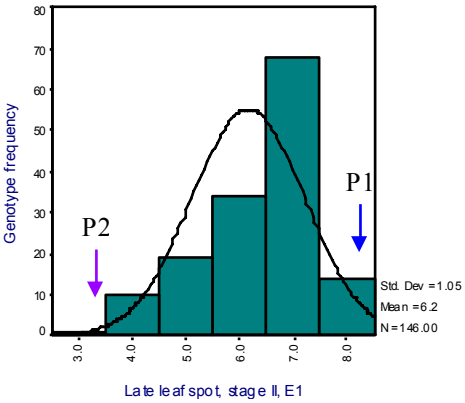
E1S1



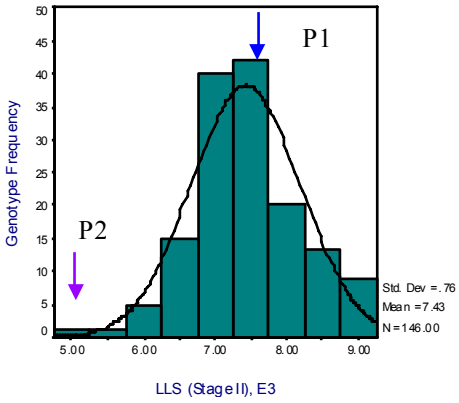
E1S2



E3S1

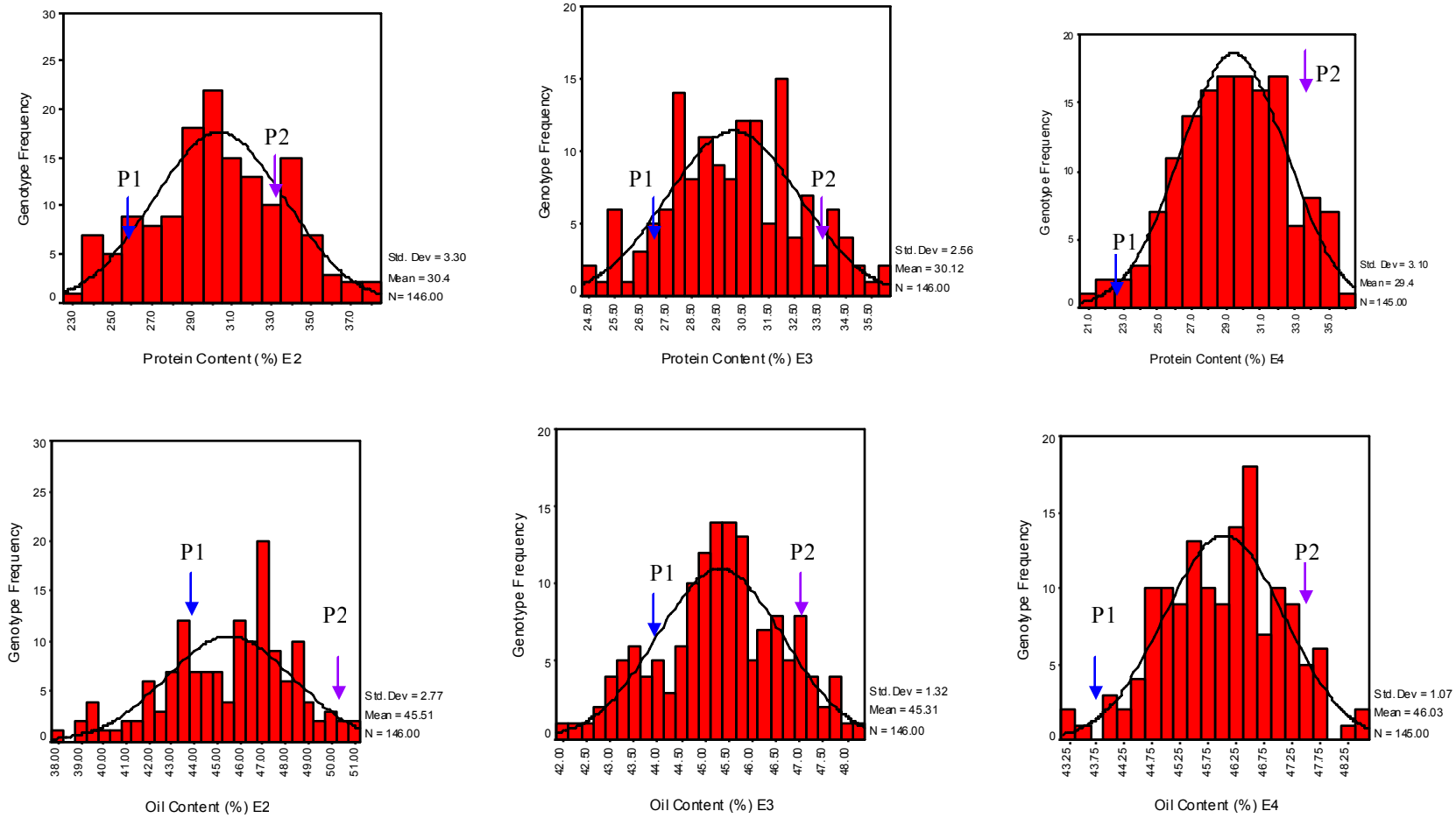


E3S2



E1 – Rainy 2005 E3 – Rainy 2007 P1 – TG 26
S1 – Stage 1 S2 – Stage 2 P2 – GPBD 4

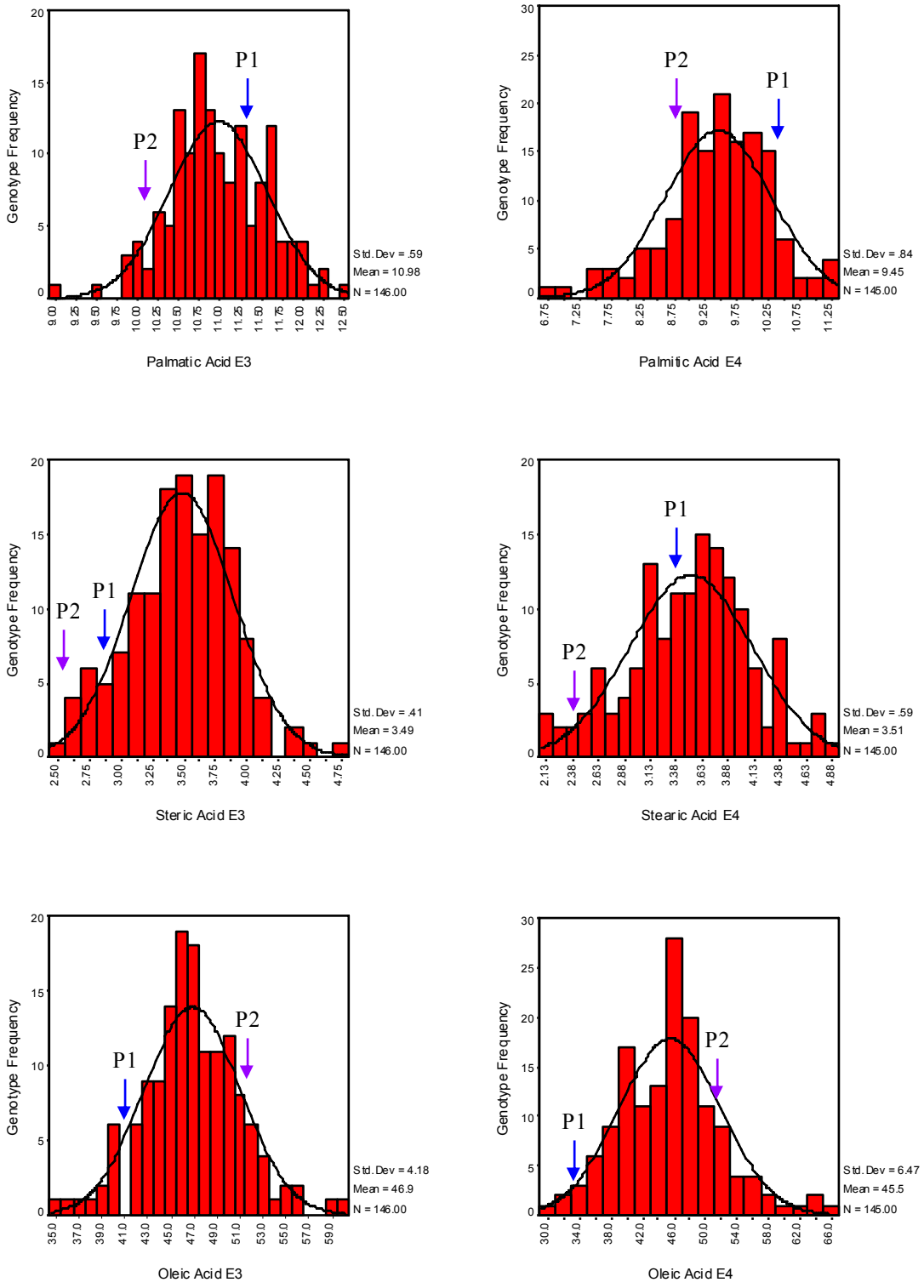
Fig. 4: Frequency distribution of RILs for LLS in TG 26 x GPBD 4 mapping population



P1-TG 26, P2-GPBD 4

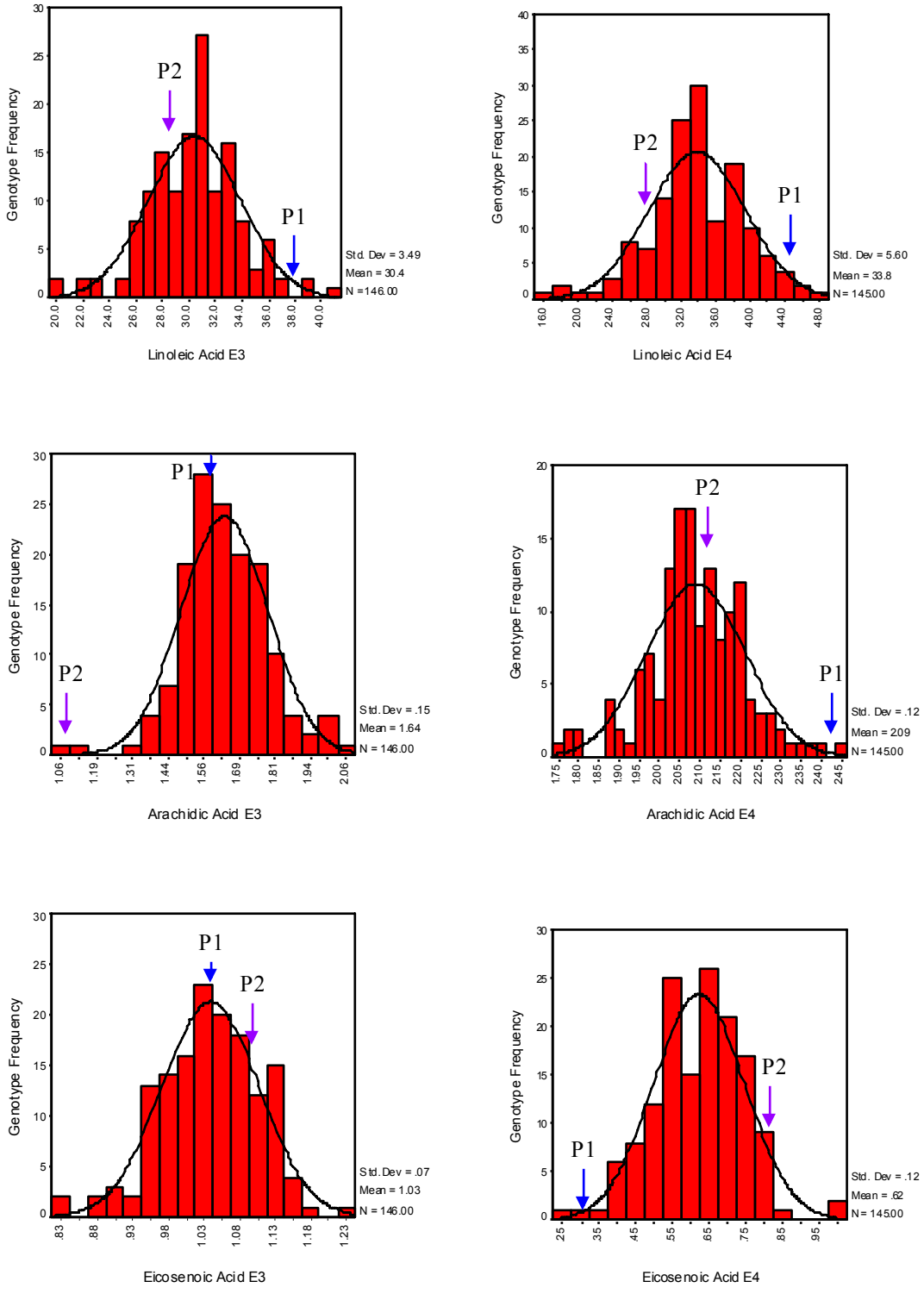
E2-Rainy 2006, E3-Rainy 2007, E4-Post rainy 2007

Fig. 5: Frequency distribution of RILs for protein and oil in TG 26 x GPBD 4 mapping population



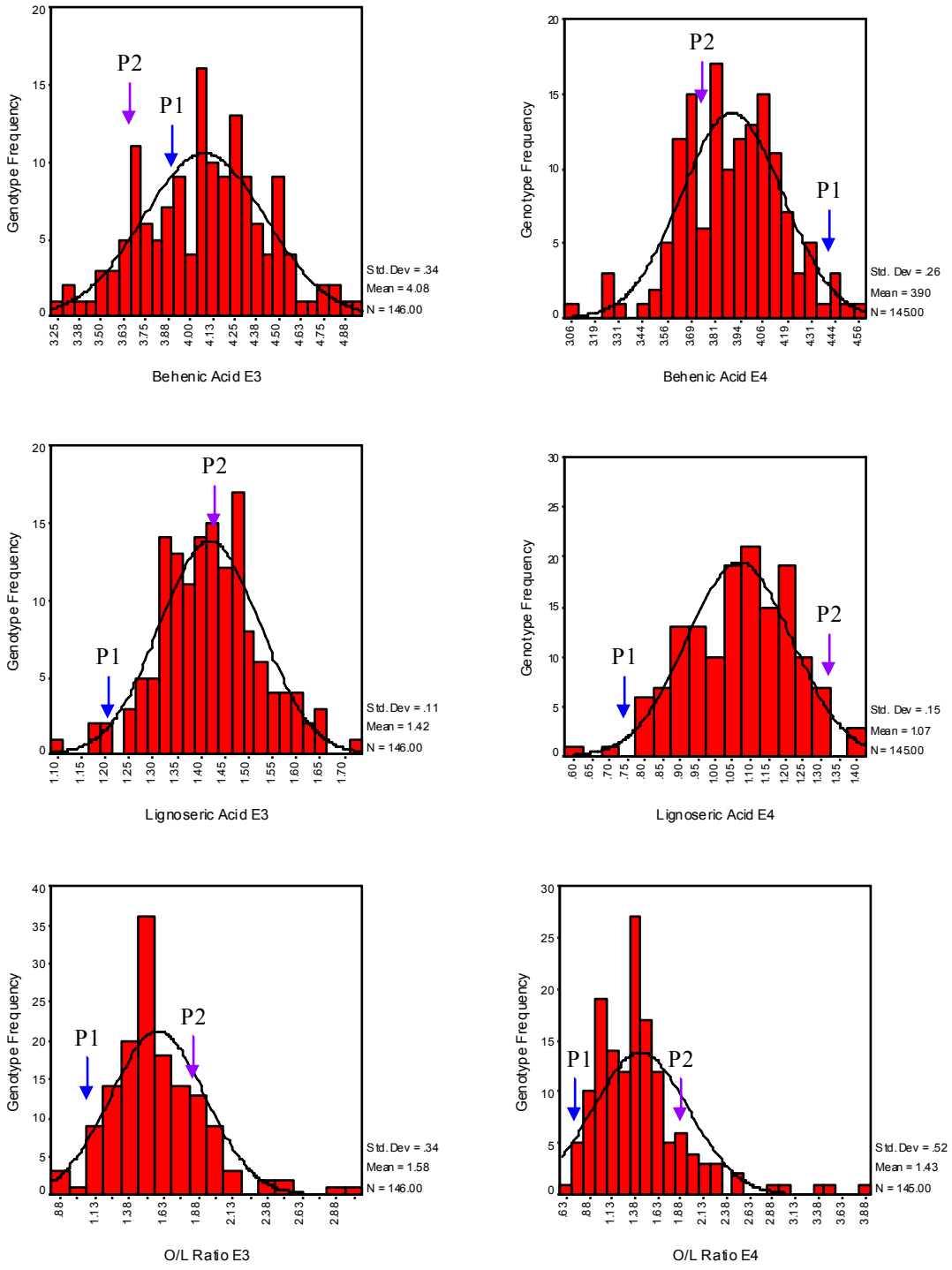
P1-TG 26, P2-GPBD 4, E3-Raiany 2007, E4-Post rainy 2007

Fig. 6 (i): Frequency distribution of RILs in for palmitic, stearic and oleic acids in TG 26 x GPBD 4 mapping population



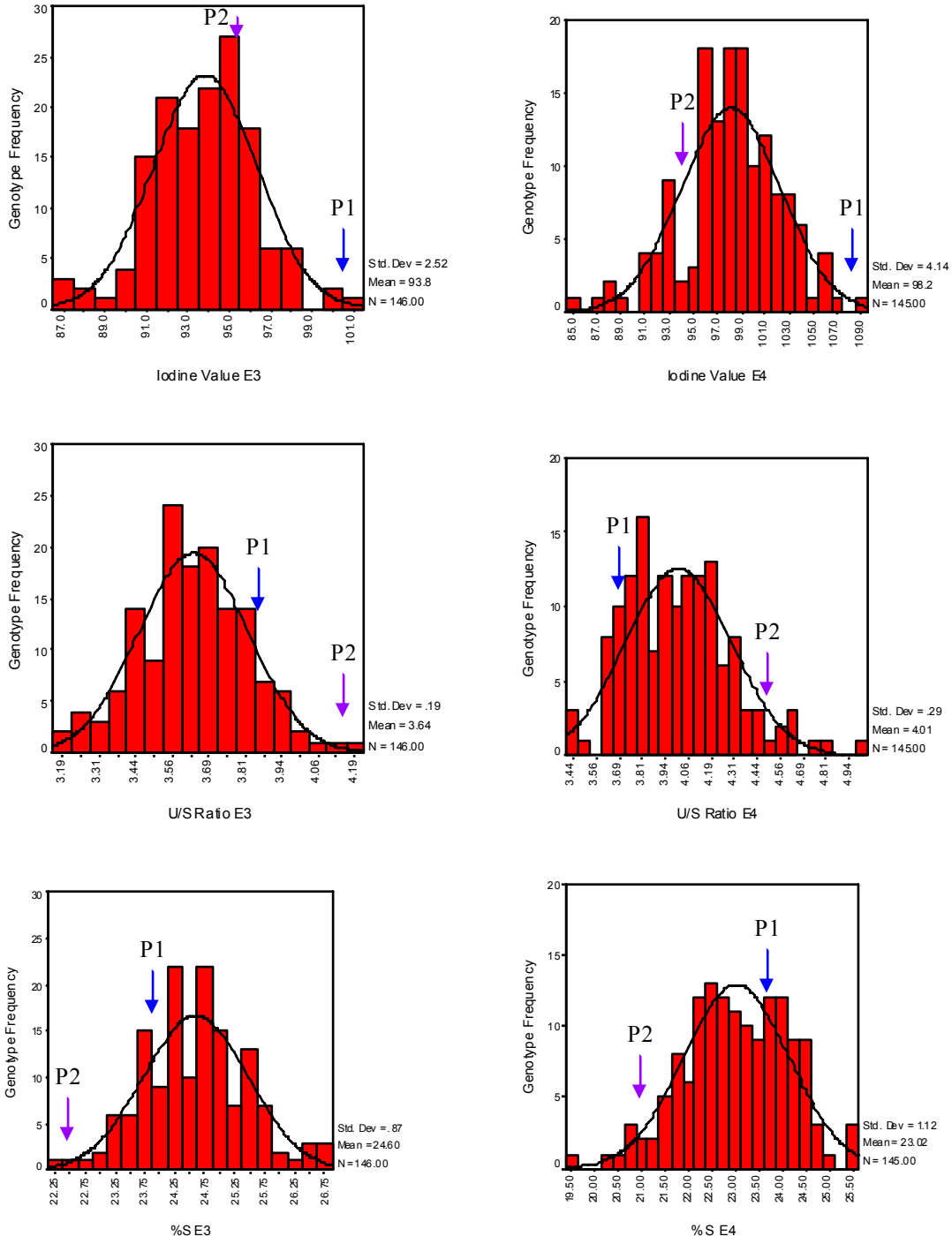
P1 – TG 26 P2 – GPBD 4

Fig. 6 (ii): Frequency distribution of RILs for linoleic, arachidic and eicosenoic acids in TG 26 x GPBD 4 mapping population



P1 – TG 26 P2 – GPBD 4

Fig. 6 (iii): Frequency distribution of RILs for behenic, lignoseric acids and O/L ratio in TG 26 x GPBD 4 mapping population



P1 – TG 26 P2 – GPBD 4

Fig. 6 (iv): Frequency distribution of RILs for iodine value, U/S ratio and % S in TG 26 x GPBD 4 mapping population

4.1.3.3 Productivity and agronomic traits

The distribution pattern was normal for all the agronomic and productivity traits in all the seasons studied except in E2 for number of branches, where it was skewed towards lower number of branches. Most of the RILs were falling within the range of parents; however, few RILs were showing transgressive segregants towards both higher (GPBD 4) and lower value (TG 26) parents in all the traits (Fig. 7 to 10).

4.1.4 Identification of superior RILs

4.1.4.1 Disease resistance to rust and LLS

Table 12A showing the prominent RILs selected for disease resistance to rust and LLS at different stages in comparison with the parents. The resistant RILs selected in different stages had a mean disease scores near to resistant parent GPBD 4 and less than the susceptible parent TG 26 for both rust and LLS in respective stages.

4.1.4.1.1 Rust

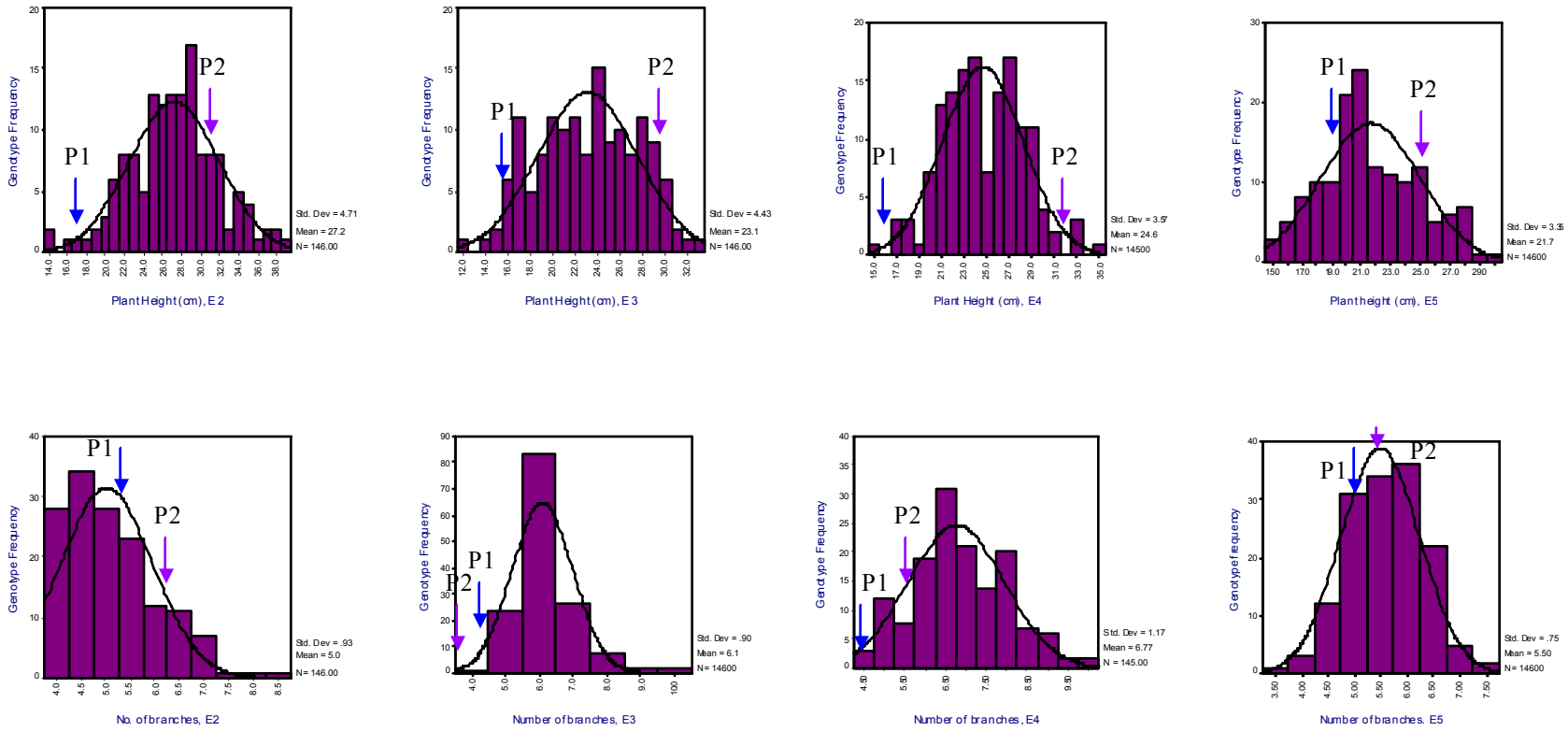
Forty five RILs in stage I, forty six in Stage II and thirty one in stage III were selected for rust resistance with the diseases scores of less than 4.5 for rust. Overall, thirty one RILs were considered to be consistent for resistance across all the stages. Among them, the disease scores of eleven RILs (61, 81, 82, 100, 121, 133, 8, 68, 72, 90 and 95) were comparable with resistant parent GPBD 4 across three stages.

4.1.4.1.2 Late leaf spot

Thirty four RILs in stage I are identified to be resistant to late leaf spot and only four RILs (83, 136, 7 and 18) were common in the stage II with the disease scores of 4.0 or less.

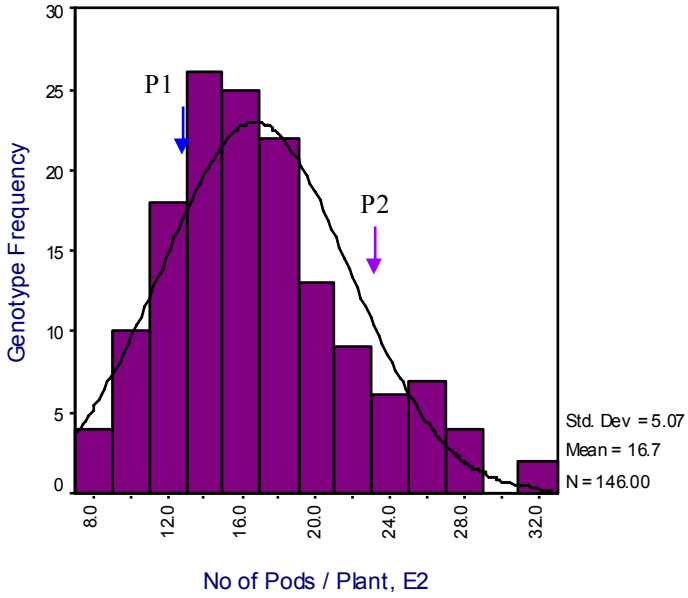
4.1.4.2 Nutritional quality traits

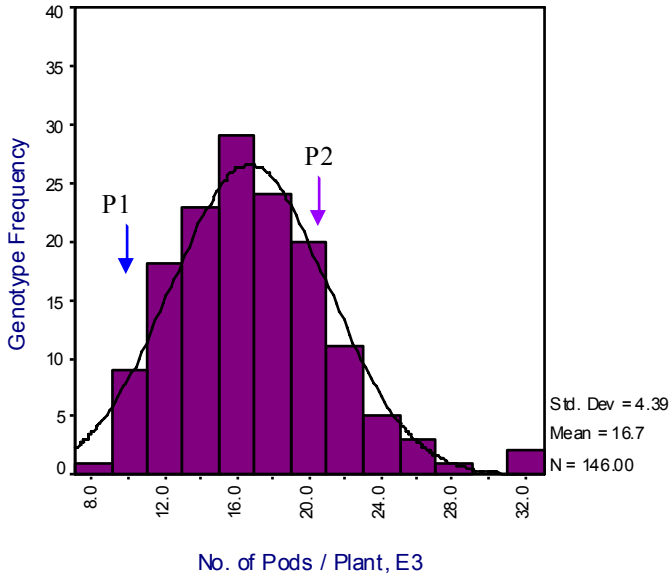
Among the nutritional quality of groundnut, protein, oil content and oil quality especially oleic/linoleic acid ratios are most preferred traits (Table 12B). Out of 146 RILs of the mapping population, seven superior RILs for high protein (higher than or equal to GPBD 4), four RILs for high oil content (higher than or equal to GPBD 4), twelve RILs for low oil (lower than or equal to TG-26), eleven for low palmitic (lower than or equal to GPBD 4),



P1 – TG 26 P2 – GPBD 4

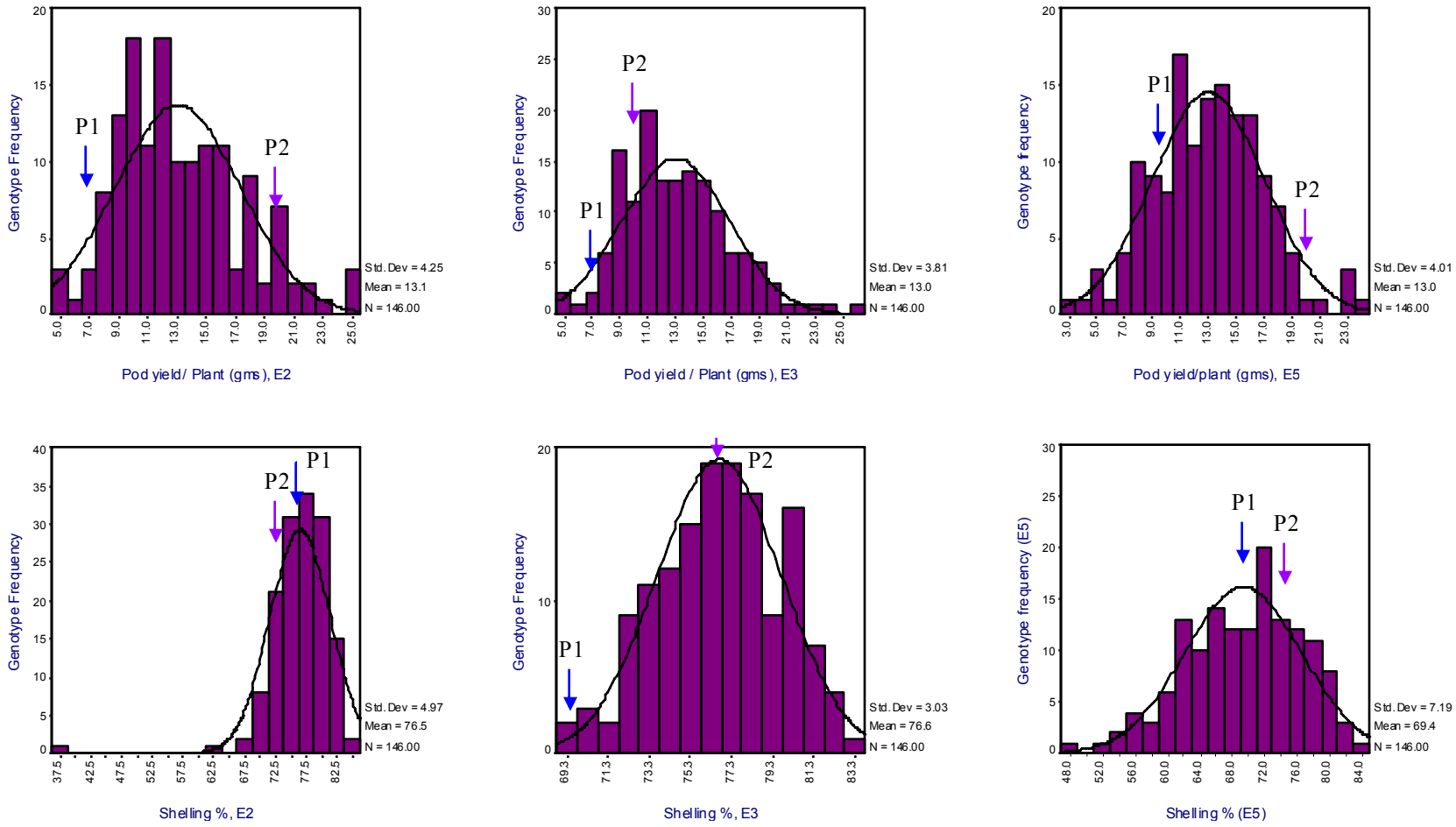
Fig. 7: Frequency distribution of RILs for plant height and number of branches in TG 26 x GPBD 4 mapping population





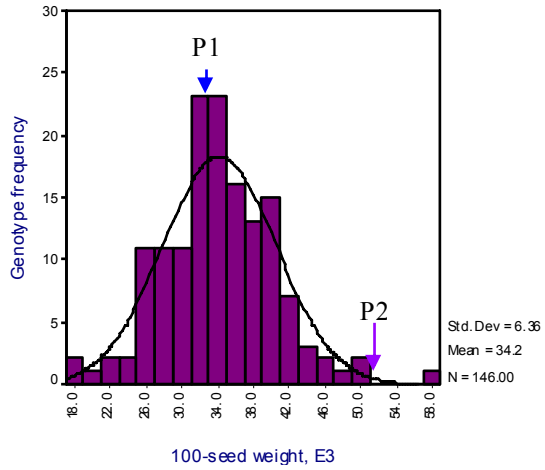
P1 – TG 26 P2 – GPBD 4 E2 – Rainy 2006 E3 – Rainy 2007

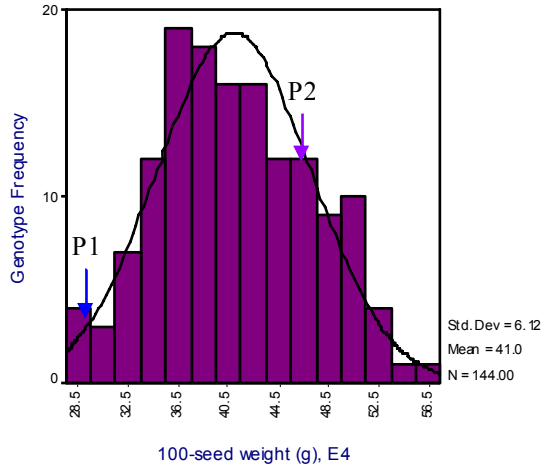
Fig. 8: Frequency distribution of RILs number of pods /plant in two seasons (E2 and E3)

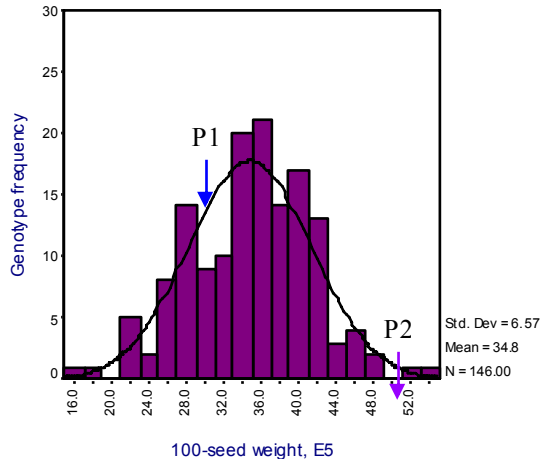


P1 – TG 26 P2 – GPBD 4 E2 – Rainy 2006 E3 – Rainy 2007 E5 – Post rainy 2008

Fig. 9: Frequency distribution of RILs in comparison with the parents for pod yield/plant and shelling %in three seasons (E2, E3 and E5)







P1 – TG 26 P2 – GPBD 4 E3 – Rainy 2007 E4 – Post rainy 2007
E5 – Post rainy 2008

Fig. 10: Frequency distribution of RILs in comparison with parents for 100-seed weight in three seasons (E3, E4 and E5)

Table 12A: Resistant RILs identified for rust and LLS at different stages in TG 26 x GPBD 4 mapping population

RILs/ Parents	Rust resistant Stage I	RILs/ Parents	Rust resistant Stage II	RILs/ Parents	Rust resistant Stage III	RILs/ Parents	LLS resistant Stage I	RILs/ Parents	LLS resistant Stage I
TG26	4.06	TG 26	5.07	TG 26	6.45	TG 26	5.50	TG 26	7.75
GPBD4	2.13	GPBD4	2.75	GPBD4	3.0	GPBD4	2.250	GPBD 4	3.50
95	2.13	100	2.75	61	3.00	32	2.75	83	4.00
100	2.13	102	2.88	81	3.25	136	2.75	136	4.00
7	2.25	122	3.00	82	3.25	18	3.00	7	3.88
107	2.25	7	3.13	100	3.25	66	3.00	18	3.88
122	2.25	82	3.13	121	3.25	10	3.25		
124	2.25	95	3.13	133	3.25	12	3.25		
133	2.25	107	3.13	8	3.50	17	3.25		
1	2.38	121	3.13	68	3.50	19	3.25		
38	2.38	24	3.25	72	3.50	28	3.25		
47	2.38	38	3.25	90	3.50	31	3.25		
50	2.38	50	3.25	95	3.50	38	3.25		
81	2.38	81	3.25	20	3.75	89	3.25		
82	2.38	90	3.25	23	3.75	96	3.25		
90	2.38	93	3.25	37	3.75	106	3.25		
93	2.38	94	3.25	38	3.75	7	3.50		
8	2.50	98	3.25	48	3.75	30	3.50		
21	2.50	103	3.25	50	3.75	44	3.50		
22	2.50	105	3.25	87	3.75	61	3.50		
23	2.50	112	3.25	93	3.75	130	3.50		
66	2.50	124	3.25	122	3.75	134	3.50		
72	2.50	1	3.38	124	3.75	4	3.75		
83	2.50	68	3.38	22	4.00	21	3.75		
94	2.50	87	3.38	47	4.00	37	3.75		
102	2.50	117	3.38	64	4.00	39	3.75		
105	2.50	8	3.50	116	4.00	74	3.75		
112	2.50	23	3.50	130	4.00	85	3.75		
24	2.63	61	3.50	7	4.25	101	3.75		
76	2.63	72	3.50	21	4.25	3	4.00		
87	2.63	83	3.50	41	4.25	13	4.00		
98	2.63	116	3.50	83	4.25	20	4.00		
103	2.63	133	3.50	102	4.25	59	4.00		
121	2.63	21	3.63			64	4.00		
128	2.63	22	3.63			78	4.00		
130	2.63	47	3.63			80	4.00		
131	2.63	128	3.63						
48	2.75	20	3.75						
54	2.75	48	3.75						
68	2.75	66	3.75						
69	2.75	69	3.75						
71	2.75	71	3.75						
116	2.75	99	3.75						
117	2.75	118	3.75						
20	2.88	130	3.75						
61	2.88	131	3.75						
146	2.88	41	3.88						
		76	3.88						

Table12B: Superior RILs identified for protein, oil, palmitic, oleic and linoleic acid in TG 26 x GPBD 4 mapping population

RILs/ Parents	High protein	RILs/ Parents	High oil content	RILs/ Parents	Low oil content	RILs/ Parents	Low Palmitic acid	RILs/ Parents	High Oleic acid	RILs/ Parents	Low Linoleic acid
TG26	25.44	TG26	43.89	TG26	43.89	TG26	10.64	TG26	37.07	TG26	41.39
GPBD4	33.51	GPBD4	47.98	GPBD4	47.98	GPBD4	9.48	GPBD4	51.65	GPBD4	28.90
9	34.97	54	48.44	113	43.10	33	8.57	95	58.88	95	21.25
2	34.33	38	48.11	104	43.02	122	8.79	122	56.83	51	23.13
62	34.30	19	47.96	57	42.94	51	8.83	51	56.65	122	23.15
3	33.95	75	47.81	78	42.74	31	8.84	33	55.99	33	23.66
120	33.76	54	48.44	127	42.27	136	8.87	60	55.88	60	23.84
142	33.68			138	43.66	95	8.90	144	55.27	144	24.76
130	33.48			135	43.65	144	8.96	136	54.75	31	24.84
133	33.48			98	43.60	56	9.11	31	54.53	136	24.95
				141	43.50	60	9.13	29	54.10	29	25.25
				139	43.48	137	9.19	91	53.67	91	25.76
				134	43.41	29	9.20	34	53.08	34	26.06
				121	43.40	35	9.27	8	52.81	8	26.52
						91	9.43	67	52.38	106	26.78
						53	9.44	106	52.34	67	26.89

fourteen RILs for high oleic acid (higher than or equal to GPBD 4), fourteen RILs for low linoleic (lower than or equal to TG-26), ten RILs for high O/L ratio (higher than or equal to GPBD 4) and eleven RILs for low iodine value (lower than GPBD 4) have been identified (Table 12C).

The superior RILs selected for oil quality traits are common between oleic, linoleic, palmitic, O/L ratio and Iodine value. The highest protein content of 34.97 % was recorded by one RIL (9) which was 1.46 % more than GPBD 4 parent. For higher oil content, not much improvement was observed among the RILs compared to GPBD 4 parent with the highest oil content of 48.44 % by RIL no. 64. For lower oil content, 42.27 % was recorded by a line 141-2 which was 1.67 % less than the lower oil content parent TG-26. No RILs were common for high protein and high/low oil content but for oil quality parameters; one recombinant inbred line 95 had a desirable traits like highest oleic acid (58.80), low linoleic acid (21.25), highest O/L ratio (2.98) and low palmitic acid (8.90) which was superior to GPBD 4 parent.

4.1.4.3 Agronomic and productivity traits

Since, most of the agronomic traits *viz*., plant height, number of branches, number of pods per plant, pod yield per plant; shelling % and 100-seed weight are having direct association with productivity, the superior RILs for each trait were selected for all these traits. GPBD 4 was a higher value parent for all these above traits. As for as plant height is concerned, dwarf plants are preferred in ideotype breeding for higher productivity, and tall plants are preferred for fodder purpose. Hence RILs showing both highest (than GPBD 4 parent) and lowest (than TG 26 parent) plant height were selected. For other productivity traits RILs showing higher values than GPBD 4 were identified (Table 12C).

Six RILs for higher plant height, three RILs for lower plant height, nine RILs for number of branches, sixteen RILs for number of pods, fourteen RILs high pod yield, nineteen for shelling % and five for 100-seed weight were identified to be superior than their parents for respective traits. Most of the RILs were common between number of pods and pod yield, among them, two RILs (99 and 125) had number of pods more than 30 and three RILS (105, 76 and 99) had >20.00gms of pod yield. Three RILs with higher

Table 12C: Superior RILs identified for O/L ratio, iodine vale and other productivity traits in TG 26 x GPBD 4 mapping population

RILs/ Parents	O/L ratio	*RILs/ Parents*	Iodine value	*RILs/ Parents*	More PLHT	*RILs/ Parents*	Less PLHT	*RILs/ Parents*	NBR
TG26	0.91	TG26	104.11	TG26	17.25	TG26	17.25	TG26	4.79
GPBD4	1.79	GPBD4	95.23	GPBD4	29.40	GPBD4	29.40	GPBD4	5.31
95	2.98	95	88.19	119	31.32	24	15.86	27	7.50
51	2.65	51	89.50	138	30.56	62	16.89	19	7.00
122	2.62	122	89.64	87	30.00	76	17.13	36	7.00
60	2.45	33	89.76	136	29.46			60	7.00
33	2.45	60	89.96	125	29.33			116	6.88
91	2.26	31	90.69	8	29.14			145	6.81
144	2.25	29	90.94					6	6.75
29	2.24	136	91.05					111	6.75
31	2.22	144	91.14					140	6.75
136	2.21	34	91.41						
		91	91.44						

RILs/ Parents	No. of pods	RILs/ Parents	Pod wt/plant	RILs/ Parents	Shelling Per cent	RILs/ Parents	100-seed wt
TG26	11.42	TG26	7.50	TG26	72.06	TG26	30.05
GPBD4	20.44	GPBD4	14.66	GPBD4	74.68	GPBD4	48.43
99	32.38	105	22.58	124	81.92	81	46.23
125	32.38	76	20.76	12	79.83	96	45.86
87	27.63	99	20.50	142	79.67	143	45.49
100	27.38	100	19.28	81	79.67	3	45.46
128	26.50	87	18.86	143	79.51	80	45.21
105	25.75	119	18.81	94	79.48		
76	24.25	77	18.80	100	79.46		
9	23.75	50	18.27	90	79.27		
108	23.75	124	18.06	83	79.10		
117	23.63	19	17.82	7	79.04		
61	23.38	125	17.67	10	78.83		
95	23.38	103	17.42	39	78.81		
79	23.25	61	17.27	130	78.69		
109	23.13	48	17.06	121	78.62		
58	23.00			122	78.48		
72	23.00			46	78.44		
				144	78.43		
				102	78.31		
				101	78.02		

plant height (119, 87 and 125) and one RIL with lower plant height (76) were also had higher pod yield. No RILs were common for higher pod yield and higher 100-seed weight.

4.1.5 Correlation studies

4.1.5.1 Among diseases

For both rust and LLS, the association was strong and positive between the stages and within the seasons indicating consistency in the reaction of RILs (Table 13A and B) but negative between rust and LLS (Table 13C).

4.1.5.2 Among quality traits

Various nutritional quality traits namely protein, oil and oil quality parameters exhibited positive and significant correlation between seasons indicating the consistency for these quality traits among the RILs (Table 14A and B). Table 14C shows phenotypic correlation between protein, oil content and oil quality parameters in individual seasons and table 14D shows phenotypic correlation between protein, oil content and oil quality parameters pooled across the seasons.

Negative association was observed between oil and protein. Oil content showed positive correlation with eicosenoic acid, lignoseric acid and negative association with stearic, arachidic and behenic acids in both the seasons studied (Table 14C).

Among the oil quality parameters, oleic acid had a strong negative correlation with palmitic acid, linoleic acid, behenic acid, iodine value and % S and it had a strong positive correlation with lignoseric acid, eicosenoic acid, O/L ratio and U/S ratio where as linoleic acid had a opposite relation for these fatty acids as both oleic and linoleic acids have a strong inverse relationship with each other. Palmitic acid was positively correlated with linoleic acid, behenic acid, lignoseric acid, iodine value and % S and it showed strong negative association with O/L ratio and U/S ratio. Stearic acid was positively correlated with arachidic, behenic and %S and negatively correlated with eicosenoic acid, lignoseric acid and U/S ratio. Arachidic acid had a negative association with eicosenoic and lignoseric acid. Eicosenoic acid was positively correlated with lignoseric acid, O/L

Table13A: Between seasons correlation for rust at various stages in TG 26 x GPBD 4 mapping population

Rust Stage I	E1	E3 (Exp I)	E3 (Exp II)	E4	Rust stage II	E1	E3 (Exp I)	E3 (Exp II)	E4	Rust Stage III	*E3 (Exp I)*	*E4*
E1 (*Rainy* 2005)	1.000				**E1 (*Rainy* 2005)**	1.000						
E3 (*Rainy* 2007, Exp I)	0.452**	1.000			**E3 (*Rainy* 2007, Exp I)**	0.437**	1.000			**E3 (*Rainy* 2007, Exp I)**	1.000	
E3 (*Rainy* 2007, Exp II)	0.593**	0.758**	1.000		**E3 (*Rainy* 2007, Exp II)**	0.529**	0.776**	1.000		**E4, Post *Rainy* 2007**	0.711**	1.000
E4, Post *Rainy* 2007	0.524**	0.607**	0.755**	1.000	**E4, Post *Rainy* 2007**	0.480**	0.705**	0.796**	1.000			

Table 13B: Between seasons correlation for LLS at various stages in TG 26 x GPBD 4 mapping population

Stage I	E1 (*Rainy* 2005)	E3(*Rainy* 2007)	Stage II	E1 (*Rainy* 2005)	E3(*Rainy* 2007)
E1 (*Rainy* 2005)	1.000		**E1 (*Rainy* 2005)**	1.000	
E3(*Rainy* 2007)	0.300**	1.000	**E3(*Rainy* 2007)**	0.257**	1.000

Table 13C: Correlation between rust and late leaf spot at various seasons

Traits	Rust Stage I	Rust Stage II	Late Leaf spot Stage I	Late Leaf spot Stage II
Rust Stage I				
E1	1.000	0.905**	-0.505**	-0.430**
E3	1.000	0.943**	-0.273**	0.105*
Rust Stage II				
E1		1.000	-0.591**	-0.526**
E3		1.000	-0.301**	0.034
Late Leaf spot Stage I				
E1			1.000	0.858**
E3			1.000	0.441**
Late Leaf spot Stage II				
E1				1.000
E3				1.000

Table 14A: Phenotypic correlations for protein content and oil content between individual seasons

	Protein content E2	Protein content E3	Protein content E4
Protein content E2	1.000		
Protein content E3	0.295**	1.000	
Protein content E4	0.233**	0.164*	1.000

	Oil content E2	Oil content E3	Oil content E4
Oil content E2	1.000		
Oil content E3	0.141*	1.000	
Oil content E4	0.071	0.411**	1.000

Table 14B: Phenotypic correlations twelve for oil quality parameters between individual seasons

Traits	**Palmitic E3**	**Palmitic E4**
Palmitic E3	1.000	
Palmitic E4	0.385**	1.000

Traits	**Stearic E3**	**Stearic E4**
Stearic E3	1.000	
Stearic E4	0.334**	1.000

Traits	**Oleic E3**	**Oleic E4**
Oleic E3	1.000	
Oleic E4	0.421**	1.000

Traits	**Linoleic E3**	**Linoleic E4**
Linoleic E3	1.000	
Linoleic E4	0.403**	1.000

Traits	**Arachidic E3**	**Arachidic E4**
Arachidic E3	1.000	
Arachidic E4	0.214**	1.000

Traits	**Eicosenoic E3**	**Eicosenoic E4**
Eicosenoic E3	1.000	
Eicosenoic E4	0.351**	1.000

Traits	**Behenic E3**	**Behenic E4**
Behenic E3	1.000	
Behenic E4	0.291**	1.000

Traits	**Lignoseric E3**	**Lignoseric E4**
Lignoseric E3	1.000	
Lignoseric E4	0.337**	1.000

Traits	**O/L ratio E3**	**O/L ratioE4**
O/L ratio E3	1.000	
O/L ratio E4	0.363**	1.000

Traits	**Iodine value E3**	**Iodine value E4**
Iodine value E3	1.000	
Iodine value E4	0.360**	1.000

Traits	**U/S ratio E3**	**U/S ratio E4**
U/S ratio E3	1.000	
U/S ratio E4	0.362**	1.000

Traits	**%S E3**	**%S E4**
%S E3	1.000	
%S E4	0.388**	1.000

* Significance at 0.05 level of probability, ** Significance at 0.01 level of probability
E2-*Kharif* 2006, E3-*Kharif* 2007, E4-Summer 2007

Table: 14C: Phenotypic correlation among nutritional quality traits in TG 26 x GPBD 4 mapping population

Traits	Protein	Oil	Palmitic	Stearic	Oleic	Linoleic	Arachidic	Eicosenoic	Behenic	Lignoseric	O/L ratio	IV	U/S	%S
***Rainy* 2006 (E2)**														
Protein content (%)	1.000													
Oil content (%)	0.124	1.000												
***Rainy* 2007 (E3)**														
Protein content (%)	1.000													
Oil content (%)	-0.390**	1.000												
Palmitic acid	0.115	-0.094	1.000											
Stearic acid	0.114	-0.370**	0.026	1.000										
Oleic acid	-0.156*	0.258**	-0.610**	0.215*	1.000									
Linoleic acid	0.146*	-0.234**	0.563**	0.167**	-0.988**	1.000								
Arachidic acid	-0.065	-0.157*	-0.496**	0.240**	-0.142*	0.151*	1.000							
Eicosenoic acid	-0.061	0.379**	-0.013	-0.442**	0.334**	-0.345**	-0.436**	1.000						
Behenic acid	0.131	-0.206**	0.007	0.227**	-0.631**	0.601**	0.470**	-0.202**	1.000					
Lignoseric acid	-0.176	0.441**	-0.037	-0.501**	0.151*	-0.154*	-0.196**	0.738**	0.055	1.000				
O/L ratio	-0.137	0.221**	-0.582**	-0.179**	0.974**	-0.977**	-0.143*	0.308**	-0.591**	0.143*	1.000			
Iodine value (IV)	0.125	-0.184**	0.479**	0.015	-0.932**	0.976**	0.148*	-0.326**	0.537**	-0.135	-0.943	1.000		
U/S ratio	-0.153*	0.305**	-0.626**	-0.597**	0.826**	-0.733**	-0.129	0.296**	-0.613**	0.163*	0.757**	-0.571**	1.000	
%S	0.155*	-0.310	0.623**	0.599**	-0.827**	0.734**	0.129	-0.302**	0.620**	-0.163*	-0.750**	0.573**	-0.998**	1.000
Post *Rainy* 2007 (E4)														
Protein content (%)	1.000													
Oil content (%)	-0.059	1.000												
Palmitic acid	0.265**	0.074	1.000											
Stearic acid	-0.079	-0.420**	-0.062	1.000										
Oleic acid	-0.234**	0.093	-0.837**	-0.182*	1.000									
Linoleic acid	0.234**	-0.076	0.809**	0.100	-0.992**	1.000								
Arachidic acid	-0.039	-0.348**	-0.381**	0.450**	0.042	-0.040	1.000							
Eicosenoic acid	-0.117	0.392**	-0.087	-0.463**	0.470**	-0.497**	-0.619**	1.000						
Behenic acid	0.166*	-0.150*	0.425**	0.412**	-0.686**	0.638**	0.084	-0.267**	1.000					
Lignoseric acid	-0.026	0.448**	0.003	-0.638**	0.394**	-0.403**	-0.583**	0.952**	-0.282**	1.000				
O/L ratio	-0.231**	0.061	-0.811**	-0.111	0.952**	-0.950**	0.099	0.410**	-0.639**	0.329**	1.000			
Iodine value (IV)	0.229**	-0.042	0.760**	-0.023	-0.958**	0.986**	-0.052	-0.503**	0.561**	-0.387**	-0.925**	1.000		
U/S ratio	-0.187*	0.185*	-0.759**	-0.557**	0.830**	-0.754**	-0.023	0.331**	-0.747**	0.351**	0.779**	-0.636**	1.000	
%S	0.182*	-0.192**	0.753**	0.570**	-0.819**	0.742**	0.027	-0.327**	0.754**	-0.350**	-0.752**	0.621**	-0.996**	1.000

* Significance at 0.05 level of probability, ** Significance at 0.01 level of probability, E2-Rainy2006, E3-Rainy 2007, E4-Post rainy 2007

Table 14D: Phenotypic correlation among quality traits pooled across the seasons in TG 26 x GPBD 4 mapping population

Traits	Protein	Oil	Palmitic	Stearic	Oleic	Linoleic	Arachidic	Eicosenoic	Behenic	Lignoseric	O/L ratio	IV	U/S	%S
Protein content (%)	1.000													
Oil content (%)	-0.199*	1.000												
Palmitic acid	0.263**	-0.071	1.000											
Stearic acid	0.069	-0.452**	-0.024	1.000										
Oleic acid	-0.302**	0.334**	-0.721**	-0.252**	1.000									
Linoleic acid	0.316**	-0.311**	0.685**	0.139*	-0.987**	1.000								
Arachidic acid	-0.102	-0.208*	-0.433**	0.342**	-0.032	0.021	1.000							
Eicosenoic acid	-0.212*	0.450**	-0.081	-0.500**	0.425**	-0.428**	-0.441**	1.000						
Behenic acid	-0.034	-0.287**	0.085	0.347**	-0.591**	0.551**	0.359**	-0.262**	1.000					
Lignoseric acid	-0.198*	0.476**	-0.030	-0.658**	0.360**	-0.343**	-0.369**	0.855**	-0.214*	1.000				
O/L ratio	-0.350**	0.298**	-0.694**	-0.172*	0.983**	-0.987**	0.004	0.406**	-0.579**	0.345**	1.000			
Iodine value (IV)	0.319**	-0.254**	0.611**	-0.036	-0.926**	0.974**	-0.009	-0.389**	0.472**	-0.283**	-0.949**	1.000		
U/S ratio	-0.189*	0.395**	-0.647**	-0.652**	0.798**	-0.693**	-0.126	0.385**	-0.593**	0.406**	0.729**	-0.514**	1.000	
%S	0.171*	-0.377**	0.647**	0.653**	-0.799**	0.693**	0.118	-0.387**	0.602**	-0.399**	-0.724**	0.513**	-0.997**	1.000

* Significance at 0.05 level of probability, ** Significance at 0.01 level of probability

ratio and U/S ratio. O/L ratio was negatively correlated with Iodine value and %S and positively correlated with U/S ratio.

4.1.5.3 Among agronomic and productivity traits

Correlation among the productivity traits has been studied in E2, E3, E4 and E5 (Table 15).

Plant height was positively correlated with number of branches (E2), number of pods per plant (E3), pod yield per plant (E3 and E5) and 100-seed weight (all the seasons). Pods per plant had a strong positive association with pod weight in all the seasons studied. Shelling % had negative association with plant height (E5) and pod yield (E5). 100-seed weight was positively correlated with pod yield (all the seasons).

4.1.5.4 Diseases with nutritional quality and productivity traits

The association of diseases on nutritional quality and productivity traits were studied (Table 16). Minor fatty acids *viz*., stearic, arachidic and behenic acids had positive correlation indicating association with susceptibility to rust. Negative association was observed for rust with oil content (both seasons) protein content (E4), eicosenoic and lignoseric acid; hence, associated with resistance to rust.

Most of the productivity traits *viz*., no. of pods per plant, pod yield, shelling % and 100-seed weight exhibited negative correlations with rust indicating favorable association with rust resistance.

In contrast, there was lack of association for many of the productivity and quality traits with late leaf spot. LLS at stage II had a negative correlation with protein and 100-seed weight indicating their association with resistance. At Stage I, positive correlation was evident with number of pods/plant and pod yield/plant revealing the association of higher productivity with susceptibility; while, it was negatively correlated with behenic acid revealing its association with LLS resistance.

4.2 Genotypic data analysis

4.2.1 Linkage map construction

Out of total of 1043 microsatellite markers screened, 954 markers were scorable and 53 found polymorphic (5.56 %) between TG 26 and GPBD 4 and the mapping

Table 15: Phenotypic correlation among productivity and other agronomic traits in TG 26 x GPBD 4 mapping population

Traits	Plant height	No. of Branches	Number of pods/plant	Pod yield/plant	Shelling %	100-seed weight
Plant height						
E2	1.000	0.149*	-0.076	-0.097	0.021	-
E3	1.000	-0.061	0.239**	0.221**	-0.013	0.165*
E4	1.000	0.000	-	-		0.183*
E5	1.000	0.069	-	0.261**	-0.161*	0.252**
No. of Branches						
E2		1.000	-0.089	-0.072	-0.056	-
E3		1.000	0.025	0.048	-0.038	0.017
E4		1.000	-	-	-0.056	0.097
E5		1.000	-	0.044	-	-0.015
Number of pods/plant						
E2			1.000	0.828**	-0.09	-
E3			1.000	0.835**	-0.032	0.074
Pod yield/plant						
E2				1.000	-0.034	-
E3				1.000	0.010	0.362**
E5				1.000	-0.237**	0.551**
Shelling %						
E2					1.000	0.085
E3					1.000	-
E5					1.000	0.035
100-seed weight						
E3						1.000
E4						1.000
E5						1.000

* Significance at 0.05 level of probability, ** Significance at 0.01 level of probabilityE2-*Rainy* 2006, E3-*Rainy* 2007, E4-Post*Rainy* 2007, E5-Post *Rainy* 2008

Table 16: Correlation for diseases (Rust and LLS) with other quality and agronomic traits in TG 26 x GPBD 4 mapping population in E3 and E4

Traits	LLS Stage I	LLS Stage II	Rust Stage I	Rust Stage II	Rust (Exp I) Stage III	Rust (Exp II) Stage I	Rust (Exp II) Stage II
Protein	-0.031	-0.167*	-0.123*	-0.103	-0.120	-0.046	-0.001
Oil	-0.012	0.084	-0.231**	-0.158*	-0.218*	-0.254**	-0.292**
Palmitic acid	0.117	0.016	-0.097	-0.106	-0.123	-0.163	-0.174*
Stearic acid	0.006	0.059	0.215*	0.190*	0.162*	0.139	0.158*
Oleic acid	0.025	0.056	-0.115	-0.133	-0.082	-0.096	-0.101
Linoleic acid	-0.033	-0.069	0.118	0.145	0.092	0.116	0.122
Arachidic acid	-0.095	-0.011	0.200*	0.221**	0.203**	0.259**	0.271**
Eicosenoic acid	0.019	-0.019	-0.313**	-0.291**	-0.263**	-0.316**	-0.343**
Behenic acid	-0.205*	-0.120	0.175*	0.172*	0.147*	0.178*	0.194*
Lignoseric acid	-0.013	0.072	-0.234**	-0.225**	-0.193*	-0.279**	-0.302**
O/L ratio	0.024	0.065	-0.106	-0.115	-0.059	-0.077	-0.080
Iodine value	-0.044	-0.086	0.114	0.152*	0.099	0.136*	0.142*
U/S ratio	0.008	0.000	-0.106	-0.088	-0.052	-0.026	-0.030
%S	-0.017	-0.002	0.111	0.097	0.063	0.037	0.044
Plant height	0.208*	-0.161	0.124	0.042	-0.034	0.004	-0.028
No. of Branches	-0.006	-0.007	-0.001	0.019	0.006	0.021	-0.002
Number of pods/plant	0.211*	0.011	-0.238**	-0.264**	-0.298**	-0.297**	-0.322**
Pod weight/plant	0.242**	0.012	-0.314**	-0.318**	-0.344**	-0.386**	-0.402**
shelling %	-0.020	-0.064	-0.314**	-0.236*	-0.265**	-0.250**	-0.179*
100-seed weight	0.145*	-0.152*	-0.288**	-0.301**	-0.296**	-0.310**	-0.303**

E3-*Rainy* 2007

Traits	Rust stage I	Rust stage II	Rust stage III
Protein	-0.192*	-0.226**	-0.240**
Oil	-0.196*	-0.223**	-0.196*
Palmitic acid	0.004	-0.019	-0.079
Stearic acid	0.224**	0.258**	0.202*
Oleic acid	-0.162*	-0.170*	-0.099
Linoleic acid	0.158*	0.168*	0.104
Arachidic acid	0.108	0.166*	0.150*
Eicosenoic acid	-0.143*	-0.184*	-0.188*
Behenic acid	0.238**	0.233**	0.159*
Lignoseric acid	-0.223*	-0.272**	-0.250**
O/L ratio	-0.154*	-0.167*	-0.088
Iodine value	0.148*	0.161*	0.106
U/S ratio	-0.163*	-0.163*	-0.065
%S	0.159*	0.158*	0.067
Plant height	0.111	0.044	0.081
no. of branches	0.102	0.105	0.091
100-seed weight	0.030	-0.041	0.017

E4-Post *Rainy* 2007

population consisting of 146 RILs. The genotypic data obtained form the polymorphic markers were subjected for linkage map construction. MAPMAKER EXPV 3.0 (Lander *et al.,* 1987 and Lincoin *et al*., 1992) software was used for linkage analysis.

The chi-square (χ^2) test was employed to test the Mendelian segregation ratio of expected 1:1 ratio. Fifteen markers showed segregation distortion. But due to less number of polymorphic markers, all the 53 markers were used for linkage map construction. A total of 45 markers were mapped on 8 linkage groups with the total span of 657.90 cM and an average marker distance was 14.62cM. Eight markers remained ungrouped. The length of the linkage group varied from 29.00 cM (LG5) to 145.3 (LG1) {Table 17}. The number of markers on each linkage group varied from 4 (LG 2, 6, 7 and 8) to 8 markers (LG1) {Fig 11}.

4.3 Analysis for marker–trait association

Phenotypic data on disease scores of rust and LLS, quality traits, agronomic and other productivity traits along with the data on 53 microsatellite markers were subjected for single marker analysis and QTL mapping to identify the putative markers associated with each trait in individual seasons.

4.3.1 Single marker analysis

Simple linear regression method (Haley and Knott, 1992) was used to identify significant marker trait association. Genstat (10^{th} edition) was used for single marker analysis.

4.3.1.1 Disease resistance

4.3.1.1.1 Rust

Single marker analysis was carried out for rust at three stages and presented in the Table 18A. A total of fifteen markers were associated with rust at different stages with the phenotypic variance ranging from 2.03 to 51.96 %. Only one marker *i.e* XIP 103 was associated with rust at different stages in all the four seasons studied with a substantial phenotypic variance ranging from 24.86 to 51.96 %. Some markers *viz*., TC1D12 (2.07-2.42 %), PM183 (2.52-5.92 %), TC4H07 (2.54-4.06 %) were observed in at least two seasons. Apart from XIP 103 and PM183 higher contribution to variance was shown by Seq19D06 (5.07 to 6.37 %) and RN16F06 (2.80 to 5.12 %).

Table 17: SSR markers assigned to linkage groups and their average distances

Linkage group	No. of SSRs	Length (cM)	Average distance (cM)
LG1	6 (+1 synthetic marker)	145.30	20.76
LG2	3 (+1 synthetic marker)	83.70	20.93
LG3	7 (+1 synthetic marker)	84.10	10.51
LG4	4 (+2 synthetic marker)	93.40	15.57
LG5	6 (+1 synthetic marker)	29.00	4.14
LG6	4 (+1 synthetic marker)	46.40	9.28
LG7	4	139.90	34.98
LG8	4	36.10	9.03
Total	**45**	**657.9**	**14.62**

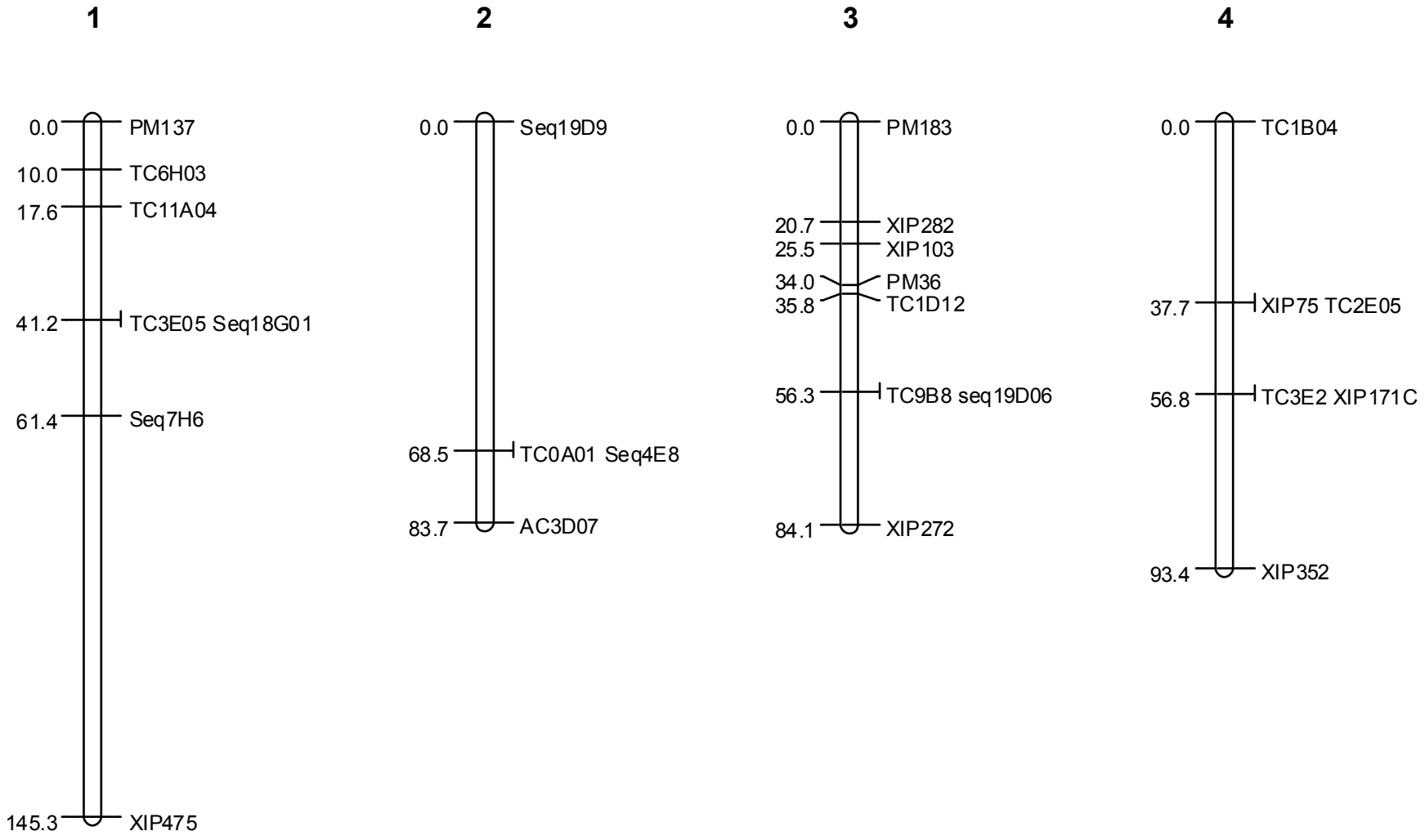
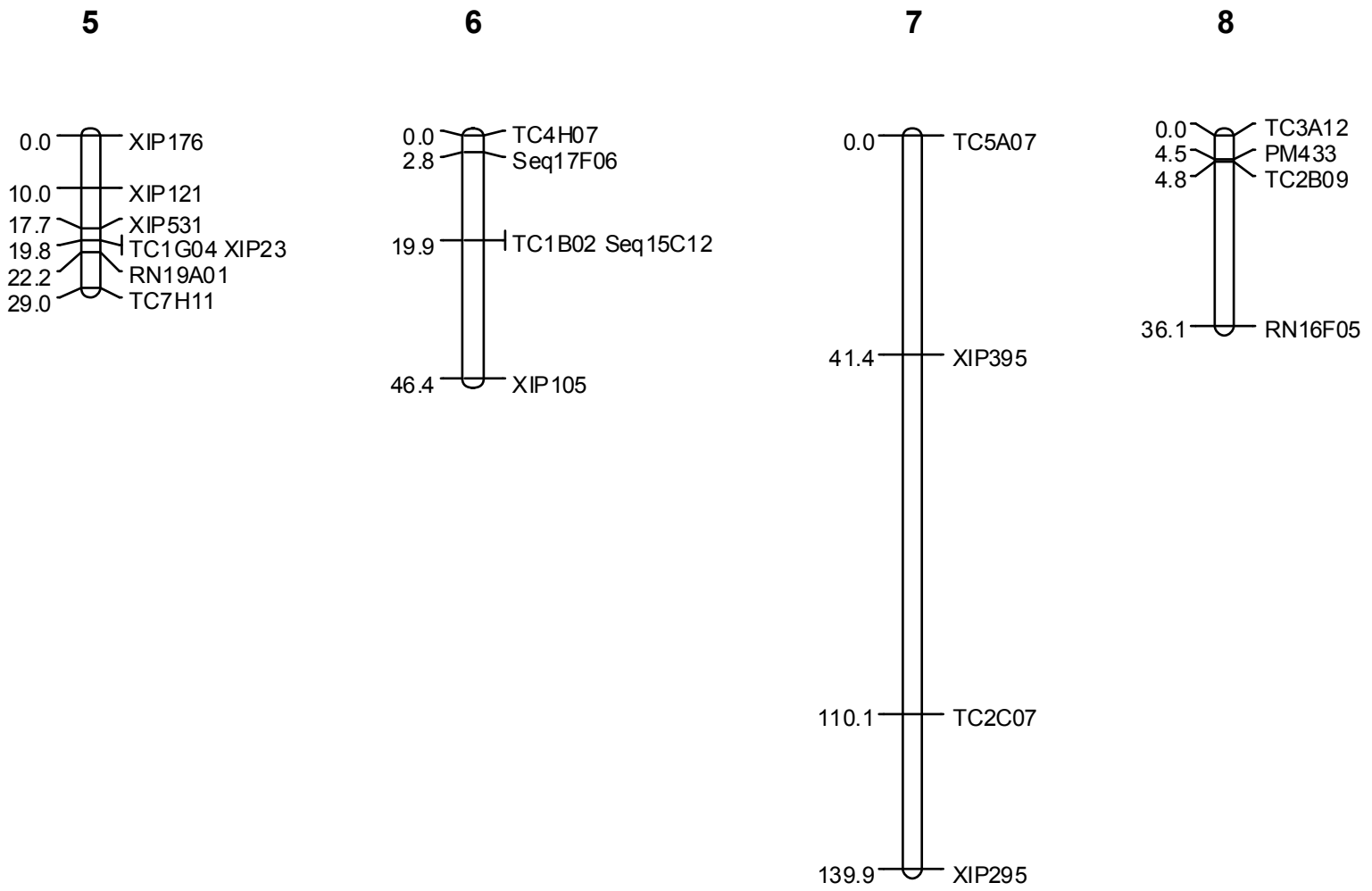


Fig. 11: Microsatellite based genetic linkage map for TG 26 x GPBD 4 mapping population of groundnut

Contd…


5
0.0 XIP176
10.0 XIP121
17.7 XIP531
19.8 TC1G04 XIP23
22.2 RN19A01
29.0 TC7H11
6
0.0 TC4H07
2.8 Seq17F06
19.9 TC1B02 Seq15C12
46.4 XIP105
7
0.0 TC5A07
41.4 XIP395
110.1 TC2C07
139.9 XIP295
8
0.0 TC3A12
4.5 PM433
4.8 TC2B09
36.1 RN16F05

4.3.1.1.2 Late leaf spot

Table 18B represents the single marker analysis for LLS at two different stages in two seasons. A total of eleven markers were identified with the phenotypic variance ranging from 2.43 (PM183) to 7.04 (Seq15C12). Thus the contribution for LLS recorded by these markers was very less compared to rust. None of the markers were observed in both the seasons but some markers (Seq3F05 and TC2B09) were found at different stages within a season.

4.3.1.2 Nutritional quality

Single marker analysis was assessed for all the fourteen quality traits in three seasons.

4.3.1.2.1 Protein

A total of twelve markers were associated with the protein content with the phenotypic variance ranging from 2.17% (TC2G05) to 9.78 % (TC6H03). Two markers, TC2C07 (4.04 to 5.88 %) TC1D12 (2.54 to 5.42) were observed in at least two seasons. Two markers *viz*., TC1B02 (8.05 %) and TC3E02 (5.87 %) had higher contribution for this trait, (Table 19).

4.3.1.2.2 Oil content

For this trait, total of eleven markers were identified with the phenotypic variance ranging from 2.18 % (TC2B09) to 6.98 % (XIP 103). Three markers *viz.,* TC3A12 (2.44-2.87 %), TC2B09 (2.18-4.18 %) and XIP 103 (5.72-6.98 %) were observed in at least two seasons (Table 19). None of the other markers had higher contribution to oil content (>5.0%) (Table 19).

4.3.1.2.3 Oil quality parameters

Single marker analysis among the eight fatty acids revealed a total of 9 markers for palmitic acid (2.28 to 3.39 %), 6 markers for stearic acid (2.15 to 8.90 %), 7 markers for oleic acid (2.20 to 3.60 %), 8 markers for linoleic acid (2.60 to 5.2 %), 6 markers for arachidic acid (2.40 to 5.34 %), 5 makers for eicosenoic acid (2.89 to 6.89 %), 8 markers for behenic acid (2.06 to 6.28 %) and 4 markers for lignoseric acid (2.73 to 4.40 %) putatively associated with different fatty acids. Some of the markers were common between the seasons for different traits *viz*., PM 137 (2.24-3.46 %) and XIP395 (3.10-3.54 %) for oleic, XIP 395 (3.29-3.59%) for

Table 18A: Single marker analysis for rust at different stages in TG 26 x GPBD 4 mapping population

Rust	E1 (*Rainy* 2005)		E3 (*Rainy* 2007, Exp I)			E3 (*Rainy* 2007, Exp II)		E4 (Post *Rainy* 2007)		
Markers	**S1**	**S2**	**S1**	**S2**	**S3**	**S1**	**S2**	**S1**	**S2**	**S3**
XIP103	32.79**	27.98**	24.86**	38.50**	36.86**	49.39**	51.96**	33.01**	37.49**	32.64**
AC3D07	2.03*	2.92*								
RN16F05	5.12**	2.8*								
Seq19D9		3.94*								
TC1D12			2.07*	2.42*	2.12*	2.14*			2.51*	
XIP295			2.26*							
PM183			3.15*	2.52*	4.02*	5.92**	3.99*			
TC2G05			2.47*	2.38*						
TC1B04				2.56*	2.22*				2.74*	
TC4H07					4.06*	2.57*	3.81*	2.54*	3.13*	
seq19D06						5.07**	6.37**			
XIP407C							4.05*			
TC3E2								3.22*	2.53*	
Seq17F06								3.02*	3.74*	
XIP121										3.63*

Table 18B: Single marker analysis for LLS at different stages in TG 26 x GPBD 4 mapping population

LLS	E1 (*Rainy* 2005)		E3 (*Rainy* 2007)	
Markers	**S1**	**S2**	**S1**	**S2**
Seq3F05	3.78*	3.17*		
PM183	2.43*			
Seq19D9	2.45*			
XIP282		3.00*		
Seq2D12B			2.91*	
TC2B09			3.06*	5.61**
XIP171C				3.7*
seq19D06				3.43*
TC3A12				5.05**
Seq15C12				7.04**
TC2C07				3.5*

S1-Stage I, S2-Stage II, S3-Stage III, E1-*Rainy* 2005, E3-*Rainy* 2007, E4-Post *Rainy* 2007

Table 19: Single marker analysis for protein and oil in TG 26 x GPBD 4 mapping population

Seasons	E2		E3		E4	
Traits	**Marker**	**R^2 adj**	**Marker**	**R^2 adj**	**Marker**	**R^2 adj**
Protein (%)	XIP108	4.34**	TC1D12	5.42**	TC1D12	2.54*
	TC7H11	4.84**	TC1B04	4.63**	TC6H03	9.78**
	PM36	2.35*	TC3E2	5.87**	TC1B02	8.05**
	TC4F10	3.87*	TC2C07	5.88**	PM36	4.31**
	TC2G05	2.17*				
	TC2C07	4.04*				
Oil (%)	XIP75	2.38**	TC3A12	2.87*	XIP475	2.77*
	TC4F10	3.60**	TC2B09	2.18*	Seq7H6	3.26*
	TC2G05	3.14**	TC6E01	2.56*	TC3A12	2.44*
			TC2C07	6.43**	PM36	2.24*
			XIP103	5.72**	TC2B09	4.18*
					XIP103	6.98**

linoleic, Seq3F05 (2.40-5.34 %) for arachidic, TC3E05 (2.21-2.97 %), XIP75 (2.13-2.49 %), TC6H03 (2.06-2.89 %) and XIP103 (4.40-6.28 %) for behenic acid, XIP176 (2.33-3.37 %), XIP395 (2.17-2.86 %) for O/L ratio, XIP395 (2.81-3.95 %) for iodine value and XIP 176 for U/S ratio (2.53-3.15 %) and %S (2.46-2.98%).

Among the derived traits, O/L ratio, was associated with a total of 11 markers (2.17 to 5.09 %) and most of them were common to oleic and linoleic acids in respective seasons. Nine markers (2.06 to 4.79 %) for iodine value, 10 markers for U/S ratio (2.06 to 4.06 %) and 9 markers (2.12 to 4.11 %) for per cent saturated fatty acids were associated with different traits (Table 20).

4.3.1.3 Productivity traits

4.3.1.3.1 Plant Height

A total of sixteen markers were associated with plant height in four seasons studied with the range of 2.11 (XIP475) to 14.72 % (TC3A12). The highest marker contribution was recorded by TC3A12 (3.78-14.72 %). The contribution of other three markers TC2B09 (4.17% to 12.32 %), PM137 (3.8-10.65 %) and XIP531 (7.24 %) were also significant for this trait (Table 21).

4.3.1.3.2 Number of branches

For number of branches, a total of eleven markers were associated with the phenotypic variance of 2.01-5.96%. Only one marker (TC2G05 with 5.96%) had a substantial contribution for this trait and none of them were observed in more than one season (Table 21).

4.3.1.3.3 Number of pods per plant

A total of eight markers were associated with number of pods per plant with the phenotypic contribution ranging from 2.26 (Seq17F06) to 5.32 % (TC2A12). Out of these, two markers *viz*., TC3A12 (5.23-5.32 %) and TC3E02 (2.47-3.28 %) were observed in both the seasons. XIP103 (4.50 %) also contributed significantly to the trait (Table 22).

4.3.1.3.4 Pod yield per plant

Single marker analysis revealed a total of eleven makers associated with pod yield with the phenotypic contribution ranging from 2.14 (XIP352) to 11.25 % (XIP103). Two markers

Table 20: Single marker analysis for oil quality parameters in TG 26 x GPBD 4 mapping population

Traits	E3		E4	
	Marker	R^2 adj	Marker	R^2 adj
Palmitic acid	XIP171C	3.01*	XIP176	2.28*
	PM137	3.06*	TC3A12	2.66*
	XIP176	2.51*	TC2B09	3.23*
	PM183	2.39*	XIP121	3.39*
			PM433	2.49*
Stearic acid	XIP352	2.64*	XIP171C	2.77*
	Seq3F05	2.15*	TC2G05	2.32*
	TC6E01	4.12*	XIP103	8.90**
Oleic acid	PM137	2.24*	PM137	3.46*
	XIP176	2.88*	TC6H03	3.42*
	Seq11G7	2.48*	XIP395	3.54*
	XIP295	2.2*		
	XIP395	3.1*		
	TC5A07	3.6*		
Linoleic acid	TC3E05	5.2**	PM137	3.81*
	XIP176	2.60*	TC6H03	3.5*
	Seq11G7	2.62*	XIP395	3.29*
	XIP295	2.61*		
	XIP395	3.59**		
	TC5A07	3.36**		
Arachidic acid	Seq19D06	5.14**	XIP295	2.74*
	Seq3F05	5.34**	Seq17F06	2.64*
	XIP 103	2.23**	Seq3F05	2.4*
			TC2C07	2.5*
Eicosenoic acid	XIP395	2.89*	XIP75	3.19*
	XIP 103	4.54**	TC6H03	6.89**
			Seq3F05	3.16*
Behenic acid	TC3E05	2.97*	TC3E05	2.21*
	XIP75	2.49*	PM137	2.39*
	TC6H03	2.89*	XIP75	2.13*
	Seq3F05	3.4*	XIP176	2.55*
	XIP103	4.40**	TC6H03	2.06*
			XIP395	2.65*
			XIP103	6.28**
Lignoseric acid	XIP103	4.40**	XIP75	2.78*
			TC6H03	4.27**
			RN19A01	2.73*

Contd..

Traits	E3		E4	
	Marker	**R^2 adj**	**Marker**	**R^2 adj**
O/L Ratio	TC3E05	5.09**	PM137	2.89*
	XIP176	3.37*	TC11A04	2.37*
	Seq11G7	3.09*	XIP176	2.33*
	XIP395	2.86*	TC6H03	3.22*
	TC5A07	4.07*	XIP395	2.17*
			Seq17F06	2.74*
			TC2B09	2.53*
Iodine value	TC3E05	4.79**	PM137	4.18**
	PM137	2.13*	TC6H03	3.31*
	XIP176	2.06*	XIP395	2.81*
	Seq11G7	2.74*	Seq17F06	2.36*
	XIP295	3.06*		
	XIP395	3.95*		
	TC5A07	2.74*		
U/S Ratio	TC3E05	3.89*	XIP475	2.06*
	XIP75	3.86*	TC11A04	2.12*
	XIP176	3.15*	XIP176	2.53*
	TC5A07	3.48**	TC6H03	2.52*
	Seq3F05	3.05*	XIP395	3.27*
			XIP103	4.06**
%S	TC3E05	4.11**	XIP475	2.12*
	XIP75	3.87*	XIP176	2.46*
	XIP176	2.98*	TC6H03	2.30*
	TC5A07	3.72*	XIP395	3.39*
	Seq3F05	3.19*	XIP103	3.87**

Table 21: Single marker analysis for plant height and number of branches in TG 26 x GPBD 4 mapping population

	Rainy 2006		*Rainy* 2007		Post *Rainy* 2007		Post *Rainy*2008	
Traits	**Marker**	R^2 adj	**Marker**	R^2 adj	**Marker**	R^2 adj	**Marker**	R^2 adj
Plant height	TC3E05	4.45**	XIP475	2.11*	XIP395	2.24*	PM137	5.22**
	PM137	10.65**	PM137	3.80*	TC3A12	3.78*	TC1D12	2.61*
	Seq7H6	4.75**	XIP395	4.64**	TC2B09	4.17*	XIP176	2.89*
	TC1G04	4.18**	TC3A12	9.89**	XIP531	7.24**	XIP395	2.48*
	TC2B09	4.37**	TC2B09	8.59**			TC3A12	14.72**
	Seq4E8	3.62**	TC6E01	2.52*			TC2B09	12.32**
			TC2C07	3.01*			RN16F05	3.27*
No. of Branches	XIP407C	2.01*	TC0A01	2.03*	TC6E01	4.10*		
	TC1B04	4.65**	XIP352	2.93*	Seq4E8	3.77*		
	Seq18G01	4.65**	Seq17F06	3.79*				
	RN19A01	2.44*	TC4H07	3.38*				
	TC2G05	5.96**						

Table 22: Single marker analysis for productivity traits in TG 26 x GPBD 4 mapping population

	E2		E3			
Traits	**Marker**	**R2**	**Marker**	**R2**		
No. of pods/plant	TC3E2	2.47*	TC3E2	3.28*		
	Seq17F06	2.26*	XIP395	2.81*		
	TC3A12	5.32**	TC3A12	5.23**		
	XIP103	4.50**	TC2B09	3.69*		
	E2		**E3**		**E5**	
Traits	**Marker**	**R2**	**Marker**	**R2**	**Marker**	**R2**
Pod yield/plant	TC3E2	3.20*	TC3E2	3.13*	TC1B02	6.33**
	Seq17F06	2.57*	XIP352	2.14*	TC3A12	4.09*
	TC4H07	3.6**	TC6H03	3.37*	TC2B09	4.62**
	Seq5C12	2.97*	XIP103	11.25**		
	TC2G05	2.27*				
	XIP103	6.40**				
Shelling %	XIP475	5.87**	XIP475	3.01*	TC11A04	2.74*
	XIP75	2.23*	TC1B04	2.86*		
	XIP103	6.20**	XIP75	5.34**		
			TC6H03	3.20*		
			TC4H07	4.93**		
			Seq15C12	5.34**		
			XIP 103	2.25*		
Traits	**E3**		**E4**		**E5**	
100-seed wt.	**Marker**	**R2**	**Marker**	**R2**	**Marker**	**R2**
	TC6H03	2.32*	AC3D07	2.08*	TC6H03	3.00*
	TC1B02	3.45*	TC1B02	10.13*	TC1B02	12.58**
	TC3A12	2.49*			XIP23	4.17**
	Seq15C12	2.21*				

(XIP103 and TC3E2) were observed in at least two seasons. Among the markers, the contribution of TC1B02 (6.33 %) was also found significant (Table 22).

4.3.1.3.5 Shelling percentage

Shelling % was associated with a total of eight markers with the phenotypic variance of 2.23 (XIP75) to 6.20 (XIP103). Three common markers (XIP 475, XIP75 and XIP103) were observed in at least two seasons. Three markers *viz.,* XIP475 (5.87 %), XIP75 (5.03 %) and TC6H03 (5.34 %) contributed significantly to shelling % (Table 22).

4.3.1.3.6 100-seed weight

A total of six markers were identified for 100-seed weight with the R^2 of 2.08-12.58 %. TC1B02 (3.45 % to 12.58 %) occurred in all the seasons with the substantial contribution and TC6H03 (2.32-3.00) was found in two seasons (Table 22).

4.3.2 QTL mapping

The foremost step towards QTL mapping is to have linkage map with good coverage of markers. The partial linkage map developed from the cross TG 26 x GPBD 4 using 45 markers along with phenotypic and genotypic data of 146 RILs was used for the study. The QTL mapping was done using software PLABQTL version 1.1 W (Utz and Melchinger, 1996) to identify putative QTLs associated with rust and LLS diseases at different stages, nutritional quality (protein, oil and fatty acids and their derivatives) and other agronomic and productivity traits.

4.3.2.1 Diseases

4.3.2.1.1 Rust

A total of five QTLs were identified for rust resistance with the phenotypic variance ranging from 1.70 to 48.90 %. One major QTL (XIP103-PM36) located on LG 3 was identified at all the stages in all the seasons with significant phenotypic variance (24.10 to 48.90 %) and very high LOD scores (8.76 to 22.28). The favorable allele was contributed by resistant parent (GPBD 4) and the additive effects increased with advancement in stages. Other QTLs were

found to be season and stage specific with lesser contribution to variance (Table 23 and Fig 12 (i) and Fig 13).

4.3.2.1.2 Late leaf spot

Only one QTL (TC2B09-RN16F05) with the contribution of 6.40% was identified for LLS resistance at stage II (E3) with the additive effect of 0.199. The favorable allele for this QTL was contributed by resistant parent, GPBD 4 (Table 23 and Fig 12 (ii)).

4.3.2.2 Nutritional quality traits

Fig 15 shows the linkage map showing QTLs identified for quality traits in TG 26 x GPBD 4 mapping population.

4.3.2.2.1 Protein and oil content

A total of seven QTLs were associated with protein content with the phenotypic variance ranging from 0.50 to 11.70 %. Two QTLs *viz.*, TC2E05-TC3E02 (10.20 %) located on LG 4 and Seq15C12-XIP105 (7.10%) located on LG 6 contributed substantially with an additive effect of 1.030 and 1.053, respectively. The favorable allele was contributed from GPBD 4 for both the QTLs. Contribution of other five QTLs came from TG 26. Among these, the contribution of QTL (TC6H03-TC11A04) was significant (10.70 %). The contribution of other QTLs *viz.,* TC3A12-PM433 (0.50 %), TC1D12-TC9B08 (4.00 %), XIP395-TC2C07 (3.90%) and TC2B09-RN16F05 (1.50%) was very small (<5.00%) {Table 24 and Fig 14 (i)}.

For oil content, a total of four QTLs (1.50 to 9.10 %) were identified in different seasons. Among them, one QTL (XIP103-PM36) located on LG3 was observed in two seasons (7.90 to 9.10 %). The additive effect for this QTL was 0.499 and 0.408, respectively and the favorable allele came from GPBD 4. However, the favorable allele for three QTLs *viz.*, TC2E05-TC3E02 (1.50%), Seq7H06-XIP475 (5.20%) and TC2B09-RN16F06 (6.80 %) came from TG 26 parent. In one of the seasons (E2) no QTLs were identified for the trait {Table 24 and Fig. 14(i)}.

4.3.2.2.2 Oil quality parameters

Total of two QTLs for palmitic (4.60 to 6.70%), one QTL for stearic (10.30 %), 4 QTLs for oleic (0.60 to 9.70%) and linoleic (0.70 to 9.00%), one QTL for arachidic (1.50 %), one QTL

Table 23: QTLs identified for resistance to rust and late leaf spot at different stages in TG 26 x GPBD 4 mapping population

Traits	LG	Marker Interval	Position (CM)	LOD	R^2 (%)	Additive effect
***Rainy* 2005 (E1)**						
Stage I	3	XIP103-PM36	26	12.74	32.30	-0.506
	8	TC2B09-RN16F05	36	3.32	5.00	-0.163
Stage II	3	XIP103-PM36	26	10.75	28.90	-0.81
***Rainy* 2007 (E3 (Exp I))**						
Stage I	3	XIP103-PM36	26	8.76	24.10	-0.297
Stage II	3	XIP103-PM36	26	15.44	35.80	-0.471
Stage III	3	XIP103-PM36	26	14.84	35.10	-0.565
***Rainy* 2007 (E3 (Exp II))**						
Stage I	3	PM183-XIP282	2	2.59	5.40	0.391
	3	XIP103-PM36	26	20.76	46.10	-1.331
	3	TC1D12-TC9B08	44	3.17	2.60	0.359
Stage II	3	XIP103-PM36	26	22.28	48.90	-1.508
Post *Rainy* 2007 (E4)						
Stage I	3	XIP103-PM36	26	14.61	32.70	-0.629
Stage II	3	XIP103-PM36	26	17.20	36.70	-0.953
	1	PM137-TC6H03	10	3.26	1.70	0.152
Stage III	3	XIP103-PM36	26	11.73	31.10	-1.053
Late leaf spot						
***Rainy* 2005 (E1)**						
Stage I	-	-	-	-	-	-
Stage II	-	-	-	-	-	-
***Rainy* 2007 (E3)**						
Stage I	-	-	-	-	-	-
Stage II	8	TC2B09-RN16F05	6	2.65	6.40	-0.199

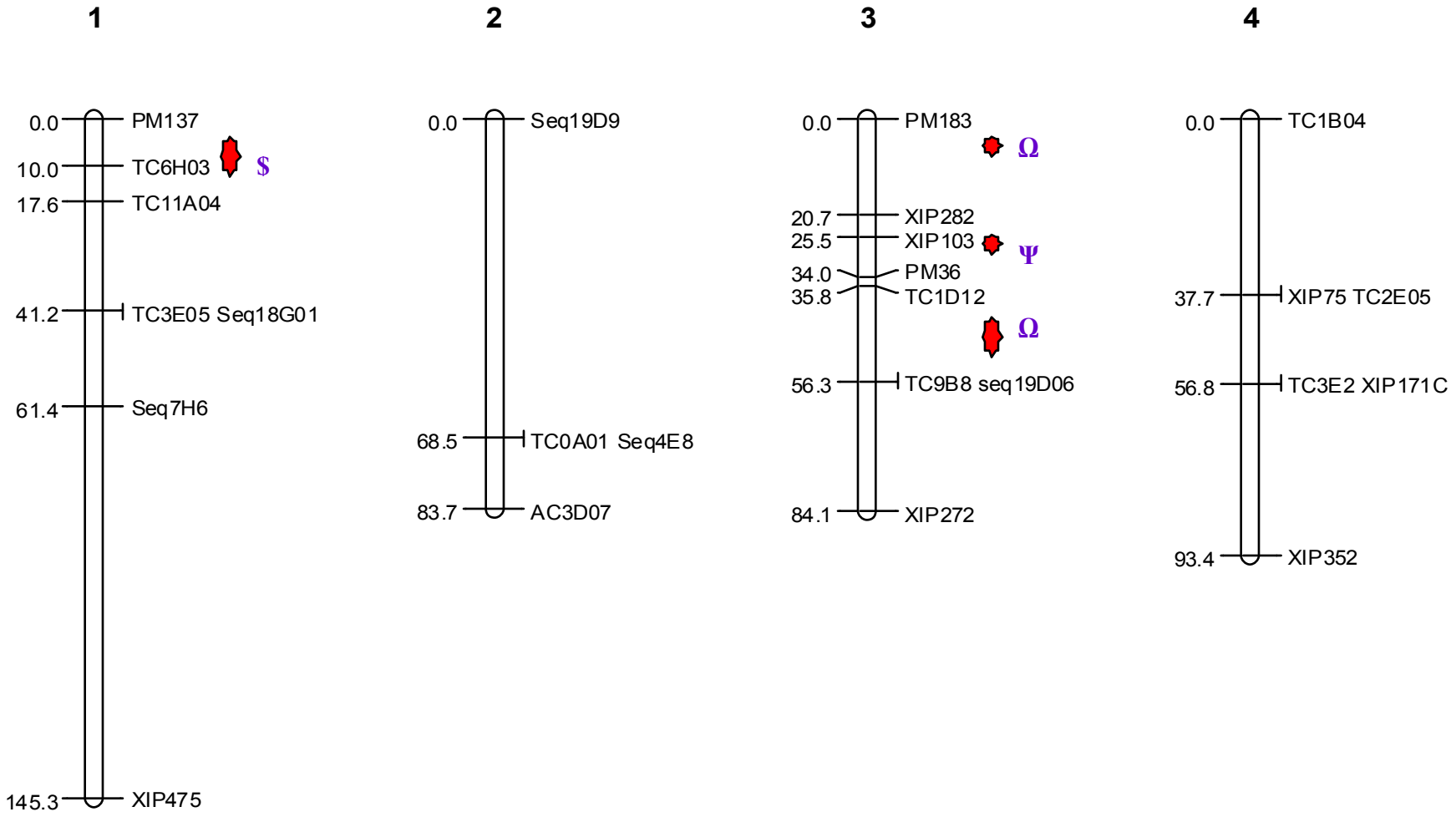


Fig. 13: Linkage map showing QTLs identified for Rust and Late leaf spot at different stages in TG-26 x GPBD-4 mapping population of groundnut

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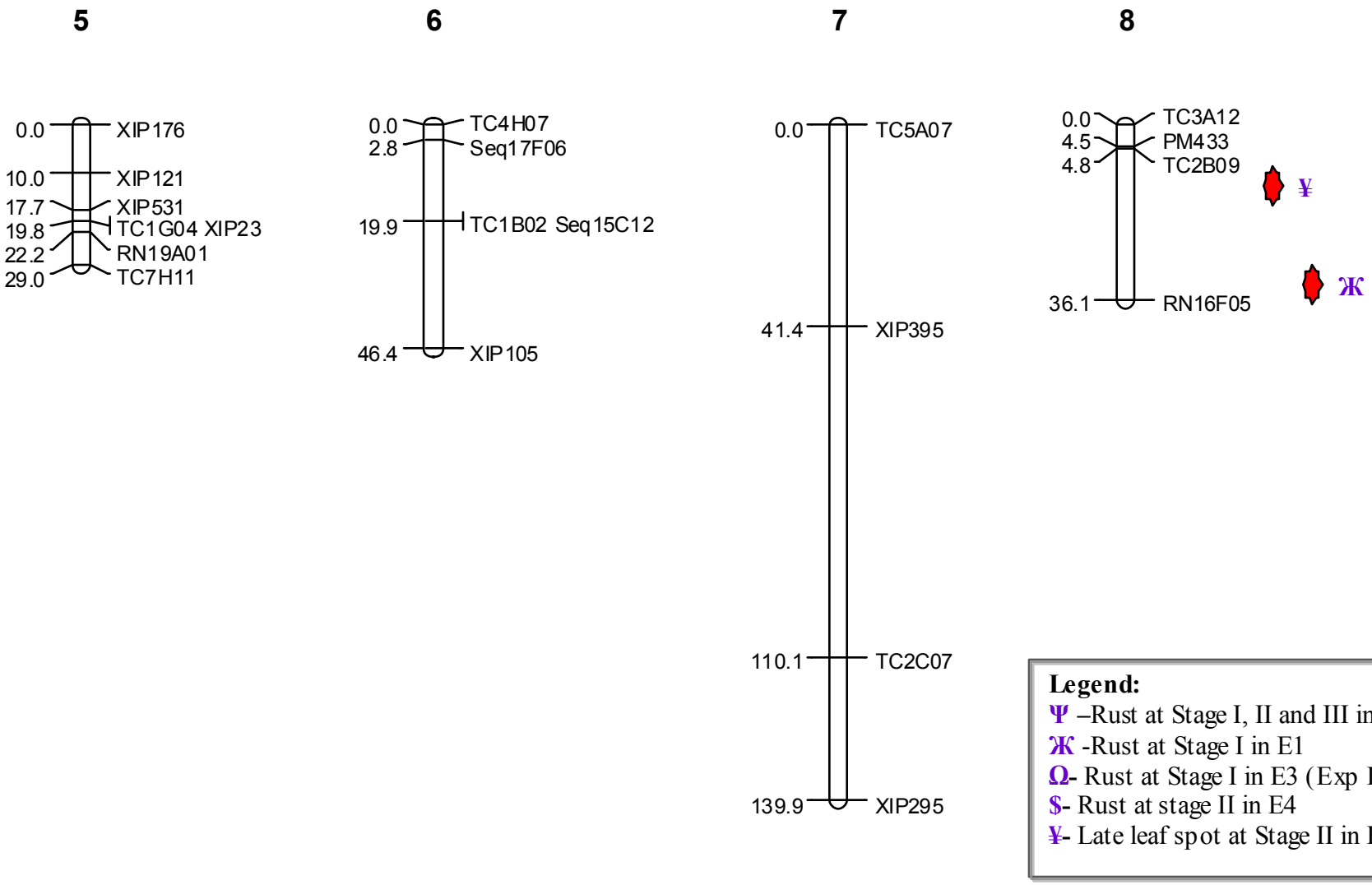
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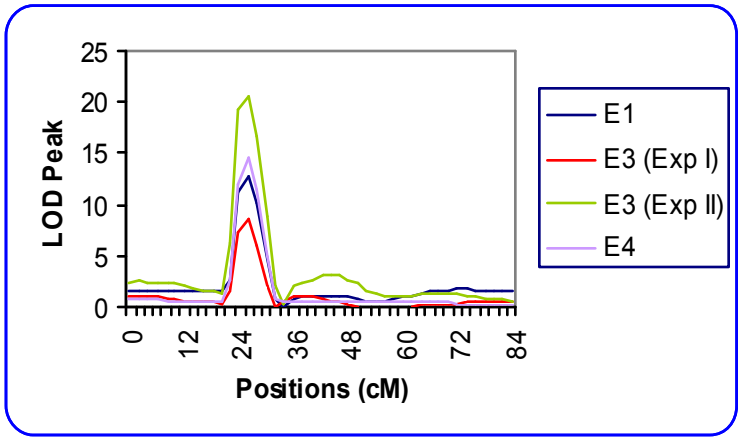
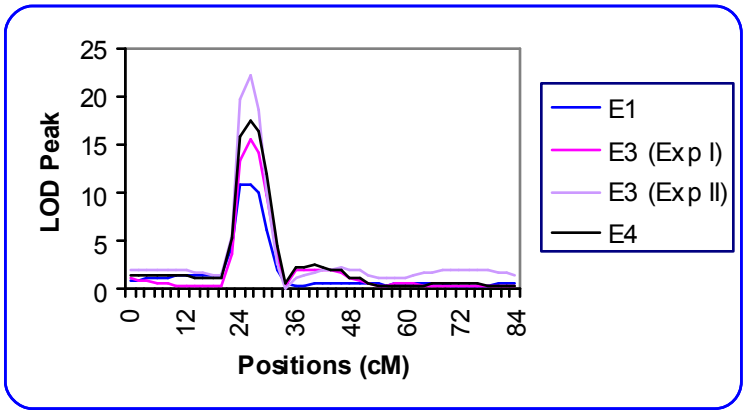
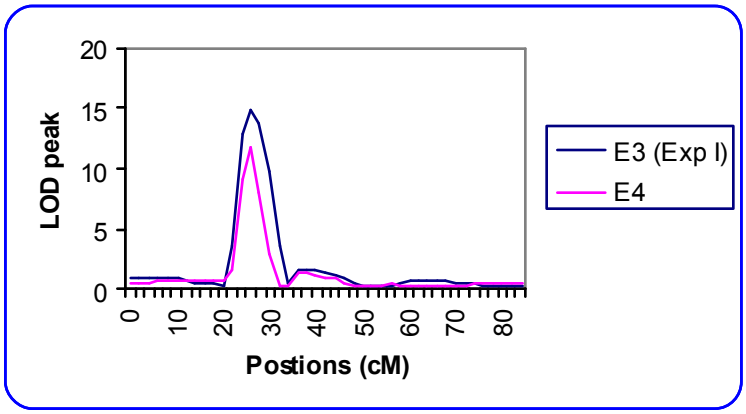
0.0 XIP176
10.0 XIP121
17.7 XIP531
19.8 TC1G04 XIP23
22.2 RN19A01
29.0 TC7H11
6
0.0 TC4H07
2.8 Seq17F06
19.9 TC1B02 Seq15C12
46.4 XIP105
7
0.0 TC5A07
41.4 XIP395
110.1 TC2C07
139.9 XIP295
8
0.0 TC3A12
4.5 PM433
4.8 TC2B09
¥
Ж
36.1 RN16F05
Legend:
Ψ –Rust at Stage I, II and III in all seasons
Ж -Rust at Stage I in E1
Ω- Rust at Stage I in E3 (Exp II)
$- Rust at stage II in E4
¥- Late leaf spot at Stage II in E3



Stage I



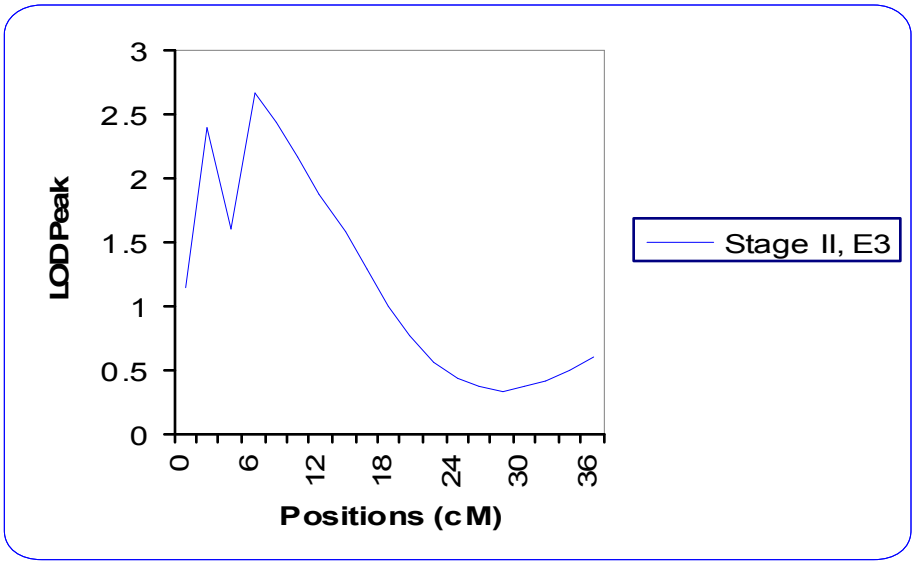
Stage II



Stage III

E1-Rainy 2005 E3- Rainy 2007 (Experiment I and II), E4-Post rainy 2007

Fig. 12(i) : LOD peak for rust at different stages on LG3 in TG 26 x GPBD 4 mapping population

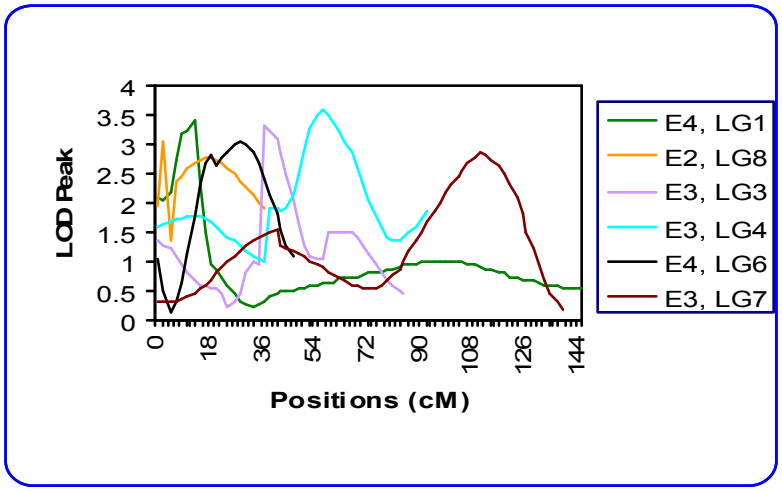


E3-*Rainy* 2007

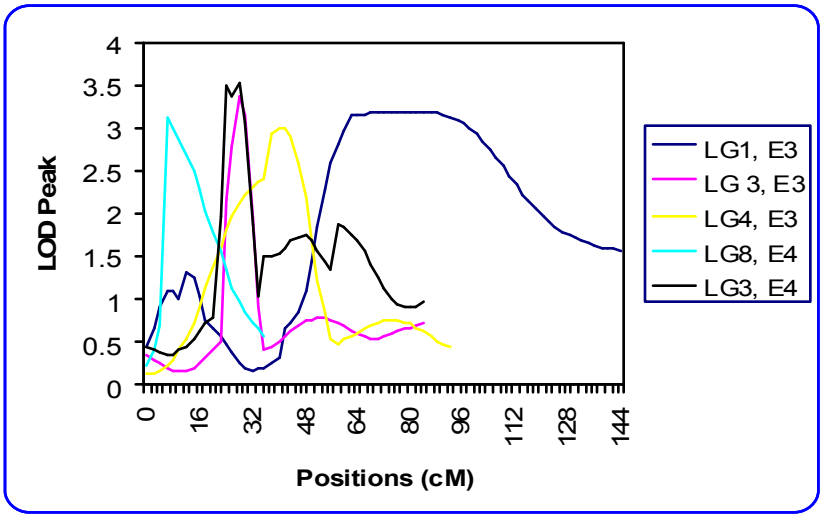
Fig. 12 (ii): LOD peak for LLS at stage II in TG 26 x GPBD 4 mapping population

Table 24: QTLs associated with protein and oil in TG 26 x GPBD 4 mapping population

Traits	LG	Marker Interval	Position (cM)	LOD	R^2 (%)	Additive effect
Protein (%)						
E2	8	TC3A12-PM433	2	3.07	0.50	-0.368
E3	3	TC1D12-TC9B08	36	3.33	4.00	-0.594
	4	TC2E05-TC3E02	56	3.62	10.20	1.030
	7	XIP395-TC2C07	110	2.87	3.90	-0.609
	8	TC2B09-RN16F05	24	2.89	1.50	-0.552
E4	1	TC6H03-TC11A04	12	3.42	10.70	-1.249
	6	Seq15C12-XIP105	28	3.04	7.10	1.053
Oil (%)						
E2	-	-	-	-	-	-
E3	3	XIP103-PM36	28	3.38	7.90	0.499
	4	TC2E05-TC3E02	42	3.01	1.50	-0.199
E4	1	Seq7H6-XIP475	80	3.20	5.20	-0.434
	3	XIP103-PM36	28	3.53	9.10	0.408
	8	TC2B09-RN16F05	6	3.12	6.80	-0.280



Protein content



Oil content

E2- *Rainy* 2006 E3-*Rainy* 2007 E4-*Post rainy* 2007 LG-Linkage Group

Fig. 14 (i): LOD peaks for protein content and oil content in TG 26 x GPBD 4 mapping population

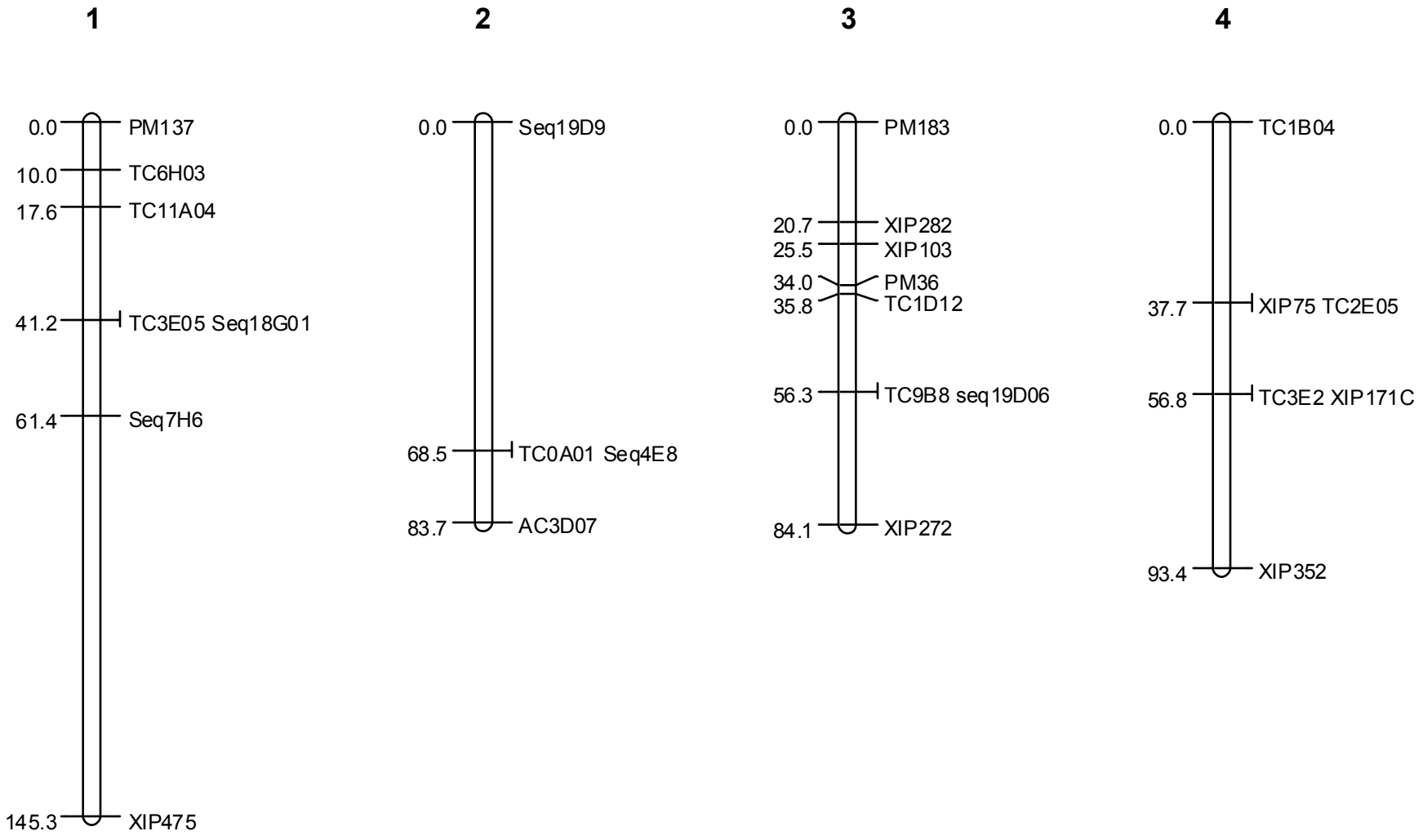


Fig. 15: Linkage map showing QTLs associated with nutritional quality traits in TG-26 x GPBD-4 population of groundnut

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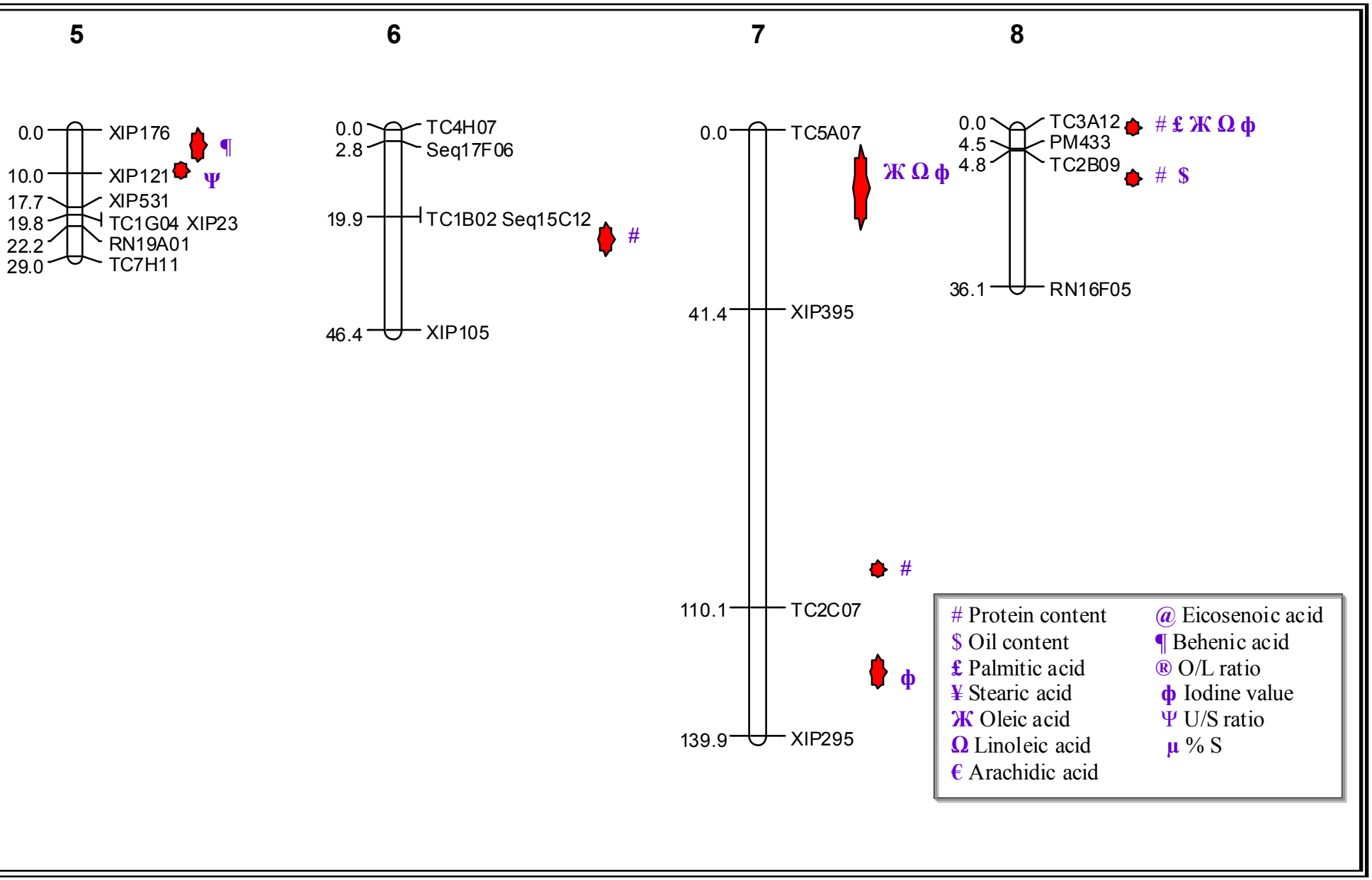
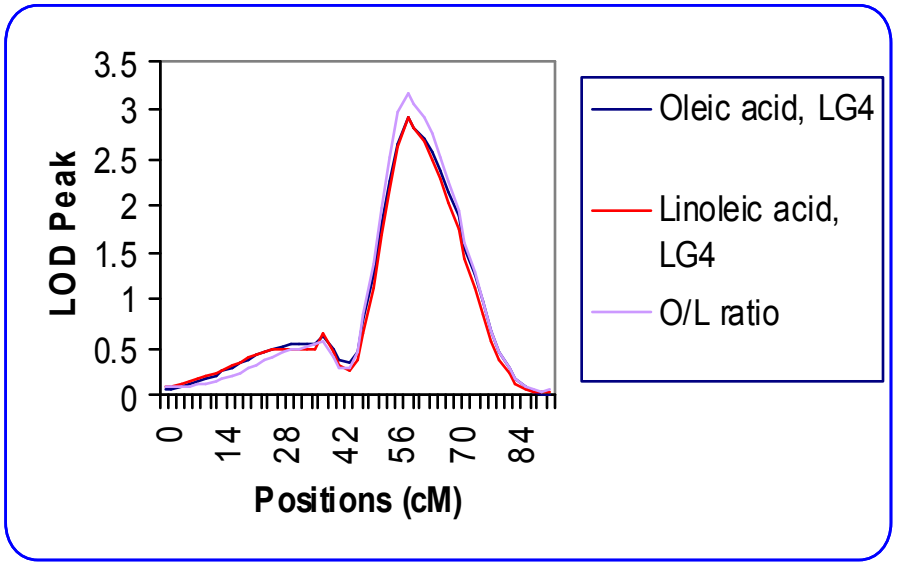
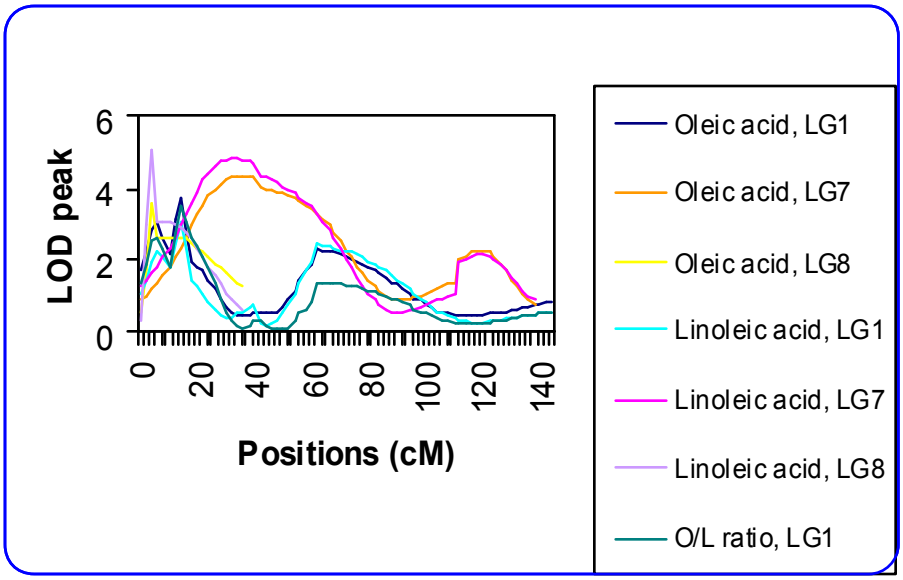

5
6
7
8
0.0 XIP176
10.0 XIP121
17.7 XIP531
19.8 TC1G04 XIP23
22.2 RN19A01
29.0 TC7H11
¶
Ψ
0.0 TC4H07
2.8 Seq17F06
19.9 TC1B02 Seq15C12
46.4 XIP105
#
0.0 TC5A07
41.4 XIP395
110.1 TC2C07
139.9 XIP295
Ж Ω ф
#
ф
0.0 TC3A12
4.5 PM433
4.8 TC2B09
36.1 RN16F05
£ Ж Ω ф
$
Protein content
$ Oil content
£ Palmitic acid
¥ Stearic acid
Ж Oleic acid
Ω Linoleic acid
€ Arachidic acid
@ Eicosenoic acid
¶ Behenic acid
® O/L ratio
ф Iodine value
Ψ U/S ratio
µ % S



E3



E4

E3-*Rainy* 2007 E4-Post rainy 2007 LG-Linkage Group

Fig. 14 (ii) : LOD peak for oleic, linoleic acids and O/L ratio in individual seasons (E3 and E4)

for eicosenoic (0.10%), two QTLs for behenic acid (3.60 to 7.70 %) two QTLs for O/L ratio (1.00 to 6.80%), 4 QTLs for iodine value (2.60 to 7.50 %), three QTLs for U/S ratio (3.20 to 7.70 %) and one QTL for %S (5.60 %) were associated with respective traits. None of the QTLs found common in both the seasons for any of the traits. One QTL flanked by markers TC3A12-PM433 located on LG8 with LOD more than 3.5 was associated with palmitic (6.7 %), oleic acid (7.2 %), linoleic acid (7.2 %) and iodine value (7.5%). Another QTL flanked by TC6H03-TC11A04 located on LG1 with the LOD of more than 3.0 was associated with oleic acid (9.70%), linoleic acid (9.0%), O/L ratio (6.8 %), U/S ratio (7.70%) and %S (5.60 %) and contributed significantly. The favorable allele for both of these QTLs was contributed by GPBD4 for all the traits. The QTLs identified for oleic, linoleic acid and O/L ratio were common in the respective seasons and the favorable allele for QTLs identified in E3 came from TG 26 and favorable allele for QTLs in E4 was contributed from GPBD 4 (Table 25).

One QTL flanked by XIP103-PM36 located on LG 3 was common for stearic acid (10.30%) and behenic acid (7.70%) with the additive effect of 0.250 and 0.077, respectively. The favorable allele for this QTL came from GPBD 4. LOD peaks for QTLs detected for oleic, linoleic acid and O/L ratio are given in the Fig 14 (ii) and for other oil quality parameters are given in Fig 14 (iii).

4.3.2.3 Productivity traits

Fig 17 showing the linkage map showing QTLs associated with agronomic and productivity traits in TG 26 x GPBD 4 mapping population.

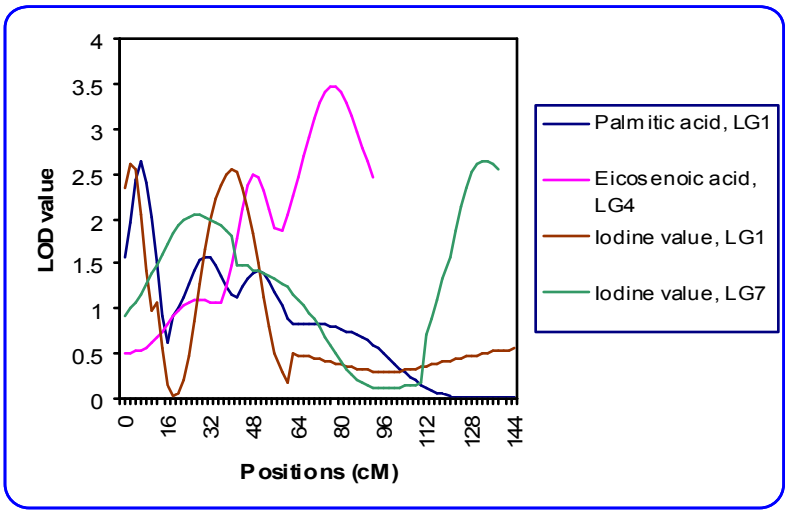
4.3.2.3.1 Plant height (cm)

A total of four QTLs were identified for plant height with the phenotypic variance ranging from 4.10 to 17.50 %. A QTL flanked by TC3A12-PM433 located on LG8 contributed maximum phenotypic variance (11.20 to 17.50 %) with higher LOD (3.47 and 6.84) in two seasons (Fig 4.3.3.1b). The favorable allele for this QTL came from GPBD 4 with an additive effect of 2.389 and 1.606, respectively. A QTL flanked by PM137-TC6H03 (11.20%) located on LG1 also contributed significantly with the favorable allele from GPBD 4 and an additive effect

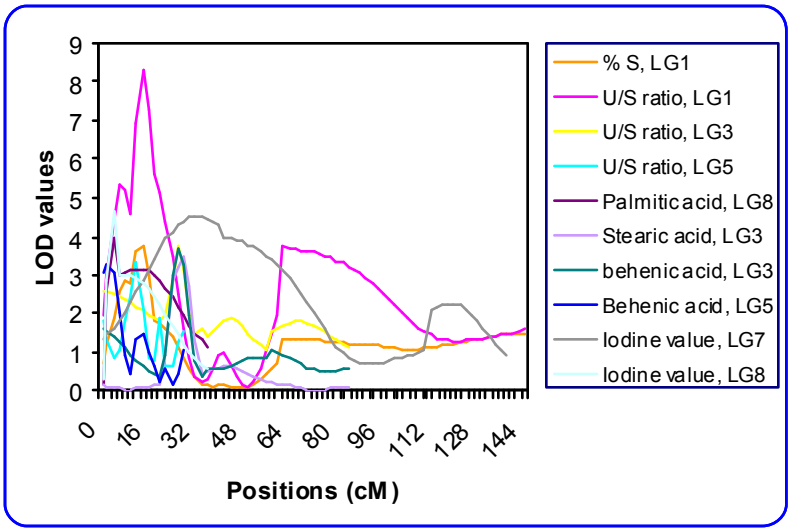
Table 25: QTLs identified for oil quality parameters in TG 26 x GPBD 4 mapping population

Traits	LG	Marker Interval	Position (cM)	LOD	R^2 (%)	Additive effect
Palmitic acid						
E3	1	PM137-TC6H03	6	2.60	4.60	-0.169
E4	8	TC3A12-PM433	4	3.95	6.70	-0.256
Stearic Acid						
E3	-	- -	-	-	-	-
E4	3	XIP103-PM36	28	3.43	10.30	-0.25
Oleic Acid						
E3	4	XIP171c-XIP352	58	2.91	0.60	-0.333
E4	1	TC6H03-TC11A04	14	3.75	9.70	2.749
	7	TC5A07-XIP395	36	4.32	5.60	1.799
	8	TC3A12-PM433	4	3.6	7.20	1.885
Linoleic Acid						
E3	4	XIP171c-XIP352	58	2.92	0.70	0.316
E4	1	TC6H03-TC11A04	14	3.04	9.00	-2.28
	7	TC5A07-XIP395	32	4.84	5.10	-1.665
	8	TC3A12-PM433	4	5.06	7.20	-1.641
Arachidic Acid						
E3	1	Seq7H6-XIP475	144	2.91	1.50	0.02
E4	-	- -	-	-	-	-
Eicosenoic Acid						
E3	4	XIP171co-XIP352	76	3.69	0.10	-0.004
E4	-	- -	-	-	-	-
Behenic Acid						
E3						
E4	3	XIP103-PM36	26	3.68	7.70	-0.077
	5	XIP176-XIP121	2	3.28	3.60	0.059
Lignoseric Acid						
E3	-	- -	-	-	-	-
E4	-	- -	-	-	-	-
O/L Ratio						
E3	4	XIP171c-XIP352	58	3.18	1.00	-0.037
E4	1	TC6H03-TC11A04	14	3.48	6.80	0.192
Iodine Value						
E3	1	TC11A04-TC3E05	40	2.54	3.60	0.686
	7	TC2C07-XIP295	132	2.65	2.60	0.694
E4	7	TC5A07-XIP395	32	4.49	3.10	-1.01
	8	TC3A12-PM433	4	4.64	7.50	-1.31
U/S Ratio						
E3	-	- -	-	-	-	-
E4	1	TC6H03-TC11A04	14	8.36	7.70	0.124
	3	PM36-TC1D12	40	3.58	8.40	0.083
	5	XIP121-XIP531	12	3.29	3.20	0.064
% S						
E3	-	- -	-	-	-	-
E4	1	TC6H03-TC11A04	14	3.84	5.60	-0.374

LG-Linkage Group, E2-*Rainy* 2006, E3-*Rainy* 2007, E4-Post *Rainy* 2007, E5-Post *Rainy* 2008



E3



E4

E3-*Rainy* 2007 E4-Post rainy 2007 LG-Linkage Group

Fig. 14 (iii) : LOD peak for palmitic, stearic, arachidic, eicosenoic acid, behenic acid and iodine value, U/S ratio and % S in individual seasons (E3 and E4)

of 1.871cm. Other two QTLs viz., TC4H07-Seq17F06 (6.30 %) and XIP176-XIP121 (4.10 %) also found prominent in a season (Table 26A and Fig.16 (i)).

4.3.2.3.2 Number of branches

For number of branches, only one QTL (TC3A12-PM433) was identified (E3). It was located on LG8 with the phenotypic variance of 2.10 %. The favorable allele was contributed by GPBD4 with an additive effect of 0.155 (Table 26A).

4.3.2.3.3 Number of pods per plant

A total of five QTLs were associated with number of pods per plant, out of which two QTLs *viz*., XIP103-PM36 on LG3 and RN19A01-TC7H11 on LG5 were observed in two seasons. The contribution of XIP103-PM36 to variance was significant (4.41 to 6.10 %) and the favorable allele came from GPBD 4. Two QTLs (TC2E05-TC3E02 on LG4 and TC3A12-PM433 on LG8) in E3 had a phenotypic variance of 8.30 %. However, their favorable allele came from different parents {Table 26B and Fig.16(i)}.

4.3.2.3.4 Pod yield per plant (g)

Three QTLs were identified for pod yield of which XIP103-PM36 located on LG3 was observed in two seasons with LOD scores of 3.64 and 7.16 and the phenotypic variance of 6.60% and 11.20%, respectively. The additive effect explained by this QTL was 1.777 to 1.296 respectively. The favorable allele for this QTL in both the seasons came from higher yielding parent (GPBD 4). The favorable allele for other two QTLs *viz*., TC2E05-TC3E02 on LG4 and XIP395-TC2C07 on LG7 with the phenotypic variance of 8.90 and 5.60 %, respectively was derived from low yielding parent (TG 26). No QTLs were identified in E4 for pod yield per plant {Table 26B and Fig 16 (i)}.

4.3.2.3.5 Shelling percentage (%)

Out of five QTLs (1.90 to 7.10 %), the QTL flanked by PM183-XIP282 located on LG3 was observed in two seasons (E2 and E3) with the phenotypic variance of 1.90 % and 7.10 %, respectively. The additive effect of this QTL was 0.789 and 0.765 respectively and the favorable allele was contributed by TG 26.

Table 26A: QTLs identified for plant height and number of branches in TG 26 x GPBD 4 mapping population

Traits	LG	Marker Interval	Position (CM)	LOD	R^2 (%)	Additive effect
Plant height (cm)						
E2	1	PM137-TC6H03	2	5.09	11.20	1.871
E3	8	TC3A12-PM433	2	3.47	12.60	2.389
E4	-	- -	-	-	-	-
E5	5	XIP176-XIP121	0	5.09	4.10	0.614
	6	TC4H07-Seq17F06	2	6.67	6.30	-0.843
	8	TC3A12-PM433	0	6.84	17.50	1.606
No. of branches						
E2	-	- -	-	-	-	-
E3	8	TC3A12-PM433	0	2.93	2.10	0.155
E4	-	- -	-	-	-	-
E5	-	- -	-	-	-	-

Table 26B: QTLs identified for productivity traits in TG 26 x GPBD 4 mapping population

Traits	LG	Marker Interval	Position (cM)	LOD	R^2 (%)	Additive effect
No. of pods/plant						
E2	3	XIP103-PM36	26	3.02	4.41	1.17
	5	RN19A01-TC7H11	28	3.31	1.20	-0.642
E3	3	XIP103-PM36	26	5.29	6.10	1.134
	4	TC2E05-TC3E02	56	3.18	8.30	-1.308
	5	RN19A01-TC7H11	28	3.58	0.10	0.12
	7	XIP395-TC2C07	80	3.05	6.10	-2.442
	8	TC3A12-PM433	2	2.72	8.30	2.217
Pod yield/plant (g)						
E2	3	XIP103-PM36	26	3.64	6.60	1.177
E3	3	XIP103-PM36	26	7.16	11.20	1.296
	4	TC2E05-TC3E02	56	4.10	8.90	-1.149
	7	XIP395-TC2C07	82	3.85	5.60	-1.149
E5	-	- -	-	-	-	-
100-Seed weight (g)						
E3	4	TC1B04-XIP75	22	2.52	1.70	1.412
E4	6	TC1B02 -XIP105	22	3.65	8.20	1.926
E5	6	TC1B02 -XIP105	22	5.01	14.00	2.701
Shelling %						
E2	3	PM183-XIP282	2	3.05	1.90	-0.789
E3	1	Seq7H6-XIP475	124	3.21	6.30	-1.398
	3	PM183-XIP282	0	2.95	7.10	-0.765
	4	TC1B04-XIP75	28	4.77	6.30	0.998
	6	TC4H07-Seq17F06	0	3.69	5.90	0.675
E5	-	- -	-	-	-	-

LG-Linkage Group, LOD-Log of odds, E2-*Rainy* 2006, E3-*Rainy* 2007, E4-Post *Rainy* 2007, E5-Post *Rainy* 2008

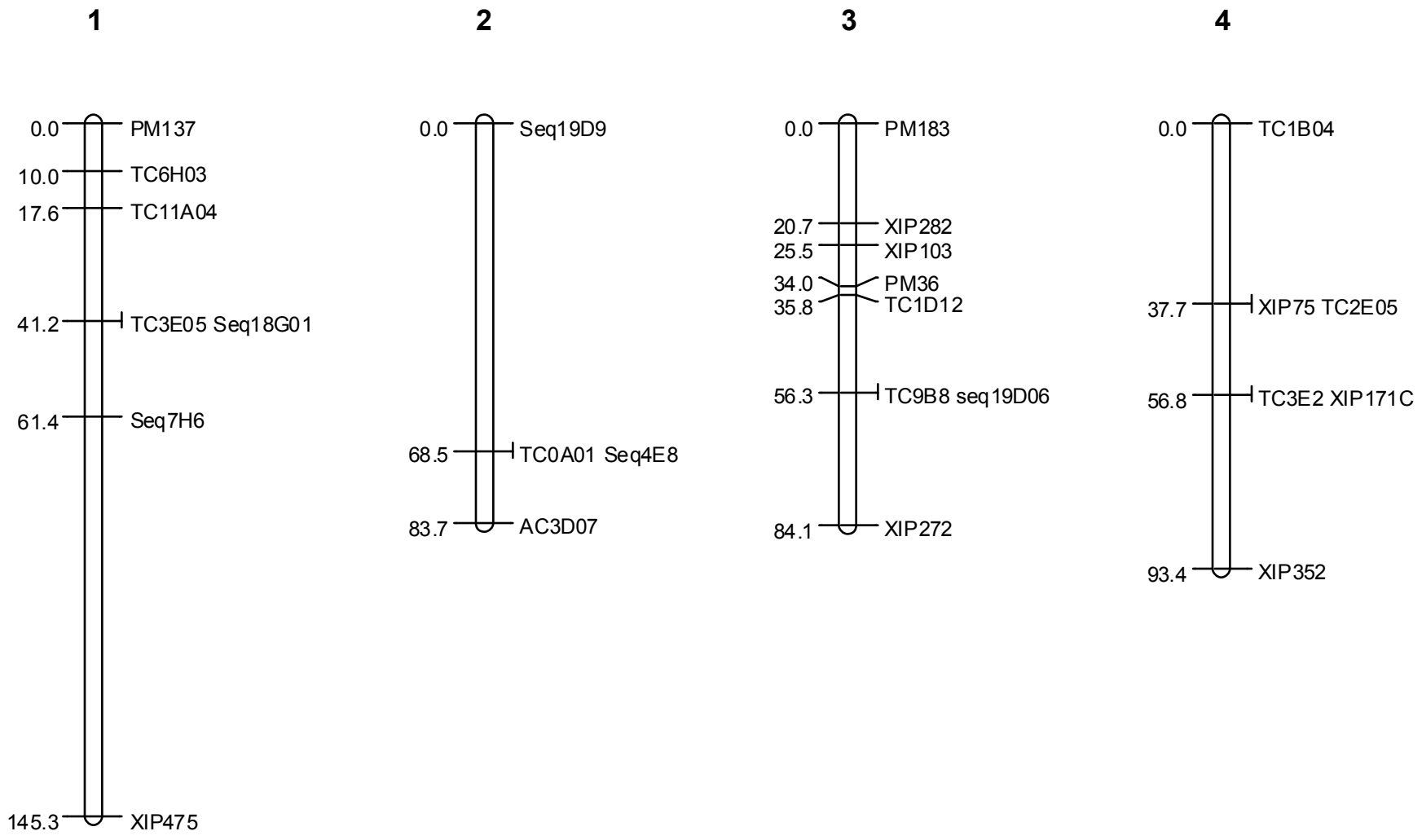


Figure 17: Linkage map showing QTLs identified for pod yield and other agronomic traits in TG 26 x GPBD 4 mapping population of groundnut

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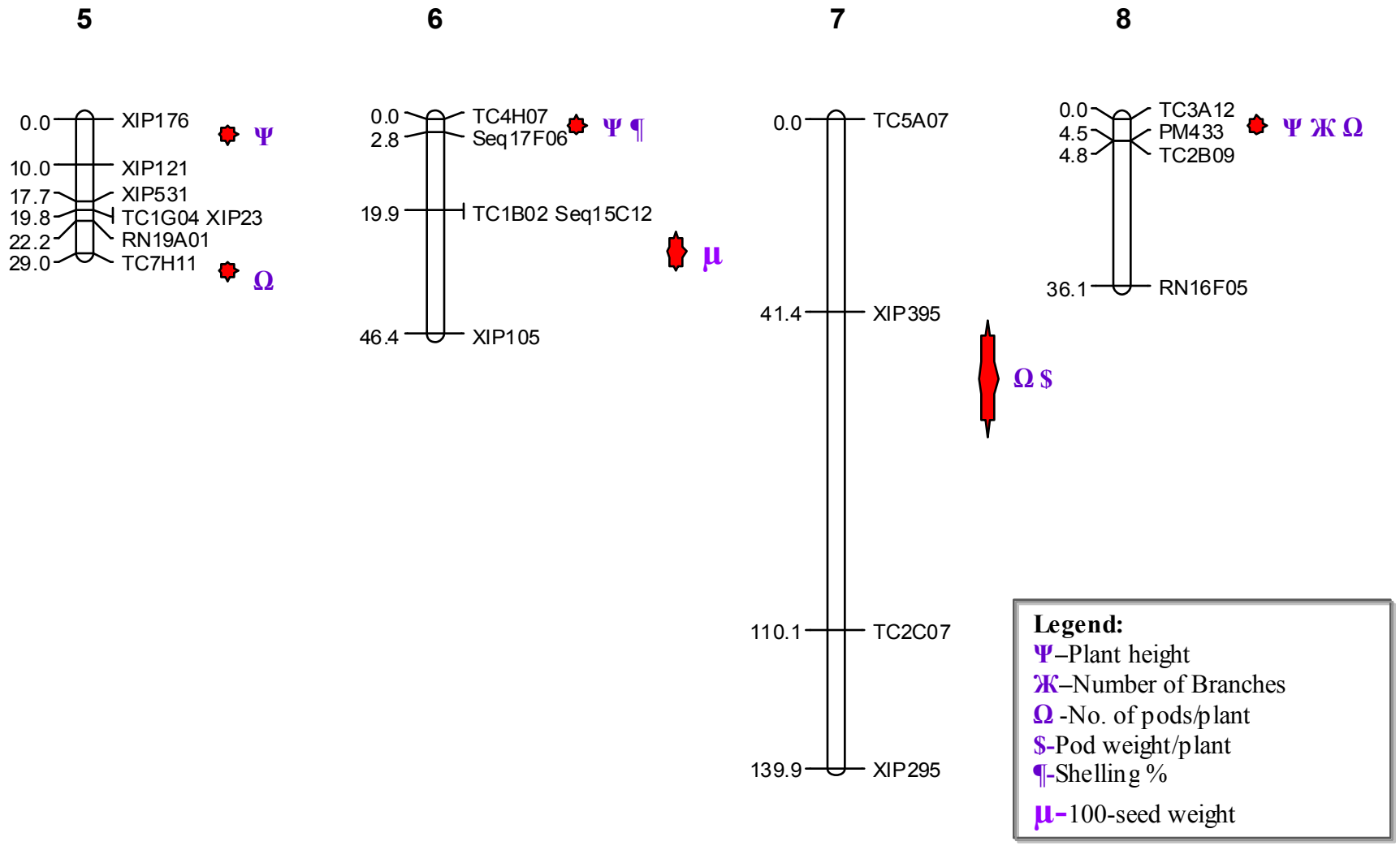
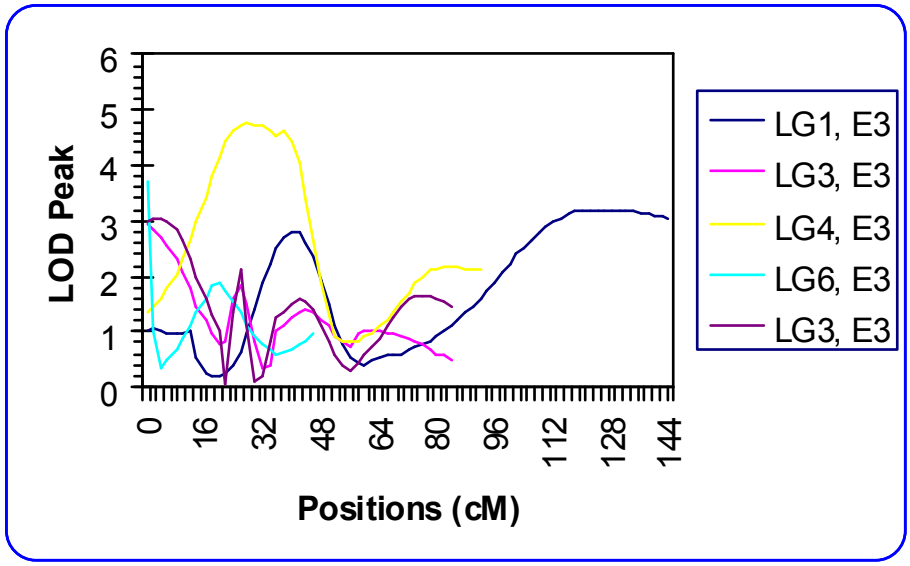

5
6
7
8
0.0 XIP176
Ψ
10.0 XIP121
17.7 XIP531
19.8 TC1G04 XIP23
22.2 RN19A01
29.0 TC7H11
Ω
0.0 TC4H07
2.8 Seq17F06
Ψ ¶
19.9 TC1B02 Seq15C12
μ
46.4 XIP105
0.0 TC5A07
41.4 XIP395
Ω $
110.1 TC2C07
139.9 XIP295
0.0 TC3A12
4.5 PM433
4.8 TC2B09
Ψ Ж Ω
36.1 RN16F05
Legend:
Ψ–Plant height
Ж–Number of Branches
Ω -No. of pods/plant
$-Pod weight/plant
¶-Shelling %
μ–100-seed weight

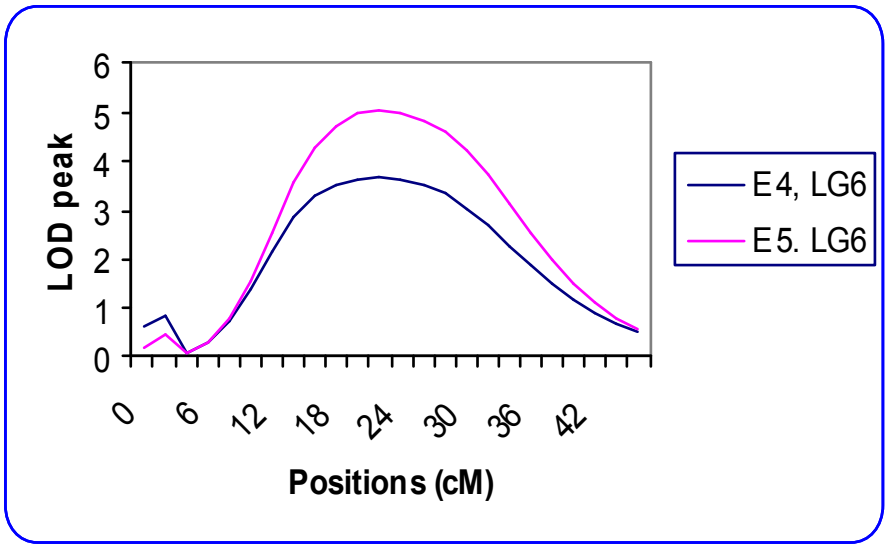
Other two QTLs *viz*., TC1B04-XIP75 (6.30 %) on LG4 and TC4H07-Seq17F06 (5.90 %) on LG6 contributed their favorable allele from GPBD 4 with the additive effect of 0.998 and 0.675, respectively {Table 26B and Fig 16 (ii)}.

4.3.2.3.6 100-seed weight (g)

One common QTL flanked by TC1B02-XIP105 located on LG 6 was associated with 100-seed weight in two seasons (E4 and E5) with significant contribution of 8.20 % and 14.00 %, respectively. The LOD scores in both the seasons were 3.65 and 5.01 with the additive effect of 1.926 and 2.701 respectively. Another QTL flanked by TC1B04-XIP75 (1.70%) contributed less to the trait. The favorable allele for both these QTLs was contributed by higher 100-seed weight parent (GPBD 4) {Table 26B and Fig 16(ii)}.



Shelling %



100-seed weight

E3-*Rainy* 2007 E4-Post rainy 2007 E5-Post rainy 2008 LG-Linkage Group

Fig. 16 (ii) : LOD peak for shelling % and 100-seed weight in TG 26 x GPBD 4 mapping population

5. DISCUSSION

Plant breeding programs aim at developing disease resistant cultivars with high productivity and improved quality for overall crop improvement. Being, one of the important sources of oil and vegetable protein, improvement for disease resistance, quantity and quality of oil and protein and higher productivity are the great challenges in groundnut breeding programs. But improving all the traits in a single cultivar is very difficult through conventional breeding techniques especially for the traits showing lower heritability and high genotype x environment interaction. Conventional breeding is time consuming, very dependent on environmental conditions and development of a new variety takes eight to twelve years and even then the release of an improved variety is not guaranteed. Hence, breeders are extremely interested in new technologies that could make their procedure more certain and efficient. Advent of modern tools like molecular markers has revolutionized the conventional breeding in gaining better success through marker-assisted selection. A large number of studies in various crop species have used molecular markers as a tool to identify major genes, QTLs or to introduce new character in elite germplasm. Knowing the location of these genes and specific alleles offers the possibility to apply MAS because one of the main objectives of plant breeder is the introgression of one or more favorable genes from a donor parent into the background of an elite variety. MAS is especially useful for traits which are controlled by recessive alleles (disease resistance), which are costly and difficult for phenotyping (nutritional quality traits) and complex traits which are polygenically controlled (productivity traits).

Hence, an attempt has been made to tag the putative microsatellite markers/QTLs with resistance to rust and LLS, nutritional quality and productivity traits in TG 26 x GPBD 4 mapping population consisting of 146 RILs segregating for the above traits.

5.1 Linkage map construction

Linkage map indicates the position and relative genetic distances between markers along chromosomes. The most important use of linkage map 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.

Very few reports on the construction of genetic linkage map (Moretzsohn *et al*., 2005; Gobbi *et al*., 2006; Khedikar 2008 and Varshney *et al*., 2008) based on SSR markers are available in groundnut. In the present study, 53 polymorphic markers (5.56 %), obtained by screening 1043 microsatellite markers were used for genotyping the population. The per cent polymorphism obtained in the present study is very less compared to earlier reports *viz*., Hopkins *et al*., 1999 (23.00%); He *et al*., 2003 (33.90%); Ferguson *et al*., 2004 (70.80-81.00 %); He *et al*., 2005 (29.23 %); Mace *et al*., 2006 (52.00 %); Moretzsohn *et al*, 2005 (47.10%); Nimmakayala *et al*., 2007 (52.08%); Khedikar 2008 (6.15 %) and Varshney *et al*., 2008 (12.60 %). In general, being a highly self pollinated plant and its origin by single event hybridization followed by polyploidization, peanut exhibits limited polymorphism (Halward *et al.,* 1991; Young *et al.,* 1996). The parents used in developing the mapping population in the present study are only two cultivars and limited polymorphism could be due to narrow genetic base of the parents compared to the reports based on the wider germplasm used. Hence, it becomes imperative to select the diverse parents for developing the mapping population.

A total of 15 out of 53 markers (28.30 %) showed segregation distortion, which is comparable to Khedikar 2008 (29.85 %) but relatively less compared to Burrow *et al*., 2001 (68.00%) and Moretzohn *et al*., 2005 (51.00%). Higher distortion in the later studies is due to use of wild species and synthetic parents leading to sterility in those studies. Segregation distortion affects the estimation of map distances and the order of markers when many distorted markers are used for linkage map construction and hence affects the QTL analysis.

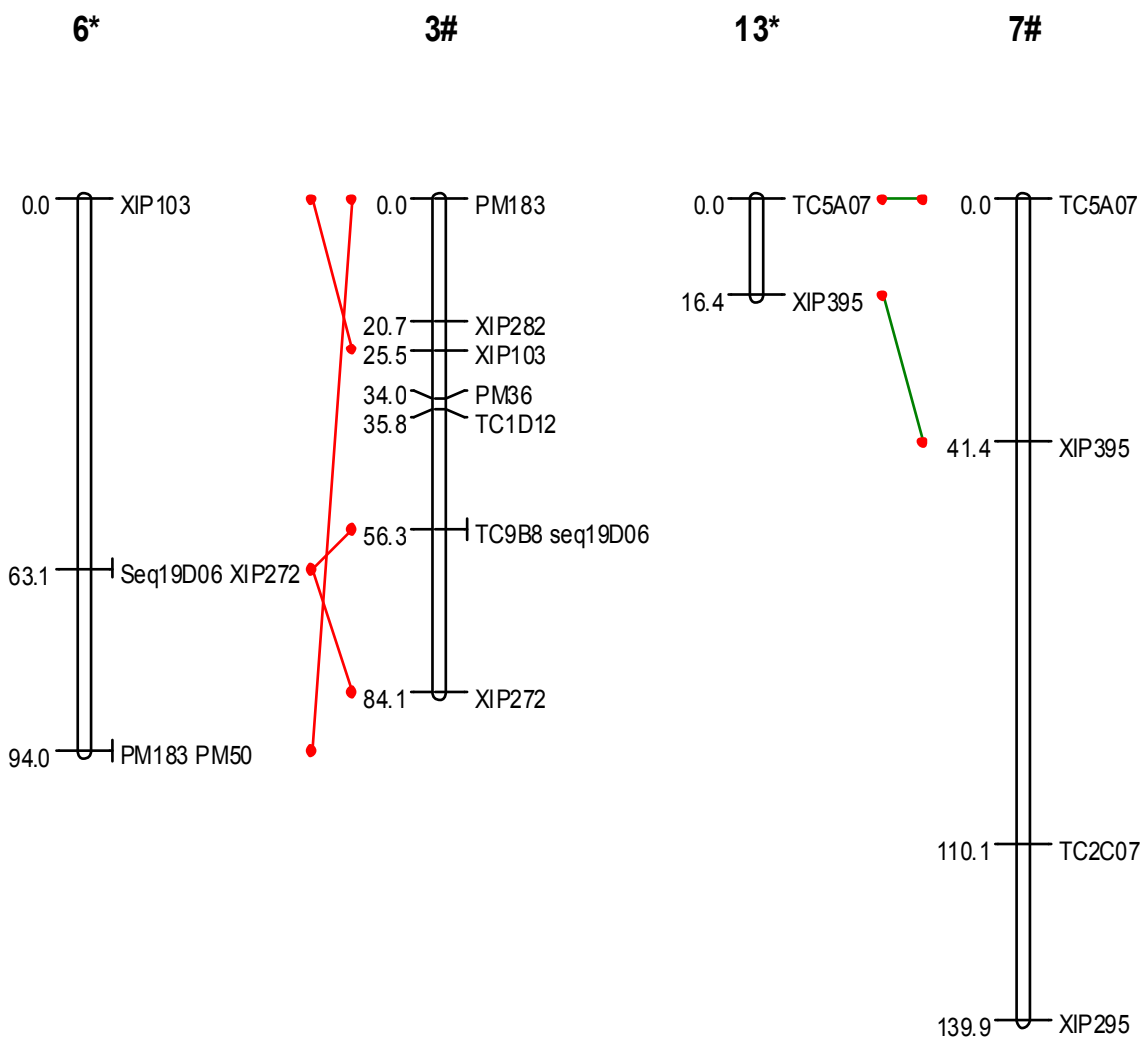
The linkage map was constructed using 53 polymorphic markers with the software MAPMAKER Version 3.0. Forty five markers could map on eight linkage groups spanning a total distance of 657.90 cM with an average marker distance of 14.62 cM and approximately 23 per cent of genome coverage and eight markers remained ungrouped (Table 17 and Fig 11).

The map coverage is much lower than Moretzsohn *et al*., 2005 (86.40%) and Gobbi *et al*., 2006 (52.97 %) but the diploid maps are of less significance to genetic improvement of cultivated groundnut. The linkage map obtained from the study is less dense than SSR map constructed by Khedikar, 2008 (909.40 cM; 13 LGs with 59 loci) and Varshney *et al*., 2008 (1270.5cM with 135 loci) and far less than RFLP map by Burrow *et al*., 2001 (2210 cM; 23LG) but it is far superior to AFLP map by Herselman *et al*., 2004 (139.4 cM; 5LGs). Although, large number of SSR markers are screened (1043) in the present study, but limited polymorphism (53) remained the biggest constraint in the construction of a dense linkage map. Since, the SSRs used were mostly of genomic origin, hence, use of genic/EST SSRs may yield better results (Varshney *et al*., 2005). Alternatively, large number of highly polymorphic markers like SNP (Single Nucleotide polymorphism) and DArTs (Diversity Array Technologies) could be utilized in the development of frame work map which could be later enriched with co-dominant SSRs (Paterson *et al*., 2004).

The linkage maps constructed in cultivated groundnut for TAG 24 x GPBD 4 (268 RILs) and TG 26 x GPBD 4 (146 RILs) were combined to designate the common markers on linkage groups using the criteria of existence of at least two common markers with the help of MAPCHART (Fig 18).

Four linkage groups, *viz*., LG3, LG7, LG5 and LG1 of present study were homologous to LG6, LG13, LG12 and LG2 of TAG 24 x GPBD 4 mapping population respectively. Although the numbers of linkage groups varied between the two populations, the order of the markers was almost same between the two populations. The difference in the number of linkage groups could be due to number of polymorphic markers used in linkage map construction and size of the population.

The linkage map was also compared with recently developed linkage map in cultivated groundnut obtained from TAG 24 x ICGV 86031 mapping population at ICRISAT using 135 SSR loci (Varshney *et al*., 2008). Two markers on LG 3 (PM183 and Seq19D06) in present study were homologous to the LG IV of the TAG 24 x ICGV 86031 population but the order was inversed. Since, the number of common markers between these populations is very less, incorporation of more number of markers on these maps would provide an opportunity to improve integration of maps and which ultimately gives valuable information about the QTL regions and further use in MAS.



* Linkage groups of TAG 24 x GPBD 4 mapping population

Linkage groups from TG 26 x GPBD 4 mapping population

Fig. 18: Combined linkage maps of TAG 24 x GPBD 4 and TG 26 x GPBD 4 mapping populations for common markers

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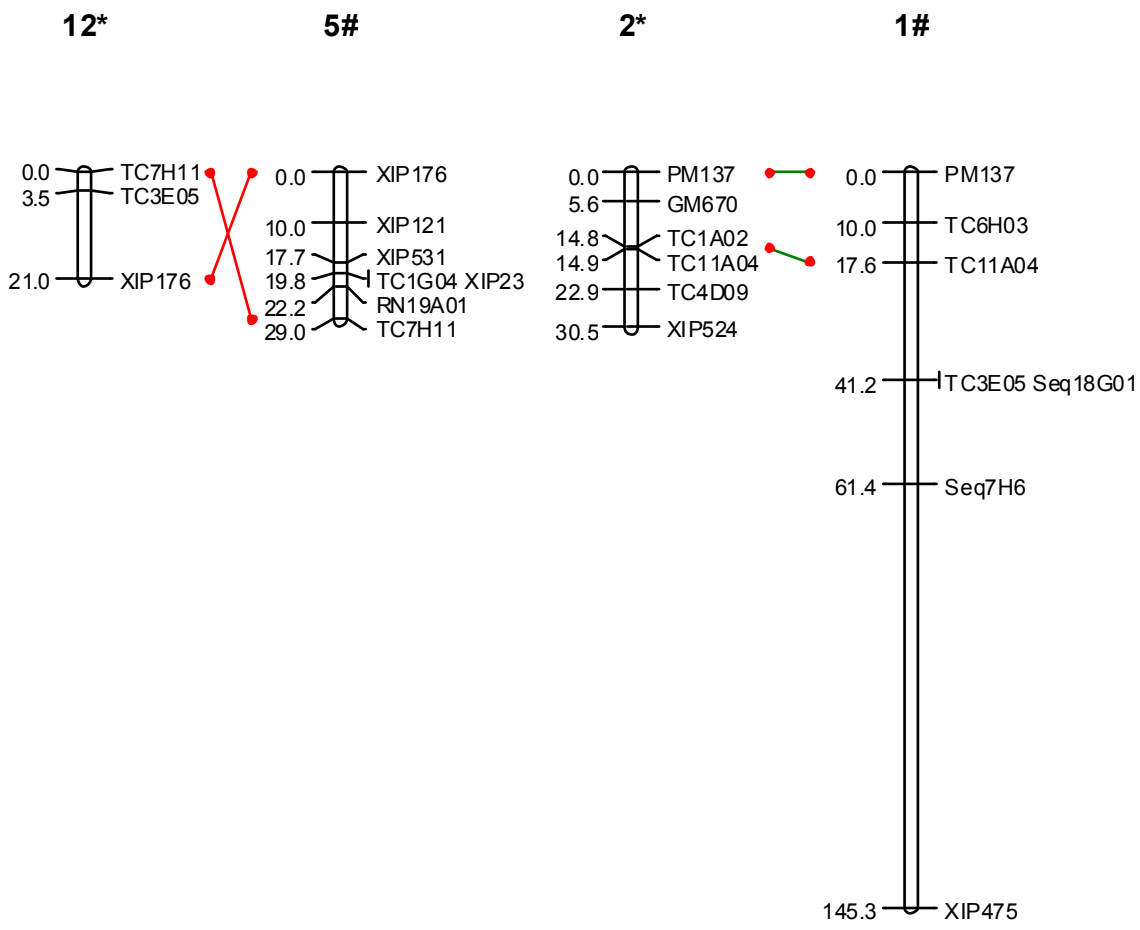


* Linkage groups of TAG 24 x GPBD 4 mapping population

Linkage groups from TG 26 x GPBD 4 mapping population

The inter marker distances of 45 markers from linkage map and the genotypic data of these 45 markers were used for QTL mapping for resistance to rust and LLS, nutritional quality and productivity traits. Before going for marker-trait association analysis, the phenotypic data for diseases, quality traits and productivity traits were subjected to data analysis *viz*., ANOVA, distribution of RILs, variability components (PCV, GCV, heritability and GAM) and association analysis.

5.2 Disease resistance to rust and late leaf spot

5.2.1 Phenotypic variation

Genetic studies on LLS and rust revealed that resistance is mostly controlled by recessive genes thus necessitating more generations and large population to identify resistant segregants (Nevill, 1982 and Kalekar *et al.,* 1984.). Further, when the diseases occur together they interfere with each other, and LLS dominates rust when both occur together, leading to difficulties in identification of resistant lines to these diseases (Subramanyam *et al*., 1984). Occurrence of these diseases is irregular most of the time. Transfer of resistance to these diseases from land races and wild relatives to cultivated background is difficult due to linkage drag *viz*., undesirable traits like thick shell, low yield, poor adaptability and long duration are associated with resistance (Singh *et al.,* 1997). Under these circumstances, newly emerging tools like marker assisted selection can play a crucial role in the success of disease resistance breeding.

Mapping population exhibited significant variation for rust and LLS as revealed by ANOVA. Significant seasonal and genotype x season interaction indicated the need for screening in multiple environments. Khedikar (2008) also found significant G x E interaction for these two diseases in a mapping population of 268 RILs obtained from the cross TAG 24 x GPBD 4.

The components of variation *viz*., PCV and GCV revealed substantial variation for both the diseases. Further, moderate to high heritability and GAM indicated highly heritable nature of the variation; thus, the population used for the study was found appropriate. The estimates of components of variation were very low in one of the seasons (E3) for LLS which could be due to predominance of rust in that season (Table 8). Usually both LLS and rust occur together but the incidence and severity vary

between localities and seasons (Subramanian *et al*., 1984) and relative occurrence of these two diseases can influence precision and assessment of diseases in the genotypes. When compared across stages, the components were low at later stage especially for LLS indicating suitability of first stage for better discrimination.

The disease scores between the stages in a season and between seasons at a particular stage were highly correlated revealing the consistency of disease reaction in the individual genotypes for both the diseases in spite of significant G x E interaction (Table 13 B and C). In contrast, correlation between rust and LLS was negative indicating differential prevalence of resistance in the RILs for the two diseases (Table 13A). However, Khedikar (2008) observed no association between the diseases in TAG 24 x GPBD 4 population indicating existence of material specific differences.

The pattern of distribution of RILs was mostly bimodal for rust and normal for LLS indicating possibility of simple inheritance with few genes for rust as compared to complex nature of inheritance for LLS. Wide distribution indicated good segregation for both the diseases. The distribution of RILs was within the range of parents for both LLS and rust indicating the possible contribution of resistance mostly by only one of the parents. Number of RILs approaching GPBD 4 for resistance was more for rust (31) as compared to LLS (4) again revealing the possibility of simple inheritance of rust in contrast to LLS. None of the RILs exhibited high level resistance to both the diseases which was also reflected by negative correlations between the traits.

5.2.2 Marker-trait association

In the present study, 53 SSR markers were used to identify putative markers associated with foliar diseases resistance. Single marker analysis was carried out using linear regression method (Haley and Knott, 1992). A total of 15 markers were associated with rust at different stages and their contribution to phenotypic variation ranged from 2.03 to 51.96 %. One of the markers (XIP103) was consistent across stages and seasons with substantial contribution (24.86 to 51.96 %) to variance. Only three other markers *viz*., Seq19D06 (5.07 to 6.37 %), PM183 (2.52 to 5.92 %) and RN16F05 (2.80 to 5.12 %) had significant (>5.00%) contribution to variance. In the earlier studies, Varma *et al*., (2005) identified 7 markers for rust resistance from two mapping populations (ICGV 99003 x TMV 2 and ICGV 99005 x TMV 2), Mace *et al*., (2006)

identified 14 SSR markers in 22 genotypes associated with LLS and rust and Khedikar (2008) identified 11 SSR markers in 268 RILs obtained from TAG 24 x GPBD 4. Among them, Seq17F06 (Varma *et al*., 2005 and Mace *et al*., 2006) and XIP 103 (Khedikar, 2008) were found common with the present study. XIP 103 with the phenotypic variance of 33.80 to 40.60 % identified in TAG 24 x GPBD 4 (Khedikar 2008) population also revealed substantial contribution to rust resistance and the marker has also been validated in different genetic backgrounds *viz.,* resistant mutants, interspecific derivatives and landraces (Gowda *et al*., 2008). Hence, this marker could be a good candidate for MAS for the development of rust resistant cultivars.

Considering the potentiality of XIP103 for MAS, a detailed analysis of RILs vis-à-vis banding pattern was undertaken. RILs were categorized in to A (Susceptible parent) and B (Resistant parent) patterns and when their mean disease scores were compared, higher disease was observed for A category compared to B at different stages in all the seasons and large difference was evident in E3 (Exp II) with maximum disease incidence (Table 28a and Fig 19). Further, to show the strength of association of this marker with rust resistance, top 20% (30 RILs) for resistance and susceptibility were assessed for the type of pattern they possessed (Table 28b). The results clearly showed a large majority of the resistant (B) and susceptible (A) RILs were observed for respective patterns (90.00 to 96.66 %) with very few false positives (A pattern with resistant scores) and false negatives (B pattern with susceptible scores) indicating very strong association of XIP103 at least in the extreme resistant and susceptible types. Hence, this marker could be efficiently employed in marker assisted breeding program especially when LLS dominates rust and even in the off-season for enriching the segregating population in early generations and if it is combined with phenotypic selection in advanced generations can improve the selection efficiency (Howes *et al*., 1998; Bonnet *et al*., 2005). MAS could also be practiced for rapid advancement of segregating material in off seasons and in seasons with low disease incidence in forward breeding programs (Holland, 2004). Since the XIP103 marker was of dominant type with presence of a band at 150bp only in the resistant types, standardizing the PCR protocols to screen in the agarose gel than in PAGE could be undertaken to make it more breeder friendly for screening large number of genotypes within a short time in laboratories with limited facilities.

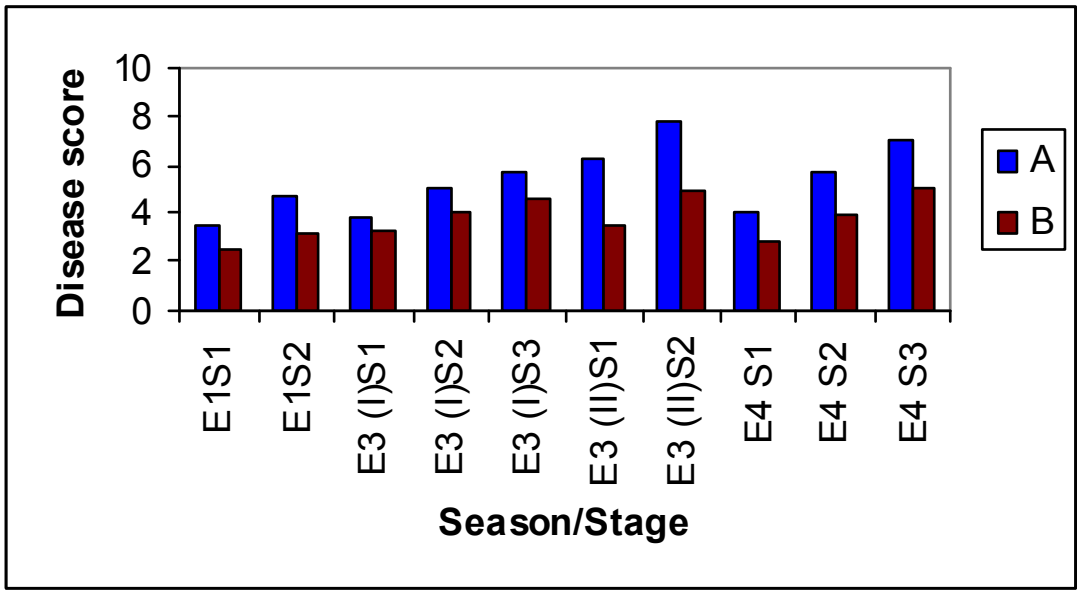
Table 28a: Comparison of disease scores in RILs with two patterns (A and B) for XIP 103 marker

RUST	TG 26 (A pattern)		GPBD 4 (B pattern)	
	Mean	Range	Mean	Range
Stage I				
Rainy 2005 (E1)	3.43	2.00-5.00	2.48	2.00-4.50
Rainy 2007 (E3 Experiment I)	3.85	3.00-5.00	3.29	2.50-4.50
Rainy 2007 (E3 Experiment II)	6.19	2.00-8.00	3.46	2.00-7.00
Post rainy 2007 (E4)	4.10	2.00-5.50	2.91	2.00-5.00
Stage II				
Rainy 2005 (E1)	4.77	2.50-7.50	3.27	2.00-7.00
Rainy 2007 (E3 Experiment I)	4.95	3.50-6.00	4.02	3.00-5.50
Rainy 2007 (E3 Experiment II)	7.79	3.50-9.00	4.91	3.00-9.00
Post rainy 2007 (E4)	5.71	2.50-7.00	3.90	2.50-6.50
Stage III				
Rainy 2007 (E3 Experiment I)	5.75	4.00-7.00	4.68	3.0-6.50
Post rainy 2007 (E4)	6.98	3.50-8.50	4.97	2.50-8.00

Table 28b: Comparison of patterns of XIP 103 in 20% of the population (30RILs) selected for resistant and susceptible types based on disease scores

20% of RILs with phenotypic extremes selected in both directions based on disease scores						
XIP103 Pattern	**Resistant (30 RILs)**			**Susceptible (30RILs)**		
Across seasons						
	Stage I	**Stage II**	**Stage III**	**Stage I**	**Stage II**	**Stage III**
A pattern	1 (3.3 %)	1 (3.3 %)	1 (3.3 %)	27 (90.0 %)	29 (96.6 %)	28 (93.3%)
B pattern	29(96.6 %)	29 (96.6 %)	29 (96.6 %)	3 (10.0%)	1 (3.3 %)	2 (6.6 %)
Best season (E3, Exp II)						
	Stage I	**Stage II**		**Stage I**	**Stage II**	
A pattern	1 (3.3 %)	2 (6.6 %)		27 (90.0 %)	28 (93.3%)	
B pattern	29(96.6 %)	28 (93.3%)		3 (10.0 %)	2 (6.6%)	

- A pattern-Susceptible
- B pattern-Resistant
- Values indicates the respective number of RILs identified out of 30RILs
- Values in bracket indicates the percentages



A-Resistant

B-Susceptible

E1-Rainy 2005, E3 (I)-Rainy 2007, Experiment I, E3 (II)-Rainy 2007, experiment II,

E4-Post rainy 2007

S1-Stage I, S2-Stage II, S3-Stage III

Fig. 19 : Comparison of mean disease scores in RILs with two patterns (A and B) for XIP 103 marker

A total of eleven markers were identified for late leaf spot with Seq15C12 (7.04 %) contributing maximum to the variance (7.04 %). Though, two markers (Seq3F05 and TC2B09) were common between the stages, none were common between seasons (Table 23). From the earlier reports, only Seq2D12B (Mace *et al*., 2006) TC2C07 (Khedikar, 2008) were found common with the present study.

Single marker analysis is the simplest tool and significance of phenotypic groups is based on regression models or ANOVA; it is preliminary and least informative and does not reveal the location and effects precisely. Hence, QTL mapping was undertaken based on the information on intermarker distance obtained in the linkage map with the help of PlabQTL software which used composite interval mapping (CIM).

One major QTL (XIP103-PM36) was identified for resistance to rust with substantial contribution (24.10 to 48.90 %) to phenotypic variance (Table 23). Very high LOD scores (10.75 to 22.28) were observed for this QTL with an additive effect ranged from 0.297 to 1.508 with its favorable allele coming from the resistant parent (GPBD 4). Hence, allelic contribution of GPBD 4 plays a major role in rust resistance. The position of the QTL was at 26cM on LG 3 which was very close to XIP103 (25.50cM). The other flanking marker of this QTL (PM36) was at 8.50cM away from XIP103 thus indicating a need for further fine mapping in this region with more markers for the ultimate purpose of map based cloning. Alternatively, marker assisted development of NILs could be undertaken for the identification of candidate genes though functional genomic approaches. Since, XIP103 alone contributed up to 50% of phenotypic variance both from single marker and QTL analysis, resistance could be assumed to be controlled by few genes with major effect. This was also supported by bimodal distribution and high frequency of resistant RILs identified in the population. Earlier reports indicated that rust resistance in groundnut is usually conferred by few recessive genes (Kalekar *et al*., 1984; Tiwari *et al*., 1984; Knauft, 1987; Paramasivam *et al*., 1990;). One major QTL (XIP103-Seq19D06) was identified for rust resistance in TAG 24 x GPBD 4 on LG 6 with the phenotypic variance ranging from 17.60 to 54.40 and additive affect of 0.179-2.270 and the favorable allele was contributed by GPBD 4. The position of QTL was very close to XIP103 and linked with Seq19D06 with the intermarker distance of 63.10 cM (Khedikar, 2008). Hence, the linkage group 3 of the

present study is an improved version of LG 6 of TAG 24 x GPBD 4 population as two more markers (PM36 and TC1D12) are incorporated between XIP 103 and Seq19D06. Hence, the intermarker distance has reduced to 8.50cM (XIP103-PM36) in the present study as compared to 63.10cM (XIP103-Seq19D06) in the other mapping population. Further, fine mapping could be possible in this region by saturation of map with more number of markers. (Fig18).

Apart from this QTL, four minor QTLs (PM183-XIP282, TC1D12-TC9B08 PM137-TC6H03 and TC2B09-RN16F05) were associated with rust resistance with phenotypic variance ranging from 1.70 to 5.40 % and they were season specific. Minor QTLs were earlier reported to be more season specific (Paterson *et al*., 1991; Lu *et al*., 1996). Among them three QTLs came from the susceptible parent, TG 26 and the left flanking marker of two QTLs (PM183 and TC1D12) were found associated with rust based on the single marker analysis. These minor QTLs need to be validated across different genetic backgrounds and locations and if confirmed, they could be used along with XIP103 for pyramiding rust resistance.

QTL mapping identified only one QTL (TC2B09-RN16F05) for late leaf spot resistance with the phenotypic variance of 6.40 % and the favorable allele was contributed by GPBD 4 (Table 23). Since no major QTL was identified for LLS in any of the seasons, suggesting a need for screening more polymorphic markers. Khedikar (2008) identified twelve QTLs for LLS and 15 QTLs for rust hence, the number of QTLs are comparatively more than the present study (one QTL for LLS and five QTLs for rust) which could be due to large size of the population (Beavis, 1994). Less number of markers used and the phenotypic difference between the parents was less in the present study compared to TAG 24 x GPBD 4.

5.3 Nutritional quality of groundnut

Although, groundnut is considered both as oil seed and food crop, it has received less attention by the breeders for improving the nutritional quality. Breeding for nutritional traits is costly, laborious and time consuming by conventional biochemical methods. Hence, it is beyond the capacity of the breeder to undertake large scale quality breeding program. Identification of molecular markers with significant phenotypic

variance to these quality traits would have great impact on the improvement of these traits using MAS.

The development of the cultivars in groundnut varies with the purpose for which they are put to use (Bandyopadhyay and Desai 2000). For edible oil purpose, cultivars having high oil with high O/L ratio are preferred; where as, the quality requirement for confectionary groundnut is more stringent and distinctly different from groundnut as an oil seed crop. This requires additional efforts to develop confectionary grade varieties with high protein and sugar, low oil, reduced aflatoxin risk and high O/L ratio (Nigam *et al*., 1989).

Since, the parents (TG 26 and GPBD 4) had significant difference for protein, oil content, oleic and linoleic acid, O/L ratio and other fatty acids, the mapping population was subjected to phenotyping for fourteen quality parameters *viz*., protein content (%), oil content (%), eight fatty acids and four derived parameters related to fatty acid composition *viz*., O/L ratio, iodine value, U/S ratio, and % S. For the first time an attempt has been made to identify QTLs associated with nutritional traits using SSR markers in peanut.

5.3.1 Phenotypic variation

Analysis of variance revealed significant variation among the RILs, seasons and RILs x Season interaction for all the fourteen quality traits. The distribution pattern for all the traits was normal in all the seasons. Transgressive segregants were observed in both the directions for all the traits indicating the contribution of favorable alleles from both the parents. Based on the mean values, GPBD 4 was higher value parent for protein, oil content, oleic acid, eicosenoic acid, lignoseric acid, O/L ratio and U/S ratio. Where as, TG 26 was a higher value parent for palmitic acid, stearic acid, arachidic acid, behenic acid, iodine value and % S. Hence, GPBD 4 is superior parent for all the important nutritional traits (Protein, oil, oleic and O/L ratio) compared to TG 26.

Genetic variability components revealed low to moderate magnitude of variation and genetic advance with very high heritability for protein but lower magnitude of variation with higher heritability and lower genetic advance for oil content. Hence, in spite of high heritability, there is better scope for selection for protein compared to oil content in this population.

Among the oil quality parameters, oleic and linoleic acids, O/L ratio, Iodine value, U/S ratio and %S exhibited low to moderate magnitude of variation with very high heritability. Hence, phenotypic selection alone would be effective for these traits but biochemical estimation of fatty acid composition is costly, cumbersome and time consuming hence, identification of efficient markers is useful for improving the traits through MAS.

Superior RILs for protein (7), oil content (7), oleic acid (14) and O/L ratio (10) more than GPBD 4 along with lower oil content (12), low linoleic acid (14), and low iodine value (11) than TG 26 and low palmitic acid (11) than GPBD 4 were identified based on the phenotypic data (Table 12B and C) as these are the important traits to be considered for developing superior quality groundnut. Among these, one RI line (95) was showing exceptionally high oleic acid (58.88 %), low linoleic acid (21.25 %), high O/L ratio (2.98), low Iodine value (88.19) and resistance to rust (2.13 to 3.50) thus combining several favorable traits and it could be used in future breeding program for developing varieties with improved nutritional quality. None of the RILs had a combination of high protein, high/low oil with high oleic acid (Table 12B &C).

Positive correlation between seasons for all the fourteen quality traits revealed the consistency among the RILs. The correlation between oil and protein was negative like in earlier studies (Kale *et al*., 1998; Parmer *et al*., 2002; Yashoda 2005 and Kavera 2008). Such a relationship could be advantageous in developing cultivars for confectionary purpose where low oil and high protein is preferred.

Before formulating suitable strategies to breed varieties for better quality, understanding the relationship among oil quality traits is of paramount importance. All the fatty acids are linked in the biosynthetic pathway through modifications such as elongation and desaturation. Hence, alteration in biosynthetic steps influences the whole fatty acid profile and determines the relationships among different fatty acids. These correlations may reflect precursor-product relations in some instances but probably also reflect genetic linkages of various enzymes involved in the conversions (Anderson *et al.,* 1998).

Among the fatty acids, oleic acid, a major fatty acid had a strong negative correlation with palmitic acid, linoleic acid, behenic acid, Iodine value and % S and it

had a strong positive correlation with O/L and U/S ratio. The inverse relationship for oleic acid with palmitic acid and linoleic acids was also evident from the earlier studies (Sekhon *et al*., 1980; Bovi *et al*., 1983; Anderson *et al*., 1998 and Kavera, 2008). The negative relationship between palmitic acid and oleic acid most likely represents an increased rate of palmitic acid elongation to stearic acid, with rapid desaturation to oleic acid via Δ-9 desaturase (Groff *et al*., 1996). The strong negative correlation between oleic and linoleic acids result from there being the chief acyl groups in the oil so that one cannot increase much without decrease in the other. Hence, increased oleic acid normally resulted in reduced palmitic acid, linoleic acid and iodine value which is desirable from the point of health and stability. Linoleic acid, a polyunsaturated fatty acid is unstable at higher temperature and has an inverse relationship with oil stability (Braddock *et al*., 1995; O'Keefe *et al*., 1993 and Holley and Hammons 1998). Stearic acid, a neutral fatty acid with respect to cardiovascular disease had significant positive association with arachidic and behenic acid (Hammond *et al*., 1997; Lukange *et al*., 2007 and Kavera, 2008) and negatively correlated with eicosenoic and lignoseric acids. Eicosenoic acid had a strong positive correlation with lignoseric acid and it also corborates with earlier reports *viz.,* Hammond *et al*., 1997 and Kavera 2008 (Table 14C).

The negative correlation for rust with oil and protein revealed the favorable association of them with rust resistance. Hence, selection for rust resistance may result in indirect improvement in protein and oil.

Among the oil quality parameters, negative correlation for eicosenoic and lignoseric acid indicated favorable association with resistance and positive correlation with arachidic and behenic acids revealed their association with susceptibility. Hence, rust is associated with only minor fatty acids and major fatty acids (Oleic and linoleic acid) are unaffected by disease reaction. Negative correlation of LLS with behenic acid (stage I) and protein (stage II) revealed their favorable association with LLS resistance (Table 16) which results in higher protein content.

5.3.2 Marker-trait association

Traits associated with seed quality are difficult and uneconomic to measure in large segregating generations. They are also substantially influenced by genotype x

environment interaction. Thus breeding progress in these traits by conventional techniques has had a limited success. Therefore, MAS is highly justified option for indirect selection of these traits in groundnut. To date, no effort has been made to identify the markers/QTLs associated with protein and oil content in groundnut. However, for high oleate, two recessive alleles ol_1 and ol_2 were identified in the Florida breeding lines in U.S.A and comparisons of encoding sequences from the high and low oleic acid genotypes revealed variation in several single nucleotide polymorphisms (Lopez *et al*., 2000).

Further, loss of function of oleoyl-PC desaturase activity is solely responsible for the high O/L trait in peanut (Ray et al., 1993) and this oleolyl-PC desaturase activity is governed by two homeologous genes, *ahFAD2A* and *ahFAD2B* (Jung *et al*., 2000). By designing the CAPS markers, mutant and wild-type *ahFAD2A* alleles were differentiated at the critical point of mutation and the mutant allele was frequent among subspecies *hypogaea* accessions but absent from subspecies *fastigiata* accessions and the putative diploid, A-genome progenitor of peanut, *Arachis duranensis* (Chu *et al*., 2007).

In the present investigation an attempt has been made to identify the microsatellite markers associated with fourteen quality traits.

Single maker analysis revealed ten markers associated with protein (2.17 to 9.78 %) and eleven markers with oil (2.18 to 6.98 %). The highest contribution was from TC6H03 (9.78 %) for protein and XIP103 (6.98 %) for oil content. Few markers *viz.,* TC2G05, TC2C07, and PM36 were found common between protein and oil contents. Further validation of these markers could be useful in MAS for improving oil and/ protein content.

QTL mapping identified seven QTLs (0.50 to 10.70%) for protein and four QTLs for oil (1.50 to 9.10%). Among them three QTLs *viz*., TC6H03-TC11A04 (10.70%), TC2E05-TC3E02 (10.20%) and Seq15C12-XIP105 (7.10%) had significant contribution to variance for protein. The favorable allele for first QTL was contributed by TG 26 and other two QTLs by GPBD 4. None of the QTLs were common between the seasons revealing seasonal sensitivity of QTLs and need for further validation of them in multiple seasons. Brummer *et al.,* (1997) identified QTL for seed protein and

oil content from eight distinct populations of soybean and among them some were sensitive to environments.

For oil content one common QTL (XIP103-PM36) was identified in two seasons with the phenotypic variance of 7.10 (E3) and 9.10 % (E4). XIP103 has been identified as a major QTL for rust resistance and the incidence of rust was very high in the two seasons (E3 and E4) indicating the impact of rust resistant QTL on oil accumulation. This was also supported by negative correlation between disease and oil content in those two seasons (Table 16).

One QTL (TC2E05-TC3E02) was common for oil and protein but the direction of favorable allele was different for oil (TG 26) and protein (GPBD 4) with the additive effect of 0.199 and 1.030, respectively. Such QTLs can lead to antagonistic relations between the traits as revealed by negative correlation between oil and protein in the present study. TC2B09-RN16F06 was also common between oil and protein but the favorable allele was contributed by TG 26 for both the traits with an additive effect of 0.280 and 0.552, respectively (Table 24).

Only minor QTLs could be identified for protein and oil which is supported by quantitative nature of inheritance of these traits as evident by the earlier reports (Tai and Young., 1975). However, few QTLs have a substantial contribution towards phenotypic variance, hence; these markers/QTLs identified can be validated in multiple seasons and in different genetic backgrounds to use them in MAS for high protein and high/low oil. Further saturation of the map may help in detecting major QTLs.

The quality of the oil depends on its fatty acid composition. Palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2) are the major fatty acids in groundnut that comprise >90 per cent of the total fatty acids (Anderson *et al*., 1998). The remaining fatty acids *viz.,* stearic (18:0), arachidic (20:0), eicosenoic (20:1), behenic (22:0) and lignoseric acid (24:0) account for remaining 10 per cent of total fatty acids. In the present study, 8 fatty acids and 4 computed derivatives of fatty acid profiles (O/L ratio, IV, U/S ratio and %S) were subjected for single marker and QTL analyses.

Among the fatty acids, it is the ratio of oleic to linoleic acid which matters a lot from the point of both oxidative stability (Holley and Hammons, 1968) and nutritional value (Lopez *et al*., 2000). Hence, importance must be given to these two fatty acids.

Seven markers (PM137, XIP176, XIP295, XIP395, TC6H03, Seq11G7 and TC5A7) were common between two major fatty acids (oleic, linoleic) and O/L ratio as revealed by SMA. Strong negative correlation (r=>0.90) existed between oleic and linoleic acids, all the four QTLs (XIP171c-XIP352, TC6H03-TC11A04, TC5A07-XIP395 and TC3A12-PM433) identified were common for these two major fatty acids. Among them, TC6H03-TC11A04 had a significant contribution to variance for oleic acid (9.70%), linoleic acid (9.0 %), O/L ratio (6.80%), U/S ratio (7.70%) and %S (5.60%). TC6H03, the left flanking marker of this QTL was associated with all the above traits as revealed by SMA. The favorable allele for this QTL was contributed by GPBD 4 for all the traits. Since this was detected in only one season, further validation is required before it is suggested for MAS application. The same QTL was also identified for protein with maximum contribution to variance (10.70%), but favorable allele came from TG 26 thus revealing, its major impact on nutritional quality. The genomic clone contributing the SSR (TC6H03) was found associated with "Ras related GTP binding protein" in Arabidopsis and with GTP-binding protein in Fabaceae (Bertioli, Personal comm.), thus it could be an important candidate gene associated with nutritional traits. The contribution of other two QTLs (TC5A07-XIP395 and TC3A12-PM433) also had significant contribution to oleic (5.60 and 7.20%) and linoleic acids (5.10 and 7.20 %) (Table 25).

Among the other fatty acids, the highest contribution was exhibited by XIP103 for stearic acid (8.90%), behenic acid (6.28 %), U/S ratio (4.06 %) and % S (3.87 %). XIP103 had significant contribution to oil and stearic acid (saturated fatty acid) and both the traits had strong inverse relationship. Reduced level of saturated fatty acid (palmitic and stearic) was also reported to be associated with significantly increased oil content (Mollers and Schierholt, 2002 in oilseed rape).

TC6H03 also contributed for eicosenoic acid (6.89 %), behenic acid (2.06-2.89 %), lignoseric acid (4.27%), iodine value (3.31 %), U/S ratio (2.52 %) and %S (2.30 %). XIP176 and PM137 were common for palmitic acid, oleic acid, linoleic acid, behenic acid, O/L ratio and iodine value. Further validation of these markers in different genetic backgrounds could give valuable information about the influence of these markers on the fatty acid profiles (Table 20).

QTL analysis identified a total of two QTLs for palmitic acid (4.60 to 6.70 %), one QTL for stearic acid (10.30 %), two QTLs for behenic acid (3.60 to 7.70 %), four QTLs for iodine value (2.60 to 7.5%), three QTLs for U/S ratio (3.20 to 7.70%) and one QTL for %S (5.60%). Most of the QTLs identified are in accordance with the single marker analysis; hence, the identified markers have some biological significance for the respective traits (Table 25). QTL flanked by XIP103-PM36 showed significant phenotypic variance for stearic acid (10.30 %) and behenic acid (7.70%). Hence, the rust resistant QTL also influenced these saturated fatty acids and the favorable allele was contributed by lower value parent (GPBD 4) indicating significant reduction in these saturated fatty acids by transferring this QTL and which is beneficial from the nutritional point of view.

5.4 Agronomic and productivity traits

The identification of QTL affecting important agronomic traits is a key step in the use of molecular markers for plant improvement and in understanding the genetic mechanisms that determine these traits. Hence, an attempt was made to identify QTLs associated with agronomic and productivity *viz*., plant height, number of branches, number of pods per plant, pod yield per plant, shelling % and 100-seed weight as the mapping population selected for the study segregated for all the above traits.

5.4.1 Phenotypic variation

Analysis of variance revealed significant variation among the RILs, seasons and also significant G x E interaction for all the traits suggesting the need to screen in multiple seasons/locations (Table 7).

The magnitude of variation as revealed by PCV was very high for all the traits except for shelling %. 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 values. As compared to season wise estimates, pooled analysis resulted in lower heritable variation thus revealing the predominance of G x E interaction. Hence, improvement through phenotypic selection will be more complex and markers are expected to improve the efficiency of selection. Frequency distribution for all the productivity and agronomic traits was mostly normal indicating quantitative

inheritance. Transgressive segregants were observed in both the directions for all the traits revealing the contribution of favorable alleles from both the parents. Several RILs superior to the best parent were identified for different traits (Table 12C). Most of the transgressive segregants for pod yield were also superior for pod number. One dwarf (76) and three tall (119, 87 and 125) transgressive segregants were also highly productive. The superior RILs could be exploited in future breeding programs.

Correlation coefficients measure the mutual relationship between various characters, which help in devising efficient strategies for indirect selection using component character and simultaneous selection of multiple traits. Pod yield had significant positive correlation with plant height, number of pods and 100-seed weight. Besides, plant height was also positively correlated with number of pods and 100-seed weight thus indicating importance of these traits for enhancing the productivity in the population (Table 15). Based on the earlier reports, pod yield possessed significant positive association with kernel yield, number of pods per plant, test weight and oil yield at both genotypic and phenotypic levels (Sah *et al.,* 2000, Laksmidevamma *et al*., 2004; Upadhyaya and Nigam, 1998).

5.4.2 Marker-trait association

Single marker and QTL analysis revealed a total of eleven markers (2.11 to 14.72 %) and four QTLs (4.10 to 17.50%) for plant height, eleven markers (2.00 to 5.96 %) and one QTL (2.10 %) for number of branches, six markers (2.26 to 5.23 %) and five QTLs (0.10 to 8.30 %) for number of pods, eleven markers (2.14 to 11.25 %) and three QTLs (5.60 to 11.20 %) for pod yield, eight markers (2.23 to 5.87 %) and four QTLs for shelling % and six markers (2.21 to 12.58 %) and two QTLs (1.70 to 14.10 %) for 100-seed weight. were identified.

Based on contribution to phenotypic variance, some prominent markers/QTLs were identified for the above traits. The marker TC3A12 contributed substantially in both SMA (3.78 to 14.72 %) and QTL analysis (12.60 to 17.50%) for plant height across the seasons. The same QTL was also associated with number of branches (2.10 %) and number of pods (5.23 to 5.32% in SMA and 8.3% for QTL), which is also supported by the positive correlation among these traits. The favorable allele came from GPBD 4.

Another marker PM137 (3.80-10.65 %) and QTL flanked by PM137-TC6H03 (11.20 %) also had substantial contribution to plant height and the favorable allele was contributed by GPBD 4 (Table 26). As plant height is an important trait and which has a direct association with pod yield; hence, these two markers/QTLs are of interest to the breeders in MAS for selection for pod as well as fodder yield. In TAG 24 x GPBD 4 population PM137 was associated with plant height, but with minor contribution (Khedikar, 2008).

Contribution of XIP103 was substantial for pod yield (6.40 to 11.25 %), shelling % (2.25 to 6.20 %) and number of pods (4.50 %) as revealed by SMA. A strong positive correlation between pod yield and number of pods (r=>0.825) identified three common QTLs for these traits; among them a stable QTL flanked by XIP103-PM36 had a significant contribution to variance for number of pods (4.41-6.10%) and pod yield (6.60 to 11.20 %). The favorable allele for this QTL was contributed by higher value parent (GPBD 4) and the position of the QTL for both the traits is also same (26cM on LG3) indicating both the traits probably are controlled by single gene due to its pleiotropic effect. Other two QTLs (TC2E05-TC3E02 and XIP395-TC2C07) also contributed significantly to number of pods (8.30 and 6.10%) and pod yield (8.90 and 5.60%). The favorable allele came from TG 26 for both the traits (1.308 and 1.149 respectively) (Table 27).

PM183-XIP282 (1.90 to 7.10%) contributed highest for shelling % in two seasons and the favorable allele came from TG 26. For 100-seed weight, TC1B02/TC1B02-XIP105 contributed substantially and also consistent across the seasons as revealed by both SMA (3.45 to 12.58 %) and QTL analysis (8.20 to 15.30 %). GPBD 4 contributed its favorable allele for higher 100-seed weight. This marker also contributed significantly to pod yield (6.33 %) as revealed by SMA and has been validated in the TAG 24 x GPBD 4 population for pod yield. Hence, this marker could be efficiently used in MAS for improved seed size and pod yield and it can also be used in developing cultivars for confectionary purpose where higher seed size is one of the criteria for selection.

Although, no major QTLs with very high phenotypic variance (>20.0%) were identified for productivity traits, but the markers such as TC3A12, PM137 and TC2B09 (plant height), XIP103 (number of pods and pod yield), TC1B02 (100-seed weight) and

QTLs flanked by respective markers will have a potential for use in MAS for enhancing the productivity, but needs further validation over seasons/locations and in different genetic backgrounds. The identification of QTL influencing the agronomically important traits in the present study should pave a way towards understanding the genetic basis of relationships among these traits in groundnut.

Based on the earlier report (Khedikar, 2008), few markers *viz.*, XIP 103 (number of pods and pod yield), XIP395 (number of pods), TC2G05, TC6H03 and TC1B02 (pod yield), PM137 (plant height), TC6H03 (shelling %) have been already validated and were common for the respective traits between TAG 24 x GPBD 4 and TG 26 x GPBD 4 mapping population. Hence, the above markers are more potential and extremely important for improving productivity traits.

5.5 Prominent markers/QTLs associated with various traits

Studies on identification of markers/QTLs associated with multiple desired traits will have an implication on overall crop improvement based on MAS and in the present study all the prominent markers were examined for this purpose (Table 29). A marker XIP103 and a QTL flanked by XIP103-PM36 located on LG3 was most prominent QTL for resistance to rust and also had a pleiotropic effect on eight quality and three productivity traits. The additive effect explained by this QTL for all the traits was contributed by GPBD 4, an interspecific variety developed by introgression of resistance from wild *spp*. (*A. cardenasii*). The favorable affects of QTL on productivity and nutritional quality reveals usefulness of wild species for traits other than resistance and it deserves further analysis for greater exploitation and the tools of genome research may unleash the genetic potential of wild species for crop improvement for the benefit of society (Tanskley and McCouch 1997)

TC3A12 marker and a QTL flanked by TC3A12-PM433 had substantial contribution to plant height as revealed by SMA (3.78 to 14.72 %) and QTL (12.60 to 17.50%). It also had and influence on late leaf spot, six quality parameters and five productivity traits, which could be due to pleiotropic or tight linkage. The favorable allele for this QTL came from GPBD 4 for all traits. Hence, this QTL is important especially for developing cultivars for fodder purpose.

The marker TC1B02 and QTL flanked by this marker TC1B02-XIP105 is extremely useful for improving 100-seed weight as revealed by both SMA (3.45-12.58 %) and QTL analyses (8.20 to 15.30 %). The favorable allele came from GPBD 4. Besides 100-seed weight, it was also associated with protein and pod yield.

Two markers *viz*., TC6H03 and PM137 and the respective QTLs *viz*., TC6H03-TC11A04 and PM137-TC6H03 both located on LG1 were particularly associated with several quality traits. Other than quality, marker PM137 had a significant contribution for plant height (3.80-10.65 %) based on SMA. Both the QTLs were associated with oleic, linoleic acids and O/L ratio with significant contribution. The marker TC6H03 and QTL flanked by this marker *i.e.,* TC6H03-TC11A04 showed substantial phenotypic variance for protein content both in the SMA (9.78 %) and QTL analysis (10.70%) and the favorable allele was contributed by TG 26 parent. For the other traits, the favorable allele for both the QTLs (TC6H03-TC11A04 and PM137-TC6H03) was contributed by GPBD 4.

A marker, TC3E02 and its QTL (TC2E05-TC3E02) has contributed significantly to protein content as revealed by both SMA (5.87 %) and QTL analysis (10.20 %) and the favorable allele was contributed by GPBD 4. It also contributed for rust resistance, oil, number of pods per plant and pod yield per plant and but the favorable allele for these traits came from TG26.

Hence, several genomic regions affected multiple traits which were generally in agreement with correlations among traits, suggesting the phenomenon of pleitropy or tight linkage. Further, detailed genetic studies are required to determine whether pleitropy or tight linkage is the genetic cause of association of multiple QTL and which will could be achieved by fine mapping of target genomic regions or by association mapping.

The markers and QTLs identified in the present study are extremely important for integrating one or more desired traits in the superior cultivar. But before using them in the breeding program, they need to be validated out side the original population. However, few markers have already been detected in the other mapping population (TAG 24 x GPBD 4) and found significant for disease resistance (XIP103) and other agronomic and productivity traits (XIP103, TC1B02, TC6H03, and PM137). Hence,

based on the results obtained from the present study, the following future line of work can be proposed.

Future line of work

- Already validated candidate marker (XIP103) could be used for development of resistant cultivars through MAS and the QTL region can be fine mapped for use in map based cloning or subjected to functional genomic approach to identify candidate genes
- Further validation of the prominent candidate markers/QTLs for various traits in different populations, across locations and/seasons is required
- Saturate linkage map with more markers so that the extensive phenotypic data could be efficiently used for further QTL detection
- Identified superior RILs for various traits can be used in the future crop improvement program
- The genomic regions contributed by wild species *A. cardenasii* could be characterized by using already available genomic resources from wild species and improve the potential of marker assisted introgression of useful alleles

Table 29: Prominent marker/QTLs identified for various traits in TG 26 x GPBD 4 mapping population

Markers/QTLs	Traits associated	Phenotypic variance		Additive effect	Favorable parent
		SMA	QTL		
XIP103/XIP103-PM36	Rust resistance	24.86-51.96	24.10-51.80	0.297-1.508	GPBD 4
	Oil (%)	5.72-6.98	7.90-9.1 0	0.408-0.499	GPBD 4
	Stearic acid	8.90	10.30	0.25	GPBD 4
	Arachidic acid	2.23	-	-	-
	Behenic acid	-	7.70	0.077	GPBD 4
	Eicosenoic acid	4.54	-	-	-
	Lignoseric acid	4.40-6.28	-	-	-
	U/S ratio	4.06	-	-	-
	% S	3.87	-	-	-
	No. of pods/plant	4.50	4.41-6.10	1.134-1.236	GPBD 4
	Pod yield/plant (g)	6.40-11.25	6.60-11.20	1.177-1.296	GPBD 4
	Shelling %	2.25-6.20	-	-	-
TC3A12/TC3A12-PM433	Late leaf spot	5.05	-	-	-
	Oil (%)	2.44-2.87	-	-	-
	Palmitic acid	2.66	6.70	0.256	GPBD 4
	Oleic acid	-	3.30-7.20	0.905-1.885	GPBD 4
	Linoleic acid	-	3.30	0.759-1.641	GPBD 4
	O/L ratio	-	1.50		GPBD 4
	Iodine value	-	7.50	1.31	GPBD 4
	Plant height (cm)	3.78-14.72	12.60-17.50	1.606-2.389	GPBD 4
	No. of branches	-	2.10	0.155	GPBD 4
	No, of pods/plant	5.23-5.32	8.30	2.217	GPBD 4
	Pod yield/plant (g)	4.09	-	-	-
	100-seed weight (g)	2.49	-	-	-
TC1B02/TC1B02-XIP105	Protein (%)	8.05	-	-	-
	Pod yield/plant (g)	6.33	-	-	-
	100-seed weight (g)	3.45-12.58	8.20-15.30	1.926-2.701	GPBD 4

Contd…

Markers/QTLs	Traits associated	Phenotypic variance			Favorable parent
		SMA	QTL	Additive effect	
TC6H03/TC6H03-TC11A04	Protein (%)	9.78	10.70	1.249	TG 26
	Oleic acid	3.42	9.70	2.749	GPBD 4
	Linoleic acid	3.50	9.00	2.280	GPBD 4
	O/L ratio	3.22	6.80	0.192	GPBD 4
	Eicosenoic acid	6.89	-	-	-
	Behenic acid	2.06-2.89	-	-	-
	Lignoseric acid	4.20	-	-	-
	Iodine value	3.31	-	-	-
	U/S ratio	2.50	7.70	0.124	GPBD 4
	%S	2.30	5.60	0.374	GPBD 4
PM137/PM137-TC6H03	Palmitic acid	3.06	4.60-5.10	0.169-0.185	GPBD 4
	Oleic acid	2.24-3.46	6.10	1.423	GPBD 4
	Linoleic acid	3.81	6.80	1.271	GPBD 4
	Eicosenoic acid	-	7.80	0.028	GPBD 4
	Behenic acid	2.39	-	-	-
	O/L ratio	2.89	5.10	0.110	GPBD 4
	Iodine value	4.18	8.10	1.015	GPBD 4
	Plant height (cm)	3.80-10.65	-	-	-
TC3E02/TC2E05-TC3E02	Rust	2.53-3.22	-	-	-
	Protein (%)	5.87	10.20	1.030	GPBD 4
	Oil (%)	-	1.50	0.239	TG 26
	No. of pods/plant	2.47-3.28	8.30	1.308	TG 26
	Pod yield/plant (g)	3.13-3.20	8.90	1.149	TG 26

*Not found, SMA-Single markers analysis, QTL-Quantitative traits loci

6. SUMMARY AND CONCLUSIONS

A mapping population consisting of 146 Recombinant Inbred Lines obtained from the cross TG 26 x GPBD 4 (F9 generation) was used in order to identify microsatellite markers/QTLs associated with foliar disease resistance, nutritional quality and productivity traits.

Phenotyping for various traits *viz*., rust (three stages), late leaf spot (LLS) (two stages), protein and oil content, eight fatty acids (Palmitic, stearic, oleic, linoleic, arachidic, eicosenoic, behenic and lignoseric fatty acids), four derived traits for fatty acids (O/L ratio, Iodine value, U/S ratio and %S), two agronomic (Plant height and number of branches) and four productivity traits (no. of pods, pod yield, shelling % and 100-seed weight) were carried out in five different seasons (*Rainy* 2005, 2006, 2007 and Post-rainy 2007 and 2008) at U.A.S., Dharwad. Parental screening (1043 SSR primers) and genotyping of 53 polymorphic markers for the mapping population was carried out at ICRISAT, Patanacheru, Andhra Pradesh. Phenotypic and genotypic data were subjected statistical analyses for establishing marker-trait association.

Analysis of variance showed significant variation among the genotypes, environments and G X E interaction for all the traits suggesting the need to screen in multi-seasons and/or multi-locations.

Phenotypic data analysis for genetic variability components revealed higher magnitude of variation with high heritability for diseases, moderate to high variability with very high heritability for nutritional quality and higher magnitude of variation but lower heritable variation for productivity traits.

Distribution of RILs was bimodal for rust and normal for LLS but within the range of parents, indicating simple inheritance for rust but complex for LLS with favorable alleles mostly contributed by the resistant parent, GPBD 4. The distribution for nutritional quality, agronomic and productivity traits was mostly normal revealing complex inheritance. Transgressive segregants in both the directions indicated the contribution of favorable alleles from both the parents.

Correlation studies revealed significant positive association between stages and seasons for both the diseases and also for nutritional quality indicating consistency

across seasons. Correlation between rust and LLS was negative revealing antagonistic nature of the diseases. Among the nutritional traits, negative correlation existed between oil and protein, oleic and linoleic acid, palmitic and oleic acid, O/L ratio and iodine value, O/L ratio and linoleic acid, O/L ratio and % saturation. Among the agronomic and productivity traits, positive correlation was observed for pod yield with plant height, number of pods, 100-seed weight and shelling per cent.

Among the parents GPBD 4 was a higher value parent for disease resistance, protein, oil, oleic, eicosenoic acid, lignoseric acid, O/L ratio and U/S ratio and all the agronomic and productivity traits and TG 26 was a higher value parent for palmitic acid, stearic acid, linoleic acid, arachidic acid, behenic acid, iodine value and per cent saturation. Several RILs superior to best parent were identified for different traits which could be utilized in future breeding program.

A partial linkage map was developed using 53 polymorphic markers using MAPMAKER version 3.0. Forty five markers mapped on eight linkage groups with the total span of 657.9 cM and an average intermarker distance of 14.62 cM with only 23 percent of genome coverage indicating a need for further saturation of the map.

Single marker analysis based on linear regression, identified a total of 15 markers (2.03 to 51.96 %) for rust and eleven markers for LLS (2.43 to 7.06 %). One marker (XIP 103) had substantial contribution to variance for rust (24.86 to 51.96 %) and consistently present in all the stages and seasons. This marker has been already validated in TAG 24 x GPBD 4 and other rust resistant germplasm and could be exploited in MAS. Few markers were common in more than one season but the contribution was less compared to XIP103. All the eleven markers identified for LLS, were found to be season specific with Seq15C12 (7.04 %) providing the highest contribution to phenotypic variance.

Among the nutritional traits, a total of sixteen markers associated with protein and oil content with the contribution to variance ranging from 2.17 to 9.78 per cent. The highest contribution was recorded by TC6H03 (9.78 %) followed by TC1B02 (8.05 %) for protein and XIP103 (5.72 to 6.98 %) followed by TC2C07 (6.43%). A total of 26 markers found associated for twelve oil quality traits with the phenotypic variance ranging from 2.06 to 8.90%. Very few markers had significant contribution (>5.0%)

viz., XIP103 for stearic (8.90%) and behenic acid (6.28%), TC3E05 for linoleic acid (5.20%), and O/L ratio (5.09%), Seq19D06 for arachidic acid (5.14%) and TC6H03 for eicosenoic acid (6.89%). Further validation of these markers will provide an opportunity to develop superior quality cultivars through MAS.

A total of 25 markers were associated with agronomic traits (plant height and number of branches) with the phenotypic variance ranging from 2.11 to 14.72%. Among them, TC3A12 (3.78 to 14.72 %), TC2B09 (4.17 to 12.32%) and PM137 (3.80 to 10.65%) for plant height and TC2G05 (5.96%) for number of branches showed significant contribution. For productivity traits, a total of eleven markers were identified with the contribution to variance ranging from 2.08 to 12.52%. Some markers contributed substantially for various traits *viz*., XIP103 (number of pods, pod yield and shelling %), TC1B02 (100-seed weight and pod yield), TC3A12 (number of pods) and XIP475, XIP75 and Seq15C12 (shelling %). These markers need to be validated in different genetic backgrounds for utilizing them in MAS.

QTL mapping using composite interval mapping (CIM) identified one major QTL (XIP103-PM36) for rust resistance which was consistent across the stages and seasons with the phenotypic variance ranging from 24.86 to 48.90 % and contribution of favorable allele came from resistant parent, GPBD 4. Very high LOD score was observed in all the seasons (8.76 to 22.28). This QTL was located at position 26cM and tightly linked to XIP 103 (25.5cM) on LG3. An inter marker distance was 8.7cM between XIP103 and PM36 suggesting a need for further saturation of the map for the purpose of cloning. Further, four minor QTLs for rust and a single minor QTL (TC2B09-RN16F06) for LLS were identified indicating the need for further saturation of map.

With respect to nutritional traits, two QTLs *viz*., TC6H03-TC11A04 (10.70 %) and TC2E05-TC3E02 (10.20%) for protein and one stable QTL, XIP103-PM36 (7.90-9.10%) for oil content contributed substantially. TC6H03-TC11A04 was common for oleic acid (9.70 %), linoleic acid (9.00 %), O/L ratio (6.80 %), U/S ratio (7.7 0%) and %S (5.6 %) with significant contribution to variance. Another QTL (TC3A12-PM433) had substantial contribution towards palmitic acid (6.70%), oleic acid (7.20%), linoleic acid (7.20%) and Iodine value (7.50%). One QTL (XIP103-PM36) contributed substantially to stearic acid (10.30%) and behenic acid (7.70%). All the above QTLs

identified are contributed by GPBD 4 except a QTL for protein content *i.e.* TC6H03-TC11A04 which was contributed by TG 26.

As for agronomic and productivity traits are concerned, four QTLs *viz*., TC3A12-PM433 (12.60 to 17.50 %) and PM137-TC6H03 (11.20%) for plant height, XIP103-PM36 for number of pods (4.41 to 6.10 %) and pod yield per plant (6.60 to 11.20%) and TC1B02-XIP105 for 100-seed weight (8.20 to 15.30 %) contributed significantly and were stable across the seasons. Eleven minor QTLs were also identified for agronomic and productivity traits.

Few prominent markers (XIP103, TC3A12, PM137, TC6H03 and TC1B02) and QTLs flanked by the respective markers contributed significantly for rust resistance, quality and productivity traits and several genomic regions/QTLs affect multiple traits. Hence, further validation of these markers outside the original population would provide a scope for use in marker assisted breeding. Among them, few markers (XIP103, TC6H03 and PM137) have already been validated out side the population and deserve serious consideration for use in MAS for crop improvement.

GPBD 4, a first interspecific variety for foliar disease resistance has contributed favorable alleles at many of the prominent QTLs for rust resistance, high protein, high oil, higher oleic acid, O/L ratio, plant height, number of pods and pod yield. Hence, a detailed analysis of genomic regions contributed by the wild species (*A. cardenasii*) will enhance their introgression into new groundnut varieties.

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Appendix I : Polymorphic SSR markers in TG26 x GPBD 4 mapping population

S. No.	Primers	Source	Ta	Base Pair		Band quality
				P1	P2	
1	XIP171c	Cuc *et al*., 2008	59	140	145	3
2	XIP475	-----"-----	59	300	310	3
3	XIP407c	-----"-----	56	150	145	4
4	XIP75	-----"-----	59	240	245	3
5	XIP352	-----"-----	59	190	200	3
6	XIP105	-----"-----	59	295	298	4
7	XIP282	-----"-----	59	180	175	3
8	XIP295	-----"-----	56	190	280	1
9	XIP108*	-----"-----	59	181	190	3
10	XIP395	-----"-----	59	210	200	3
11	XIP272	-----"-----	59	175	170	4
12	XIP23	-----"-----	59	150	155	3
13	XIP531*	-----"-----	59	305	300	3
14	XIP121	-----"-----	59	150	160	4
15	XIP103	-----"-----	59	140	150	1
16	XIP176*	-----"-----	59	180	190	4
17	TC3E05*	Moretzsohn *et al*., 2005	59	345	350	3
18	TC11A04	-----"-----	59	210	205	3
19	TC0A01	-----"-----	59	310	310	3
20	TC1D12	-----"-----	59	210	208	3
21	TC1B04	-----"-----	59	260	250	3
22	TC2E05	-----"-----	59	215	210	3
23	TC9B8*	-----"-----	59	120	110	1
24	TC3E2	-----"-----	59	160	155	3
25	TC6H03	-----"-----	59	220	215	2
26	TC1G04	-----"-----	59	280	280	3
27	TC7H11	-----"-----	59	290	300	3
28	TC1B02	-----"-----	56	300	290	4
29	TC5A07	-----"-----	59	140	145	3
30	TC3A12	-----"-----	59	190	185	3

Contd….

S. No.	Primers	Source	Ta	Base Pair		Band quality
				P1	P2	
31	TC4H07	Moretzsohn *et al.*, 2005	59	200	205	3
32	TC4F10	-----"-----	59	230	235	3
33	TC2B09*	-----"-----	59	200	205	3
34	TC6E01	-----"-----	59	170	172	3
35	TC2G05	-----"-----	59	305	300	3
36	TC2C07	-----"-----	59	160	170	3
37	PM137	He *et al.*, 2003	59	150	155	3
38	PM36	-----"-----	59	220	225	3
39	PM183	-----"-----	59	130	120	3
40	PM433	-----"-----	59	110	120	2
41	Seq7H6	Ferguson *et al.*,2001	59	315	325	3
42	Seq11G7	-----"-----	59	490	500	3
43	Seq18G01	-----"-----	59	280	290	1
44	Seq17F06	-----"-----	59	130	125	3
45	seq19D06	-----"-----	56	270	260	3
46	Seq3F05	-----"-----	59	290	280	3
47	Seq2D12B	-----"-----	59	320	330	3
48	Seq15C12*	-----"-----	59	300	310	3
49	Seq19D9*	-----"-----	59	154	179	3
50	Seq4E8	-----"-----	56	310	315	4
51	RN19A01	Bertioli *et al.* (Unpublished)	59	320	322	1
52	RN16F05	-----"-----	59	500	510	3
53	AC3D07	-----"-----	59	220	218	3

* Labeled primers
Ta-Annealing Temperature
1-Single and strong band
2-Single and weak band
3-Multiple and strong band
4-Multiple and weak band