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# Validation of SSR markers linked to the rust and late leaf spot diseases resistance in diverse peanut genotypes

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**Abstract**

The low productivity of peanut (*Arachis hypogaea* L.) in India and several African countries is ascribed to many biotic stresses of which, two foliar fungal-diseases namely, late leaf spot (LLS) and rust are widespread and economically most important. Partial saturation of peanut linkage map and use of different mapping populations have led to the identification of various SSR markers linked to these diseases along with their linkage groups. In the present investigation, 22 SSR markers linked to rust and LLS diseases resistance were tested on 95 diverse genotypes for marker validation, of which 16 SSRs could be validated. Among rust resistant varieties and germplasm lines, nearly perfect marker validation was recorded but for 30 wild *Arachis* species, marker validation was very poor. Maximum numbers of rust and LLS linked-markers were found to be present on the linkage group 03 (eight SSRs) followed by linkage group 04 (three SSRs) which is an important step towards identification of more closely linked markers to the rust and LLS resistance QTLs and its cloning in future. Cluster analysis also grouped these foliar fungal disease resistant and susceptible genotypes separately except for those resistant genotypes where marker amplification was very poor. The results of principal coordinate analysis (PCoA) were comparable to the cluster analysis. This study will help in selection of suitable parents and marker combination for marker assisted breeding for foliar disease resistance in peanut.

**Keywords**: Groundnut; *Puccinia arachidis*; *Phaeoisariopsis personata*; SSR validation; foliar fungal-diseases.
**Abbreviations**: BC_Backcross; DGR_Directorate of groundnut research; LG_Linkage groups; LLS_Late leaf spot; MAS_Marker assisted selection; PCoA_Principal coordinate analysis; QTLs_Quantitative trait loci; RILs_Recombinant inbred lines; SNP_Single nucleotide polymorphism; SSR_Simple sequence repeat; UPGMA_Unweighted pair group method with arithmetic means.

## Introduction

Peanut (*Arachis hypogaea* L.) also known as groundnut is cultivated in the semi-arid tropical and sub-tropical regions of the world between 40° N and 40° S, mainly in developing countries of Africa and Asia, (Shoba et al., 2012). It is an important oilseed, food and feed crop grown on about 20.88 million ha throughout the world (FAOSTAT, 2012). In India, it occupied an area of 4.20 million ha with a production of 6.9 million tons in 2011, which accounted for a productivity of 1655 kg $ha^{-1}$ (FAOSTAT, 2012). Peanut yield is constrained due to foliar fungal-diseases in most areas of the world. Rust (caused by *Puccinia arachidis* Speg.) and late leaf spot (caused by *Phaeoisariopsis personata* Ber. and M A Curtis) are economically very important foliar fungal-diseases of cultivated peanut and together they can reduce the yield by more than 50% (Waliyar, 1991) along with an adverse effect on seed and fodder quality. The regular incidence of these diseases warrants the development of resistant cultivars by which we can control not only these diseases but also improve the production and quality besides reducing the adverse effects of chemicals on our ecosystem (Shoba et al., 2012). Genetics of rust resistance revealed recessive digenic inheritance (Vindhiyavarman et al., 1993) and dominant single gene resistance (Singh et al., 1984) whereas LLS resistance genetics recorded its complex and polygenic nature (Dwivedi et al., 2002) and recently, a combination of both nuclear and maternal gene effect (Pasupuleti et al., 2013). Complex nature of these disease resistances makes the identification of resistant and susceptible lines cumbersome through conventional screening techniques (Leal-Bertioli et al., 2009). Generally, abundant DNA polymorphism in wild *Arachis* species has been observed whereas little variation has been reported in cultivated peanut (Herselman, 2003; He and Prakash, 2001). Recently, many DNA markers were found to be putatively linked with rust (Varma et al., 2005; Mace et al., 2006; Khedikar et al., 2010; Sujay et al., 2012) and LLS resistance gene(s) (Mace et al., 2006; Shoba et al., 2012; Sujay et al., 2012). Validation of these markers will speed up the process of introgression of rust and LLS resistance gene(s) into preferred peanut genotypes through its planned deployment in molecular breeding programme (Sujay et al., 2012). In the present investigation, we have attempted to validate the linkage of 22 SSR markers for rust and LLS disease complex as reported by different workers in a group of diverse resistant and susceptible genotypes so as to find the answers to the following questions:

a) What is the polymorphism status for SSR marker(s) (linked to the rust and LLS resistance genes) in our selected lines?

b) How effectively these SSRs can be used for its deployment in molecular breeding programme for imparting resistance to rust and LLS diseases in peanut?

c) Which linkage group(s) is/are most important for the identification of more closely linked marker(s) for these foliar fungal-disease resistance gene(s) in peanut?

**Result and Discussion**

A few years back MAS for foliar diseases in peanut was not expected because very little information on marker linkage was available (Varma et al., 2005; Mace et al., 2006) and a good saturated map was also lacking. Recently, different reports on linked markers with these diseases are available (Khedikar et al., 2010; Shoba et al., 2012; Mondal and Bhdigannavar, 2010; Shirasawa et al., 2013) which along with the previously reported markers (Varma et al., 2005; Mace et al., 2006) were used in the present investigation for its validation (Table 1) since efficient utilization of these markers, in any resistance breeding programme necessitates their validation in other genetic backgrounds (Mondal et al., 2007). The results of marker validation are presented and discussed below.

***Marker validation for rust resistant and susceptible varieties***

Thirty-one resistant and 03 rust susceptible peanut varieties and germplasm lines were used for marker validation. Among the resistant genotypes, except one (i.e. PI 393531), all other amplified at least one SSR marker. Non-amplification of markers could be because of the absence of marker locus in the genotype used in the study (Mondal et al., 2007) or it could be due to the occurrence of crossing-over between the marker and the resistance gene (Mondal et al., 2012). It could also be explained by the presence of other genetic factors conditioning rust resistance in this genotype, as found in common bean (Young and Kelly, 1997). Marker validation details of all the rust resistance and susceptible varieties are presented in the Fig. 1. Three varieties *viz*. GBPD4, ICGV86590 and R2001-03 amplified 12, 11 and 11 SSRs, respectively, revealed the suitability of these varieties as foliar fungal-disease resistant parents in the marker assisted breeding programme. Number of markers amplified ranged from 1-12 in different genotypes which mean approximately 97% of the rust-resistant varieties tested have confirmed the linkage of at least one marker. In our group of genotypes, only 16 markers could be validated (Fig. 1-4) while 6 markers did not produce any specific amplification pattern associated with resistant or susceptible genotypes (Fig. S1). Therefore, these 16 identified SSRs should be the first choice of any marker-aided backcross program for the improvement of rust and LLS resistance, in peanut. Non-specific amplification which was recorded for the 6 SSRs could be due to the allele specificity to the parental genotypes in which they are identified or they are not very tightly linked to the gene of interest. These markers may not be of good choice for future MAS in the lines selected for the present investigation as also reported by Mondal et al. (2007) for the repulsion phase linked RAPD marker J7.

***Marker validation for rust and LLS resistant and susceptible genotypes***

Five LLS resistant, 03 rust+LLS resistant and 09 rust+LLS susceptible peanut varieties and germplasm lines were analyzed for SSR validation. In LLS resistant lines, maximum 03 SSRs were validated in PI476164 of which 01 is for LLS and other 02 are for rust resistance. In the genotype EC76446 single validation (PM50) specific to the rust and no LLS specific validation was observed. However, for 03 rust+LLS resistant genotypes, 05 SSRs could be validated and genotype PI393641 recorded maximum of 03 SSRs validation. Non-validation of other known linked markers in these resistant lines could be due to the presence of some new resistant gene(s) or there is absence of tested linked SSR polymorphism in these genotypes (Mondal et al., 2007, 2012). Marker validation details of all the resistant and susceptible varieties are presented in the Fig. 2. Among 09 rust and LLS susceptible varieties and germplasm lines tested, in a variety (i.e. TAG24) and a two germplasm lines (i.e. CS19 and Chico) all the known SSRs could be validated whereas, in other 06 varieties except one other SSR marker could be validated. The amplification of disease resistance specific marker(s) in susceptible varieties could be due to the presence of same locus for respective markers in these lines. This information can be used while selecting the resistant and susceptible parents for future crossing plan for MAS (Ma et al., 2011).

***Marker validation for rust and/or LLS resistant wild Arachis species***

Thirty wild *Arachis* species (07, 08 and 15 - resistant to LLS, rust and rust+LLS respectively) were selected for marker validation as they represented different peanut genomes i.e. 'A' and 'B'-genome and are quite frequently used in the crossing programme for the development of inter-specific hybrids for further varietal development. High level of rust resistance from *Arachis cardenasii* (Pande and Rao, 2001) was transferred to *A. hypogaea* which has resulted in a resistant breeding line VG 9514 (Varman, 1999). A rust resistant variety, GBPD4 is an inter-specific derivative, and is parent of many mapping population which are used for the identification of linked markers (Gowda et al., 2002; Khedikar et al., 2010; Sujay et al., 2012). Extensive variation for morphological and physiological traits has been observed in both wild and cultivated species of peanut (Varman, 1999). However, in the present investigation only 14 of the 30 wild-species validated at least one marker and, a maximum of 03 markers each were validated in 03 species (*A. appressipila, A. hagenbeckii* and *A. pintoi*). In addition, out of the 22 markers tested, only 10 SSRs gave the desired amplification in any of the wild-species tested. The non-validation of markers in the wild-species indicated that SSR motifs which are present in the cultivated genotypes are probably not so common in wild-species (Mondal et al., 2007, 2012). However a more elaborate marker survey is required to reveal the specific reasons of non-amplification of SSR markers in wild species. Marker validation details of all the wild-species used in this study is presented in the Fig. 3.

***Parent marker validation in advanced breeding lines and $F_6$ progenies of promising crosses***

Nine promising advanced breeding lines and 05 crosses (derived from resistant and susceptible parents, in $F_6$ generation) were studied for finding the efficiency of trait-marker selection under conventional breeding approaches. Marker validation details of these lines are presented in the Fig. 4. When these genotypes were compared with their parents, it is observed that maximum 08 SSRs in PBS22101 and minimum 03 SSRs in PBS22096 advanced breeding line have shown expected amplification. Although all these lines

**Table 1.** SSR markers linked to the rust and LLS resistance used for validation.

| S. No. | Primers | Linkage Group | Cross/ genotypes | Reference | Remarks |
|---|---|---|---|---|---|
| 1 | seq3A01$_{238*}$ | a07 | ICGV 99003 x TMV 2 | Varma et al., 2005 | Rust resistance |
| 2 | seq5D05$_{274}$ | b07and a07 | TMV 2 x COG 0437 ($F_2$) and ICGV 99005 x TMV 2 | Shoba et al., 2012; Varma et al., 2005; Shirasawa et al., 2013 | Rust and LLS resistance |
| 3 | seq16F01$_{271}$ | b03 | ICGV 99005 x TMV 2 | Varma et al., 2005 | Rust resistance |
| 4 | seq17F06$_{152}$ | b04 | ICGV 99005 x TMV 2 and 22 genotypes | Varma et al., 2005; Mace et al., 2006; Shirasawa et al., 2013 | Rust and LLS resistance |
| 5 | seq13A07$_{265}$ | b01 | ICGV 99005 x TMV 2 and 22 genotypes | Varma et al., 2005; Mace et al., 2006; Shirasawa et al., 2013 | Rust and LLS resistance |
| 6 | seq2F05$_{280}$ | b02 | 22 genotypes | Mace et al., 2006; Shirasawa et al., 2013 | Rust and LLS resistance |
| 7 | seq8E12$_{200}$ | a01 | 22 genotypes | Mace et al., 2006 | Rust resistance |
| 8 | seq16C06$_{263}$ | b03 | 22 genotypes | Mace et al., 2006 | Rust resistance |
| 9 | seq13A10$_{250}$ | b04 | 22 genotypes | Mace et al., 2006 | Rust resistance |
| 10 | seq2B10$_{290}$ | b03 | 22 genotypes | Mace et al., 2006 | LLS resistance |
| 11 | IPAHM103$_{160}$ | a03 and b03 | TAG 24 xGPBD 4 | Khedikar et al., 2010 | Rust resistance |
| 12 | PM384$_{100}$ | - | TMV 2 x COG 0437 ($F_2$) | Shoba et al., 2012 | LLS resistance |
| 13 | PM137$_{150}$ | b06 | TMV 2 x COG 0437 ($F_2$) | Shoba et al., 2012; Shirasawa et al., 2013 | LLS resistance |
| 14 | PM03$_{168}$ | a03 and b03 | TMV 2 x COG 0437 ($F_2$) | Shoba et al., 2012; Shirasawa et al., 2013 | LLS resistance |
| 15 | PMc588$_{183}$ | - | TMV 2 x COG 0437 ($F_2$) | Shoba et al., 2012 | LLS resistance |
| 16 | PM375$_{102}$ | a04 | TMV 2 x COG 0437 ($F_2$) | Shoba et al., 2012 | LLS resistance |
| 17 | seq8D09$_{190}$ | b10 and a09 | TAG 24 x GPBD 4 | Sujay et al., 2012; Shirasawa et al., 2013 | LLS resistance |
| 18 | GM1536$_{410}$ | b03 | TG 26 xGPBD 4 | Sujay et al., 2012 | Rust resistance |
| 19 | GM2301$_{137}$ | b03 | TG 26 xGPBD 4 | Sujay et al., 2012 | Rust resistance |
| 20 | GM2079$_{418}$ | b03 | TG 26 xGPBD 4 | Sujay et al., 2012 | Rust resistance |
| 21 | PM50$_{110}$ | b05 | 20 genotypes | Mondal and Badigannavar, 2010 | Rust resistance |
| 22 | PM35$_{124}$ | a06 and b04 | 20 genotypes | Mondal and Badigannavar, 2010; Shirasawa et al., 2013 | Rust and LLS resistance |

*Subscript numerical values are for linked band size

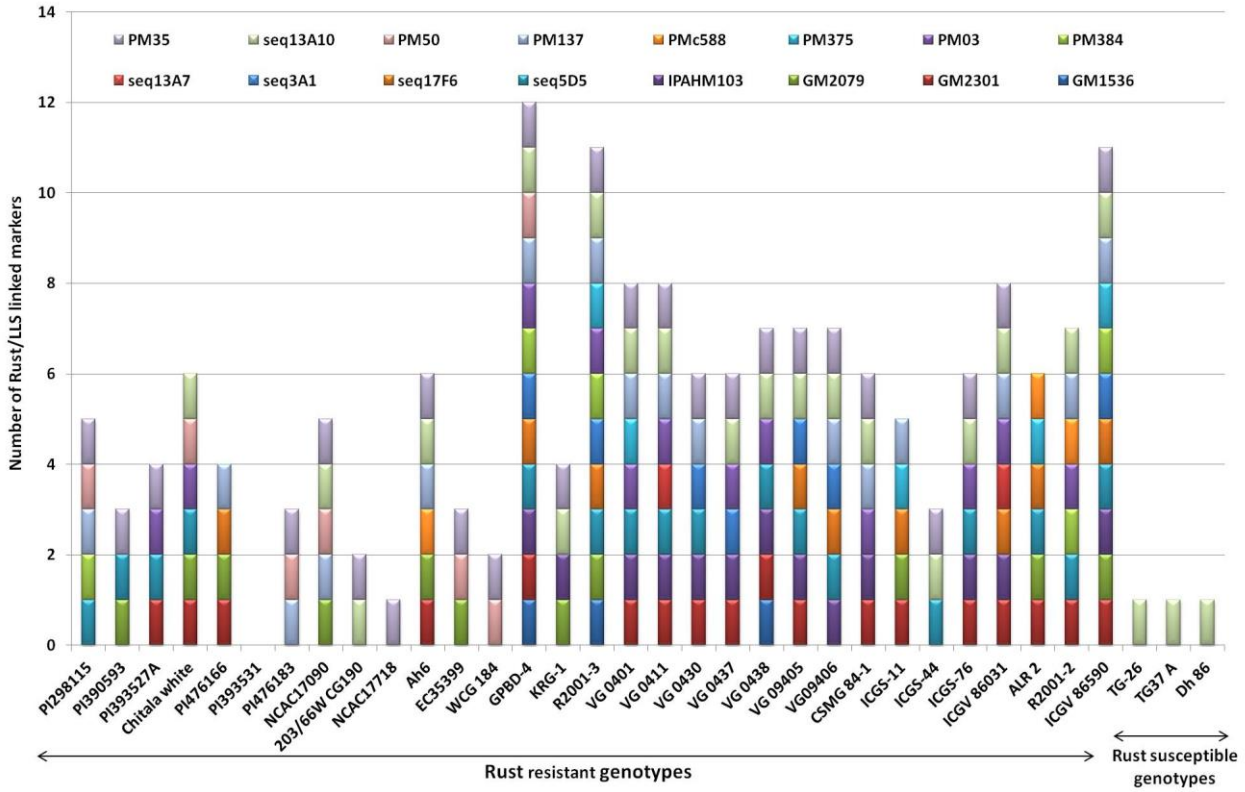


**Fig 1.** SSR marker validation in known rust resistant and susceptible genotypes.

has shown resistant reaction to the foliar disease at DGR, Junagadh but it needs further fine scoring at different locations so as to ascertain their exact disease score. In all the crosses 03-08 markers were missing though were present in the parents used for the crossing programme. This shows the importance of use of molecular markers in marker assisted breeding (Fig. 4). Our results have indicated that, in future during the development of advanced breeding lines and advancement of promising crosses in peanut, MAS should be used to increase the chance of getting more number of QTLs in one background which otherwise is not easily possible through conventional breeding approach.

***Foliar disease linked marker frequency among all the genotypes***

When linked SSR marker frequency was calculated across all the 95 genotypes studied, it was observed that maximum 43 genotypes could be validated by the SSR marker PM35 followed by seq13A10, GM2301, and seq5D5 where- 39, 30 and 26 genotypes, respectively, could be validated. However, least validation was observed with the seq13A7, where only two genotypes (i.e. VG0411 and ICGV 86031) gave the desired amplification. SSR validation details are presented in Fig. 5. Although selection and use of foliar diseases linked marker(s) is based on parents used in any breeding programme but, from Fig. 5 it is evident that markers such as PM35, seq13A10, GM2301 and seq5D5 can be of great use whereas, seq13A7, GM1536 and PM384 may not be of significant assistance across a range of genotypes while practicing the MAS.

***Marker linkage-group association***

Genome mapping and trait mapping is still in its infancy in peanut because SSR-based genetic linkage map, using recombinant inbred line (RIL) mapping population of cultivated peanut has been developed only recently (Varshney et al., 2009) and a few trait mapping have been reported (Herselman et al., 2004; Varshney et al., 2009). In terms of mapping resistance to LLS and rust, so far only a few studies have been conducted in cultivated peanut, based on a partial genetic map (Khedikar et al., 2010; Sujay et al., 2012; Mondal et al., 2012; Shirasawa et al., 2013). In order to ascertain the location of molecular markers on the linkage groups (LG), we used most comprehensive consensus genetic maps published till date (Gautami et al., 2012; Shirasawa et al., 2013) and of 22 SSRs, except two (i.e. PM384 and PMc588) LGs of all SSRs could be ascertained (Table 1). It is found that 04, 13 and 03 SSRs were present on 'A', 'B' and both 'A' and 'B' genomes, respectively (Gautami et al., 2012). While compiling the LG, we found more than one LG for two SSRs i.e. seq8D09 is found to be present on LG_b10 (Sujay et al., 2012) and LG_a09 (Shirasawa et al., 2013) while PM35 on LG_a06 (Mondal and Badigannavar, 2010) and LG_b04 (Shirasawa et al., 2013). For two markers i.e. IPAHM 103 and PM 3, Gautami et al. (2012) found that these were located on both LG_a03 and LG_b03 whereas seq5D05 was present on both LG_a07 and LG_b07. This discrepancy in the location of different SSRs on more than one LG again reiterate the fact that even integrated maps available till date are not yet fully saturated. When all the SSRs were compared together, maximum number of loci were found to be present on LG_03 (eight SSRs) followed by LG_04 (three SSRs), which could be due to the presence of a few major QTLs for foliar fungal-disease resistance on these LGs. It is therefore recommended to use the markers present on these two LGs for parental polymorphism survey and linkage analysis while doing the mapping studies. Thus, in future, towards identification of more closely linked markers to the rust and LLS resistance QTLs and its cloning LG_03 would be the first choice. Since linked SSR markers are distributed on

**Table 2.** List of germplasm and varieties used which differ for their reaction to Rust and LLS diseases.

| S. No | Genotypes | Type | S. No | Genotypes | Type |
|---|---|---|---|---|---|
| LLS resistant | | | Rust resistant | | |
| | PI 476164 | Germplasm | 28. | VG 09406 | Cultivar |
| | PI 476195 | Germplasm | 29. | CSMG 84-1 | Cultivar |
| | EC 76446 | Germplasm | 30. | ICGS 11 | Cultivar* |
| | PI 341879 | Germplasm | 31. | ICGS 44 | Cultivar* |
| | PI 215696 | Germplasm | 32. | ICGS 76 | Cultivar |
| Rust resistant | | | 33. | ICGV 86031 | Cultivar |
| | PI 298115 | Germplasm | 34. | ALR 2 | Cultivar |
| | PI 390593 | Germplasm | 35. | R 2001-2 | Cultivar |
| | PI 393527A | Germplasm | 36. | ICGV 86590 | Cultivar |
| | Chitala White | Cultivar | Rust and LLS resistant | | |
| | PI 476166 | Germplasm | 37. | PI 393641 | Germplasm |
| | PI 393531 | Germplasm | 38. | PI 468363 | Germplasm |
| | PI 476183 | Germplasm | 39. | PI 259747 | Germplasm |
| | NCAc17090 | Germplasm | Rust Susceptible | | |
| | 203/66W CG190 | Germplasm | 40. | TG 26 | Cultivar |
| | NCAC 17718 | Germplasm | 41. | TG 37A | Cultivar |
| | Ah 6 | Cultivar | 42. | Dh 86 | Cultivar |
| | EC 35399 | Germplasm | Rust and LLS Susceptible | | |
| | WCG 184 | Germplasm | 43. | GG 2 | Cultivar |
| | GPBD 4 | Cultivar | 44. | JL 24 | Cultivar |
| | KRG 1 | Cultivar | 45. | GG 20 | Cultivar |
| | R2001 3 | Cultivar | 46. | CS 19 | Cultivar* |
| | VG 0401 | Cultivar | 47. | GG 11 | Cultivar |
| | VG 0411 | Cultivar | 48. | TMV 2 | Cultivar |
| | VG 0430 | Cultivar | 49. | SG 99 | Cultivar |
| | VG 0437 | Cultivar | 50. | TAG 24 | Cultivar |
| | VG 0438 | Cultivar | 51. | Chico | Germplasm |
| | VG 09405 | Cultivar | | | |

*There are different reports regarding their reaction to rust (medium to low resistance).

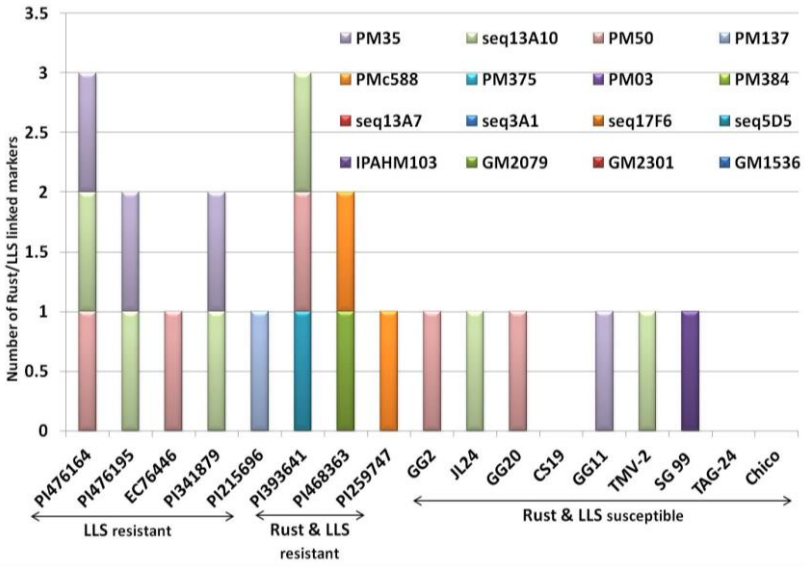


**Fig 2.** SSR marker validation in LLS, rust+LLS resistant and susceptible genotypes.

different LGs, it implies that there are many different QTLs imparting resistance against foliar fungal-diseases complex in different genotypic background. It is expected that increasing the number of resistance imparting QTLs using pyramiding approach would significantly improve the resistance level of any breeding line. Therefore, pyramiding or combination of resistance QTLs to virulent pathotypes should be considered to impart durable resistance in breeding programme (Hua et al., 2009). Thus, LGs identified can provide an opportunity to develop a marker-assisted recurrent selection approach to pyramid different locus (Kannan et al., 2014) controlling rust and LLS resistance during the development of improved peanut populations. There is need of further in-depth studies on the effect of pyramiding of different QTLs in various genotypic backgrounds.

***Linkage between rust and LLS resistance gene(s)?***

In past, elite cultivars and varieties resistant to LLS and/or rust have been developed world-wide through conventional breeding, but co-occurrence of these two diseases and the defoliating nature of LLS poses serious challenges to the breeding community in phenotypic selection (Varshney et al., 2005; Leal-Bertioli et al., 2009). Moreover, out of 22 SSRs,

**Table 3.** Wild *Arachis* species with their section, genome and foliar disease reaction.

| S. No. | Wild species | Section | Genome | S. No. | Wild species | Section | Genome |
|---|---|---|---|---|---|---|---|
| | LLS resistant | | | | Rust and LLS resistant | | |
| 1. | *A. appressipila* | *Procumbentes* | P | 16. | *A. cardenasii* | *Arachis* | A |
| 2. | *A. correntina* | *Arachis* | A | 17. | *A. stenosperma* | *Arachis* | A |
| 3. | *A. pusilla* | *Heteranthae* | H | 18. | *A. cryptopotamica* | *Erectoides* | E |
| 4. | *A. villosa* | *Arachis* | A | 19. | *A. marginata* | *Extranervosae* | Ex |
| 5. | *A. glabrata* | *Rhizomatosae* | RR | 20. | *A. pintoi* | *Caulorrhizae* | C |
| 6. | *A. hagenbeckii* | *Rhizomatosae* | R | 21. | *A. kempff-mercadoi* | *Arachis* | A |
| 7. | *A. paraguariensis* | *Erectoides* | E | 22. | *A. sylvestris* | *Heteranthae* | H |
| | Rust resistant | | | 23. | *A. matiensis* | *Procumbentes* | P |
| 8. | *A. duranensis* | *Arachis* | A | 24. | *A. valida* | *Arachis* | B |
| 9. | *A. stenophylla* | *Erectoides* | E | 25. | *A. prostrata* | *Extranervosae* | Ex |
| 10. | *A. kretschmeri* | *Procumbentes* | E | 26. | *A. magna* | *Arachis* | B |
| 11. | *A. monticola* | *Arachis* | AB | 27. | *A. oteroi* | *Erectoides* | E |
| 12. | *A. hermannii* | *Erectoides* | E | 28 | *A. cruziana* | *Arachis* | B |
| 13. | *A. diogoi* | *Arachis* | A | 29. | *A. dardani* | *Heteranthae* | H |
| 14. | *A. benensis* | *Arachis* | A | 30. | *A. triseminata* | *Triseminata* | T |
| 15. | *A. rigonii* | *Procumbentes* | E | | | | |

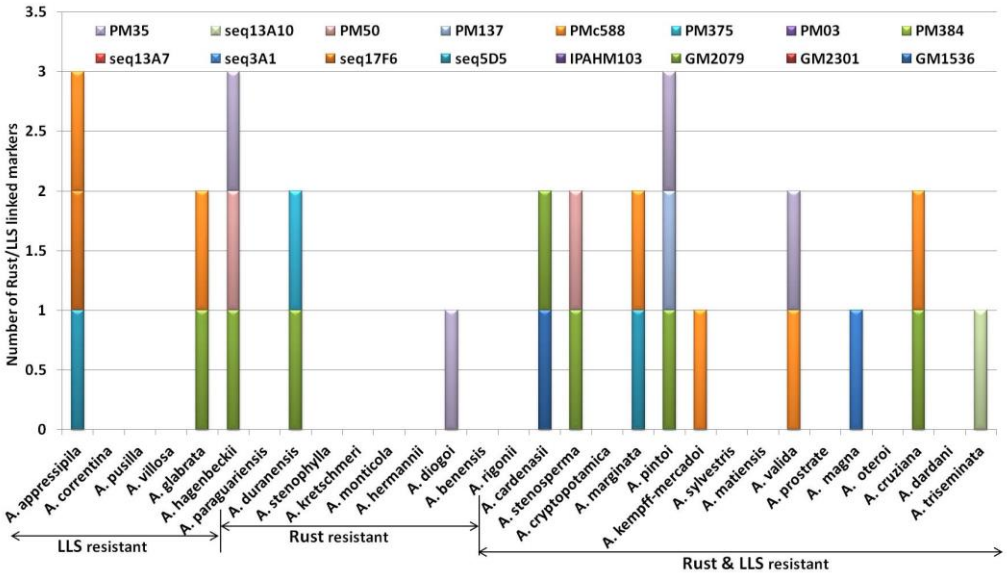


**Fig 3.** SSR marker validation in the related wild *Arachis* species.

five *viz*. seq5D05, seq17F06, seq13A07, seq2F05 and PM35 were found to be linked with both rust and LLS diseases (Table 1). It indicates the possibility of some sort of linkage between these two foliar diseases, or it could also be due to the co-occurrence of these diseases as complex which needs further conclusive confirmation.

***Cluster analysis***

Cluster analysis was performed using amplification data of 16 SSRs, which could be validated and all the 95 genotypes were broadly grouped into 6 major clusters. Cluster I comprised of susceptible genotypes and wild species whereas, cluster II consisted of most resistant genotypes. Cluster III composed of mostly susceptible genotypes and those resistant genotypes where very few amplicons were recorded. Cluster IV was constituted of mostly resistant genotypes and their crosses including $F_6$ progenies. Cluster V and VI consisted of mostly wild-species (Fig. 6). Overall clustering pattern showed that the resistant and susceptible genotypes do form separate clusters except for those resistant lines where no or very little amplicons were recorded. In case of wild-species, we did not find any clear clustering pattern; this could be due to the fact that very poor validation was observed across these wild-genotypes. The results of PCoA were comparable to the cluster analysis (Fig. 7) where, resistant genotypes grouped together on the one side whereas susceptible lines and wild-genotypes (with very poor marker validation) fell on distant grouping. However, resistant genotypes such as R2001-3, ICGV86590 and GPBD4 clearly fell apart in which maximum number of linked marker amplification was recorded.

**Materials and Methods**

***Plant materials***

The seeds of 95 genotypes of cultivated varieties, wild relatives, advanced breeding lines, and promising crosses ($F_6$ progenies) differing in disease resistance to the foliar diseases were used in the present study (Table 2-5). These genotypes were already screened by previous workers for their rust and LLS scores (Varma, 2002; Rajgopal et al., 2002; Bera et al., 2004; Mondal and Badigannavar, 2010; Mondal et al., 2007, 2012; Shoba et al., 2012). Subrahmanyam et al. (1995) reported extensive screening of these genotypes using a modified 9-point scale, where 1= no disease, 2=1-5%, 3=6-

**Table 4.** Rust resistant breeding lines with their pedigree.

| S. No | Breeding lines | Pedigree | Rust resistant parent |
|---|---|---|---|
| 1. | PBS 12187 | CS19 x GPBD 4 | GPBD 4 |
| 2. | PBS 12189 | ICGV 86590 x CS19 | ICGV 86590 |
| 3. | PBS 12191 | GG 20 x GPBD 4 | GPBD 4 |
| 4. | PBS 12192 | PBS 24030 x GPBD 4 | GPBD 4 |
| 5. | PBS 12194 | TG 37A x GPBD 4 | GPBD 4 |
| 6. | PBS 22096 | GG 20 x ICGV 86590 | ICGV 86590 |
| 7. | PBS 22099 | ICGV 86590 x PBS 24030 | ICGV 86590 |
| 8. | PBS 22101 | PBS 24030 x ICGV 86590 | ICGV 86590 |
| 9. | PBS 24030 | M 13 x R 33-1 | R 33-1 |

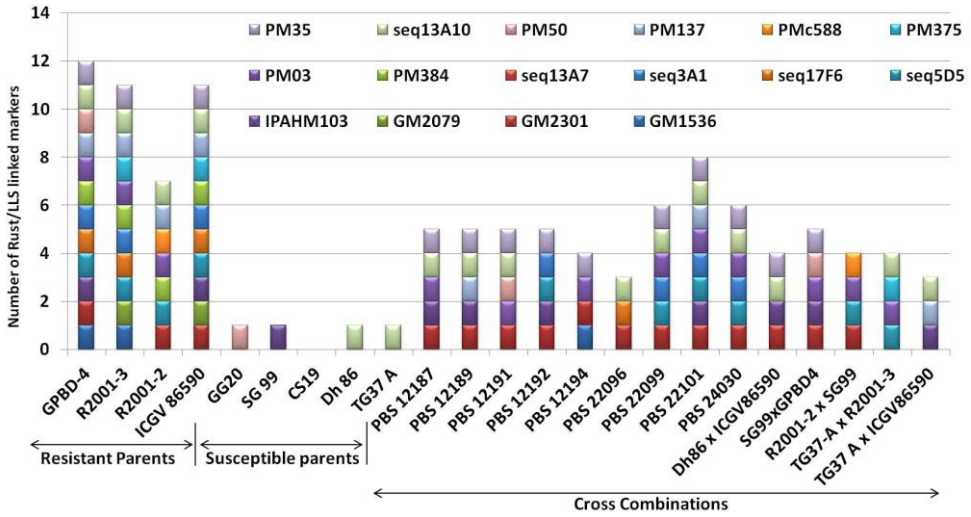


**Fig 4.** SSR marker validation and its transfer from rust and LLS resistant and susceptible parents to their crosses in advanced generations.

**Table 5.** $F_6$ progenies of resistant promising crosses with their rust resistance parent.

| S. No. | Crosses | Generation | Rust resistant parent |
|---|---|---|---|
| 1. | Dh 86 x ICGV 86590 | $F_6$ | ICGV 86590 |
| 2. | SG 99 x GPBD 4 | $F_6$ | GPBD 4 |
| 3. | R 2001-2 x SG99 | $F_6$ | R 2001-2 |
| 4. | TG 37A x R 2001-3 | $F_6$ | R 2001-3 |
| 5. | TG 37A x ICGV 86590 | $F_6$ | ICGV 86590 |

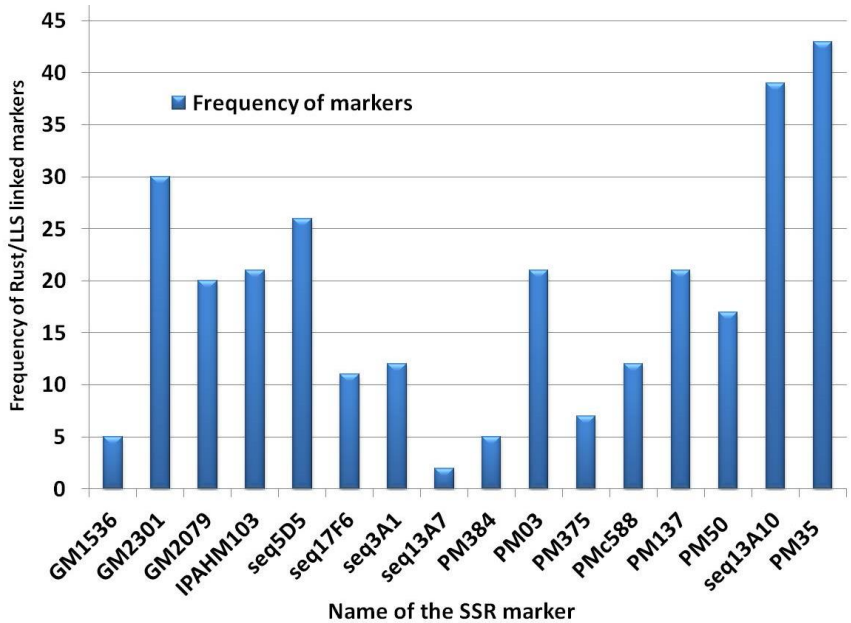


**Fig 5.** Frequency of linked and associated SSR markers in all the genotypes selected.

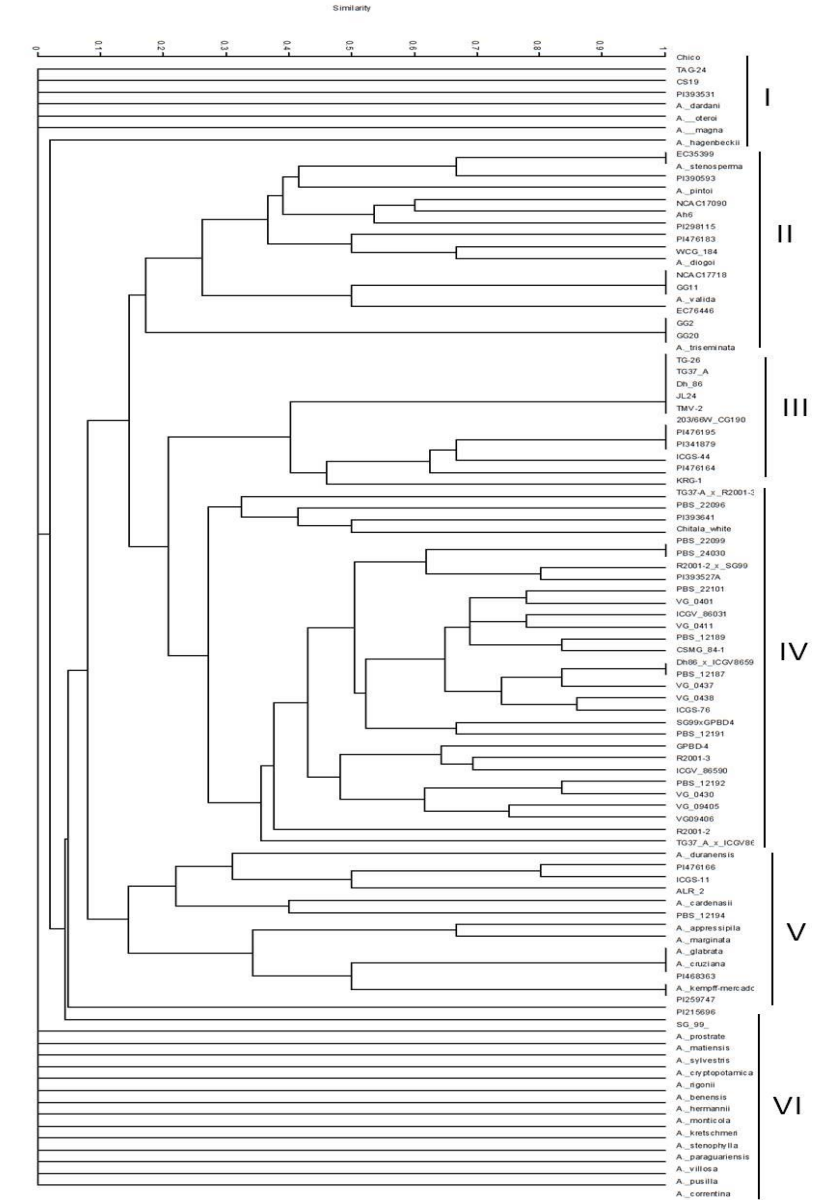


**Fig 6.** Dendrogram generated using unweighted pair of group method with arithmetic average analysis, showing relationship between 95 peanut foliar diseases resistant and susceptible genotypes.

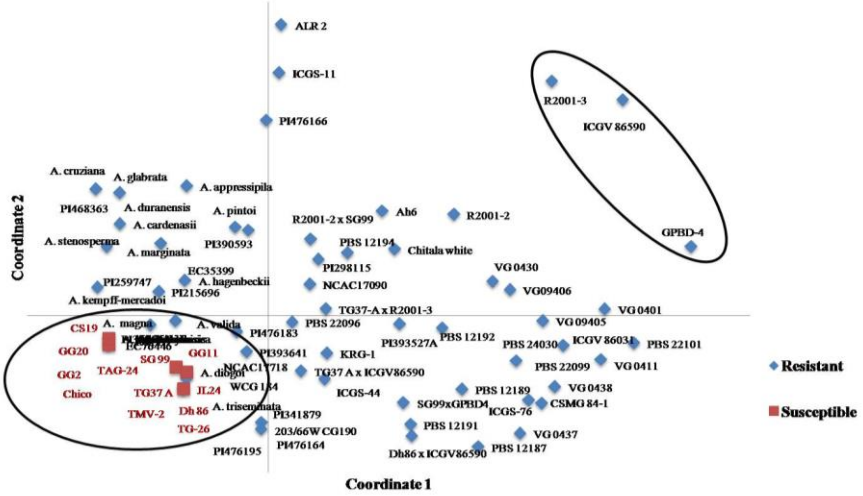


**Fig 7.** Two-dimensional plot of principal coordinate analysis of 95 peanut foliar fungal-disease resistant and susceptible genotypes using SSR analysis.

10%, 4=11-20%, 5=21-30%, 6=31-40%, 7=41-60%, 8=61-80% and 9=81-100% (% values are disease severity), to record % leaf area damaged, % leaf defoliation (for Leaf spots), and disease scored at 15-days intervals from 35 days after inoculation until 1 week before harvest.

***SSR analysis***

Two seeds of each accession were grown in plastic pots filled with sand, in a greenhouse. Genomic DNA was extracted from fresh leaves of one week old plants by CTAB method (Doyle and Doyle, 1987). The quality of DNA was checked on 0.8% (w/v) agarose gel with λ DNA as standard and quantified using NanoDrop. Twenty-two SSR primer pairs known to be linked or associated with foliar diseases in the cultivated peanut were used for polymerase chain reaction (PCR) (Table 1). The PCR mixtures (10 µl) contained 1µl template DNA (20 ng), 1 µl of 10x Taq buffer + $MgCl_2$(15 mM), 0.8 µl of dNTP (2 mM), 1.0 µl of primers (10 p moles each, Forward and Reverse), 0.1 µl of Taq polymerase (Genei 5U) and 5.1 µl of sterile double distilled water. Amplification was performed in 0.2 ml (each tube) thin walled PCR plates (96 wells $plate^{-1}$) in a thermal cycler (Eppendorf). The samples were initially incubated at 94.0 ºC for 3 min and then subjected to 5 times of the following cycle: 94.0 ºC for 30 s (−1.0 ºC reduction per cycle), 61.0-56.0 ºC for 30 s and 72.0 ºC for 1 min. This was followed by another 35 cycles of 94.0 ºC for 30 s, 56.0 ºC for 30 s and 72.0 ºC for 1 min. Final Extension was 72.0 ºC for 10 min. Amplification for each SSR marker was performed twice and amplified products were analyzed using 5% non-denaturing poly-acrylamide gel at constant power 225 volts for about 2.5-3.0 h and stained with Ethidium Bromide (Benbouza et al., 2006). The gels were documented in automated gel documentation system (Fujifilm FLA-5000) and scored.

***Data analysis***

The similarity matrix prepared based on the PCR amplicon data was subjected to cluster analysis by unweighted pair group method with arithmetic means (UPGMA) and a dendrogram was generated using PAST version 1.81 software. This was also used to perform principal coordinate analysis (PCoA) which plots the relationship between distance matrix elements based on their first two principal coordinates (Hammer et al., 2008).

**Conclusions**

For powdery mildew resistance, molecular markers have been validated in different varieties of crops like wheat. In rice, marker assisted improvement have been used for the QTL transfer in multiple genetic backgrounds. However, in peanut very limited reports of marker validation are available primarily due to relatively low level of genetic diversity in cultivated peanut and non-availability of large number of adequate molecular markers e.g., SNPs. Therefore, validation of SSR markers linked to the foliar diseases in a range of genotypes will facilitate both, identification of resistant parent(s) and resistant line(s) from a segregating population, even in the absence of disease epiphytotic and low molecular diversity conditions. Positioning of linked SSRs to the rust and LLS resistance on different LGs and its validation in a wide range of genotypes provided a strong thrust to employ these genomic regions further to answer the following questions (Yadav et al., 2011). What are the underlying mechanism(s) associated with different locus? Is disease resistance phenotype in different genotypes is associated independently or in combinations with which locus? Finding answers to these queries would enhance both our understanding and the deployment of these markers for imparting effective foliar fungal-disease resistance in peanut improvement programmes.

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