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Genotype × Environment Studies on Resistance to Late Leaf Spot Disease and Pod Yield and Identification of Stable Recombinant Inbred Lines in Groundnut (*Arachis hypogaea* L.)

N. Shilpa[1], N. Marappa[1], Navjot Singh Sidhu[1], C.R. Jahir Basha[2], H.B. Mahesh[1]

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ABSTARCT

Background: Groundnut being one of the important leguminous oilseed crops, the objective of groundnut breeding lies in development disease resistance cultivars with higher productivity. Late leaf spot (LLS) disease is one of the most devastating disease in groundnut. Therefore, identification of resistant genotypes is of prime importance in resistance breeding.

Methods: Present investigation was carried out to identify stable Recombinant Inbred Lines (RILs) resistant to LLS disease in 94 RIL mapping population that was developed from the cross TMV 2 × GPBD 4 using site regression analysis (GGE biplot). RILs were evaluated during *kharif* 2022 under three environments and were evaluated for LLS disease reaction, pod yield and its related traits.

Result: Pooled ANOVA recorded significant variation among RILs (genotypic difference) and also significant environment and genotype × environment (G × E) interaction effects at p<0.001 for PDI at all the intervals and also for pod yield under disease pressure and disease-free condition. GGE biplot analysis reported stable RILs resistant to LLS across three locations. In comparison with parents and checks, 10 superior RILs were identified that were resistant to LLS disease across three locations. Stable RILs identified across the environments in the current study can be farther evaluated for resistance reaction and can be released after evaluation using a popular national check for comparison and also can be used in development of resistant groundnut cultivars for LLS disease with high pod yield.

Key words: GGE biplot, Groundnut, Late leaf spot disease, RILs.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the principal oil seed crops cultivated traditionally since the origin of humanity. It is high energy value crop rice in oil (44-53 %), protein (23-25 %) carbohydrate (20%), fibre (3%), calcium, phosphorus, iron, thiamine (B1), riboflavin (B2) and niacin. It is widely grown in tropics and subtropics and is important to both small and large commercial producers. Globally, area under groundnut cultivation is 32.7 mha with a production of 53.9 mt and productivity of 1,648 kg/ha. Average productivity of India is about 988 kg/ha as against 2995 kg/ha in USA, 2688 kg/ha in China, 1379 kg/ha in Brazil, 1360 kg/h in Indonesia and 1145 kg/ha in Nigeria (Anonymous, 2023).

Biotic and abiotic stresses are the major constraints that affect quantity and quality of the groundnut. Majority of the commercially grown varieties are highly susceptible to foliar diseases namely, rust caused by *Puccinia arachidis*, early and late leaf spots, stem rot (*Sclerotium rolfsii*) and collar rot (*Aspergillus niger*). Late leaf Spot (LLS) disease is caused by *Pheoisariopsis personata* (*Cercosporidium personatum*) and is of significant concern for peanut growers. LLS disease typically manifests as small, circular to irregularly shaped lesions on the leaves. The lesions are initially small, dark brown to black that enlarge to about 3-8 mm diameter. Infection starts at around 55-57 days after sowing and results in premature senescence and shedding of leaves. LLS disease in severe cases causes complete defoliation and yield losses linearly increases at the rate of 2.2 to 2.8% per 10% increase in defoliation (Anco *et al.,* 2020). Thus, development of resistant cultivars using plant breeding approaches (Woodward *et al.,* 2014) is an eco-friendly approach for long term sustainability of agriculture and also reduces financial burden for small and marginal farmers.

[1]Department of Genetics and Plant Breeding, College of Agricuture, Gandhi Krishi Vigyan Kendra, University of Agricultural Sciences, Bangalore-560 065, Karnataka, India.
[2]Department of Plant Pathology, College of Agricuture, Gandhi Krishi Vigyan Kendra, University of Agricultural Sciences, Bangalore-560 065, Karnataka, India.

Corresponding Author: N. Marappa, Department of Genetics and Plant Breeding, College of Agricuture, Gandhi Krishi Vigyan Kendra, University of Agricultural Sciences, Bangalore-560 065, Karnataka, India. Email: nmaars@gmail.com

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Genetic studies on LLS resistance suggested that resistance mechanism to this fungal disease is complex, polygenic in nature and sensitive to environments (Motagi *et al.,* 2001; Dwivedi *et al.,* 2002 and Sujay *et al.,* 2012). It has been long known that environmental factors like temperature and humidity evoke infection and development of disease. Land races and wild species of groundnut possess high level of resistance to foliar diseases, but the resistance is generally linked to low productivity, late maturity, poor adaptability and undesirable pod. Genetic barriers and linkage of undesirable traits prevents the usage of wild ancestors in breeding for LLS resistant cultivars (Pandey *et al.,* 2012). Undesirable linkages of resistance with other traits (Subrahamanyam *et al.,* 1995) makes selection of superior genotypes laborious during the breeding programmes.

Molecular breeding had offered several advantages in groundnut breeding over last two decades, particularly in biotic stress resistance (Mishra *et al.,* 2015). Therefore, use of suitable mapping population for the identification of molecular markers that are associated with this quantitative trait loci (QTL) and implementation in marker assisted selection (MAS) will be advantageous in developing LLS disease resistant cultivars. QTL mapping uses maker and phenotyping data to identify QTLs for a particular trait and multilocation evaluation of recombinant inbred lines (RILs) aids in identifying stable QTLs that can later be utilised in fine mapping of it. Therefore, the present study was aimed to evaluate RILs for resistant to LLS across different environments which will be used for construction of linkage map and locating QTLs for LLS disease resistance. The screening of these RILs also identifies RILs resistant to LLS disease which can be used in future breeding programs.

MATERIALS AND METHODS

Planting material and experimental design

The plant material in the present study comprised of 94 F6 RIL population derived from the cross TMV 2 and GPBD 4, developed from single seed descent method. The research work was carried out at three locations in *kharif* 2022. Experimental plots, K-block, University of Agricultural Sciences, GKVK, Bengaluru (E1) of zone 5. Agricultural Research Station (ARS), Pavagada (E2) belongs to zone 4 and College of Agriculture, VC Farm, Mandya (E3) of zone 5 of Karnataka. Data was recorded for all plants from each line for LLS disease reaction, pod yield and its attributing traits. Experiments were laid out in Alpha Lattice Design with two replications at all the three environments. Two separate experiments were conducted for LLS disease scoring and recording observations related to yield traits. In one experiment natural disease epiphytotic condition was created for recording LLS disease reaction and pod yield per plant, while another experiment was laid out to record yield related observations by controlling LLS disease by spraying Mancozeb 0.2% after the 45 days after sowing at an interval of 20 days until maturity.

Disease scoring

For disease scoring natural epiphytotic condition was created by practising Spreader row method and LLS disease reaction was recorded. The genotype TMV 2 was used as a spreader row, since the variety is highly susceptible to create natural epiphytotic condition for the spread of the disease. It was replicated after an interval of four lines. Late leaf spot (LLS) disease incidence scoring was performed by using a modified 9-point scale (Subramanyam *et al.,* 1995) during the *Kharif* season. During LLS disease severity, the incidence of disease was recorded on the 60th, 70th, 90th and 105 days after sowing. The severity of the disease was converted into *per cent* disease index (PDI) using formula and the RILs were classified based on disease reaction (Table 1). GGE analysis was carried out in GenStat software.

Percentage disease index will be calculated using formula:

$$PDI = \frac{\text{Sum of individual raings}}{\text{No. of leaves observed} \times \text{Maximum scale}} \times 100$$

Statistical analysis

Replicated data from different trials were subjected to combined analyses of variance (ANOVA). Site regression analysis (commonly known as GGE biplot) was used to illustrate the genotype plus genotype-by-environment variation using principal components (PC) scores from singular value decomposition (SVD) (Yan *et al.,* 2000). GGE biplot with average-environment coordination (AEC) and polygon view was drawn to examine the performance of all genotypes within a specific environment and to simultaneous select genotypes based on stability and mean performance. The model for the GGE based on SVD of first two PCs is given by:

$$Yij - \mu - \beta_j = \lambda_1 \xi j_1 \, nj_1 + \lambda_2 \xi j_2 \, nj_2 + \varepsilon_{ij}$$

Where,

Yij = Mean performance of genotype i in environment *j*.

μ = Grand mean.

β_j = Environment *j* main effect.

λ_1 and λ_2 = Singular values of the first and second PC.

ξj_1 and ξj_2 = Eigenvectors for genotype i.

nj_1 and nj_2 are the eigenvectors for environment *j*.

ε_{ij} = Residual effect.

Simple scatter plot was also plotted for comparing environment-centered incidence score of genotypes in two environments. All analyses were performed using GenStat software 15th edition (VSN International, Hemel Hempstead, UK).

RESULTS AND DISCUSSION

Analysis of variance and disease reaction of RILs against LLS

Pooled ANOVA recorded significant variation among RILs (genotypic difference) and also significant environment and genotype × environment (G × E) interaction effects at $p<0.001$ for PDI at all the intervals and also for pod yield

under disease pressure and disease-free condition. Significant G and GEI effects suggest that the disease reaction varies with respect to the three environments under study and the possibility of identifying resistant genotypes that is suitable for specific target environment (Table 2). The PDI scores of RILs for LLS at the three environments varied considerably. This variation also accounts for the significant role of GEI and polygenic nature of LLS disease. Significant GEI was reported by Chaudhari *et al.* (2019) and Shaibu *et al.* (2020) in genomic selection training population and minicore collection, respectively. Study conducted by Vishuvardhan *et al.* (2013) and Zanjare *et al.* (2020) also had similar results. Out of 94 RILs, 18 RILs exhibited resistant reaction, 12 RILs were classified as moderately resistant, 34 as moderately susceptible and 30 as susceptible at 90 DAS in GKVK; at Mandya environment, 18 RILs were categorized as R, 9 as MR, 31 as MS and 36 RILs as (S) environments whereas, due to high disease pressure at Pavagada location only 11 RILs were resistant to LLS disease, 17 were MR, 14 were MS and 52 RILs were showing susceptibility reaction (Fig 1). Significant differences in genotype, environment and GEI for PDI ta different intervals indicate the polygenic nature of the disease infection. As the prime objective of the present study is to identify stable RILs resistance across the environments, wide range of variation and significant GEI explains that there is influence of environment in severity of disease and RILs that are adoptable to specific environment need to be identified for better management of LLS disease.

Table 1: Classification based on the reaction to LLS disease.

Disease reaction	Score
Immune	0
Resistance	0-3.50
Moderately resistance	3.51-4.50
Moderately susceptible	4.51-5.60
Susceptible	Above 5.60

Stability of disease reaction across the environments

Ninety-four RILs evaluated for resistance to LLS were subjected to stability analysis to identify RILs that area stable for LLS disease resistance and pod yield by GGE biplot technique using average environment coordinate (AEC) method (Yan, 2001 and Yan, 2002). GGE biplot uses first two principal components (PC1 and PC2) derived from SVD of the environment- centered data and graphically explains genotype main effect along with G × E interaction. The first two PCs in the biplot explained 86.09% of the total variation due to genotype main effect and GEI for LLS at 90 DAS (Fig 2).

Table 2: Combined analysis of variance for disease score of LLS across environments during *kharif*, 2022.

Source	df	PDI@60	PDI@75	PDI@90	PDI@105	PYP[a]	PYP[b]
Environment	2	11.5**	11.45**	9.913**	8.67**	23.545**	17.23**
Replication (ENV)	3	0.34**	0.086**	0.045**	0.056**	0.145**	1.24**
Block (ENV × REP)	54	0.004	0.005**	0.005**	0.004**	0.017**	1.45**
Genotypes	93	0.045**	0.023**	0.035**	0.02**	0.045**	0.034**
Genotypes × Environment	186	0.005**	0.005**	0.004**	0.005**	0.013**	0.011*
Error	225	0.003	0.005	0.006	0.003	0.004	0.008

df = Degree of freedom; PDI@60 = Per cent disease incidence at 60 DAS; PDI@75= Per cent disease incidence at 75 DAS; PDI@90 = Per cent disease incidence at 90 DAS; PDI@105= Per cent disease incidence at 105 DAS; PYP = Pod yield per plant (g).

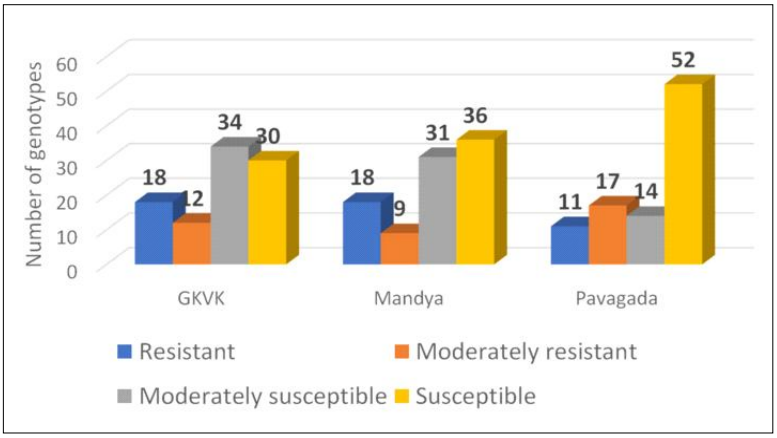


Fig 1: Categorization of RILs based on disease reaction across the environments.

Polygon view of GGE Biplot for PDI scores for LLS disease at 90 DAS

GGE biplot using polygon view helps in visualization of different interaction patterns between genotypes and environments which indicates the presence or absence of crossover GEI. Information graphs of which won who" pattern in the multi-environment trails (MET) data aids in observing the possible mega-environments that are possible in the target environments. In the biplot presented in Fig 2A and 2B, the vertex genotypes are connected with straight lines to form a polygon and remaining RILs fall within the polygon. For PDI at 90 DAS the vertex genotypes are 35, 92, 74, 17, 34, 70 and 8 (Fig 2A). These genotypes are the resistant or the susceptible genotypes for LLS in one of the three or all the three environments as they fall farthest from the origin of the biplot. The GGE biplot, the genotypes that fall on the right side of the polygon will be having higher mean performance whereas, the genotypes on the left side of the biplot will be having low mean performance foe the concerned trait. Since while selecting genotypes for disease resistance the one which is having low disease scores will be selected. Therefore, in our study the RILs that are on the left side of the biplot are the resistant RILs for LLS. In the polygon view of biplot analysis, the RILs fall under four sections and the three environments fall in two sections. The first section contains test environments GKVK and Pavagada and the vertex RILs were 35 which was susceptible to LLS whereas, RIL 8, 54, 9, 45, 48 plotted on the left side indicate the resistant RIL with lowest PDI score. The second section contains third environment Mandya (E3) with RIL 92 on the vertex as the high PDI score for LLS.

Similarly, for pod yield the first two PCs in the biplot explained 85.26% of the total variation due to genotype main effect and GEI. For pod yield, the vertex genotypes were 70, 8, 39, 50 and 35 (Fig 2B). these are the best a poor performing RILs for pod yield under disease pressure condition. The RIL 8, 70 plotted farthest on the right side indicates higher pod yield, whereas, RILs 35, 50 which are plotted farthest on the left side of the biplot are low yielders across the environments.

Mean performance and stability of RILs for LLS at 90 DAS

Fig 2C and 2D represents the ranking of 94 RILs based on their PDI score and stable performance for LLS pod yield, respectively. In ranking biplot the line that is passing through the origin of biplot is defined by the average PC1 and PC2 of all the environments and is called as average environment axis (AEA). Average environment circle (AEC) is the small concentric circle present of AEA. The stability of the genotypes is represented by the line which passes through the biplot and is perpendicular to the AEA axis. Genotypes which fall on either side of the origin that are away from AEA will be showing higher GEI and lower stability. The genotypes on the right side of the biplot have higher mean performance for a trait and genotypes on the left side of the biplot will be having lower mean performance. Since for resistant reaction to be shown by the RILs, those RILs which have low mean performance which are falling on the left side of the biplot will be having higher disease resistance in ranking biplot and RILs having shortest vector from the AEA show stability for resistance reaction. Therefore, the stable resistant RILs are the one which are showing low mean performance and shortest vector from AEA. RILs such as, 9, 45, 8, 54 91, 48 are said to be showing lower disease severity and shorter vector length and thus these are the stable resistant RILs for LLS disease. RILs 74, 92, 17, 34, 30, 35, 89 have high mean of PDI scores and show susceptibility reaction for LLS (Fig 2C). RIL 76 had low mean performance for PDI but showed higher vector length indicating less stable nature.

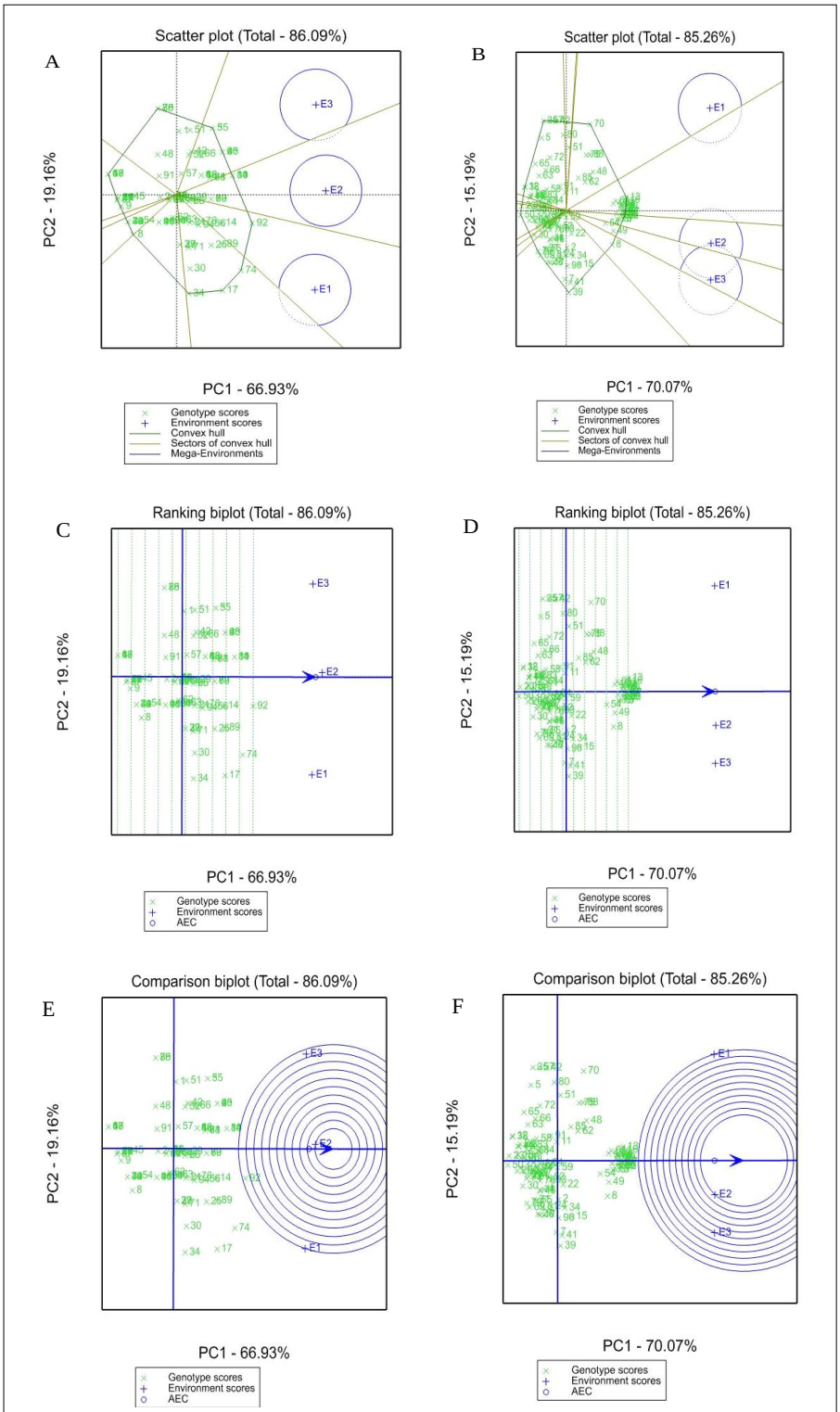
On the other hand, for pod yield genotypes on the right side of the biplot should be selected as they have higher mean performance (Fig 2D). RILs such as 13, 54, 49, 8 62, 16 recorded higher mean and were stable. RILs 70, 48, 39, 41 recorded high mean performance but the vector length from AEA was higher indicting their unstable nature.

Relationship among test environments

Comparison biplot (Fig 2E and 2F) summarizes the interrelationships among the test environments for LLS and pod yield, respectively. Environment vectors are the one which connects biplot origin and the markers of environment. The angle between the vectors of the two test environments depicts the correlation coefficient between them. Acute angle between the two test environments indicate positive correlation, obtuse angle relates to negative correlation and right angle indicate no correlation. Based on the angle between the test environments, all the three test environments *viz*., GKVK, Mandya and Pavagada were positively correlated with each other for LLS as the angle between them was <90°. All the environments are most discriminative as they plotted farthest from the origin of the biplot on the right side. The environment which plots away from the biplot origin are the ideal environment for screening. In this view all the environments are suitable has they record high disease scores. Also, ranking of environments with respect to ideal test environments revealed that Pavagada plotted on the inner circle of the biplot indicates that E2 was having highest disease pressure and is ideal for evaluation of LLS disease. Similarly, for pod yield, all the three environments were positively correlated and Pavagada (E2) is the best environment as it lies in the inner circle and maximum phenotyping expression can be seen, followed by Mandya (E3) and GKVK (E1).

Identification of stable RILs for LLS disease with higher pod yield

GGE biplot analysis reported RILs such as RIL 9, RIL 45, RIL 8 and RIL 54 as stable RILs resistant to LLS across



(A and B) polygon view of scattered biplot showing ranking of RILs based on which won where pattern for disease severity against late leaf spot and pod yield, respectively; (C and D) GGE biplot showing ranking of genotypes for mean and stability of disease severity scores of LLS and pod yield, respectively; (E and F) Comparison of environments with respect to ideal test environment for disease severity of LLS and pod yield, respectively.

Fig 2: GGE biplot analysis for disease severity scores to LLS at 90 DAS and pod yield.

Table 3: Superior RILs of groundnut from cross TMV 2 × GPBD 4 for LLS disease resistance, pod yield and related traits across three environments selected based on per se performance.

RIL no.	Pods per plant	Pod yield per plant (g) under disease	Pod yield per plant (g) under control	Kernels per plant	Kernel yield per plant (g)	Sound mature kernel (per cent)	Shelling per cent
19	43.5	44.5	43.9	88.4	30.7	79.5	69.9
8	41.6	41.6	43.2	77.3	27.8	75.6	64.3
67	39.8	43.8	42.8	72.8	28.9	69.7	67.5
13	44.6	43.7	42.1	81.6	27.9	77.4	66.2
16	38.5	41.0	41.6	72.5	26.9	71.4	64.6
45	43.4	39.7	41.3	80.2	27.8	69.3	67.3
9	38.6	42.0	41.2	77.9	26.8	61.6	65.0
48	40.5	42.6	41.2	74.1	26.4	73.7	64.0
28	42.6	41.7	40.8	79.3	27.6	69.3	67.6
54	40.5	39.6	40.7	76.7	22.7	73.7	55.7
TMV 2	23.7	13.8	17.8	38.9	7.90	69.1	67.8
GPBD 4	35.8	24.9	29.8	68.8	14.9	73.6	65.7
GKVK 27 (C)	38.9	23.9	25.8	73.7	17.8	72.7	68.9
CD @ 5%	4.28	-	4.77	2.18	3.58	16.55	12.21
S.Em. ±	1.50	-	1.67	2.60	1.26	5.81	4.28

Table 4: Mean performance of RILs for LLS disease and pod yield per plant in disease pressure and control condition.

Trait	Disease condition	GKVK (E1)	Mandya (E2)	Pavagada (E3)	TMV 2 (P1)	GPBD 4 (P2)
PDI @ 90	Disease pressure	4.8	4.7	5.5	6.5	2.8
PDI @ 105	Disease pressure	5.7	5.4	5.9	8.5	3.4
Pod yield per	Disease pressure	26.37	25.96	23.67	8.9	29.8
plant (g)	Disease free	35.96	31.55	28.88	15.6	34.6

three locations. In comparison with parents and checks, 10 superior RILs were identified that were resistant to LLS disease across three locations (Table 3) *viz.*, RIL 19, RIL 8, RIL 67, RIL 13, RIL 16, RIL 45, RIL 9, RIL 48, RIL 28 and RIL 54. These were selected based on biplot method and ranking them based on their *per se* performance for pod yield per plant. In these resistant RILs, pods per plant ranged from 43.5 (RIL 19) to 40.5 (RIL 54). The result revealed that Pavagada (E2) is the best environment for screening for LLS as it induces maximum disease pressure which is also seen in decrease in mean performance of RILs when compared to other locations (Table 4). This could be attributed to favourable environmental components such as high humidity and rainfall during disease infection.

CONCLUSION

Prime objective of resistance breeding in a self-pollinated crop like groundnut lies in development of disease resistance cultivars with higher productivity. Late leaf spot (LLS) is one of the most devastating disease in groundnut. Genotype × Environment interaction plays crucial roles in the LLS disease reaction and expression of pod yield. In the present study, we identified elite stable RILs resistance to LLS across the three environments. Stable genotypes identified across the environments can be used as parents in breeding programs and also can be released after evaluation and comparison with popular national checks. Further, the RILs with these resistance genes aids in QTLs mapping for identification of markers and genomic regions governing LLS disease resistance in groundnut.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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