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Screening of Recombinant Inbred Lines (RILs) and Identification of Stable RILs Resistant to Late Leaf Spot Disease in Groundnut (*Arachis hypogaea* L.)

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ABSTRACT

Groundnut is one of the important leguminous oilseed crops. Prime objective of groundnut breeding lies in development of Late Leaf Spot (LLS) disease resistance cultivars with higher productivity as LLS is one of the most devastating diseases in groundnut. In the present study, 94 RILs were phenotyped during *kharif* 2022 under three environmental conditions and were screened for LLS disease reaction, pod yield and its related traits. ANOVA explained significant difference between the RILs, which accounted for sufficient amount of variation. Mean performance of the RILs under three locations were used to identify the resistant RILs with higher pod yield using Principal Component (PC) approach and biplot was drawn to group the RILs based on disease reaction. PC1 and PC2 explained 89.39 *per cent* variance out of total variability. Sixteen RILs exhibited stable performance for disease resistance and pod yield. The identified RILs shall be further evaluated for their stability and can be utilised as resistance sources in groundnut breeding programs.

Keywords : Biplot, LLS disease, PCA approach, RILs, Resistance breeding

GROUNDNUT (*Arachis hypogaea* L.) is one of the principal oil seed crops in the world. It is popularly known as 'King of oil seeds' because of its high edible oil content and utilized for human consumption as vegetable oil, table purpose and confectionery, while fodder for livestock. It contains edible 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. Groundnut is a self-pollinated crop belonging to the family *Fabaceae* and ploidy level is allotetraploid (2n = 4x = 40).

In India, among the oil seed crops grown such as sunflower (905 kg/ha), rapeseed and mustard (14.58 kg/ha) and soyabean (10.59 kg/ha), productivity of groundnut is highest which makes it as the leading oil seed crop. The trend of groundnut productivity since 1950, reports that the productivity has increase by three times but at present yield plateau is reached. This allows one to look into the constraints present in groundnut cultivation.

Biotic and abiotic stresses are the major constraints that affect quantity and quality of the groundnut. Several other reasons are attributed for low yield levels in India *viz*., lack of improved high yielding cultivars, cultivation under shallow soils of low fertility, uneven rainfall distribution, continuous cropping without rotation of crop, low plant population and incidence of foliar diseases and pests. Majority of the commercially grown varieties belong to Spanish bunch types (*Arachis hypogaeas* sp. *fastigiata*) and they 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, resulting in over 50 per cent yield loss (Waliyar, 1991). LLS disease in severe cases causes complete defoliation and yield losses linearly increases at the rate of 2.2 to 2.8 per cent per 10 per cent increase in defoliation (Anco *et al*., 2020). Yield loss due to rust and LLS can go up to 70 per cent in India when fungicides are not applied (Subrahmanyam *et al*., 1995). In Karnataka major hotspot regions for LLS disease are Tumkur district majorly Pavagaga and Sira taluks. The present status of yield loss due to LLS diseased in reported to be 13.5 per cent in Karnataka (Anonymous, 2022).

Majority of farmers depend on chemical control, as it is the available option to combat the disease and reduce yield losses. But usage of fungicides comes with ecological and human health concerns and also increases cost of production by 10 per cent (Monyo *et al*., 2009). In addition, the quality of groundnut is adversely affected by fungicides when compared with non-fungicide treated groundnut (Hammonds *et al*., 1976). Chemical control is not an eco-friendly approach for long term sustainability of agriculture, and also creates financial burden for small and marginal farmers (Coffelt and Porter, 1986). Therefore, an alternate strategy to control LLS disease in groundnut, resistant cultivars has to be developed using plant breeding approaches (Woodward *et al*., 2014).

Screening and identification of resistant cultivars is the primary objective of resistance breeding. In the present study attempt was made to screen recombinant inbred lines (RILs) for LLS disease resistance and identify resistant RILs under field conditions.

MATERIAL AND METHODS

The present research work was carried out at three locations in *kharif* 2022. Experimental plots, K-block, University of Agricultural Sciences, GKVK, Bengaluru (E1), College of Agriculture, VC Farm, Mandya (E2) and Agricultural Research Station (ARS), Pavagada (E3). The plant material in the present study comprised of 94 F_6 RIL population derived from the cross TMV 2 and GPBD 4. Data was recorded for all plants from each line for LLS disease reaction and yield related traits. The observations were recorded in augmented design along with checks and parents. 'Spreader row' method was used for recording LLS disease reaction. 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 when disease incidence more. The scale had a 1-9 disease score and extent of leaf area destroyed (0-100%) which showed the linear relationship among each other depending on the percentage of the disease-damaged leaf region. During LLS disease severity, the incidence of disease was recorded on the 60, 70, 90 and 105th days after sowing. Along with this pod yield per plant was also recorded. The severity of the disease was converted into *per cent* disease index (PDI) using formula (Table 1).

Percentage disease index will be calculated using formula :

$$\text{PDI} = \frac{\text{Sum of individual ratings}}{\text{No. of leaves observed x maximum scale}} \times 100$$

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

Principal Component Analysis (PCA) was computed based on the correlation matrix using data from PDI at different intervals along with pod yield in XLSTAT 2014, Copyright Addinsoft 1995-2014 (http://www.xlstat.com) as followed by Iqbal *et al*. (2014). RILs were categorized into four groups (A, B, C and D) based on their performance for disease resistance. Group A - accessions expressing resistance, Group B - accessions that are moderately resistant, Group C - accessions that are moderately resistant and Group D - accessions that are susceptible.

Results and Discussion

Analysis of variance (ANOVA) F_6 RILs of cross TMV 2 × GPBD4

The ANOVA for disease reaction at different intervals (Table 2) of F_6 generation revealed the significant differences among the RILs for the trait under consideration This indicates presence of greater amount of variability even after achieving maximum homozygosity. The results were further supported by the fact that the range for the traits was quite wide suggesting presence of extreme RILs which can be further improved following simple selection. Gopinath *et al*. (2008); Savita (2012); Mallikarjun, (2014); Bhavya (2015) and Jambagi *et al*. (2020) have also reported significant differences among RILs for pod yield related traits in groundnut. The mean performance of RILs for pod yield under disease stress and control conditions across three locations is graphically presented in Fig. 1. It is worth noticing that pod yield of few RILs such as RIL 9, RIL, RIL 13, RIL 19, RIL 67, RIL 88 were high and comparable under both the conditions.

Biplot Analysis Based on Principal Component Analysis

Principal Component analysis is a method to find the linear combination that accounts for as much as variability as possible. In the present study principal component (PC) analysis was carried out to group genotypes of groundnut into different categories based on the mean performance for reaction to LLS. This method distinguishes resistant RILs on the bases of disease reaction. Principal component (PC) analysis had been performed and the corresponding biplot were

Table 2

Mean sum of squares of F_6 RIL population derived from the cross TMV 2 × GPBD 4 in groundnut for late leaf spot disease reaction

Source	Df	PDI@ 60 DAS	PDI@ 75 DAS	PDI@ 90 DAS	PDI@ 105 DAS	PYP
Treatment (ignoring Blocks)	96	0.76 *	2.6 **	1.45 *	2.45 **	32.15 *
Check	2	3.11 **	18.11 **	33.17 *	28.14 *	43.45 **
Test vs. Check	1	19.46 **	7.62 **	5.67	6.69	12.67 **
Test	93	1.3	2.04 *	3.24 *	5.43 **	34.67 *
Block (eliminating Treatments)	6	0.47	0.56	0.67	0.18	21.56 *
Error	12	0.6	0.16	0.34	0.18	12.45

Df = Degrees of freedom; *significant @P = 0.05

PWP = Pod weight per plant (g); *significant @P = 0.01

PDI = per cent disease incidence; DAS = Days after sowing; PYP = Pod yield per plant

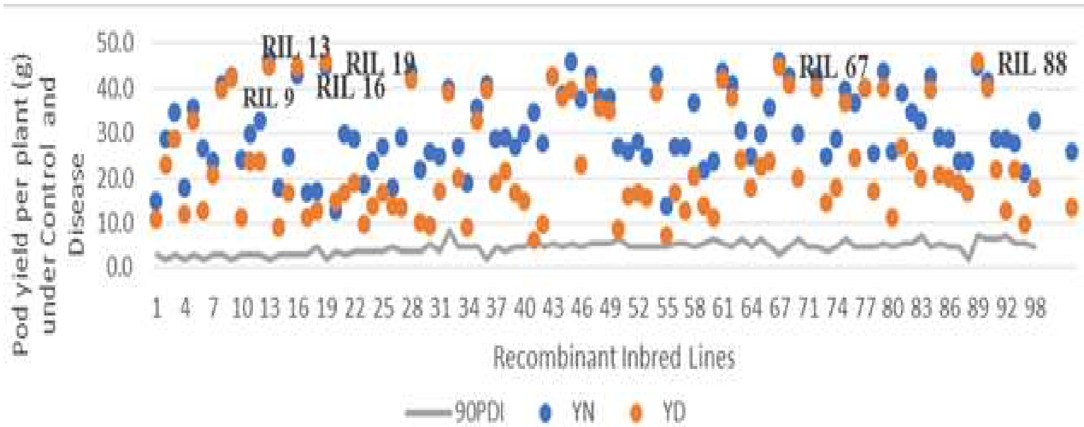

Fig. 1 : Responses of the recombinant inbred lines for mean pod yield per plant under control and disease pressure condition of three locations

Note : 90PDI: LLS disease reaction at 90 DAS; YN : Pod yield per plant under control condition; YD : Pod yield per plant under disease condition

drawn. The first two PC accounted for maximum variation of 89.39, 91.91 and 97.76 per cent of the total variance in the data under disease stress and control conditions. Our results were in accordance with the findings of Makinde and Ariyo (2010). Anthony *et al*. (2011) also reported that the first four PCs accounted for 76.92 per cent of the total variation among 50 genotypes. The results showed high positive loadings for pod yield per plant for PC1 and negative loadings were noticed for disease reaction at different intervals for PC2. Thus, biplot was drawn based on first two principal components, which shows overview of inter-relationships among pod yield and disease reaction.

The relationships among pod yield and disease reactions were graphically presented I biplot of first (PC1) and second (PC2) principal component analysis of 94 RIL population. The relationships, similarities and dissimilarities among disease reactions and pod yield per plant was explained based on indices correlation matrix from PCA using XLSTAT *ver*. 2022 (XLSTAT solutions).

The cosine of the angle between the index vectors represents their approximate positive (acute angle) or negative (obtuse angle) correlation. As disease and yield are negatively correlated they fall in different quadrant in the biplot. The biplot analysis is a useful tool often used by many researches in plant breeding (Adalid *et al*., 2010; Joshi *et al*., 2011; Panthee *et al*., 2013; Hernandez *et al*., 2014 and Thi, 2016). In the present study, biplot was drawn using pooled data of three locations. Based on resistance and susceptibility reaction, RILs were grouped into four groups. RILs that fall in group A quadrant is considered as resistant and RILs in group D is considered as susceptible. PC1 and PC2 explained 89.39 per cent variance that accounted for total variation (Table 3).

TABLE 3

Estimates of eigen values and percentage of variation under GKVK of RIL population developed from the cross TMV 2 × GPBD 4

Principal components (PCs)	Eigen value	Variability (%)	Cumulative (%)
1	4.897	54.58	54.58
2	1.467	34.81	89.39
3	0.390	6.503	95.89
4	0.127	2.122	98.01
5	0.066	1.108	99.12
6	0.052	0.874	100.00

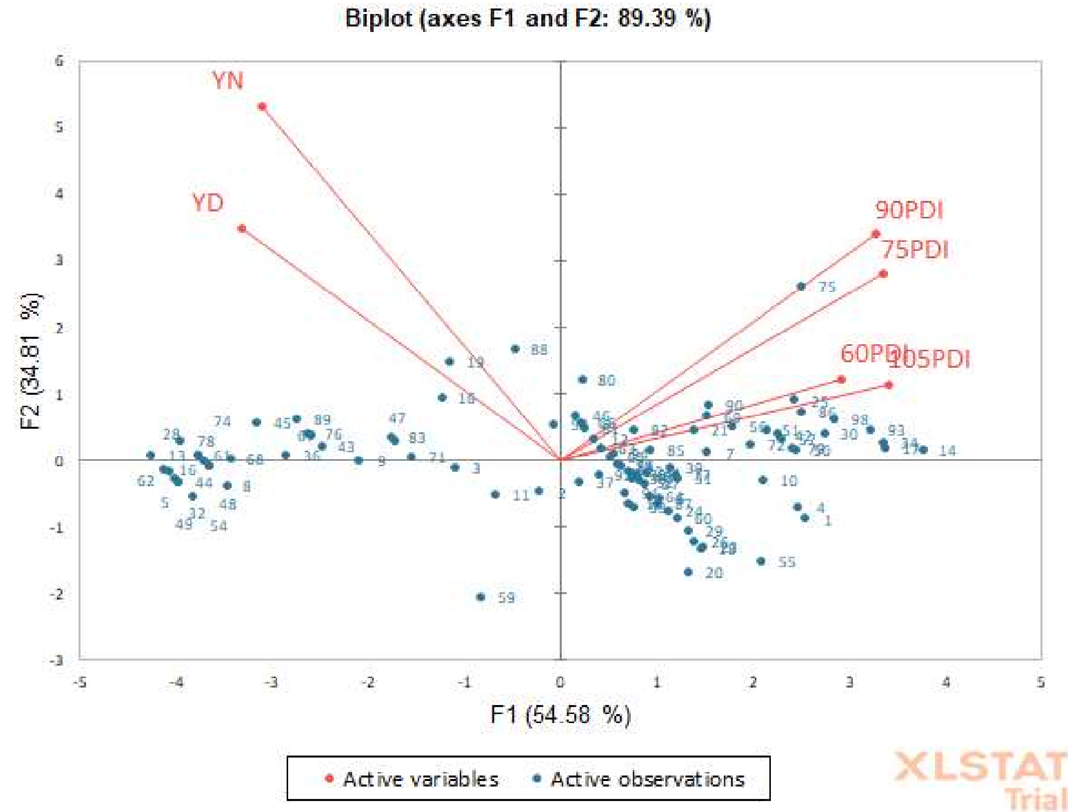

Fig. 2 : Identification of LLS resistant F_6 RILs using Biplot analysis combining three locations

Note : YN : Pod yield per plant under control condition; YD : Pod yield per plant under disease condition

TABLE 4

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	Kernel 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
88	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
43	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
36	40.5	39.6	40.7	76.7	22.7	73.7	55.7
83	39.6	39.9	40.3	69.6	21.9	64.9	54.3

Continued....

TABLE 4 Continued....

RIL No.	Pods per plant	Pod yield per plant (g) under disease	Pod yield per plant (g) under control	Kernel per plant	Kernel yield per plant (g)	Sound Mature Kernel *per cent* (%)	Shelling *per cent*
78	41.6	39.3	40.1	75.3	25.9	62.7	64.6
47	42.5	42.7	40.0	79.2	24.6	69.7	61.4
61	41.6	41.6	39.8	76.9	27.9	63.4	70.4
74	40.9	39.6	38.3	72.1	20.7	66.2	54.0
68	39.8	38.9	36.8	76.7	24.5	65.5	66.5
TMV 2	23.7	13.8	17.8	38.9	7.90	69.1	67.8
GPBD 4	35.8	29.9	24.8	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

Note : C-Check

When biplot was drawn eighteen RILs were found to be resistant and also ranked highest with regard to pod yield per plant (Fig. 2). Zare, (2012) used the same method to identify drought tolerant genotypes in barley. Similarly, in safflower by Bahrami *et al*. (2014), in Tomato by Thi *et al*. (2016) and Suresh, (2018) and in Groundnut by Savita *et al*. (2014), Jambagi *et al*. 2020a, Pooniya *et al*. (2020) and Shilpa *et al*. (2023). The results of the present study were comparable with Fernandez (1992), Kaya *et al*. (2002), Golabadi *et al*. (2006), Farshadfar *et al*. (2012), Zare (2012), Bahrami *et al*. (2014) and Brdar-Jokanovic *et al*. (2014 and 2017).

Identification of superior RILs resistant to LLS disease

In comparison with parents and checks, 16 superior RILs were identified that were resistant to LLS disease across three locations (Table 4) *viz*., RIL 19, RIL 88, RIL 67, RIL 13, RIL 16, RIL 45, RIL 9, RIL 43, RIL 28, RIL 36, RIL 83, RIL 78, RIL 47, RIL 61, RIL 74 and RIL 68. These were selected based on biplot method and ranking them based on its *per se* performance for pod yield per plant. In these resistant RILs, pods per plant ranged from 43.5 in RIL 19 to 39.8 in RIL 68. Shelling per cent in these RILs ranged from 69.9 to 66.5 per cent. The performance was these RILs should be confirmed by evaluating them under multi locations/ years for LLS disease resistance and pod yield related traits. Late these superior RILs can be exploited in future groundnut breeding programs.

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