Variability and Trait Association Studies for Late Leaf Spot Resistance in a Groundnut MAGIC Population

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Abstract: Globally, late leaf spot (LLS), a foliar fungal disease is one of the most important biotic constraint in groundnut production. Multi-Parent Advanced Generation Inter Cross (MAGIC) groundnut population was developed in a convergent crossing scheme using eight founder parents to develop a mapping population for multiple traits includes LLS. The experiments conducted in light chamber using detached leaf assay, and disease field screening nurseries at two locations (ICRISAT and ARS, Kasbe Digraj) showed significant variability for LLS resistance and component of resistance traits. Total 10 MAGIC lines with longer incubation (>11.0 days) and two MAGIC lines with longer latent period (>27 days) than the resistant parent, GPBD 4 were identified. The MAGIC lines, ICGR 171443 and ICGR 171413, with lesion diameter of <1 mm and 4.10–5.67% of leaf area damage can be valuable sources for the alleles limiting the pathogen severity. Further studies to determine the type and number of genes controlling the LLS component traits in groundnut will be useful for improvement of resistance to LLS. Genomic selection approach can be valuable in groundnut breeding to harness the minor alleles contributing to the component traits of LLS resistance.

Keywords: groundnut; MAGIC population; late leaf spot; detached leaf assay

1. Introduction

Groundnut (Arachis hypogaea L.), or peanut, is an important food and oilseed crop cultivated on 29.59 million hectares (m ha) across the world. Africa and Asia together contribute to ~91% of global groundnut production of 48.75 million tonnes (m t). The major groundnut producing countries are China, India, Nigeria, the United States of America, Sudan, Indonesia and Myanmar. China is the leading producer of groundnut with a production of 17.51 m t contributing to ~36% percent of the world groundnut production [1]. Groundnut kernels contain 20–25% protein and 48–50% oil and is rich in several vitamins, minerals and dietary source of biologically active polyphenols, flavonoids, and isoflavones [2]. The kernels contain several health enhancing compounds such as antioxidants (p-coumaric acid, resveratrol), vitamin E, and B-complex vitamins (thiamine, pantothenic acid, vitamin B-6, folates, and niacin). Groundnut haulms are valued for its protein content (8–15%) and complements the cereal fodder to increase the livestock productivity. The digestibility of nutrients in groundnut haulm is around 53% and releases energy up to 2.337 cal kg−1 of dry matter. Thus, groundnut haulms are economically important, particularly in dry tropics ecologies of Africa and Asia. Groundnut is a legume and fixes the nitrogen from...
atmosphere through biological nitrogen fixation (BNF) recruiting Rhizobium. The BNF enhances soil fertility, environmentally sustainable and economically beneficial through the savings from the purchase of nitrogenous fertilizers.

Groundnut production is constrained by several biotic stresses such as, early and late leaf spots (LLS), rust, stem rot and *Alternaria* leaf blight etc. Among the diseases, LLS, caused by *Phaeoisariopsis personata* (Berk. & Curt.) van Arx. is known to be one of the most destructive disease. It produces lesions on leaves, stem, petioles and pegs and causes premature leaf defoliation. It causes yield loss up to 70% in susceptible genotypes as a consequence of shedding of leaves resulting in complete defoliation [3]. Co-occurrence of LLS along with other foliar diseases such as rust can reduce the groundnut pod yield to an extent of 50–70% [4]. The foliar fungal diseases such as LLS reduces the yield and seed quality and grades. It also declines the quantity and quality of haulm to be used as animal fodder [5].

Fungicides can be used to manage the LLS disease; however, it increases the input cost for smallholder farmers and is not an environmentally sustainable approach. Host-resistance to manage diseases in general is economically feasible and an environmentally sustainable approach. Resistance to LLS in groundnut is reported in cultivated [6] and wild species [7] and resistance levels are high in wild diploid species. Introgression lines from wild diploid species are derived to use in breeding to develop varieties such as GPBD 4 [8] and Mutant (28-2) [9]. The introgression lines from *Arachis cardinasii* L. have been extensively used to develop improved cultivars with moderate resistance to LLS. Using marker assisted selection (MAS), a major quantitative trait loci (QTL) that explains >80% of phenotypic variance (PV) for rust resistance and 67.98% PV for LLS resistance were introgressed into three popular susceptible cultivars [10].

Bi-parental mapping populations are extensively used in groundnut and several other crops to develop the genomic tools for a marker-aided selection in breeding program. In recent years, multi-parent populations such as MAGIC and Nested Associated Mapping (NAM) populations are used in several crops. In groundnut, NAM population was used to identify QTLs for pod and seed weight [11]. In many crops, MAGIC populations have been developed and deployed in the QTLs mapping underlying important economic traits viz. wheat [12]; cotton [13]; cowpea [14]; maize [15]; rice [16]; sorghum [17] etc. A MAGIC population provides three principal advantages over a bi-parental mapping population for mapping the QTLs: higher genetic diversity, less population structure, and high resolution [18]. Apart from the use as mapping population, a MAGIC population also provides valuable germplasm for a breeding program. MAGIC population combines several useful traits from multiple elite parents [19]. Phenotypic diversity is expanded from genetic recombinations, thus allowing desirable segregants in the MAGIC populations that can be used by the breeding programs. For example, wider phenotypic variations were observed for cooking and eating quality traits in indica rice in a MAGIC population as compared to their eight founder parents [20]. A MAGIC population of groundnut developed at ICRISAT using eight elite parents was screened in the experiments conducted in field disease screening nurseries at two locations in India, and in a light chamber (controlled facility) to understand the variability for LLS resistance component traits and disease score, and their associations.

2. Materials and Methods

2.1. Study Population

A MAGIC population comprising 600 recombinant inbred lines (RILs) together with eight founder parents, and 13 resistance/ susceptible checks constituted the study population (621). The magic population was developed at ICRISAT by making all possible two-way inter-crossovers between eight diverse parents (Table 1), followed by intercressing the F₁’s from the two-way crosses, and then intercrossing the F₁’s four-way crosses. The F₁’s obtained from four-way intercrosses were advanced to F₂ generation, and the generation advancement from F₂ to F₈ was carried out following single seed descent (SSD).
approach, which involved advancement of one seed from each plant to the next generation, consequently the population size of F$_2$ was maintained in F$_8$.

Table 1. List of founder parents used in the development of MAGIC population and standard checks.

| Sr. No | List of Parents | Pedigree | Traits | Source of Origin |
|--------|-----------------|----------|--------|------------------|
| 1      | ICGV 91114      | ICGV 86055 × ICGV 86533 | Water-deficit tolerant, early maturing (90–95 days) [21], susceptible to LLS and rust [10] | ICRISAT |
| 2      | ICGV 06040      | (ICGV 92069 × ICGV 93184) × (NC Ac 343 × ICGV 86187) | High Fe and Zn Content (56 mg/kg Fe and 80 mg/kg Zn) [22] | ICRISAT |
| 3      | 55-437          | Unknown: ancient selection (1955) | Water-deficit and heat stress tolerant, tolerance to A. flavus infection [23] | Isra-Cirad, Senegal |
| 4      | ICGV 00440      | (ICGV 88386 × ASHFORD) × ICGV 95172 | Large-seeded high yielding variety, 100—seed weight (75 g) and low oil content (45%) | ICRISAT |
| 5      | ICGV 00308      | (ICGV 95244 × ICGV 96223) | Water deficit tolerant, short duration, susceptible to LLS and rust | ICRISAT |
| 6      | GPBD 4          | KRG 1 × CS 16 | Resistant to LLS and rust (LLS score 4 at 90 days) [24] | UAS, Dharwad, India |
| 7      | ICGV 05155      | (ICGV 99160 × ICGV 99240) | High oil content (55 %) [25] and high oil yield | ICRISAT |
| 8      | ICGV 88145      | (PI337409 × FESR-12B6-B1-B1-B) | Tolerance to Aspergillus infection under field [26] | ICRISAT |

Standard Checks

| Sr. No | List of Parents | Pedigree | Traits | Source of Origin |
|--------|-----------------|----------|--------|------------------|
| 1      | ICGV 02266      | ICGV 94143 × ICGV 94136 | Drought tolerant; high yielding variety, moderate resistance to LLS | ICRISAT |
| 2      | ICGV 03042      | ICGV 99160 × (ICGV 93124 × (LI × ICGS 44)) | High oil (51%) [25], tolerance to LLS | ICRISAT |
| 3      | ICGV 03043      | ICGV 99160 × (ICGV 93124 × (LI × ICGS 44)) | High oil (50%) content; early maturity (110 days), tolerance to LLS | ICRISAT |
| 4      | ICGV 06420      | ICGV 87846 × ICGV 99240 | High oil (56%) [25], tolerance to LLS | ICRISAT |
| 5      | ICGV 14421      | ICGV 91114 × GPBD 4 | LLS and rust resistant | ICRISAT |
| 6      | ICGV 86105      | (Nc Ac 537) | High yielder across international environment | ICRISAT |
| 7      | GG 20           | GAUG-10 × R-33–1 | High yielding early maturity (110–115 days) | JAU, Gujrat |
| 8      | JL 24           | Selection from EC-94943 | LLS susceptible [27], early maturity, wider adaptability | MPKV, Jalgaon, India |
| 9      | M 335           | M-13 × F-7 | Tolerant to ELS and LLS | PAU, Ludhiana, India |
| 10     | ICGS 76         | ICGV 87141 (TMV 10 × Chico) | Tolerance to bud necrosis disease | ICRISAT |
| 11     | ICGV 171179     | ICG 9930 × ICG 13585 | Red seeded | ICRISAT |
| 12     | ICGV 171174     | ICG 9930 × ICG 13585 | Red seeded | ICRISAT |
| 13     | TMV 2           | Mass selection from “Gudhiatham bunch” | Susceptible to LLS and rust [4] | TNAU, Coimbatore |

ICGV, ICRISAT groundnut variety; ICG, ICRISAT groundnut; ICGS, ICRISAT groundnut selection; TNAU, Tamil Nadu Agriculture University; JAU, Junagadh Agriculture University; UAS, University of Agriculture Sciences; MPKV, Mahatma Phule Krishi Vidyapeeth; PAU, Punjab Agriculture University; GG, Gujarat groundnut; ELS, early leaf spot; LLS, late leaf spot.
2.2. Screening for LLS Resistance Using Detached Leaf Assay in Light Chamber

In post-rainy 2018, the population (620) was screened in light chamber (Controlled facility) using the detached leaf assays which is a rapid screening technique for LLS [27,28]. A completely randomized (CRD) experimental design with two replications was used. The fully expanded two quadrifoliate (third or fourth from top) leaves from 45-day-old plants were excised through pulvinus and planted in a culture of sterile sand and vermiculite mixture (50:50) in plastic trays. The leaves in the tray were sprayed with LLS inoculum (~30,000 conidia mL$^{-1}$). The trays were covered with plastic bags and incubated in the light chamber at a temperature of 24 °C and 85% relative humidity. Incubation was performed by spraying water once a day on the leaves for 5 days to maintain the high humidity to create the congenial environment for the growth of pathogen. The LLS disease component traits such as incubation period (IP), latent period (LP), lesion number (LN), lesion diameter (LD), leaf area damage (LAD) were recorded every alternate day from 5 to 30 days after inoculation (DAI).

IP is defined as number of days from inoculation to the appearance of the first lesions, while LP is number of days from inoculation to the appearance of the first sporulating lesion. Both IP and LP are recorded on each leaf every alternate day from 5 to 30 DAI. LD is the average diameter of four lesions in mm randomly selected on each leaflet. It is measured at 25 DAI using Vernier calipers under a magnifying glass. The LN is the average of number of lesions on two randomly selected leaflets at 25 DAI. The percent LAD was assessed by comparing the leaves with diagrams depicting leaves with a known percentage of their areas affected at 30 DAI. The disease score recorded at 15 days after inoculation (DS$_{15\_DAI}$), and at 30 days after inoculation (DS$_{30\_DAI}$) [29] based on percent leaf area damage on 1–9 scale given as disease severity (Table 2) and area under the disease progress curve in light chamber (AUDPC$_{LC}$) was calculated using a formula suggested by [30].

Table 2. Modified 9-point scale for LLS used for field screening of groundnut.

| Disease Score | Phenotype 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, 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 leaflets 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%                |

* Percentage leaf area damaged by LLS (late leaf spot).
2.3. Screening for LLS Resistance in Field Disease Screening Nursery

Field screening was conducted at two locations in India, viz., International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru and Agriculture Research Station, Kasbe Digraj, Maharashtra (ARS-KD). Screening of study population comprising 620 genotypes was conducted in rainy 2019 in the alpha lattice design with two replications at both locations. The experiment was planted in a single row of four-meter length at both locations with spacing of 30 and 10 cm between rows and plants, respectively. At ICRISAT, artificial disease screening nursery was established using infector rows, spraying inoculum and maintaining favorable microclimate for disease development. ARS-KD is an LLS disease hotspot location, where the disease spread is complimented by planting infector rows. At both the locations in the field nursery, after every 10 rows of test material, an infector row of susceptible variety, TMV 2 was planted to ensure the uniform spread of disease inoculum. Rust was controlled by spraying Calixin @ 1.5 ml L$^{-1}$ of water regularly to avoid its interference with reaction to LLS. At ICRISAT, artificial inoculation was performed by planting the LLS infected potted plants from glasshouse throughout the infector rows of experimental plot at 30 days after planting (DAP). Subsequently, at 50 DAP the test plants and infector rows of the experimental plot were sprayed with a conidial suspension of LLS pathogen to ensure uniform disease pressure in the disease nursery. After inoculation at 30 DAP, light irrigation was provided daily for 15 min in the evening hours for 30 days to promote disease development. Disease score was recorded at 75 DAP (LLS_75_DAP_I) and 105 DAP (LLS_105_DAP_I) at ICRISAT location, and at 90 DAP for ARS-KD location (LLS_90_DAP_KD). A modified 9-point scale [29] (Table 2) was followed to record disease score in field nursery [29].

2.4. Statistical Analysis

The data collected from experiments conducted in light chamber and field disease screening nursery at two locations was analyzed separately. The effect of replication, block, and genotypes factor was kept random. The individual variances were estimated and modeled to error distribution using residual maximum likelihood (REML) procedure using ASREML-R v4 [31]. BLUPs (best linear unbiased predictors) were estimated for genotypes and calculated pair wise comparisons using t-statistic (LSD) for significant effects. Heritability for an individual environment was calculated using formula proposed by [32]. Karl Pearson’s correlation coefficient between traits was calculated using PROC CORR procedure in SAS v9.4 (SAS Institute, Cary, NC, USA, 2018).

The least significant difference (LSD) at 5% of significance was calculated by following equation

$$\text{LSD} = t(\text{5\%}, \text{Edf}) \times \text{SED}$$

where $t$ is the cumulative Student’s $t$ distribution, 0.05 is selected $\alpha$ level (5%), Edf is the error degrees of freedom from the ANOVA model, and SED is standard error of differences of the mean.

The coefficient of variation in % was calculated by following equation

$$\text{CV} (%) = \left(\frac{\sigma}{\overline{x}}\right) \times 100$$

where $\sigma$ is standard deviation, and $\overline{x}$ is mean.

3. Results

The data obtained from disease-screening nurseries of ARS-KD, ICRISAT and light chamber was subjected to ANOVA (Table 3). ANOVA revealed significant differences among the genotypes for all the traits, IP, LP, LN, LAD%, LD, LLS_15_DAI, LLS_30_DAI and AUDPC_LC recorded in light chamber. Similarly, the ANOVA for the traits recorded from the field experiment conducted at ICRISAT revealed significant differences between genotypes for disease scores, LLS_75_DAP_I and LLS_105_DAP_I and ANOVA for disease score recorded at ARS-KD, LLS_90_DAP_KD showed significant difference among the
genotypes. Coefficient of variation (CV) between 4.58% to 14.66% was observed for various traits recorded in light chamber and CV was high for IP (14.66%) and LN (14.45%) and low for LLS_105_DAP_I (4.35%). High heritability of >60% was recorded for all the traits under this study. The highest heritability value of >90% was observed for LAD% (97.00) and LLS_15_DAI (93.00).

Table 3. ANOVA and descriptive statistics for LLS resistance component across light chamber and disease screening nursery.

| Trait              | Genotypic Variance | SE (±) | Mean      | Range     | LSD   | CV (%) | H² (%) |
|--------------------|--------------------|--------|-----------|-----------|-------|--------|--------|
| **Light Chamber**  |                    |        |           |           |       |        |        |
| IP                 | 2.04 **            | 0.178  | 9.35      | 7.40–14.93| 1.49  | 14.66  | 68     |
| LP                 | 6.46 **            | 0.436  | 22.54     | 17.40–27.61| 1.65  | 6.69   | 85     |
| LN                 | 1.73 **            | 0.123  | 38.75     | 30.00–83.00| 1.25  | 14.45  | 82     |
| LD                 | 0.27 **            | 0.018  | 2.33      | 0.74–3.69 | 0.69  | 11.06  | 89     |
| LAD                | 0.04 **            | 0.002  | 39.35     | 3.58–81.38| 0.31  | 7.22   | 97     |
| LLS_15_DAI         | 0.16 **            | 0.01   | 5.33      | 2.16–8.71 | 0.54  | 6.83   | 93     |
| LLS_30_DAI         | 0.02 **            | 0.002  | 7.24      | 2.93–8.56 | 0.45  | 4.58   | 76     |
| AUDPC_LC           | 1272.16 **         | 88.07  | 172.54    | 66.37–241.18| 6.36  | 13.12  | 83     |
| **ICRISAT**        |                    |        |           |           |       |        |        |
| LLS_75_DAP_I       | 0.04 **            | 0.003  | 3.68      | 1.28–4.88 | 0.5   | 6.98   | 82     |
| LLS_105_DAP_I      | 0.02 **            | 0.001  | 7.94      | 5.60–8.83 | 0.44  | 4.35   | 67     |
| **ARS (Kasbe Digraj)** |                        |        |           |           |       |        |        |
| LLS_90_DAP_KD      | 0.09 **            | 0.006  | 5.98      | 2.33–8.62 | 0.53  | 6.17   | 87     |

** significance at 1% level of probability.

The trait mean values of parents, checks and MAGIC population and the trait range in the MAGIC population are summarized in Table 4. Among the parents, the genotype ICGV 91114, which is a LLS susceptible parent showed shortest IP (7.74 days) and LP (17.40 days) highest values for LN (41.06), LD (2.66 mm), LAD (52.05%), LLS_15 DAI (7.77), LLS_30 DAI (8.19), AUDPC_LC (222.45), LLS_105 DAP_I (8.74) and LLS_90 DAP_KD (7.38). Conversely, the resistant parent, GPBD 4, recorded long IP (11.51 days) and LP (27.61 days) and low values for LN (5), LD (1.68 mm), LAD (3.94%), LLS_15 DAI (2.61), LLS_30 DAI (3.36), AUDPC_LC (72.61), LLS_75 DAP_I (2.26), LLS_105 DAP_I (6.89) and LLS_90 DAP_KD (4.53). Among the checks, GG 20 and JL 24 were susceptible and recorded an IP of 8.42 days. The disease scores of GG 20 were: LLS_15 DAI (7.80), LLS_30 DAI (7.82), and LLS_105 DAP_I (7.14), and high AUDPC_LC (216.21). Disease score in the susceptible parents, ICGV 91114, GG 20 and JL 24 indicated good disease pressure in the experiments conducted in light chamber and field disease nursery at both the locations.

Large variation for all the traits measured in light chamber and field conditions was observed in the MAGIC population (Table 4). The IP and LP among the 600 MAGIC lines ranged from 7.40 to 14.93 days, and from 17.40 to 27.61 days, respectively indicating large variability for appearance of first symptom and first sporulation lesion. The lesion numbers varied from 5.51 to 83.06 per leaf, and the lesion diameter varied from 0.73 to 3.69 mm. Similarly, large variation was observed for LAD, which varied from 3.58% to 81.38%. The AUDPC_LC values among the MAGIC population ranged from 66.37–241.18. The LLS scores at different days after inoculation (DAI) at light chamber and different days after planting (DAP) at field such as LLS_15 DAI, LLS_30 DAI, LLS_75 DAP_I, LLS_105 DAP_I and LLS_90 DAP_KD were in the range of 2.16–8.56, 2.93–8.56, 2.15–4.88, 5.60–8.83 and 2.83–8.62, respectively.
| Designation | IP  | LP  | LN  | LD  | LAD | LLS_15_DAI | LLS_30_DAI | AUDPC_IC | LLS_75_DAP_I | LLS_105_DAP_I | LLS_90_DAP_KD |
|-------------|-----|-----|-----|-----|-----|------------|------------|----------|--------------|----------------|----------------|
| **Parents** |     |     |     |     |     |            |            |          |              |                |                |
| ICGV 91114 | 7.74| 17.40| 41.06| 2.66| 52.05| 7.77       | 8.19       | 222.45   | 4.33         | 8.74           | 7.38           |
| ICGV 06040 | 8.42| 21.66| 33.36| 1.90| 47.21| 5.48       | 7.07       | 172.51   | 3.42         | 8.01           | 3.69           |
| 55-437      | 9.11| 23.36| 25.82| 2.04| 18.04| 3.58       | 7.03       | 147.53   | 3.08         | 6.78           | 4.26           |
| ICGV 00440 | 8.77| 20.80| 57.38| 2.48| 50.60| 7.77       | 7.08       | 216.21   | 3.83         | 7.00           | 5.11           |
| ICGV 00308 | 8.42| 19.53| 40.33| 2.35| 39.76| 6.41       | 7.44       | 191.24   | 3.96         | 8.54           | 4.77           |
| GPBD 4      | 11.51| 27.61| 5.00| 1.68| 3.94| 2.16       | 3.36       | 72.61    | 2.26         | 6.89           | 4.53           |
| ICGV 05155  | 8.42| 25.06| 8.14| 1.01| 19.22| 6.41       | 7.07       | 184.99   | 2.69         | 7.98           | 4.65           |
| ICGV 88145  | 10.48| 22.51| 32.28| 2.39| 27.83| 3.04       | 7.44       | 147.53   | 4.36         | 8.38           | 7.28           |
| **Checks**  |     |     |     |     |     |            |            |          |              |                |                |
| ICGV 02266 | 9.45| 25.06| 37.78| 2.04| 13.09| 3.12       | 7.44       | 147.53   | 2.21         | 6.71           | 4.10           |
| ICGV 03042 | 8.77| 18.25| 50.61| 2.53| 52.05| 6.41       | 7.44       | 191.24   | 2.28         | 7.74           | 2.39           |
| ICGV 03043 | 11.51| 27.61| 23.20| 2.13| 23.46| 2.62       | 6.67       | 128.80   | 2.65         | 7.60           | 2.44           |
| ICGV 06420 | 11.16| 27.61| 64.06| 2.21| 40.93| 2.62       | 7.44       | 141.29   | 4.06         | 8.26           | 6.64           |
| ICGV 14421 | 11.16| 25.91| 27.34| 2.04| 29.31| 2.16       | 6.67       | 122.56   | 3.45         | 8.08           | 6.05           |
| ICGV 86105 | 11.51| 25.06| 22.61| 1.68| 16.63| 3.58       | 7.07       | 147.53   | 3.22         | 8.37           | 6.36           |
| GG 20      | 8.42| 20.80| 45.63| 3.02| 56.90| 5.94       | 7.44       | 184.99   | 4.38         | 7.92           | 7.78           |
| JL 24      | 8.42| 22.08| 52.14| 2.66| 45.28| 7.80       | 7.82       | 216.21   | 1.28         | 7.14           | 4.26           |
| M 335      | 9.79| 21.66| 29.20| 2.44| 25.43| 6.89       | 7.44       | 197.48   | 3.94         | 7.65           | 6.69           |
| ICGS 76    | 11.51| 25.91| 17.37| 3.06| 10.21| 3.12       | 6.67       | 135.05   | 3.15         | 7.36           | 5.99           |
| ICGV 17179 | 10.82| 25.91| 27.00| 1.95| 18.04| 3.12       | 6.26       | 128.80   | 3.08         | 8.10           | 4.64           |
| ICGV 171174| 9.79| 26.76| 33.73| 2.13| 24.45| 4.08       | 7.82       | 166.26   | 3.11         | 7.94           | 7.26           |
| **MAGIC**  |     |     |     |     |     |            |            |          |              |                |                |
| Population-Mean | 9.33| 22.50| 38.95| 2.34| 40.05| 5.35       | 7.25       | 172.85   | 3.70         | 7.94           | 6.00           |
| Population-Range | 7.40–14.93 | 17.40–27.61 | 5.51–83.06 | 0.74–3.69 | 3.58–81.38 | 2.16–8.71 | 2.93–8.56 | 66.37–241.18 | 2.15–4.88 | 5.60–8.83 | 2.83–8.62 |
The results of correlation studies among the LLS disease related traits are presented in Table 5. The AUDPC_LC showed significant and positive correlation with all traits, whereas it showed a negatively significant relationship for IP (r = −0.70 **) and LP (r = −0.65 **). Incubation period showed significant and positive association with only LP (r = 0.53 **) and significant negative association with all other traits. Latent Period showed significant and negative correlation with all traits. Lesion number showed significant positive correlation with all other traits and no association with LD and significant negative association with IP and LP. The trait lesion diameter showed positive significant association with all the traits except LP, IP and LLS_15_DAI. The traits LAD%, LLS_15_DAI, LLS_30_DAI, LLS_75_DAP_I and LLS_105_DAP_I showed significant positive correlation with all remaining traits and significant negative association with IP and LP.

4. Discussion

The LLS disease pressure in the light chamber was adequate, evident from the LLS disease scores and higher area under the disease progress curves (AUDPC_LC); shortest incubation and latent periods; large number and diameter of the lesions; and leaf area damage of the susceptible parent, ICGV 91114, and the susceptible checks, JL 24 and GG 20. Similarly, the LLS disease scores at 75 DAP and 105 DAP at ICRISAT and at 90 DAP at ARS-KD indicated good disease pressure in both the locations where the field experiments were conducted.

The MAGIC population of groundnut showed significant variability for LLS disease resistance. ANOVA suggests that the study population comprising 600 MAGIC lines, eight founder and 12 checks showed significant variation for all the 12 traits recorded in the light chamber and disease field nurseries at ICRISAT and ARS-KD (Table 3). Earlier variability was reported for leaf spot disease traits in F2 population [33–35], in F3 population [36], in inter-specific lines [37], in recombinant inbred lines (RILs) [38] and in advanced breeding lines [39] of groundnut.

The incubation period is the time taken from inoculation to appearance of first symptoms, which varied from 7.40 days to 14.93 days in the study population. The genotypes with longer incubation period would take more time to develop the symptoms, thus delaying the infection and thus will be able to tolerate LLS disease. The incubation period was reported to be longer in resistant cultivars [40,41] and in wild species of groundnut [42]. The resistant parent, GPBD 4 recorded an incubation period of 11.53 days, which was the longest duration for the appearance of first symptoms in the study population and the other moderately resistant parent, ICGV 88145 recorded an incubation period of 10.48 days. Among the checks, ICGV 14421 and other moderately resistant checks, ICGVs 03043, 06420, 86105 and ICGS 76 recorded more than 11.0 days of incubation period. Sixty-three MAGIC lines also recorded an incubation period of more than 11.0 days. Incubation period was reported to be the most frequent component of resistance involved in resistant genotypes [42]. In the present study, incubation period is negatively associated with all other disease component traits including disease score and is positively associated with latent period.

Table 5. Correlation among LLS resistance component across light chamber and disease screening nursery.

| Trait          | IP   | LP   | LN   | LAD  | LD   | LLS_15_DAI | LLS_30_DAI | LLS_75_DAP_I | LLS_105_DAP_I | LLS_90_DAP_KD |
|----------------|------|------|------|------|------|------------|------------|--------------|---------------|---------------|
| AUDPC_LC       | −0.70 ** | −0.65 ** | 0.53 ** | 0.70 ** | 0.12 ** | −0.85 ** | 0.80 ** | 0.16 ** | 0.19 ** | 0.19 ** |
| IP             | 0.53 ** | −0.36 ** | −0.54 ** | −0.11 ** | −0.70 ** | −0.52 ** | −0.25 ** | −0.25 ** | −0.22 ** | −0.22 ** |
| LP             | −0.53 ** | −0.46 ** | −0.09 ** | −0.07 ** | −0.43 ** | −0.17 ** | −0.18 ** | −0.18 ** | −0.19 ** | −0.19 ** |
| LN             | 0.69 ** | 0.08 | 0.44 ** | 0.54 ** | 0.16 ** | 0.13 ** | 0.18 ** | 0.18 ** | 0.20 ** | 0.20 ** |
| LAD            | 0.16 ** | 0.08 | 0.44 ** | 0.54 ** | 0.16 ** | 0.13 ** | 0.18 ** | 0.18 ** | 0.20 ** | 0.20 ** |
| LD             | 0.06 | 0.23 ** | 0.12 ** | 0.12 ** | 0.09 ** | 0.16 ** | 0.16 ** | 0.16 ** | 0.16 ** | 0.16 ** |
| LLS_15_DAI     | 0.06 | 0.62 ** | 0.10 ** | 0.11 ** | 0.13 ** | 0.23 ** | 0.35 ** | 0.35 ** | 0.35 ** | 0.35 ** |
| LLS_30_DAI     | 0.06 | 0.62 ** | 0.10 ** | 0.11 ** | 0.13 ** | 0.23 ** | 0.35 ** | 0.35 ** | 0.35 ** | 0.35 ** |
| LLS_75_DAP_I   | 0.06 | 0.62 ** | 0.10 ** | 0.11 ** | 0.13 ** | 0.23 ** | 0.35 ** | 0.35 ** | 0.35 ** | 0.35 ** |
| LLS_105_DAP_I  | 0.06 | 0.62 ** | 0.10 ** | 0.11 ** | 0.13 ** | 0.23 ** | 0.35 ** | 0.35 ** | 0.35 ** | 0.35 ** |
| LLS_90_DAP_KD  | 0.06 | 0.62 ** | 0.10 ** | 0.11 ** | 0.13 ** | 0.23 ** | 0.35 ** | 0.35 ** | 0.35 ** | 0.35 ** |

* significance at 5% level of probability; ** significance at 1% level of probability; IP, incubation period; LP, latent period; LN, lesion number; LD, lesion diameter (mm); LAD, leaf area damage (%); LLS_15_DAI, late leaf spot score at 15 days after inoculation; LLS_30_DAI, late leaf spot score at 30 days after inoculation; AUDPC_LC, area under disease progress curve at light chamber; LLS_75_DAP_I, late leaf spot score at 75 days after planting at ICRISAT; LLS_105_DAP_I, late leaf spot score at 105 days after planting at ICRISAT; LLS_90_DAP_KD, late leaf spot score at 90 days after planting at Kasbe Digraj.
The latent period is an important trait in screening fungal diseases and defined as the period between infection and onset of sporulation from the infection [43]. It is an aggressive trait in plant pathology and pathogens with longer latent period are said to be more tolerant than the pathogens with shorter latent period [44,45]. Similar to the incubation period, the lines with longer latent period that delay sporulation are more tolerant. The resistant parent, GPBD 4 recorded longest latent period of 27.61 days, and two checks ICGVs 03043 and 06420 and twenty-two MAGIC lines recorded a latent period of >27 days (Table 4). During sporulation, the lesions on the abaxial side of the leaflets are covered with conidiophores that act as secondary inoculum and spread the disease. Under favorable conditions, the leaf spot disease spreads and increases rapidly with multiple secondary cycles during a single season. Thus, a delay in sporulation can reduce the secondary cycles and thus the severity of the LLS disease in tolerant genotypes. The MAGIC lines with longer incubation and latent periods than the resistant parent, GPBD 4, indicates the contribution of minor alleles from the other parents for these two traits of LLS resistance. High heritability of 98% for incubation period and 85% for latent period suggests the possible genetic gains through selection and thus, incubation and latent period can be a valuable trait for identifying the resistant genotypes for LLS in groundnut. Besides the major QTL explaining > 80% phenotypic variance that was selected through marker assisted selection, the role of background genotype contributing minor alleles to resistance to rust was reported in introgression lines of groundnut [10].

Lesion diameter is often used to assess the pathogen virulence, disease severity and host susceptibility in foliar leaf diseases. The amount of leaf area damage is the primary measure of disease severity; hence, both lesion number and area should characterize to evaluate the host susceptibility [46]. The parent GPBD 4 showed lower number of lesions (5.00) and diameter (1.68 mm), and a leaf area damage of 3.94% (Table 4). Among the checks, ICGV 86105 recorded lesion diameter of 1.68 mm similar to GPBD 4, but the number of lesions was 22.08. Although ICGS 76 showed a leaf area damage of 10.21% which is low when compared to other checks, the number of lesions was more, 17.36 per quadrifoliate leaf. The MAGIC lines namely ICGR 171425, ICGR 171590, ICGR 171215, and ICGR 171418 recorded less than 10 lesions per quadrifoliate leaf at 25 DAI; of which ICGR 171425, ICGR 171590 and ICGR 171215 had a leaf area damage of 3.94–5.67%. The MAGIC lines namely ICGR 171413, ICGR 171443, ICGR 171546 and ICGR 171175 recorded small lesions, with a lesion diameter of <1 mm as compared to GPBD 4 (1.68 mm lesions), of which ICGR 171413 and ICGR 171443 recorded 4.10% and 5.67% of leaf area damage. Ten lines recorded a leaf area damage of <10%. The heritability for the component traits, lesion number and diameter and leaf area damage were high (82–97%), suggesting genetic control of these traits. In the previous studies on LLS resistance related traits, high [5,39,40] to moderate [36,47] heritability was reported. The MAGIC lines with low lesion number, smaller lesions and low leaf area damage will be valuable for alleles restricting the disease severity in the resistant and moderately resistant genotypes of groundnut.

LLS disease score measures overall disease severity and is an important parameter to identify tolerant genotypes using a modified disease rating scale suggested by [29], which was extensively used by the researchers. Besides, the disease score, AUDPC is useful as it gives quantitative summary of disease intensity over time for comparison across locations, years or any other management tactics. The most commonly used method is the trapezoidal method, and it considers variability in time (hours, days, weeks, months or years) and evaluates average disease intensity between two points [48]. AUDPC is reported to be a best indicator to identify resistant mechanism in whole plant over a period of time [49] and lower AUDPC values shows more resistance. In the light chamber, the disease score at 15 and 30 days after inoculation in the MAGIC population varied from 2.16 to 8.71 and 2.93 to 8.56, respectively. The resistant parent, GPBD 4 showed least AUDPC value of 72.61 under light chamber and disease score of 3.36 at 30 DAI. Among parents, ICGV 14421 showed least AUDPC value of 122.56 in light chamber; however, the disease score was 6.67 at 30 DAI. The MAGIC line, ICGR 171546 recorded a AUDPC value of 66.37 which
is significantly lower than that of resistant parent GPBD 4 and two MAGIC lines, ICGR 171459 and 171531 recorded 72.61 and 78.85 that are on par with GPBD 4. The field disease score at 75 and 105 DAP at ICRISAT varied from 2.93 to 8.56, respectively, and at 90 DAP at ARS-KD varied from 2.83–8.62. The LLS disease scores recorded high heritability of 67–87% indicating possible gains through selection in segregating populations. The resistant parent, GPBD 4 recorded the lowest disease score in both the locations of the field experiment at all the three stages of crop viz., 75, 90 and 105 DAP. GPBD 4 recorded a disease score of 2.26 and 6.89 at 75 and 105 DAP, respectively at ICRISAT, and 4.53 at 90 DAP at ARS-KD. Higher disease scores at later stages of crop growth explain that the degree of susceptibility is more prominent in later stages of crop, and it may also be due to an increase in magnitude of inoculum at later stages. Total 20 MAGIC lines, ICGR 171039, 171472, 171520, 171242, 171423, 171490, 171291, 171061, 171537, 171073, 171479, 171491, 171299, 171575, 171433, 171268, 171014, 171281, and 171296 recorded significantly superior disease score at 105 DAP (5.60–6.37) as compared to resistant check, GPDB 4.

The incubation and latent periods are significant and negatively correlated with all other disease component traits viz., lesion number and diameter, and leaf area damage; and disease scores in light chamber and field conditions, viz., LLS_15_DAI, LLS_30_DAI, LLS_90_DAP_KD, LLS_75_DAP_I, LLS_105_DAP_I, and AUDPC_LC. This indicates that genotypes having longer incubation period and latent period tend to have reduced sporulation and enhanced resistance to LLS [27,42,50,51]. The lesion number and lesion diameter are positively correlated with leaf area damage which is a basic indication that increases in lesion number and diameter reflects more area on leaf with disease severity. The LLS scores at 15 DAI and 30 DAI, are positively correlated (r = 0.62**) with each other which indicate LLS screening at any one stage after inoculation will enough to reflect the disease severity at light chamber. For the field screening LLS score at 75, 90 and 105 days after planting, they were positively associated, but the r values were low (r = < 0.5).

The MAGIC lines recorded higher levels of resistance compared to the resistant check, GPBD 4 indicating contribution of minor alleles by other parents to the component traits of resistance, particularly incubation and latent period, lesion diameter, and leaf area damage. Earlier studies reported QTLs for LLS disease score [24,52], and the markers developed using these major QTLs in marker assisted selection (MAS) have thus far resulted in limited progress in the improvement of resistance to LLS. Consequently, the level of resistance for LLS in the improved breeding lines with superior agronomic traits did not cross the resistance levels of GPBD 4 that was developed over two decades ago. The resistant MAGIC lines identified in the study offer the opportunity to further improve the level of resistance in the breeding pipeline. Given the importance of component traits in LLS disease resistance, and possible role of minor alleles determining these component traits, genomic selection (GS) can be a better approach as compared to the selection of a major QTL through MAS to improve resistance to LLS. The incubation and latent periods are significantly associated with disease scores in both light chamber and field disease nursery that measure the disease severity and thus can be valuable component traits for LLS resistance in groundnut. The selected MAGIC lines are recycled in the groundnut breeding program as parents to improve the frequency of desirable alleles in the breeding populations, and they can also be advanced to multi-location trials for testing their agronomic performance for their release as commercial cultivars.

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