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**Research Article**

# Development of superior introgression lines for resistance to foliar diseases and productivity in groundnut (*Arachis hypogaea* L.)

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## Abstract

Seventy eight introgression lines (ILs) from three populations (ICGS 76 × ISATGR 278-18, DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5B) involving late leaf spot (LLS) and rust susceptible varieties (ICGS 76 and DH 86) and disease resistant synthetic allotetraploids (ISATGR 278-18 and ISATGR 5B) were screened for LLS and rust resistance and productivity traits. ILs showed considerable variability, high heritability and genetic advance over mean for disease resistance and productivity traits. In total, two lines superior to ICGS 76, and five lines superior to DH 86 were selected. Of the seven, three ILs were also superior over the national check variety, GPBD 4. The selected superior lines carried resistant allele at majority of the LLS and rust resistance-linked marker loci. These ILs with high level of resistance to LLS and rust, and high productivity can be considered for variety release trials or as donors in breeding programmes.

**Key words**
Groundnut, synthetic allotetraploids, introgression lines, late leaf spot, rust resistance

## Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed, food and feed legume crops belonging to Fabaceae. Several biotic and abiotic constraints limit the quantity and quality of the groundnut yield. Cultivated groundnut varieties, especially those belonging to Spanish bunch types are highly susceptible to foliar diseases namely, rust caused by *Puccinia arachidis* Speg. and late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk. & Curt.) Van Arx. (McDonald *et al*., 1985; Subrahmanyam *et al*., 1985). The yield loss due to the co-occurrence of rust and LLS can go up to 70% in India when fungicides are not applied (Subrahmanyam *et al*., 1984; Subrahmanyam et al., 1985). Considerable efforts to breed for foliar disease resistant cultivars have been made through conventional breeding approaches; however, the success has been limited. GPBD 4 (Gowda *et al*., 2002) and G 2-52 (Nadaf *et al*., 2009) varieties developed from University of Agricultural Sciences, Dharwad, Karnataka, and a few varieties developed from ICRISAT, Patancheru (Singh et al., 2003) are resistant to LLS and rust. The major limiting factors include narrow genetic base of groundnut leading to low variability for disease resistance, association of disease resistance with undesirable pod features, kernel features, long duration and low productivity (Subrahamanyam *et al*., 1993), difficulty in selecting the desirable recombinants due to interference among the foliar diseases and complex inheritance pattern (Bromfield and Bailey, 1972; Tiwari *et al*., 1984; Paramasivam *et al*., 1990). However, a few improved genotypes showed foliar disease resistance along with desirable pod and kernel features (Gowda *et al*., 2002; Nadaf *et al*., 2009; Gajjar *et al*., 2014).

Use of wild diploids, which are foliar disease resistant, in the breeding programme through synthetics is known to broaden the genetic base in many crops (Gur and Zamir, 2004; Fernie et al., 2006; Fu *et al*., 2010; Nevo and Chen, 2010) including groundnut (Mallikarjuna *et al*., 2011; Varshakumari *et al*., 2014). Integration of genomic tools like markers and marker assisted selection with conventional breeding approaches might enhance the precision and speedy development of improved groundnut cultivars for LLS and rust resistance. In this regard, an effort was made to develop introgression populations by crossing LLS and rust susceptible varieties (ICGS 76 and DH 86) with disease resistant synthetic allotetraploids (ISATGR 278-18 and ISATGR 5B), and backcrossing the progenies twice with respective recurrent parent (ICGS 76 or DH 86) (Varshakumari et al., 2014). Our recent studies have identified quantitative trait loci (QTL) and linked markers for late leaf spot and rust resistance from introgression line (IL) and recombinant inbred line (RIL) mapping populations (Sujay *et al*., 2012; Varshakumari, 2013). Also, these markers were validated using various other genetic resources like germplasm and breeding lines (Khedikar *et al*., 2010), other RIL populations

(Sukruth *et al*., 2015) and near-isogenic lines (NILs) (Yeri *et al*., 2014). In the present study, highly resistant introgression lines (with score ≤4.0) from three backcross populations involving cultivated varieties (ICGS 76 and DH 86) and synthetic amphidiploids groundnut (ISATGR 278-18 and ISATGR 5B) were screened for LLS and rust resistance along with the productivity traits and pod features with an objective of identifying superior ILs. Based on their phenotypic evaluation and allele type at LLS and rust resistance-linked marker loci, ILs combining high level of resistance and productivity along with desirable pod features were selected for further varietal release trials.

## Materials and methods

A total of 78 ILs ($BC_2F_5$) with high level of resistance to LLS and rust (with score ≤4.0) were selected based on the performance in the previous generations from three (ICGS 76 × ISATGR 278-18, DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5B) populations, and the seeds of these ILs were obtained from the Department of Genetics and Plant Breeding, UAS, Dharwad. They were grown at IABT Garden of the Department of Biotechnology, UAS, Dharwad, India during the rainy season of 2013 in randomized block design with two replications. Each replication consisted of 2 rows of 2.5 mt length with a spacing of 30 × 10 cm.

Genotypes were evaluated for pod yield, number of pods per plant, test weight and shelling percentage using “Groundnut descriptors” (IBPGR/ICRISAT, 1992). The genotypes were subjected to field screening for rust and LLS reaction using spreader row technique (Subrahmanyam et al., 1995) in which the disease spreader plants [TMV 2 (susceptible to both LLS and rust) and Mutant 28-2 (susceptible to rust)] were planted at regular interval of 10 rows. Disease scoring for both rust and LLS was done at 90 days after sowing (DAS) according to modified 9-point scale (Subbarao et al., 1990).

Phenotypic data analyses like analysis of variance (ANOVA), estimation of phenotypic and genotypic coefficients of variation (GCV and PCV), heritability ($h^2_{bs}$), phenotypic correlation and genetic advance as percent of mean (GAM) were carried out for all the traits using Windostat version 8. The lines showing significantly higher mean for disease resistance and productivity traits were selected as superior lines. Genomic DNA was isolated from the young leaves of superior lines and their parents by following CTAB method (Sambrook and Russell, 2001). PCR was carried out (Sujay et al., 2012) for markers linked to LLS and rust resistance in order to check the type of allele at these loci among the superior lines.

## Result and discussion

Sixty introgression lines from ICGS 76 × ISATGR 278-18, 14 from DH 86 × ISATGR 278-18 and four from DH 86 × ISATGR 5B along with the parents, ICGS 76 and DH 86, and a national check variety, GPBD 4 (Gowda *et al*., 2002) were evaluated for productivity traits and resistance to LLS and rust. Introgression lines showed significant differences for all the traits recorded in this study. The nature and magnitude of variability was assessed by phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV). LLS score, rust score and pod yield (kg/ha) recorded high PCV and GCV (Table 1), indicating the scope for selecting the superior ILs for these traits. Heritability and genetic advance over mean (GAM) were also high for reaction to late leaf spot and rust, and pod yield (kg/ha), indicating the possibility of exercising fairly easy selection and the extent of improvement that can be brought about through selection in these traits.

Selection of an IL based on a trait might influence its performance for other traits depending upon the extent of correlation between the traits under consideration. An effort was made to study the correlation between various productivity traits and disease resistance. Occurrence of LLS and rust were significantly and positively correlated (Table 2). Pod yield per plant, number of pods per plant and pod yield (kg/ha) showed significantly negative correlation with the severity of LLS and rust. Pod yield (kg/ha) recorded a significantly positive correlation with other productivity traits. Early backcross progenies possessed high pod constriction and reticulation (Varshakumari et al., 2014). In contrast, majority of the advanced ILs across the crosses showed moderate pod constriction and moderate pod reticulation along with slight pod beak, indicating that the ILs had acceptable pod features in spite of enhanced resistance to LLS and rust resistance. This observation could be significant considering the strong linkage between disease resistance and poor pod features as reported earlier (Singh et al., 1997). An attempt was made to select the ILs that were superior over ICGS 76 and DH 86 for disease resistance as well as productivity traits. Most of the ILs (76 out of 78) which were selected as resistant to LLS and rust in their previous generation ($BC_2F_5$), were resistant in the subsequent generation ($BC_2F_6$) also, indicating their true breeding behavior for resistance. Two ILs from ICGS 76 × ISATGR 278-18 and five from DH 86 × ISATGR 278-18 were selected as superior over respective recurrent parent for pod yield as well as LLS and rust resistance (Table 3). Apart from resistance to LLS and rust, and pod yield (kg/ha), significant superiority for other productivity traits like number of pods per plant, test weight and shelling percentage were also checked among the selected lines. IL 49 (22-3) from ICGS 76 × ISATGR 278-18 exhibited marginal superiority for number of pods per plant, test weight and shelling percentage over ICGS 76. Of the five ILs that were superior to DH 86 for LLS and rust resistance and pod yield (kg/ha), all except IL 8 (8-11), exhibited superiority for test weight over DH 86. ILs 8 (5-7), 8 (8-11) and 9 (11-1) recorded superiority for shelling percentage also. Pods of all the selected seven superior lines showed moderate constriction, reticulation and slight beak (Figure 1), which were in the acceptable category.

An attempt was also made to compare the performance of selected ILs with GPBD 4, an improved variety and national check for late leaf spot and rust resistance with high productivity (Gowda et al., 2002). Three lines [IL 4 (8-2), 12 (8-10) and 5 (5-7) from DH 86 × ISATGR 278-18] were significantly superior to GPBD 4 pod yield (kg/ha). They were comparable with GPBD 4 for resistance to LLS and rust. ILs 4 (8-2) and 12 (8-10) were significantly superior for test weight over GPBD 4.

These superior introgression lines were checked for the type of allele at LLS and rust resistance-linked marker loci (Mondal *et al*., 2012; Sujay *et al*., 2012; Kolekar *et al*., 2015). All the superior lines carried favorable (resistant) allele at majority of the marker loci linked to LLS and rust resistance (Figure 2). These introgression lines with high level of resistance to LLS and rust, and desirable productivity traits along with acceptable pod features can be considered for multi-location trials or as donors in future breeding programmes.

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**Table 1. Mean, range and genetic variability components for late leaf spot and rust resistance and productivity traits among the introgression lines of groundnut**

| Trait | Mean | Range | | Coefficient of variation (%) | | $h^2_{bs}$ | GA | GAM |
|---|---|---|---|---|---|---|---|---|
| | | Min | Max | PCV | GCV | | | |
| LLS | 3.28 | 2.99 | 8.50 | 30.00 | 28.57 | 90.6 | 1.84 | 56.04 |
| Rust | 3.21 | 3.00 | 7.00 | 20.90 | 19.94 | 90.9 | 1.25 | 39.18 |
| PYP | 15.12 | 9.35 | 21.58 | 15.40 | 12.90 | 70.0 | 3.36 | 22.28 |
| NPP | 27.69 | 9.10 | 31.60 | 16.51 | 15.31 | 86.0 | 8.10 | 29.24 |
| PY | 2393.00 | 549.80 | 3881.60 | 29.17 | 27.06 | 86.0 | 1238 | 51.72 |
| TW | 38.00 | 27.50 | 48.50 | 13.07 | 12.12 | 85.9 | 8.79 | 23.14 |
| SP | 66.84 | 30.25 | 76.50 | 11.02 | 10.15 | 84.0 | 12.87 | 19.25 |

LLS: Late leaf spot score; Rust: Rust score; PYP: Pod yield per plant (gm); NPP: Number of pods per plant; PY: Pod yield (kg/ha); TW: Test weight (gm); SP: Shelling percentage; PCV: Phenotypic coefficient of variation; GCV: Genotypic coefficient of variation; $h^2_{bs}$: Heritability in broad sense; GA: Genetic advance and GAM: Genetic advance over mean (%)

**Table 2. Association between late leaf spot and rust resistance and productivity traits among the introgression lines of groundnut**

| | LLS | RUST | PYP | NPP | TW | SP |
|---|---|---|---|---|---|---|
| LLS | 1 | | | | | |
| RUST | 0.8096$^{**}$ | 1 | | | | |
| PYP | -0.2797$^{**}$ | -0.4133** | 1 | | | |
| NPP | -0.4913$^{**}$ | -0.5840$^{**}$ | 0.2104** | 1 | | |
| TW | 0.2765$^{**}$ | 0.1918* | 0.0032 | -0.3342$^{**}$ | 1 | |
| SP | -0.0614 | -0.1317 | 0.0672 | 0.0919 | 0.2237** | 1 |
| PY | -0.2547$^{**}$ | -0.3526$^{**}$ | 0.3735$^{**}$ | 0.1755* | 0.4094$^{**}$ | 0.3405$^{**}$ |

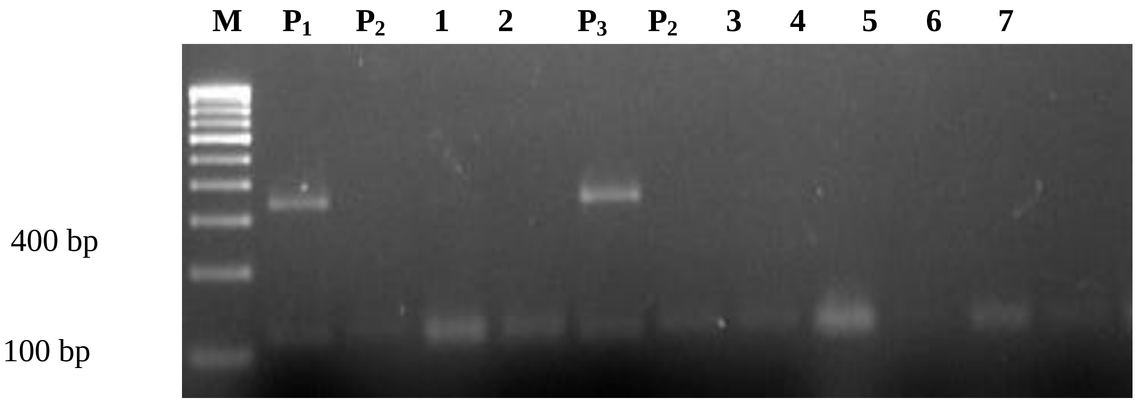
*, **: Significant at 5% and 1% level of probability, respectively. LLS: Late leaf spot; PYP: Pod yield per plant; NPP: Number of pods per plant; PY: Pod yield (kg/ha); TW: Test weight (gm); and SP: Shelling percentage

**Table 3. Performance of the superior introgression lines of groundnut**

| Cross | Genotypes | PY | NPP | TW | SP | LLS | RUST | PC | PR | PB |
|---|---|---|---|---|---|---|---|---|---|---|
| ICGS 76 × ISATGR 278-18 | 41 (19-4) | 3306.7 | 25.2 | 31.8 | 72.0 | 3.0 | 3.0 | 5 | 5 | 3 |
| | 49 (22-3) | 3568.3 | 31.4 | 43.5 | 65.0 | 3.0 | 3.0 | 5 | 5 | 3 |
| DH 86 × ISATGR 278-18 | 4 (8-2) | 3881.6 | 23.6 | 48.5 | 56.5 | 3.0 | 3.0 | 5 | 5 | 3 |
| | 12 (8-10) | 3720.0 | 25.6 | 47.5 | 63.5 | 3.0 | 3.0 | 5 | 5 | 3 |
| | 5 (5-7) | 3653.3 | 30.1 | 42.0 | 68.0 | 3.0 | 3.0 | 5 | 5 | 3 |
| | 8 (8-11) | 3456.7 | 26.0 | 41.0 | 76.5 | 3.0 | 3.5 | 5 | 5 | 3 |
| | 9 (11-1) | 3376.7 | 27.7 | 45.0 | 70.5 | 3.0 | 3.5 | 5 | 5 | 3 |
| Checks | ICGS 76 | 2410.0 | 28.1 | 40.5 | 62.5 | 5.0 | 5.5 | 5 | 5 | 3 |
| | DH 86 | 2603.4 | 26.0 | 42.0 | 66.5 | 7.0 | 4.0 | 5 | 5 | 5 |
| | GPBD 4 | 2872.9 | 17.4 | 43.5 | 74.2 | 3.0 | 3.0 | 5 | 5 | 3 |
| | CD (5%) | 733.4 | 4.8 | 5.2 | 8.0 | 0.8 | 0.5 | - | - | - |
| | CV (%) | 15.4 | 8.7 | 6.9 | 6.0 | 12.9 | 8.8 | - | - | - |

PY: Pod yield (kg/ha); NPP: Number of pods per plant; TW: Test weight (gm); SP: Shelling percentage; LLS: Late leaf spot score and RUST: Rust score; PC: Pod constriction (5: Medium); PR: Pod reticulation (5: Medium) and PB: Pod beak (3: Slight)

**Figure 1. Pod and kernel features of a few superior introgression lines**



M: 100 bp DNA ladder; $P_1$: ICGS 76; $P_2$: ISATGR 278-18; $P_3$: DH 86; 1: 41 (19-4); 2: 49 (22-3); 3: 4 (8-2); 4: 12 (8-10); 5: 5 (5-7); 6: 8 (8-11) and 7: 9 (11-1)

**Figure 2. Allele pattern at a LLS and rust resistance-linked marker locus (AhTE498) among the superior introgression lines**