

Introgression of Foliar Disease Resistance into Cultivated species by Backcross Breeding from Synthetic Amphidiploids in Groundnut (*Arachis hypogaea* L.)

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ABSTRACT: In order to incorporate disease resistance from wild species into cultivated groundnut, the cultivated variety ICGS 76 was crossed with a synthetic amphidiploid, ISATGR 278-18. This resulted in the development of a BC₂F₄ introgression line population, ICGS 76 × ISATGR 278-18, which segregated for late leaf spot and rust, two significant foliar fungal diseases that cause significant yield losses to the crop. With the exception of the number of major branches, phenotypic data demonstrated strong heritability for resistance to both diseases, agronomic, and productivity traits, as well as significant variance for genotype, environment, and genotype × environment interaction.

Keywords: Groundnut, Synthetic amphidiploids, introgression line(ILs), Rust, Late Leaf Spot (LLS).

INTRODUCTION

Groundnuts (*Arachis hypogaea* L.) are grown in tropical and subtropical regions of India and around the world, are an important oilseed and legume crop. 45.95 million tons of pods were produced by it in 2022 on 28.5 million hectares of cultivation (FAOSTAT. 2022). Nigeria and the United States are the next two biggest producers of peanuts, behind China and India. Groundnuts are high in calories and comprise 48–50% oil, 20–25% easily digested protein, and 10–25% carbohydrates. Products made from groundnut protein are being explored in peanut milk to provide children's meals more nutrients. Calcium, magnesium, Vitamin A, E, phosphorus, iron, thiamine, niacin, riboflavin, zinc and potassium are among the many nutrients found in peanuts (Savage and Keenan 1994). In addition to being consumed directly, groundnuts are utilized in the confectionary industry and also as a protein source for animals.

Despite India being the world's largest grower of groundnuts, the productivity is incredibly poor (1.00 t ha⁻¹) in contrast to (4.50 t ha⁻¹) with China. This is explained by crop damage brought on stresses viz., biotic and abiotic. The main foliar diseases, late leaf spot and rust are the most significant biotic stresses economically and can lessen groundnut yield by up to 50–70%. For farmers with limited resources, controlling the disease with fungicide treatment is not a

practical solution because it may contaminate groundwater and other environmental resources, increasing production costs and posing additional risks. Therefore, the creation of resistant cultivars is thought to be the most effective method of controlling these foliar diseases (Subramanyam *et al.*, 1984; Mallikarjuna *et al.*, 2010).

Because of their great genetic diversity, wild diploid *Arachis* species are valuable genes reservoirs that have been effectively used to increase resistance to disease and productivity. Nevertheless, the utilization of wild diploid cousins in peanut breeding efforts has been hindered by the genetic barrier that results from ploidy differences between cultivated and wild peanut species. In this sense, creating synthetic amphidiploids is a practical way to get over the genetic barrier. By crossing several diploid species and then treating them with colchicine to duplicate their genomes, many synthetic amphidiploids have been created (Stalker *et al.*, 1991; Simpson, 2000; Holbrook and Stalker 2010; Mallikarjuna *et al.*, 2004a and b).

MATERIAL AND METHODS

Phenotyping: Recurrent parents of cultivated groundnut varieties sensitive to foliar diseases were crossed with donors who were resistant synthetic amphidiploids to create BC₂F₄ introgression lines. The F₁ offspring were then backcrossed with the recurrent

parents.

A hybrid between TMV10 × CHICO produced the enhanced, high-yielding virginia bunch variety ICGS 76 (Mallikarjuna and Hoisington 2009). It contains 45% oil and oleic/linoleic acid ratios are high, as is its shelling turnover (73%). It shows resilience against bud necrosis disease and a strong recovery from mid-season dryness. However prone to late leaf spot and rust diseases. Synthetic amphidiploid, ISATGR 278-18 ($2n=4x=40$), was created by doubling the F_1 chromosome obtained from crossing two wild diploid species, A genome donor *A. duranensis* and B genome donor *A. batizocoi*. To create F_1 seeds, the cross ICGS 76 × ISATGR 278-18 was conducted. To obtain BC_1F_1 s, hybrid were backcrossed to the corresponding recipient parent (Burow *et al.*, 2001).

In a randomized block design, total of 184 introgression lines in BC_2F_4 were grown out in two replications. Each genotype was planted in a 2.5-meter bed with a 30-centimeter intrarow and a 10-centimeter interplant spacing. Its resistance to late leaf spot rust and its agronomic and productive attributes, were assessed in the field over the course of three seasons: kharif 2011, summer 2012, and kharif 2012. The following traits viz., plant height (cm), leaf length, leaf width, number of branches, and total pod weight (g), shelling percentage, 100-seed weight (g), rust (70 DAS), (80 DAS), (90 DAS) and late leaf spot (70 DAS), (80 DAS), and (90 DAS) were observed in two plants were randomly selected from both replications in each of the ILS and its parents.

RESULTS

For the LLS, rust, agronomic and productivity attributes for the population of ILSs, the data gathered from each environment separately and the combined data across environments were subjected to an analysis of variance. Among the ILSs of ICGS 76 × ISATGR 278-18, variances due to genotypes were significant in all three seasons for rust and LLS at all three phases (70, 80, and 90 DAS). Significant differences in the genotypes of the ILSs population were found by doing an analysis of variance for eight agronomic characteristics over the course of the three seasons. With the exception of the number of primary branches, resistance to rust and LLS, agronomic and productive characteristics were found to be significantly difference among genotypes, seasons and genotype × season in pooled analysis of variance (Table 1).

Components of variation for introgression line population: For each trait under study, the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H^2), and genetic advance as a percentage mean (GAM) were used to evaluate the kind and amount of variation.

Rust and late leaf spot: In every season except rust in kharif 2011, there were moderate changes in both the phenotypic and genotypic coefficients of variation for resistance to rust and LLS. All seasons showed strong heritability and genetic advance for both diseases, demonstrating the high heritable nature of the genetic variance. When estimating across the seasons, the

components of variability were, however, comparatively smaller for all the attributes (Table 2).

Agronomic traits: All agronomic traits, with the exception of leaf attributes, generally showed a moderate to high phenotypic and genotypic coefficient of variation. In kharif 2011, heritability and genetic advancement were high for every attribute; however, in kharif 2012 and summer 2012, they were only high for plant height and the number of secondary branches, and moderate for leaf length, leaf width, and the number of main branches (Table 2).

Productivity traits: With the exception of shelling percentage in the summer of 2012, all productivity traits showed substantial magnitude variation throughout the three seasons, according to the phenotypic and genotypic coefficients of variation. All of the variables showed excellent heritability and genetic advance throughout the year, with the exception of the shelling % in kharif 2011 and kharif 2012. For every attribute, there was a decrease in the magnitude of components of variation when measured over the seasons (Table 2).

Frequency distribution, mean and range of introgression population: For the pooled mean data of all three seasons for all disease and other traits the frequency distribution among the ILSs in comparison to parents was provided in the figures (Fig. 1). The Y-axis shows the genotype frequencies for the corresponding traits, and the X-axis shows the traits that are split into equal class intervals. Table 2 displayed the average and range of the ILSs for productivity, agronomic, disease resistance traits.

Rust and late leafspot: Rust and late leaf spot: In all three seasons, the most of the ILSs lies within the parents' range, and the frequency distribution for rust followed a normal distribution. The distribution of late leaf spot was bimodal and biased in favour of susceptibility. While the most of the ILSs lies within their parents' range in all seasons, several of the ILSs showed greater susceptibility than their inferior parent (Fig. 1).

DISCUSSION

Phenotypic evaluation of ILSs: During kharif 2011, summer 2012, and kharif 2012, three years of phenotyping on diseases (rust and LLS), morphological, and productivity features were conducted.

Resistance to rust and late leaf spot: Resistance to late leaf spot and rust: An ANOVA analysis showed that introgression line populations differed significantly in all three seasons for rust and LLS based on genotypes. Notable variations were also discovered across genotypes, season, and genotype × season, suggesting the necessity of screening in various settings. Khedikar (2008); Sarvamangala (2009) also noted significant differences due to $G \times E$ among the RILs of TAG 24 × GPBD 4 and TG 26 × GPBD 4 for LLS and rust, respectively.

The distribution pattern of ILSs for LLS was bimodal, meaning that while most ILSs fell within the parental range, some ILSs showed higher susceptibility

(transgressive segregation) than the susceptible parent ICGS 76. This suggests that susceptibility may be the result of simple inheritance with few genes, and that both parents may have contributed to the susceptibility. The emergence of individuals in populations that deviate from their parental phenotypes is known as a transgressive segregation. Because of the de novo mutation caused by the wide cross itself and the complementary action of genes from the two parental species, it is frequently seen in the progeny resulting from interspecific matings (Vega and Frey 1980; Rick and Smith 1953).

The PCV and GCV components of variation showed significant variation for the LLS throughout all seasons. Furthermore, the highly heritable nature of the variation for LLS in populations was revealed by high heritability and GAM. Nevertheless, because of the strong $G \times E$ interactions, the components of variability were comparatively smaller when evaluated throughout the seasons. Despite a large $G \times E$ interaction, the disease scores for the LLS showed a strong correlation between seasons at a specific stage, indicating the consistency of disease reactions in the individual genotype.

The distribution of ILs for rust resistance was typical, suggesting that inheritance is complex and that only one parent may have contributed resistance. There was significant variance for the rust in all seasons, according to the components of variation, PCV and GCV. Furthermore, the variance for rust in the populations was highly heritable, as demonstrated by high heritability and GAM. Due to substantial $G \times E$

interactions, the variability components for rust were comparatively lower when calculated across seasons. Despite a large $G \times E$ interaction, the disease scores for rust showed a strong correlation between seasons in a specific stage, indicating the constancy of disease reactions in the individual genotype.

Agronomic and productivity traits. With the exception of the number of primary branches, which suggests the need to screen in different seasons/locations, the analysis of variance for the ILs of ICGS 76 $\times$ ISATGR 278-18 revealed significant variations among genotypes, seasons, and genotype $\times$ season for all the traits. With the exception of leaf characteristics, all of the traits had moderate to high levels of variation as indicated by GCV and PCV. With the exception of leaf length and leaf breadth, which were found to be low to moderately heritable, higher heritability and GAM suggested more heritable variation for the majority of these characteristics.

Pooled analysis showed decreased heritable variation compared to season-wise estimates, indicating that $G \times E$ interaction predominates for the populations. As a result, phenotypic selection will require more work to enhance, and markers should increase selection efficiency. The majority of the productivity and agronomic trait frequency distributions were normal, suggesting quantitative inheritance. For leaf length, leaf breadth, total pod weight, and test weight, transgressive segregants were seen in both directions, indicating the contribution of advantageous alleles from both parents.

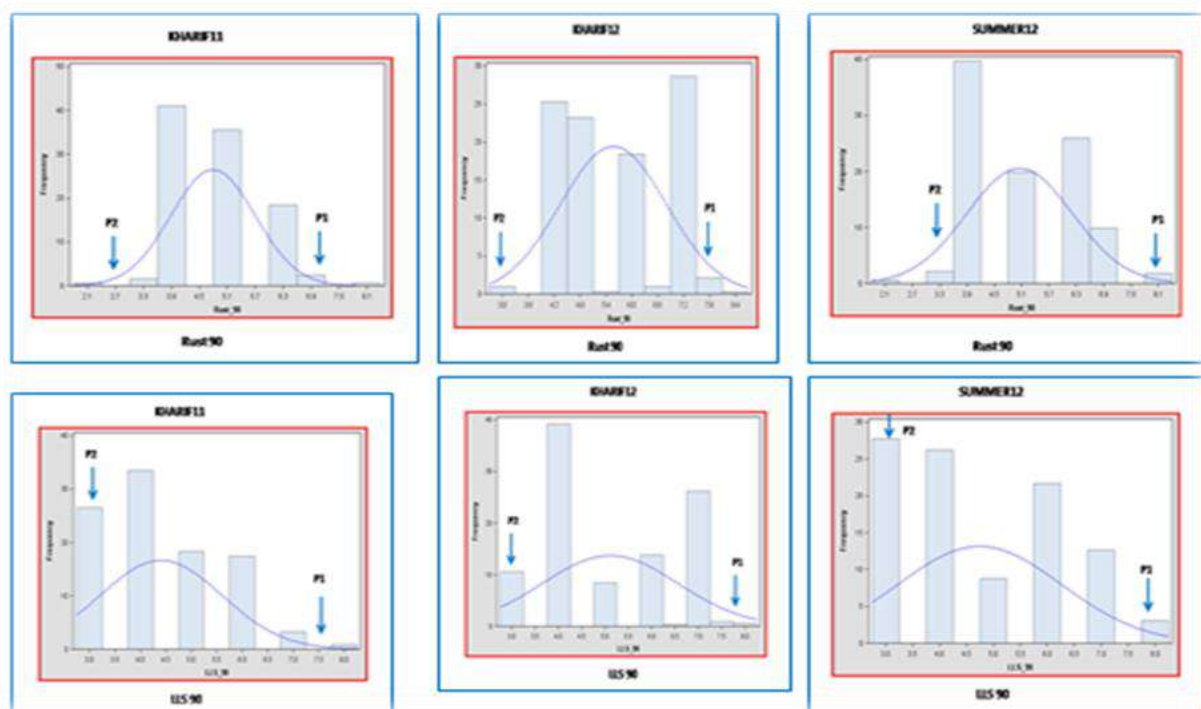


Fig. 1. Frequency distribution for rust and LLS in ICGS 76 $\times$ ISATGR 278-18 introgression population.

Table 1: Pooled ANOVA for agronomic, productivity and disease resistance traits in ICGS 76 × ISATGR 278-18 introgression population.

Source of variation	D.F.	F value									
		PLHT	LL	LW	NOPB	NOSB	TPW (g)	TW (g)	SP (%)	Rust90	LLS90
Season	2	33.21**	74.44**	4.59**	0.39	734.71**	14895.8**	9617.66**	603.33**	530.51**	393.48**
Replication × Season	3	87.53	5.95	29.38	12.80	33.53	17.28	127.23	10.93	6.19	18.63
Genotype	165	40.47**	13.26**	6.97**	7.51**	42.99**	184.85**	15.44**	3.23**	47.09**	65.96**
Season × Genotype	330	4.02**	2.48**	1.85**	2.34**	11.84**	92.82**	12.46**	2.19**	6.79**	8.79**
Pr > F		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
CV		14.46	8.11	11.21	18.33	15.86	7.47	10.54	16.91	6.70	7.84
S.E _d		2.47	0.36	0.249	1.09	1.50	13.37	2.74	12.49	0.34	0.3696

*, **: Significant at 5% and 1% level of probability respectively

PLHT- Plant height

Sp-Shelling per cent

LL-Leaf length

Rust 90- Rust score after 90 days of sowing

LW-Leaf width

LLS 90- late leaf spot score after 90 days of sowing

NOPB-Number of primary branches

NOSB-Number of secondary branches

TPW-Total pod weight (g)

TW-Test weight or 100-seed weight

Table 2: Mean, range and genetic variability components for agronomic, productivity and disease traits in ICGS 76 × ISATGR 278-18 introgression population.

Traits	MEAN	Range	PCV	GCV	h ² b.s %	GAM
Kharif 2012						
PLHT	17.57	11.75-24.88	27.12	21.61	65.70	41.64
LL	4.42	3.35-6.53	7.79	4.04	58.31	18.64
LW	2.26	1.43-3.30	5.43	4.32	39.00	12.41
NOPB	6.06	3.75- 13.25	39.46	14.52	53.79	18.21
NOSB	11.05	1.25- 31.50	28.36	24.75	63.21	47.08
TPW(g)	123.59	20.00-460.12	47.82	46.33	78.42	22.58
TW(g)	33.56	8.75-45.00	42.34	41.41	72.81	21.98
Sp%	65.52	15.50- 83.00	34.24	22.64	53.94	15.35
Rust90	5.56	3.0-8.0	27.39	25.93	87.26	44.34
LLS90	5.10	3.0-7.5	41.49	39.68	87.77	57.31
Kharif 2011						
PLHT	18.42	10.03-31.75	33.55	33.12	69.35	47.19
LL	4.69	2.35-8.25	9.75	8.20	64.04	33.12
LW	2.31	1.26-3.88	6.90	6.34	65.78	34.04
NOPB	6.02	3.0- 13.0	59.39	49.76	71.17	58.89
NOSB	7.89	1.0- 25.5	41.61	40.97	69.22	24.81
TPW(g)	259.01	20.00-667.50	67.49	56.63	79.50	21.89
TW(g)	14.78	0.85- 43.83	53.91	53.77	79.87	22.40
Sp%	24.60	1.75-73.50	32.35	28.26	56.75	10.05
Rust90	4.79	2.0-8.0	17.00	14.52	82.13	35.67
LLS90	4.40	3.0-8.0	32.34	29.71	75.77	53.36
Summer 2012						
PLHT	17.20	10.50-25.13	28.07	22.69	67.41	44.27
LL	4.42	2.50-6.48	8.11	4.43	30.68	19.68
LW	2.27	1.45-3.28	5.17	4.20	37.61	11.67
NOPB	5.98	3.25-12.75	32.72	16.59	55.95	10.75
NOSB	11.84	1.00-31.00	30.45	28.16	66.09	30.02
TPW(g)	125.04	25.00-217.0	65.81	55.40	71.41	43.12
TW(g)	34.45	8.75-46.8	48.71	32.95	73.25	25.86
Sp%	54.62	15.50-78.9	19.73	10.16	74.90	37.83
Rust90	5.05	2.0-8.0	26.89	23.83	73.96	44.56
LLS90	4.74	3.0-8.0	48.49	44.10	85.26	62.62
Across seasons						
PLHT	17.73	10.76-27.25	24.23	13.60	63.88	41.36
LL	4.51	2.73-7.08	6.11	5.61	53.37	19.95
LW	2.28	1.38-3.48	3.28	2.80	33.61	20.59
NOPB	6.02	3.33-13	22.85	19.46	46.61	20.69
NOSB	10.26	1.08-29.33	18.99	18.57	44.26	28.38
TPW(g)	169.21	21.66-448.20	24.84	15.74	43.13	21.96
TW(g)	27.59	6.11-45.21	37.41	28.42	18.48	4.98
Sp%	48.24	10.91-78.46	11.77	24.57	12.95	4.20
Rust90	5.13	2.33-8.0	20.13	22.10	52.37	25.01
LLS90	4.74	3.0-7.83	28.93	30.32	74.21	43.74

CONCLUSIONS

The introgression of foliar disease resistance through crossing cultivated and synthetic amphidiploids in groundnut shows promise for enhancing crop resilience. This approach combines the desirable traits of both parental lines, potentially resulting in improved groundnut varieties with heightened resistance to foliar diseases, contributing to sustainable agriculture and increased yields. Further research and field trials are crucial to validate and optimize the outcomes of this introgression strategy.

FUTURE SCOPE

Investigating the genetic basis of foliar disease resistance in both cultivated and synthetic amphidiploid varieties to identify specific genes or loci associated with resistance. To validate the effectiveness of introgression in conferring resistance to foliar diseases, ensuring robust and consistent results can be conducted thorough field trials and laboratory assessments. Molecular markers can be utilized to track and verify the introgression of disease resistance genes in subsequent generations, aiding in the selection of superior lines.

Conflict of interest. None.

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