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RESEARCH

Harnessing Genetic Diversity of Wild *Arachis* Species for Genetic Enhancement of Cultivated Peanut

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ABSTRACT

Peanut (*Arachis hypogaea* L.) is an important self-pollinating tetraploid (AABB, $2n = 4x = 40$) legume grown for the high-quality edible oil and easily digestible protein in its seeds. Enormous genetic variability is present in the genus *Arachis* containing 79 wild species and cultivated peanut. Wild species offer significant variability, particularly for biotic and abiotic stresses, and can be used to develop cultivars with enhanced levels of resistance to key stresses. However, utilization of these species requires use of ploidy manipulations, bridge crosses, and embryo or ovule rescue. For efficient use of diploid wild species from section *Arachis*, several synthetics (amphidiploids and autotetraploids) have been developed using A- and B-genome accessions with high levels of resistance to multiple stresses. These synthetics are used in crossing programs with cultigens to develop prebreeding populations and introgression lines (ILs) with high frequency of useful genes and alleles into good agronomic backgrounds. Evaluation of two such populations derived from ICGV 91114 × ISATGR 121250 (a synthetic derived from *A. duranensis* Krapov. & W.C. Greg. × *A. ipaensis* Krapov. & W.C. Greg.) and ICGV 87846 × ISATGR 265-5 (*A. kempf-mercadoi* W.C. Greg. & C.E. Simpson × *A. hoehnei* Krapov. & W.C. Greg.) resulted in the identification of ILs with high levels of late leaf spot (LLS) and rust resistance and significant genetic variability for morphoagronomic traits. Genotyping of these ILs with markers linked to rust and LLS resistance provided evidence that introgression of possible novel alleles and resistance sources from different wild species other than the commonly used *A. cardenasii* Krapov. & W.C. Greg. will be beneficial for peanut improvement.

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Abbreviations: DAS, days after sowing; g × e, genotype × environment; IL, introgression line; LLS, late leaf spot; RBD, randomized block design; REML, residual maximum likelihood.

Peanut or groundnut (*Arachis hypogaea* L.), an important self-pollinating tetraploid (AABB, $2n = 4x = 40$) oilseed crop, is grown in more than 108 countries representing tropical, subtropical and warm temperate regions of the world, extending from 40° N to 40° S. It ranks sixth among the oilseed crops and is cultivated on 25.7 million ha area, with a total production of 42.4 million t and average productivity of 1.65 t ha^{-1} globally (FAO, 2014). Peanut is mainly cultivated for its seeds, which are rich in oil, protein, minerals, and vitamins and are consumed in a variety of forms. About two-thirds of global produced peanut is crushed for extracting vegetable oil, whereas the remaining one-third is used in the form of edible products. Peanut cake obtained after oil extraction is used as protein-rich meal for livestock or for making other food products.

The peanut production is adversely affected by biotic stresses such as diseases [rust (*Puccinia arachidis* Speg.), early leaf spot (*Cercospora arachidicola* S. Hori), late leaf spot [LLS; *Phaeoisariopsis personata* (Berk. & M.A. Curtis) Arx], *peanut bud necrosis virus*, rosette disease, and bacterial wilt (*Pseudomonas solanacearum* (Smith) Smith)], insect pests [leaf miner (*Aproaerema modicella* Deventer), tobacco caterpillar/tobacco armyworm (*Spodoptera litura* Fab.), cotton leafworm (*Spodoptera littoralis* Boisduval), termites (*Microtermes* spp., *Odontotermes* spp., *Macrotermes* spp., *Ancistrotermes latinotus* Holgren), corn earworm (*Helicoverpa zea* Boddie), lesser cornstalk borer (*Elasmopalpus lignosellus* Zeller) and southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber)], abiotic stresses (drought,

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salinity, and high temperature), and aflatoxin contamination. Among diseases, foliar fungal diseases such as LLS and rust are widespread and major constraints for production, resulting in yield losses and poor seed quality (Subrahmanyam et al., 1980; McDonald et al., 1985; Miller et al., 1990; Grichar et al., 1998). The severity of other diseases varies across regions. For instance, *peanut bud necrosis virus* is important in South Asia, rosette disease in Africa, and bacterial wilt in Southeast Asia. Both LLS and rust, due to chlorotic lesions, result in the reduction of the green leaf area available for photosynthesis and stimulate leaflet abscission leading to extensive defoliation (McDonald et al., 1985), thereby affecting the seed quantity, quality, and fodder value of the plants (Gupta et al., 1987).

Although chemical control measures are available to control these diseases, they increase the cost of production and thus are not economical to the smallholder farmers. Host-plant resistance to develop resistant cultivars is the most effective and economic way to minimize the yield losses due to these diseases. Breeding efforts have led to the development of high-yielding peanut cultivars with moderate levels of resistance to LLS. However, developing new high-yielding cultivars with high levels of LLS resistance and acceptable market traits remains a focus for peanut improvement globally. Although sources of resistance have been identified for foliar diseases, complete or high levels of resistance to these diseases are not available in cultivated genepool (Waliyar et al., 1993; Singh et al., 1997; Fávero et al., 2009; Sudini et al., 2015). The genus *Arachis*, containing 80 species classified into nine sections, provides sufficient genetic variability and new and diverse sources of resistance genes, such as immune to highly resistance sources for LLS and rust (Subrahmanyam et al., 1985; Stalker and Moss, 1987; Pande and Rao, 2001; Fávero et al., 2009; Michelotto et al., 2015) for peanut improvement. A few interspecific derivatives with high levels of LLS resistance were developed, most of which carry LLS resistance derived from *A. cardenasii* Krapov. & W.C. Greg. Keeping in mind the frequent breakdown of foliar disease resistance, such as the breakdown of the *Yr17* gene conferring resistance against yellow rust (caused by *Puccinia striiformis* f. sp. *tritici*) in wheat (*Triticum aestivum* L.) cultivars (Bayles et al., 2000; El-Jarroudi et al., 2011), it would be necessary to include other species as donors to broaden the genetic base of foliar disease resistance in peanut.

In the genus *Arachis* containing cultivated peanut, its tetraploid progenitor *A. monticola* Krapov. & Rigoni, and 29 diploid wild *Arachis* species (including two diploid progenitor species, *A. ipaensis* Krapov. & W.C. Greg. and *A. duranensis* Krapov. & W.C. Greg.) (Krapovickas and Gregory, 1994; Valls and Simpson, 2005) is of particular interest to peanut breeders, as the wild species in this section are crossable with cultivated peanut. However, frequent use of these wild species for peanut improvement is hindered due to the differences in ploidy levels. Such crossing programs involving tetraploid cultivated peanut and diploid wild *Arachis* species would require several generations of selfing in the segregating material to select desirable tetraploid recombinants. To overcome these difficulties, synthetic peanuts have been developed by doubling the chromosome number of the hybrid derived from two diploid ($2n = 2x = 20$) wild *Arachis* species (Mallikarjuna et al., 2011). These tetraploid synthetic peanuts ($2n = 4x = 40$) can be used freely in the crossing programs to transfer useful genes and alleles from wild species into cultivated genetic backgrounds. In previous studies, the development and use of synthetic amphidiploids such as TxAG-6 (Simpson et al., 1993) in breeding programs has resulted in the release of two cultivars, Coan (Simpson and Starr 2001) and NemaTAM (Simpson et al., 2003), carrying genes for root-knot nematode (*Meloidogyne arenaria*) resistance from *A. cardenasii* (Simpson and Starr, 2001, Simpson et al., 2003), as well as led to the development of backcross progenies having high yield and higher seed weight (up to 95 g) (Upadhyaya 2008). Use of the synthetics derived from different wild *Arachis* species having resistance or tolerance to important biotic and abiotic stresses would not only help in diversifying the sources of resistance but would also enable the pyramiding of resistance genes from different species into a common genetic background to develop new cultivars with enhanced levels of resistance or tolerance to stresses.

The present investigation was undertaken to use synthetics derived from LLS- and rust-resistant diploid wild *Arachis* accessions for introgressing high-level resistance into peanut cultivars. The objectives of the study were to develop introgression lines (ILs) having high levels of foliar disease resistance and to diversify the sources of resistance in cultivated backgrounds for peanut improvement. The present study holds a great significance in enhancing and diversifying the sources of LLS and rust resistance using synthetics derived from different diploid wild *Arachis* species. Such an attempt provides sufficient useful genetic variability in peanut breeding pipelines to develop new cultivars with a broad genetic base and enhanced levels of foliar disease resistance, especially for diversifying LLS resistance derived from different wild species other than the commonly used *A. cardenasii*.

MATERIALS AND METHODS

Plant Material and Population Development

Two peanut cultivars, ICGV 91114 and ICGV 87846, and two synthetic tetraploids were used in this study. The amphidiploid ISATGR 121250 (AABB, $2n = 4x = 40$) (Mallikarjuna et al., 2011) was derived from a cross between LLS- and rust-resistant A-genome species *A. duranensis* (ICG 8123) and B-genome species *A. ipaensis* (ICG 8206) (Subrahmanyam et al., 1985; Pande and Rao, 2001). The autotetraploid ISATGR 265-5 (AAAA;

$2n = 4x = 40$) was derived from a cross between two LLS- and rust-resistant A-genome species, *A. kempf-mercadoi* (ICG 8164) and *A. hoehnei* (ICG 8190) (Subrahmanyam et al., 1985). ICGV 91114 is a Spanish bunch-type (*A. hypogaea* ssp. *fastigiata* var. *vulgaris*), widely adapted, high-yielding, drought-tolerant, LLS- and rust-susceptible, early-maturing (90–95 d) peanut cultivar. ICGV 87846 is a Virginia bunch-type (*A. hypogaea* ssp. *hypogaea* var. *hypogaea*), drought-tolerant, moderately resistant to LLS (score 5.0 at 90 d after sowing [DAS]) and rust (score 4.0 at 90 DAS), dual-purpose cultivar having high pod yield and released as Co 6 in Tamil Nadu, India. Using these two peanut cultivars as recipients and synthetics as donors for LLS and rust resistance, two F_1 crosses, ICGV 91114 × ISATGR 121250 and ICGV 87846 × ISATGR 265-5, were generated. Both F_1 crosses were backcrossed twice with respective cultivated recipient parents to generate two advanced backcross (BC_2F_1) populations, followed by selfing for three seasons to generate BC_2F_4 populations. The BC_2F_4 population derived from ICGV 91114 × ISATGR 121250 containing 437 ILs was designated as Pop I, and the population from ICGV 87846 × ISATGR 265-5 containing 598 ILs was designated as Pop II.

Evaluation for LLS, Rust, and Yield-Related Traits

Pop I containing 437 ILs, cultivated parent ICGV 91114, Pop II containing 598 ILs, and cultivated parent ICGV 87846 were screened in a disease screening nursery in an Alfisols (Alfisol-Patancheru soil series: Udic Rhodustolf) precision field under an infector row system during the 2014 rainy season at ICRISAT, Patancheru, India. The disease screening experiments were conducted in augmented designs. Six peanut cultivars—GPBD 4, ICGS 76, J 11, JL 24, Tifrunner, and ICG 1311—were used as checks, and each check was repeated three times after 25 entries in Pop I and 34 entries in Pop II. Of these, the GPBD 4 is LLS and rust resistant and is used as a national resistance check for both the diseases in field trials of the All India Coordinated Research Projects (AICRP) on peanut. This cultivar was derived from the cross KRG 1 × CS 16 (ICGV 86855) and is a second-cycle derivative of interspecific hybridization between *A. hypogaea* and *A. cardenasii*. ICGS 76 is a high-yielding, medium-duration cultivar with moderate resistance to LLS (score 5.0) and rust (score 4.0); J 11 and JL 24 are high-yielding, short-duration cultivars and both are susceptible to LLS and rust; ICG 1311 is a germplasm line susceptible to LLS and moderately resistant to rust; and Tifrunner is a runner cultivar and is moderately resistant to both LLS and rust (score 5.0). In each population, ILs, cultivated parents, and checks formed the test material. After every five rows of test material, an infector row of susceptible cultivar TMV 2 (national susceptibility check for LLS and rust in field trials of the AICRP on peanut) was planted to ensure uniform spread of disease inoculum. In both populations, the plot size was a single 4-m-long row per genotype in a ridge-furrow system. Row-to-row distance was 60 cm, and plant-to-plant distance within a row was 10 cm. Standard package of practices were adopted to raise a healthy crop that included 60 kg P_2O_5 as basal application, seed treatment with Mancozeb at 2 g kg^{-1} seed before sowing, pre-emergence application of Pendimethalin at 1 kg a.i. ha^{-1}, irrigation soon after planting, gypsum application at 400 kg ha^{-1} at the peak flowering stage, and protection against insect pests throughout the crop growth. Subsequent irrigations were provided as and when needed in the rainy season. At 30 DAS, LLS- and rust-infected potted plants of TMV 2 from greenhouse were placed randomly throughout the infector rows of the experimental plot. Further, artificial inoculation was done at 50 DAS by spraying the test plants and infector rows with a conidial suspension of LLS and urediniospores of rust pathogens at a concentration of 5×10^4 spores mL^{-1} to ensure uniform and heavy disease pressure in the experimental plot. After inoculation, sprinkler irrigation was provided daily, for 30 min in the evening for 30 d, to create a congenial environment for disease development. Observations on diseases score for LLS and rust were recorded on 75 and 90 DAS on each row following a 1-to-9 scale (where 1 = no disease and 9 = 81–100% disease severity), as described by Subrahmanyam et al. (1995). Besides test material, two synthetics and four wild parents were also screened under field as well as greenhouse conditions for confirming LLS and rust resistance. The synthetics and wild *Arachis* parents have small pods, and it is difficult to plant them directly in the field along with cultivated material. Therefore, plants of each synthetic and its wild parent were raised in pots with one plant per pot. For each synthetic and wild parent, five pots were used for screening. These pots were placed at random in rows in the field, along with the test material, in the 2014 rainy season for screening against LLS and rust. Besides field screening, these synthetics and their diploid wild parents were also screened for LLS in the greenhouse in the 2014 rainy season, following similar methodology and two to three artificial inoculations at a weekly interval to ensure very high disease pressure. Observations on disease score in the greenhouse were recorded 75 d after first inoculation following a 1-to-9 scale. The genotypes were classified as resistant (score of 1–3), moderately resistant (score of 4–5), susceptible (score of 6–7), and highly susceptible (score of 8–9) (Sudini et al., 2015).

The ILs in both populations had one or more undesirable traits, such as procumbent growth habit, late flowering and maturity, and small, highly beaked and reticulated pods. Therefore, within LLS- and rust-resistant ILs (score 2.0–3.0), further selection was made to select the plants with the lowest LLS and rust scores coupled with acceptable plant type, such as erect growth habit, early maturity, and acceptable pod traits. Using these criteria, 17 LLS- and rust-resistant ILs with good agronomic backgrounds were selected. Single-plant progenies of these selected 17 ILs were reevaluated to confirm resistance during the 2015 and 2016 rainy seasons in a randomized block design (RBD) with three replications, along with four peanut cultivars, GPBD 4, ICG 1311, J 11, and JL 24, as checks using the infector-row technique. The methodology used in the 2014 rainy season screen was followed to confirm resistance under similar field conditions in both years. The plot size was a two-row plot per genotype in the 2015 rainy and a four-row plot per genotype in the 2016 rainy season. Each row was 4 m long in a ridge-furrow system. The infector row, TMV 2, was repeated after every five rows (Fig. 1). In both seasons, observations on diseases score were recorded on 75 and 90 DAS for each genotype in each replication, following a 1-to-9 scale as described by Subrahmanyam et al. (1995). In the 2015 rainy season, besides disease score, data were also recorded for morphoagronomic traits on five randomly selected competitive plants per genotype per replication. Data on days to first flowering, days to 50% flowering, and growth

Fig. 1. Confirmation of late leaf spot and rust resistance in selected introgression lines and the resistant and susceptible control cultivars using an infector-row technique during the 2016 rainy season at ICRISAT, Patancheru, India.

habit were recorded on plot basis, while branching pattern, number of pods per plant, pod yield per plant, number of seeds per plant, seed yield per plant, 100-seed weight, shelling percentage, pod reticulation, pod constriction, and pod beak were recorded on five selected plants. Morphoagronomic performance of these selected 17 ILs, along with four peanut cultivars (GPBD 4, ICG 1311, J 11, and JL 24) as checks, was reevaluated during the 2015–2016 post-rainy season in RBD with three replications, following the same methodology under similar field conditions.

DNA Isolation and Genotyping with Linked Markers to LLS and Rust Resistance

Fresh leaves from 25-d-old seedlings of the selected 17 ILs were collected, and DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) extraction method (Cuc et al., 2008). After isolation of DNA, its quality and quantity was checked on 0.8% agarose gels, and DNA concentration, followed by normalization to ~5 ng μl^{-1}, was used in genotyping with linked markers. IPAHM103, GM1536, GM2301 and GM2079 for both rust and LLS resistance and Seq8D09, GM1009, GM1573, and GM2032 for LLS resistance were used for genotyping the selected ILs. These markers were used for amplification with polymerase chain reaction (PCR) following conditions mentioned in Khedikar et al. (2010) and Sujay et al. (2012). Genotyping of eight linked markers for LLS and rust resistance was performed on a total 17 selected ILs in the genetic backgrounds of ICGV 91114 (PBGNIL-1, PBGNIL-2, PBGNIL-3, PBGNIL-4, PBGNIL-5, and PBGNIL-6) and ICGV 87846 (PBGNIL-7, PBGNIL-8, PBGNIL-9, PBGNIL-10, PBGNIL-11, PBGNIL-12, PBGNIL-13, PBGNIL-14, PBGNIL-15, PBGNIL-16, and PBGNIL-17). In addition to the above ILs, two cultivated parents (ICGV 91114 and ICGV 87846), two synthetics (ISATGR 121250 and ISATGR 265-5), four diploid wild *Arachis* accessions (ICG 8164, ICG 8190, ICG 8123, and ICG 8206), and four peanut cultivars (J 11, JL 24, ICG 1311, and GPBD 4) were also included in the genotyping panel.

Statistical Analysis

The disease score data for LLS and rust for Pop I and Pop II screened in the 2014 rainy season and the replicate-wise data on disease score of LLS and rust and agronomic traits, such as days to first flowering, days to 50% flowering, number of pods per plant, pod yield per plant, number of seeds per plant, seed yield per plant, 100-seed weight, and shelling percentage, were used for statistical analysis of each environment and season using the residual maximum likelihood (REML) method and considering genotypes as random effects using GenStat software (VSN International, 2015). Variance components due to genotypes (σ^2_g) and their standard errors were determined. Environment-wise best linear unbiased predictors for the genotypes were calculated. The significance of variance components was tested using respective standard errors. For the pooled analysis, seasons were considered as fixed effects. The variances due to genotype (σ^2_g) and genotype × environment (g × e) interaction ($\sigma^2_{g \times e}$) and their standard errors were determined. The significance of environment was assessed using the Wald statistic that asymptotically follows a χ^2 distribution.

RESULTS

Late Leaf Spot Resistance

The REML analysis indicated significant variations ($P \leq 0.05$) for LLS resistance among genotypes in Pop I at 90 DAS and in Pop II at both stages (75 and 90 DAS) (Table 1). In both populations, most of the ILs and all control cultivars had a disease score of ≤3.0 at 75 DAS, whereas the disease score was greater as the plants grew older, and considerable variability was observed at 90 DAS. At 90 DAS in Pop I, only 18 ILs were found resistant (score 2–3) and 260 ILs were found moderately resistant (score 4–5) (Table 2). This was probably due to the increase of inoculum rather than the age of the plants (Sudini et al., 2015). Similarly, in Pop II at 90 DAS, 335 ILs were found resistant (score 2–3) and 238 ILs were

Table 1. Variance components due to peanut genotypes (σ^2_g), and their SEs for late leaf spot (LLS) and rust in Pop I and Pop II, screened during the 2014 rainy season at ICRISAT, Patancheru, India.

		Pop I				Pop II			
Disease	Stage	Range	Mean	σ^2_g	SE	Range	Mean	σ^2_g	SE
	DAS†								
LLS	75	1–4	2.6	0.09	0.055	2–3	2.0	0.02*	0.004
	90	2–8	5.1	0.50*	0.134	2–7	3.4	1.01*	0.105
Rust	75	1–4	2.2	0.16*	0.039	1–3	1.1	0.07*	0.008
	90	2–7	3.6	0.68*	0.235	1–7	1.9	0.51*	0.049

* Significant at the 0.05 probability level.

† DAS, days after sowing.

Table 2. Number of introgression lines (ILs) having late leaf spot (LLS) and rust resistance in Pop I and Pop II, screened during the 2014 rainy season at ICRISAT, Patancheru, India.

Population	No. of ILs screened	Disease	Stage	Resistant	Moderately resistant	Susceptible	Highly susceptible
			DAS†				
Pop I	437	LLS	75	431 (1–3, 2.6)‡	6 (all 4.0)	–	–
			90	18 (2–3, 2.9)	260 (4–5, 4.7)	156 (6–7, 6.1)	3 (all 8.0)
		Rust	75	431 (1– 3, 2.2)	6 (all 4.0)	–	–
			90	240 (2–3, 2.6)	164 (4–5, 4.4)	33 (6–7, 6.2)	–
Pop II	598	LLS	75	598 (2–3, 2.0)	–	–	–
			90	335 (2–3, 2.6)	238 (4–5, 4.3)	25 (6–7, 6.1)	–
		Rust	75	598 (1–3, 1.1)	–	–	–
			90	573 (1–3, 1.8)	24 (4–5, 4.0)	1 (7.0)	–

† DAS, days after sowing.

‡ Range and average disease score given in the parenthesis.

found moderately resistant (score 4–5) (Table 2). Among the control cultivars, GPBD 4 was resistant (score 2.0–3.0), ICGS 76 and Tifrunner were moderately resistant (score 5.0), and the remaining were susceptible (score 6–7). The cultivated parent ICGV 91114 was susceptible (score 6) and ICGV 87846 was moderately resistant (score 5). Synthetics ISATGR 1212 and ISATGR 265-5 and their diploid parents (ICG 8123 and ICG 8206 and ICG 8164 and ICG 8190, respectively) were found resistant for LLS (score 2–3) under field conditions. Under very high disease pressure in the greenhouse, however, ISATGR 265-5 was moderately resistant (score 5.0), whereas its diploid wild parents, ICG 8164 (score 3.0) and ICG 8190 (score 2.0), continued to be LLS resistant. Another synthetic, ISATGR 121250 (score 5.0), and its diploid wild parents, ICG 8123 (score 5.0) and ICG 8206 (score 4.0), were found moderately resistant under greenhouse conditions.

Rust Resistance

For rust resistance, REML analysis indicated significant variation ($P \leq 0.05$) among the genotypes in Pop I and Pop II at both stages (Table 1). In both populations, disease score varied from 1.0 to 4.0 at 75 DAS. At 90 DAS, 240 ILs in Pop I and 573 ILs in Pop II were found resistant (score 1–3), 164 ILs in Pop I and 24 ILs in Pop II were moderately resistant (score 4–5), and the remaining ILs in Pop I and Pop II were susceptible (Table 2). Among the control cultivars, GPBD 4 was resistant (score 2–3); ICGS 76, Tifrunner, and ICG 1311 were found moderately resistant (score 4–5); and the remaining all were susceptible (score 6–7) in both populations. The cultivated parent ICGV 91114 was susceptible (score 6) and ICGV 87846 was found moderately resistant (score 4). Synthetics ISATGR 1212 and ISATGR 265-5 and their diploid parents, ICG 8123 and ICG 8206 and ICG 8164 and ICG 8190, respectively, were highly resistant for rust (score 1–2).

Confirmation of LLS and Rust Resistance

Given the disease score and morphoagronomic traits, such as growth habit and pod traits, six ILs from Pop I and 11 ILs from Pop II possessing low disease score for LLS (score 2–3) and rust (score 1–3) at 90 DAS were selected for rescreening to confirm the resistance. The REML analysis indicated significant variation among genotypes for LLS and rust resistance at both stages in both years separately, as well as in pooled data ($P \leq 0.05$) (Table 3). Significant g × e interactions were observed for LLS and rust. Given the pooled disease score, LLS and rust resistance were confirmed in all of these selected accessions at 75 and 90 DAS, wherein 15 ILs were found resistant for LLS (score 2–3 at both 75 and 90 DAS) and rust (score 1–3 at both 75 and 90 DAS) (Fig. 2), and two ILs (IL PBGNIL-2 and IL PBGNIL-6) were moderately resistant to LLS and rust (Table 4). The best LLS- and rust-resistant

Table 3. Variance components due to genotype (σ^2_g), genotype × environment ($\sigma^2_{g\times e}$) interactions, and their standard errors (SE) for late leaf spot (LLS) and rust in selected introgression lines rescreened during the 2015 and 2016 rainy seasons at ICRISAT, Patancheru, India.

		2015 rainy		2016 rainy		Pooled			
Traits	Stage	σ^2_g	SE	σ^2_g	SE	σ^2_g	SE	$\sigma^2_{g\times e}$	SE
	DAS†								
LLS	75	1.01*	0.330	1.02*	0.346	0.84*	0.304	0.16*	0.072
	90	1.61*	0.535	2.54*	0.825	1.73*	0.616	0.35*	0.134
Rust	75	0.54*	0.182	0.91*	0.297	0.56*	0.210	0.16*	0.063
	90	1.92*	0.612	2.86*	0.916	1.88*	0.684	0.51*	0.169

* Significant at the 0.05 probability level.

† DAS, days after sowing.

control cultivar, GPBD, was also found resistant at 75 and 90 DAS (Table 4) (Fig. 2).

Agronomic Evaluation

The REML analysis indicated significant ($P \leq 0.05$) variations among genotypes for days to first flowering in both seasons separately, as well as in the pooled data (Table 5). In the 2015 rainy season, genotypic variance (σ^2_g) was significant for most of the traits except 100-seed weight and shelling percentage, whereas in the 2015–2016 post-rainy season, σ^2_g was significant for four traits (days to first flowering, days to 50% flowering, number of pods per plant, and 100-seed weight) (Table 5). The Wald statistics indicated a nonsignificant effect of seasons for most of the traits except seed yield per plant, 100-seed weight, and shelling percentage. Significant g × e interactions were observed for most of the traits except 100-seed weight and shelling percentage.

Agronomic performance of these selected LLS- and rust-resistant ILs was compared with the best LLS-resistant control cultivar, GPBD 4. Promising ILs with superior performance over GPBD 4 for various yield-related traits in the 2015 rainy and 2015–2016 post-rainy seasons and across seasons is given in Table 6. Most of the ILs performed better than or at par with the control cultivar. In the 2015 rainy season, seven ILs flowered at par with GPBD 4, of which two ILs (PBGNILs 2 and 4) flowered earlier than GPBD 4 on per-se basis. Five ILs (PBGNILs 7, 11, 15, 1, and 8) were significantly superior (36–43 pods plant^{-1}) to GPBD 4 (26 pods) for pod number, one (PBGNIL-1) for seed number (53 vs. 36 seeds plant^{-1} in GPBD 4), and one (PGNIL-10) for 100-seed weight (51.5 vs. 35 g in

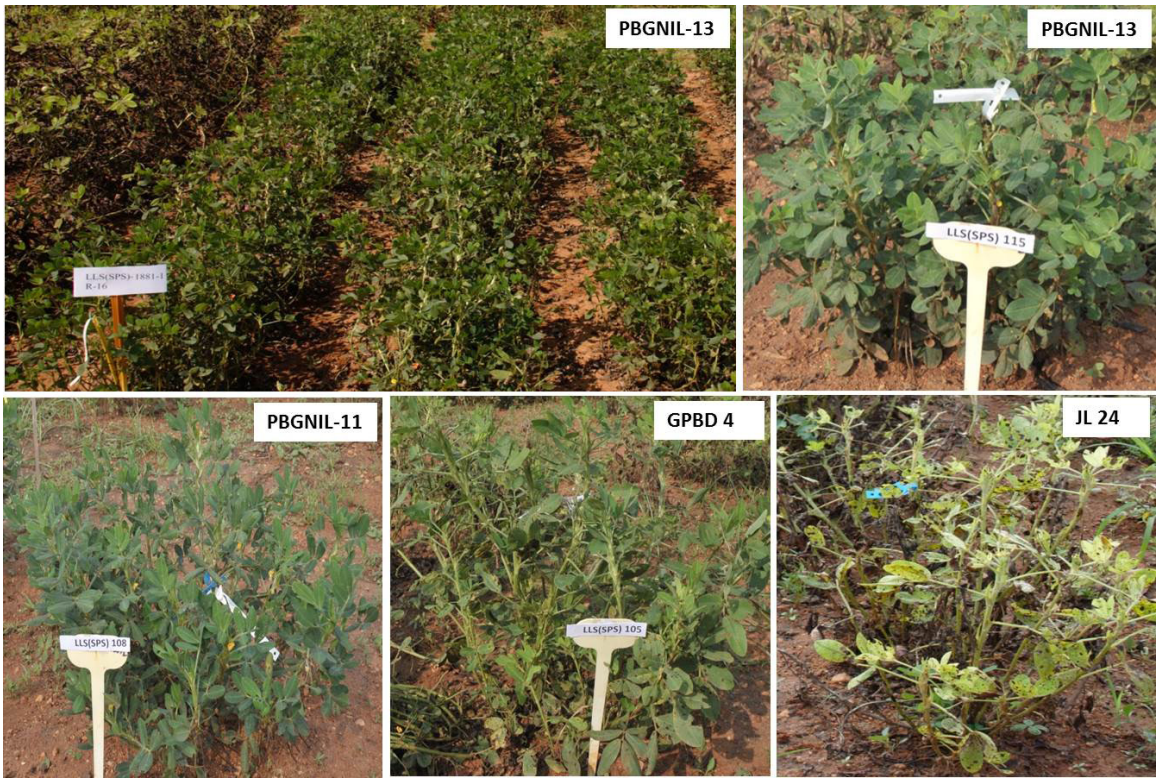

Fig. 2. Symptoms of late leaf spot and rust resistance in the introgression line (top and bottom left) and the resistant (bottom middle) and susceptible control cultivars (bottom right) at ICRISAT, Patancheru, India.

Table 4. Disease reaction and allelic pattern for selected introgression lines and cultivated and wild parents for late leaf spot (LLS) and rust at ICRISAT, Patancheru, India.

Genotypes/ introgression lines	Pedigree information	LLS disease reaction		Rust disease reaction		Linked markers for rust and LLS resistance†		
		LLS 75 DAS‡	LLS 90 DAS	Rust 75 DAS	Rust 90 DAS	GM1536	GM2079	SEQ8D09
		1–9 scale§						
ICGV 91114	72-R Virginia × Chico) F2-P1-B1-NIB1-B1-B1-NIB1-B1-B1-B1-B1-B1) × [(Robut 33-1-18-17) × NC Ac 1705]	3	6	4	6	–	–	–
ISATGR 121250	ICG 8123 × ICG 8206	3	5	1	2	–	–	+
ICG 8123	*A. duranensis*	2	5	1	1			
ICG 8206	*A. ipaensis*	2	5	1	1	–		–
PBGNIL-1	ICGV 91114 × [ICGV 91114 × (ICGV 91114 × 265-5)]	2	3	2	2	+	+	+
PBGNIL-2		4	5	3	4	–	–	–
PBGNIL-3		2	3	2	2	+	+	+
PBGNIL-4		2	3	2	2	+	+	+
PBGNIL-5		2	3	2	2	+	+	+
PBGNIL-6		3	5	2	4	–	+	–
ICGV 87846	CS 9 × [(Robut 33-1 × NC Ac 316) F2-B2-B1-B1-NIB1-B1-B1-B1-B1-B1-B1-B1)]	4	5	3	4	–	–	
ISATGR 265-5	ICG 8164 × ICG 8190	3	5	1	1	–	–	+
ICG 8164	*A. kempf-mercadoi*	2	3	1	1		+	
ICG 8190	*A. hoehnei*	2	2	1	1			+
PBGNIL-7	ICGV 87846 × [ICGV 87846 × (ICGV 87846 × 265-5)]	2	2	1	2	+	+	+
PBGNIL-8		2	2	1	2	+	+	+
PBGNIL-9		2	3	2	2	+	+	+
PBGNIL-10		2	3	1	2	+	+	+
PBGNIL-11		2	2	1	2	+	+	+
PBGNIL-12		2	3	2	2	+	+	+
PBGNIL-13		2	3	2	2	+	+	+
PBGNIL-14		2	3	2	2	+	+	+
PBGNIL-15		2	3	2	2	+	+	+
PBGNIL-16		2	3	1	2	+	+	+
PBGNIL-17		2	3	1	2	+	+	+
GPBD4	KRG 1 × CS 16 (ICGV 86855)	2	3	2	2	+	+	+
J11	Ah 4213 × Ah 4354	5	6	3	6	–	–	–
JL24	Selection from EC 94943 (introduction from Taiwan)	5	7	3	6	–	–	–
ICG 1311	A germplasm line	5	6	3	5	–	–	+

† (–) indicates susceptible allele similar to JL 24, (+) indicates resistance allele similar to GPBD4, and no symbol indicates a different allele than JL 24 and GPBD 4

‡ DAS, days after sowing.

§ Disease reaction score, where 1 = no disease and 9 = 81–100% disease severity.

Table 5. Variance components due to genotypes (σ^2_g), genotype × environment ($\sigma^2_{g \times e}$) interactions and their standard errors (SE) for yield-related traits in the selected introgression lines evaluated during the 2015 rainy and 2015–2016 post-rainy seasons at ICRISAT, Patancheru, India.

Trait	2015 rainy season		2015–2016 post-rainy season		Pooled			
	σ^2_g	SE	σ^2_g	SE	σ^2_g	SE	$\sigma^2_{g \times e}$	SE
Days to first flowering	4.29*	1.434	1.10*	0.528	1.12*	0.537	1.62*	0.401
Days to 50% flowering	7.46*	2.427	1.34*	0.647	1.30	0.690	2.79*	0.599
No. of pods per plant	83.08*	30.280	65.30*	26.570	26.57	15.700	58.97*	16.200
Pod weight per plant (g)	43.03*	17.670	11.74	8.990	1.96*	5.030	25.66*	9.340
No. of seeds per plant	116.80*	48.500	79.40	42.000	36.2	25.600	88.00*	33.100
Seed weight per plant (g)	18.47*	8.000	4.92	3.210	2.47	2.630	8.59*	3.940
100-seed weight (g)	55.50	29.300	22.43*	9.770	23.66	12.540	6.75	10.810
Shelling (%)	7.74	9.970	22.98	16.400	24.01	12.390	6.32	13.090

* Significant at the 0.05 probability level.

Table 6. Late leaf spot (LLS)- and rust-resistant introgression lines better or significantly better for yield-related traits compared with LLS-resistant control cultivar, GPBD 4, in the 2015 rainy and 2015–2016 post-rainy seasons and pooled analysis.

Traits	2015 rainy season	2015–2016 post-rainy season	Pooled
Days to first flowering	PBGNIL-2, PBGNIL-4	PBGNIL-2, PBGNIL-1, PBGNIL-15	PBGNIL-2
Days to 50% flowering	PBGNIL-2	PBGNIL-2, PBGNIL-1, PBGNIL-15, PBGNIL-11	PBGNIL-2, PBGNIL-1
Pod no.	PBGNIL-7, PBGNIL-11, PBGNIL-15, PBGNIL-1, PBGNIL-8, PBGNIL-13, PBGNIL-12	PBGNIL-4, PBGNIL-13, PBGNIL-5, PBGNIL-11, PBGNIL-1, PBGNIL-3, PBGNIL-17, PBGNIL-8, PBGNIL-6, PBGNIL-2, PBGNIL-15, PBGNIL-7, PBGNIL-12	PBGNIL-11, PBGNIL-4, PBGNIL-13, PBGNIL-1, PBGNIL-7, PBGNIL-15, PBGNIL-8, PBGNIL-12, PBGNIL-5
Pod yield per plant (g)	PBGNIL-8, PBGNIL-7, PBGNIL-1, PBGNIL-15, PBGNIL-13, PBGNIL-11, PBGNIL-14, PBGNIL-9, PBGNIL-12	PBGNIL-4, PBGNIL-13, PBGNIL-5, PBGNIL-15, PBGNIL-6, PBGNIL-3, PBGNIL-1, PBGNIL-11	PBGNIL-13, PBGNIL-15, PBGNIL-1, PBGNIL-8, PBGNIL-7, PBGNIL-11, PBGNIL-4, PBGNIL-14, PBGNIL-5, PBGNIL-12
Seed no.	PBGNIL-1, PBGNIL-7, PBGNIL-8, PBGNIL-11, PBGNIL-13, PBGNIL-15, PBGNIL-12	PBGNIL-4, PBGNIL-13, PBGNIL-5, PBGNIL-1, PBGNIL-11, PBGNIL-6, PBGNIL-3, PBGNIL-7, PBGNIL-2, PBGNIL-8, PBGNIL-15, PBGNIL-12, PBGNIL-17, PBGNIL-9	PBGNIL-1, PBGNIL-13, PBGNIL-4, PBGNIL-7, PBGNIL-11, PBGNIL-8, PBGNIL-5, PBGNIL-15, PBGNIL-12
Seed yield per plant (g)	PBGNIL-13, PBGNIL-7, PBGNIL-1, PBGNIL-15, PBGNIL-8, PBGNIL-11, PBGNIL-12	PBGNIL-13, PBGNIL-5, PBGNIL-4, PBGNIL-15, PBGNIL-2, PBGNIL-6, PBGNIL-14, PBGNIL-12, PBGNIL-1, PBGNIL-3	PBGNIL-13, PBGNIL-15, PBGNIL-1, PBGNIL-7, PBGNIL-12, PBGNIL-8, PBGNIL-11, PBGNIL-5
100-seed weight (g)	PBGNIL-10, PBGNIL-13, PBGNIL-15, PBGNIL-12, PBGNIL-14, PBGNIL-8, PBGNIL-7, PBGNIL-11, PBGNIL-6, PBGNIL-9	PBGNIL-14, PBGNIL-15, PBGNIL-2, PBGNIL-13, PBGNIL-10	PBGNIL-10, PBGNIL-13, PBGNIL-15, PBGNIL-14, PBGNIL-12, PBGNIL-6
Shelling (%)	PBGNIL-13, PBGNIL-10	PBGNIL-10, PBGNIL-2, PBGNIL-9, PBGNIL-15, PBGNIL-14, PBGNIL-13, PBGNIL-5, PBGNIL-12, PBGNIL-1	PBGNIL-10, PBGNIL-13, PBGNIL-2, PBGNIL-9

GPBD 4). Similarly, in the 2015–2016 post-rainy season, almost all of the ILs performed better than or similar to GPBD 4, and a few ILs were significantly better than the control cultivar. For example, three ILs (PBGNILs 4, 13, and 5) were significantly superior (38–53 pods plant^{-1}) to GPBD 4 (27 pods) for pod number, and one (PBGNIL-4) for seed number (62 vs. 39 seeds plant^{-1} in GPBD 4) (Table 6). In combined analysis, seven ILs (PBGNILs 11, 4, 13, 1, 7, 15, and 8) were significantly better than GPBD 4 for pod number (32–40 vs. 26 pods plant^{-1} in GPBD 4), one (PBGNIL-13) for pod yield per plant (25 vs. 19 g in GPBD 4), one (PBGNIL-1) for seed number per plant (49 vs. 37 seeds plant^{-1} in GPBD 4), one (PBGNIL-13) for seed yield per plant (17 vs. 12 g in GPBD 4), and one (PBGNIL-10) for 100-seed weight (42 vs. 31 g in GPBD 4).

Confirmation of Resistance Alleles and Identification of Novel Alleles for Resistance

Eight linked markers to LLS and rust resistance were genotyped among a set of 29 genotypes to check the loci variation for these marker alleles among the newly developed ILs. Three markers—GM1536, GM2079, and Seq8D09—gave clear peak pattern and amplified in all genotypes. The markers GM1536 and GM2079 were found to control both the foliar fungal diseases, whereas the marker Seq8D09 represents another genomic region controlling only LLS resistance (Sujay et al., 2012). The markers GM1536, GM2079, and Seq8D09 produced resistance alleles of 473, 403, and 132 bp, respectively, in GPBD 4, a resistant parent of the mapping population (TAG 24 × GPBD 4) used earlier to conduct genetic mapping. A majority of the ILs had similar markers alleles to GPBD 4 except two ILs, PBGNIL-2 and PBGNIL-6. The IL PBGNIL-2 showed moderate resistance to both the diseases but carried susceptible alleles (485 bp from GM1536, 409 bp from GM2079, and 135 bp from Seq8D09). Similarly, another IL, PBGNIL-6, carried susceptible alleles for markers GM1536 and Seq8D09 and a novel allele of 418 bp from marker GM2079, despite having a moderate level of disease resistance. Therefore, these two ILs could be important sources of novel alleles for resistance to both the foliar fungal diseases and should be used in further genetic and breeding applications.

DISCUSSION

Lack of availability of high levels of resistance for foliar fungal diseases in cultivated peanut necessitates the exploitation of new sources to enhance the levels of resistance in new peanut cultivars. Several studies have reported high levels of resistance against two of the most devastating foliar fungal diseases, LLS and rust, in wild *Arachis* species (Subrahmanyam et al., 1985; Pande and Rao, 2001). The currently well-exploited resistance alleles present in popular peanut cultivar GPBD 4 were traced back to

A. cardenasii (Varshney et al., 2014). Keeping in mind the presence of enormous genetic variability and high levels of resistance or tolerance to important biotic and abiotic stresses in different wild *Arachis* species, it is important to exploit these diverse sources to develop new peanut cultivars. Such an attempt would play an important role in improving peanut production and productivity globally, especially under disease pressure by avoiding disease development and epidemics. In peanut, a few successful examples are available wherein genes from wild *Arachis* species were successfully used for genetic improvement, such as introgression of root-knot nematode resistance from the amphidiploid TxAG-6 (Burow et al., 2014), rust and LLS resistance in GPBD 4 from CS 16 (ICGV 86855) (Gowda et al., 2002), and identification of quantitative trait loci for rust resistance (Leal-Bertioli et al., 2015). In the present study, we aimed to introgress high levels of LLS and rust resistance from diploid wild *Arachis* species into cultivated peanut to develop ILs with enhanced levels of resistance in good agronomic backgrounds and to diversify the sources of LLS and rust resistance by using wild species, rather than the commonly used *A. cardenasii*, for peanut improvement. To overcome the ploidy level difference between the diploid wild *Arachis* species and tetraploid cultivated peanut, two tetraploid synthetics derived from the chromosome doubling of F_1 crosses between diploid wild *Arachis* species were used as donors. Further, to minimize the linage drag, an advanced backcross approach was used for population development, with the aim to recover the recombinants with a small segment introgressed from wild species in the genetic background of cultivated types.

The synthetic ISATGR 121250 was derived from F_1 crosses between *A. duranensis* (ICG 8123) and *A. ipaensis* (ICG 8206), the two progenitor species of cultivated peanut. Another synthetic, ISATGR 265-5, was derived from F_1 crosses between nonprogenitor species, *A. kempf-mercadoi* (ICG 8164) and *A. hohnei* (ICG 8190) (Mallikarjuna et al., 2011). These four accessions (ICG 8123, ICG 8206, ICG 8164, and ICG 8190) were reported to possess high levels of LLS and rust resistance (Subrahmanyam et al., 1985; Pande and Rao, 2001). In this study, two advanced backcross populations were developed using two synthetics, ISATGR 121250 and ISATGR 265-5, as donors for introgressing LLS and rust resistance into two popular peanut cultivars, ICGV 91114 and ICGV 87846. Screening of these two advanced backcross populations, followed by rescreening, resulted in the identification of 15 ILs with high levels and two ILs with moderate levels of LLS and rust resistance in acceptable agronomic backgrounds. These results indicated that LLS and rust resistance in these ILs were introgressed from wild *Arachis* species *A. duranensis*, *A. ipaensis*, *A. kempf-mercadoi*, and *A. hohnei*. Because the levels of LLS and rust resistance in these ILs was found better than GPBD 4 and were derived from wild *Arachis* species other than the commonly used *A. cardenasii*, these ILs provide new and diverse sources of LLS and rust resistance for peanut improvement.

Besides LLS and rust resistance, these accessions also exhibited good agronomic performance, such as early flowering, high number of pods per plant, pod yield per plant, number of seeds per plant, seed yield per plant, 100-seed weight, and shelling percentage, compared with the best LLS- and rust-resistant peanut cultivar, GPBD 4, in rainy and post-rainy seasons. However, the variable performance of these ILs in rainy and post-rainy seasons was mainly due to the significant g × e interaction observed for most traits. Most of the ILs were erect (15 ILs) and decumbent (2 ILs) in growth habit, with sequential (14 ILs) and alternate (3 ILs) branching patterns, slight (9 ILs) to moderate (8 ILs) pod beaks, slight (13 ILs) to moderate (4 ILs) pod constriction, and moderate (all 17 ILs) pod reticulations (data not given). It is interesting to note that the majority of favorable alleles conferring disease resistance were contributed by the wild *Arachis* species through synthetics, whereas favorable alleles for agronomic traits were mostly contributed by the cultivated parent after backcrossing and recombination. All of these ILs exhibited high LLS and rust resistance and good agronomic performance and could be evaluated across multilocations to identify promising and stable high-yielding LLS- and rust-resistant ILs, either for direct release as cultivars in specific regions or for use as new and diverse sources of variation in the breeding programs developing new disease-resistant peanut cultivars with a broad genetic base.

An effort was made to genotype these promising ILs by using linked markers for LLS and rust resistance. These markers were identified in the mapping population developed from the cross TAG 24 × GPBD 4 (Khedikar et al., 2010; Sujay et al., 2012) and have also been deployed successfully in breeding programs to improve rust resistance in three elite and popular cultivars using a marker-assisted backcrossing (MABC) approach (Varshney et al., 2014). In these studies, the resistance source for LLS and rust was GPBD 4, and the resistance alleles were traced back to *A. cardenasii*. In the present study, genotyping results showed that two ILs, namely PBGNIL-2 and PBGNIL-6, carry different alleles than the known resistance source, GPBD 4, and showed moderate levels of resistance for both foliar fungal diseases. Because these two ILs were derived from a cross involving LLS- and rust-susceptible peanut cultivar ICGV 91114, and ISATGR 121250 derived from LLS- and rust-resistant *A. duranensis* (ICG 8123) × *A. ipaensis* (ICG 8206), the results indicated that the resistance in these two ILs may be different from the commonly used *A. cardenasii*, and they therefore provide novel sources introgressed from *A. duranensis* and *A. ipaensis*. However, it is important to mention that the markers used for genotyping were associated markers identified in a genetic

study and not the gene-based functional markers, which provide allele mining very precisely. Nevertheless, the genome sequence availability of both the diploid progenitors (Bertioli et al., 2016; Chen et al., 2016) may facilitate detailed genetic and sequence analysis of the highly resistant ILs, along with other sources of resistance, for faster discovery of genes using next-generation sequencing approaches (Pandey et al., 2016). Pyramiding of diverse resistance alleles will enhance the stable performance of newly developed cultivars in farm fields, leading to greater adoption by the peanut farming community.

In summary, this research reports development of highly resistant ILs using diverse genetic sources that so far remained unused in the peanut breeding programs. These ILs will not only serve as an alternate source of resistance in breeding but also help towards diversifying the currently narrow genetic base of cultivated peanut varieties. In addition, these ILs may also facilitate further genetic, breeding, and genomics studies hoping to identify the right allelic combinations to provide sustained resistance to LLS and rust in peanut.

Conflict of Interest

The authors declare there to be no conflict of interest.

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