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Diversification of primary gene pool through introgression of resistance to foliar diseases from synthetic amphidiploids to cultivated groundnut (*Arachis hypogaea* L.)

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is widely grown and consumed around the world and is considered to have originated from a single hybridization event between two wild diploids. The utilization of wild germplasm in breeding programs has been restricted by reproductive barriers between wild and cultivated species and technical difficulties in making large numbers of crosses. Efforts to overcome these hurdles have resulted in the development of synthetic amphidiploids, namely ISATGR 278-18 (*Arachis duranensis* × *Arachis batizocoi*) and ISATGR 5B (*Arachis magna* × *A. batizocoi*), which possess several desirable traits, including resistance to foliar diseases that generally cause huge yield losses annually in groundnut growing areas of Asia, America, and Africa. With an objective to improve foliar disease resistance, the primary gene pool was diversified by introgressing foliar disease resistance in five cultivated genotypes (ICGV 91114, ICGS 76, ICGV 91278, JL 24, and DH 86) from synthetic amphidiploids using a backcross breeding approach. Several introgression lines with resistance to two foliar diseases (rust and late leaf spot) were identified with levels of resistance equal to the donors. These backcross derived lines have shown a wide range of variation for several morphological and agronomic traits. These lines, after further evaluation and selection, can serve as donors in future breeding programs aimed at

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developing improved cultivars with desirable agronomic traits, high resilience to biotic/abiotic stresses and a broadened genetic base.

1. Introduction

Cultivated groundnut (*Arachis hypogaea* L.), also known as peanut, is grown on nearly 24 million hectares of land area globally with an annual production of 38 million tons (Mt) [1]. Although it originated in South America, the vast majority of groundnut is produced in Asia (68%, 23 Mt) and Africa (24%, 8 Mt), whereas the remaining (8%, 3.5 Mt) comes from North America, Caribbean countries, Europe and Oceania [1]. Besides being a major source of vegetable oil and providing several confectionary preparations, this crop is also a principal source of nutrition by providing human dietary protein, oil/fat, and vitamins such as thiamine, riboflavin and niacin in parts of Asia and Africa [2]. Additionally, it provides an important livestock feed along with improving soil fertility through contributing up to 60 kg ha^{-1} of nitrogen to the soil [3].

Surmounting biotic and abiotic pressure along with the narrow genetic base of the cultivated gene pool has seriously reduced the crop potential and hampered the possibility of meeting future demands of continuously increasing human and animal populations [4,5]. Control of drought stress and foliar diseases requires urgent attention in order to sustain productivity in the fields of resource-poor farmers. Foliar diseases such as late leaf spot (LLS) caused by *Cercosporidium personatum* and leaf rust caused by *Puccinia arachidis* are important diseases of groundnut in Africa, Asia, and the Americas [6,7]. The extent of economic loss due to LLS [8] may be much higher than the reported global yield loss of 600 million US$. Disease management through application of fungicides is not a viable option for resource-poor farmers; also, fungicides may pollute the environment and ground water besides causing greater risk and damage to crop [7]. Hence, the only eco-friendly approach is to equip popular cultivars with resistance genes that will ensure sustainable resistance against foliar fungal pathogens.

Molecular analysis has shown that cultivated groundnut possesses a narrow genetic base [9,10] due to a single hybridization event that occurred ~3500 years ago [11]. The genus *Arachis* has a total of nine sections possessing different genomes. Earlier reports have indicated the existence of a large range of variability among these sections. However, this variability cannot be exploited in a direct way because of ploidy or genome differences among the species [12,13]. In order to overcome the genetic bottleneck of restricted gene flow, the development of synthetic amphidiploids is an effective option to diversify the cultivated gene pool. To date, several synthetics have been developed by using different diploid species through colchicine-mediated genome duplication [14–17]. These highly diverse synthetics provide an opportunity for introgression of some important traits to cultivated germplasm. However, limited success has been achieved so far in using the wild species as genetic resources for the development of resistant cultivars. Nevertheless, release of an Indian variety (GPBD 4) containing resistance to foliar diseases in chromosome segments from *Arachis cardenasii* is an example of success. GPBD 4 is an improved variety developed as a second cycle derivative of an interspecific cross and is grown in several states in India for its desirable traits such as foliar disease resistance and high yield. Because of its high levels of resistance, *A. cardenasii* Krapov. & W. C. Greg. is the most widely used wild species in groundnut breeding programs aimed at improving foliar disease resistance. However, it is always better to look for alternative sources of resistance in order to diversify the cultivated gene pool [4]. Realizing the great potential of synthetic amphidiploids for enhancing the richness of the gene pool, this study was undertaken to broaden the genetic base of cultivated groundnut by introgressing resistance genes into five cultivated genotypes. We report the development of diverse genetic materials in groundnut with potential for several genetic and breeding applications.

2. Materials and methods

Synthetic amphidiploids ISATGR 278-18 [ICG 8138 (*Arachis duranesis* Kaprov. & W. C. Greg.) × ICG 13160 (*Arachis batizocoi* Kaprov. & W. C. Greg.)] and ISATGR 5B [ICG 8960 (*Arachis magna* Kaprov., W. C. Greg. & C. E. Simpson) × ICG 8209 (*A. batizocoi* Kaprov. & W. C. Greg.)] with $2n = 2x = 40$ were generated at ICRISAT (Hyderabad, India). Seeds from these amphidiploids were planted in a glasshouse at the University of Agricultural Sciences (UAS), Dharwad, India. Both amphidiploids were used to generate backcross populations with five elite varieties/genotypes, namely ICGV 91114, ICGS 76, ICGV 91278, JL 24, and DH 86 after making two backcrosses.

Flowers of cultivated genotypes were emasculated a day before pollination. Cross pollination was carried out before 10:00 a.m. on the following day by using the synthetic amphidiploids as pollen parents. Cotton swabs impregnated with gibberellic acid (GA_3) (0.5 mL; 75 mg L^{-1}) were wrapped around the base of pollinated pistils. Flowering was generally observed on recurrent parents about 45 days after sowing (DAS) and continued, allowing crossing for the next 30 days. The pods were harvested and percentages of crossed pods were calculated. In the next season, the F_1 plants were used as pollen parents for the first backcross to each recurrent parent. Pods of BC_1F_1 generation from all crosses were harvested and grown in the next season. These plants were then used to make second backcrosses. The BC_2F_1s were grown and selfed thrice to produce BC_2F_4 population after three seasons (Fig. 1).

Both amphidiploids were evaluated for component traits of rust and late leaf spot (LLS) resistances using a detached leaf technique [18]. On the 40th DAS, tetrafoliate leaves were excised from the pulvinous regions and arranged in plastic trays containing autoclaved sand in a randomized block design with two replications. In order to compare the disease

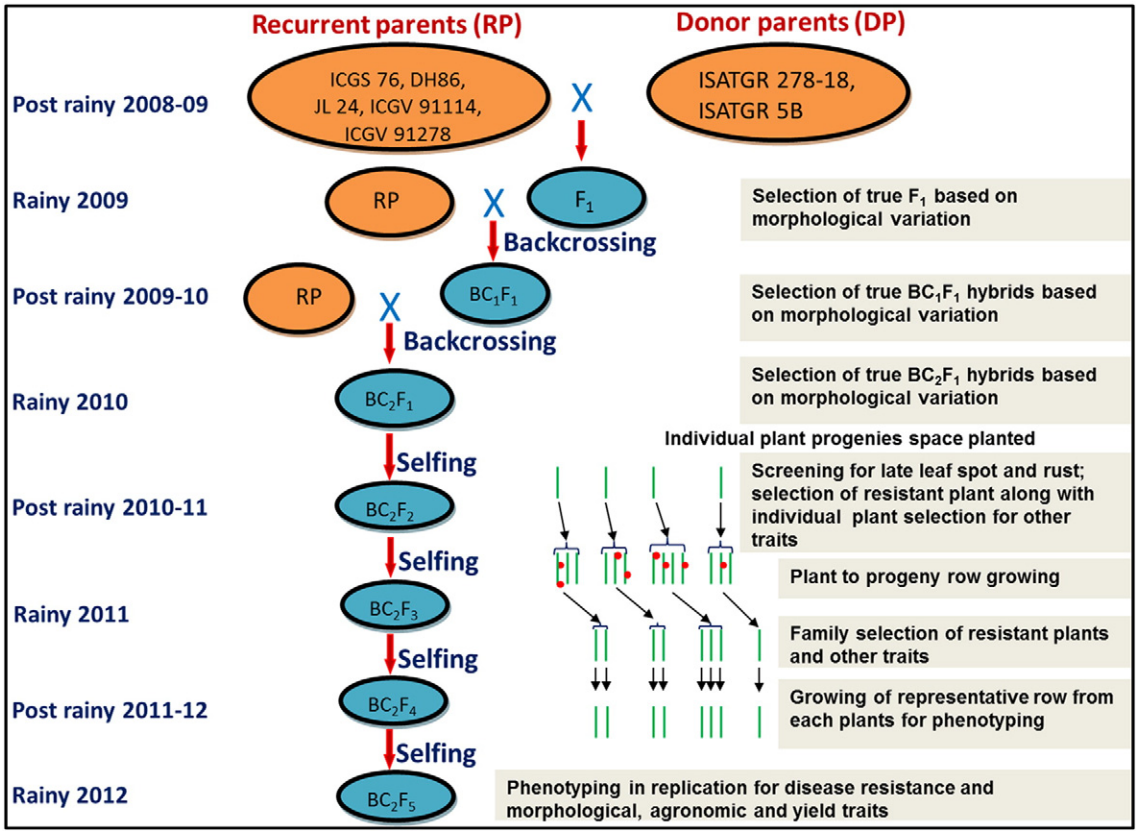

Fig. 1 – Development of introgression lines by backcrossing and self-pollination.

severities, a susceptible check (variety "TMV 2") was used for both the diseases. *P. arachidis* urediniospores and *C. personatum* conidia were initially produced on susceptible cultivar TMV 2 and harvested with a cyclone spore collector. The concentrations of the spore suspensions were set to 20,000 spores mL^{-1} using a hemocytometer by adding sterile distilled water containing a few drops of Tween-80 (polyoxyethylene sorbitan mono-oleate) in order to promote adhesion. Spore suspensions of both the pathogens were sprayed on to the leaves by using an atomizer, and the trays were kept in a growth chamber at 23–25 °C immediately after the inoculation to ensure leaf moisture during the night. Two weeks after inoculation, leaves were inspected for symptoms and time to sporulation. Damage due to rust and LLS was determined after 30 days based on these parameters. Cultivar TMV 2 was used as the susceptible check and cultivar GPBD 4 was used as resistant check in all disease screening experiments.

Plants of BC_2F_2 generation generated from each of the nine crosses were screened for disease resistance during the rainy season of 2011 following the protocol of Subrahmanyam et al. [19]. Seeds were treated with seed protectant and sown in the field with 45 cm and 10 cm inter- and intra-row spacing, respectively. The parental genotypes were sown once as controls and TMV 2 (susceptible variety for both diseases) was planted at every 10th row as well as a border around the field to maintain an effective inoculum load. Uniform inoculation across the field was performed in the evening of 45th DAS. Disease scoring for LLS and rust occurred on the 80th and 90th DAS using a 0–9 scale of disease severity (%) on the leaves for lesions and defoliation in the case of LLS, and on pustules and necrosis in the case of rust. Scores were as follows: (i) 1.0, no disease; (ii) 2.0, 1%–5% severity, lesions/pustules on lower leaves; (iii) 3.0, 6%–10% severity, lesions/pustules mostly on lower leaves and very few on middle leaves along with defoliation or necrosis of lower leaves; (iv) 4.0, 11%–20% severity, lesions/pustules on lower and middle leaves but severe on lower leaves with defoliation/necrosis of some leaflets on lower leaves; (v) 5.0, 21%–30% severity, lesions/pustules on all lower and middle leaves along with defoliation/necrosis of >50% lower leaves; (vi) 6.0, 31%–40% severity, severe lesions/pustules on lower and middle leaves, few symptoms on top leaves along with extensive defoliation/necrosis of lower leaves and some middle leaves; (vii) 7.0, 41%–60% severity, lesions/pustules present on all leaves but less severe on the top leaves along with complete defoliation/necrosis of lower leaves and some middle leaves; (viii) 8.0, 61%–80% severity, lesions/pustules fully covering lower and middle leaves and severe lesions on top leaves along with some defoliation/necrosis of top leaves; and (ix) 9.0, 81%–100% severity, almost complete defoliation/necrosis for lower, middle and top leaves leaving bare stems.

The final introgression lines were characterized for morphological traits such as plant height, leaf features (length, width, color, and shape), stem features (pigmentation, pubescence) secondary branching, flower color, growth habit and branching pattern. Growth habit was scored as "Erect" (main

Table 1 – Disease response scores for synthetic amphidiploids and cultivated parents used for introgression of disease resistance.

Genotype	Type	Mean rust score	Mean LLS score	Number of LLS lesions/leaf	Number of days to sporulation	Number of days to 50% sporulation for LLS	% leaf infected for LLS	Lesion diameter for LLS (cm)
JL 24	Susceptible check	6.3	7.2	23.6	20	25	80	0.194
TMV 2	Susceptible check	5.4	7.1	23.2	21	27	60	0.187
ISATGR 278-18	Synthetic parent	2.1	2.9	6.8	41	47	13	0.091
ISATGR 5B	Synthetic parent	2.2	2.3	5.6	39	43	22	0.083

stem erect), "Decumbent-1" (completely spreading, primary branches at 90° angles with the main stem), "Decumbent-2" (semi spreading, primary branches at 60° to the main stem) and "Decumbent-3" (semi erect, primary branches at 45° to the main stem). Similarly, branching pattern was recorded as "Sequential" (flowers on main stems and primary branches, but not on secondary branches), "Irregular with flower on main stem" (flowers on main stems, primary branches and secondary branches), and "Irregular without flower on main stem" (no flowers on main stems, but present on primary and secondary branches).

3. Results

3.1. *Development of introgression lines*

Disease screening was carried out for foliar disease responses (leaf rust and LLS) among the parental genotypes (ICGV 91114, ICGS 76, ICGV 91278, JL 24, DH 86, ISATGR 278-18, and ISATGR 5B). Both amphidiploids (ISATGR 278-18 and ISATGR 5B) showed high levels of resistance (disease scores 2.0–3.0) to both rust and LLS whereas the cultivated parental genotypes were susceptible (disease scores 6.0–7.0) (Table 1). Five crosses were achieved for ISATGR 278-18 (ICGV 91114 × ISATGR 278-18, ICGS 76 × ISATGR 278-18, ICGV 91278 × ISATGR 278-18, JL 24 × ISATGR 278-18, and DH 86 × ISATGR 278-18), and ISATGR 5B (ICGV 91114 × ISATGR 5B, ICGS 76 × ISATGR 5B, ICGV 91278 × ISATGR 5B, JL 24 × ISATGR 5B, and DH 86 × ISATGR 5B). Peg formation began about 25 days after pollination. From the 597 buds pollinated, 198 pods were harvested with percentage seed set ranging from 15 (DH 86 × ISATGR 278-18) to 47% (JL 24 × ISATGR 5B) (Table 2).

All 212 potential F_1 seeds from 198 pods were planted and examined for hybridity based on morphological attributes. A total of 51 plants from ten crosses were confirmed to be hybrids. Hybrids had a spreading growth habit along with distinctive leaf morphology, flower color and pod morphology similar to the synthetic parents.

True hybrids from all the nine crosses were used as pollen parents to make the first backcross with the respective recurrent parents (Tables 2 and 3). Of the 673 buds pollinated in all the backcrosses, 293 mature pods were harvested. The mean percentage of seed set ranged from 38% (ICGV 91114 × ISATGR 5B) to 50% (DH 86 × ISATGR 278-18) (Table 2). The average percentage of seed set was higher in BC_1F_1 generation (44.0%) than that achieved in F_1 generation (31.7%) plants.

All 320 potential BC_1F_1 seeds obtained from backcrossed plants were planted and subjected to phenotypic screening. A total of 84 BC_1F_1 plants were confirmed for hybridity based on morphological traits and disease reaction (Table 4). Confirmed BC_1F_1 plants were again backcrossed with the recurrent

Table 2 – Outcomes of attempted crosses between cultivated varieties and amphidiploids.

Cross combinations	Generation of F_1 seeds			Generation of BC_1F_1 seeds			
	Number of buds pollinated	Number of pods harvested	% crossed pods obtained	Confirmed F_1 plants	Number of buds pollinated	Total number of pods harvested	% crossed pods obtained
JL 24 × ISATGR 278-18	89	40	45	6	90	37	42
JL 24 × ISATGR 5B	51	24	47	13	95	42	41
DH 86 × ISATGR 278-18	47	7	15	7	40	20	50
DH 86 × ISATGR 5B	49	10	21	5	52	23	45
ICGS 76 × ISATGR 278-18	59	17	29	2	76	31	41
ICGS 76 × ISATGR 5B	58	9	16	3	83	37	45
ICGV 91114 × ISATGR 278-18	78	32	41	4	97	45	47
ICGV 91114 × ISATGR 5B	60	25	41	5	87	33	38
ICGV 91278 × ISATGR 278-18	63	24	39	6	53	25	47
ICGV 91278 × ISATGR 5B	43	10	23	–	–	–	–

Table 3 – Numbers of backcross introgression lines possessing resistance to rust and late leaf spot (LLS) and their mean disease scores (0–9 scale).

Parental genotypes and cross combinations	Number of resistant BC_1F_4 plants identified	Number of resistant BC_2F_4 plants identified	Mean disease score for rust	Mean disease score for LLS
DH 86 (recurrent parent)			8.0	8.0
DH 86 × ISATGR 278-18	8	18	3.0	3.0
DH 86 × ISATGR 5B	8	10	3.5	3.5
ICGS 76 (recurrent parent)			8.0	6.0
ICGS 76 × ISATGR 278-18	10	90	5.0	3.5
ICGS 76 × ISATGR 5B	6	–	4.0	5.0
JL 24 (recurrent parent)			8.0	8.0
JL 24 × ISATGR 278-18	6	–	2.5	2.5
JL 24 × ISATGR 5B	–	–	–	–
ICGV 91114 (recurrent parent)			7.0	7.0
ICGV 91114 × ISATGR 278-18	–	2	2.5	2.5
ICGV 91114 × ISATGR 5B	–	–	–	–
ISATGR 278-18 (male parent)			2.0	3.0
ISATGR 5B (male parent)			2.0	2.0

parents and BC_2F_1 pods were harvested. In the next season, BC_2F_1 seeds were planted and BC_2F_1 plants were again confirmed by morphological characters and disease response. Selected BC_2F_1s in each of the seven crosses were selfed and the progenies were screened for reaction to rust and LLS during the rainy season of 2011.

Table 4 – Morphological descriptions of parental genotypes and introgression lines.

Parental genotypes and cross combinations	Plant height range (cm)	Leaf length range (cm)	Leaf width range (cm)	No. of secondary branches range	Growth habit	Branching pattern	Leaf shape	Leaf color	Stem color
JL 24 (female parent)	26.0	5.8	2.6	–	Erect	Sequential	Wide elliptical	Green	Green
JL 24* × ISATGR 278-18	14.0–45.0	2.6–8.5	1.8–4.5	1–23	D1–D3	Sequential	Oblong elliptical	LG–DG	LP
JL 24* × ISATGR 5B	12.0–46.0	2.7–7.3	2.0–3.5	1–13	D1–D3	Sequential	Narrow elliptical	LG–DG	Green
DH 86 (female parent)	20.0	4.2	2.0	–	Erect	Sequential	Oblong elliptical	DG	Green
DH 86* × ISATGR 278-18	12.0–29.0	2.5–6.1	1.0–2.7	1–12	D1–D3	Sequential	Oblong elliptical	LG–DG	LP
DH 86* × ISATGR 5B	13.0–33.0	3.0–6.4	1.5–3.6	1–11	D1–D3	Sequential	Narrow elliptical	LG–DG	LP
ICGS 76 (female parent)	25.0	4.5	2.2	6–12	D2	Irregular without flower on main stem	Oblong elliptical	DG	LP
ICGS 76* × ISATGR 278-18	14.1–34.0	3.5–8.1	1.6–3.9	1–31	Erect–D3	Irregular with flower on main stem	Obovate-wide elliptical	LG–DG	Green-DP
ICGS 76* × ISATGR 5B	13.0–26.5	3.8–7.7	1.7–3.5	6–30	D1–D3	Irregular without flower on main stem	Narrow elliptical–oblong elliptical	LG–DG	LP–DP
ICGV 91114 (female parent)	32.0	5.5	2.2	Absent	Erect	Sequential	Wide elliptical	Green	Green
ICGV 91114* × ISATGR 278-18	22.0–37.0	3.8–7.7	2.1–3.8	2–10	Erect	Sequential	Wide elliptical	Green	Green
ISATGR 278-18 (male parent)	95.5	3.1	2.7	Present	D1	Irregular with flower on main stem	Oblong elliptical	DG	LP
ISATGR 5B (male parent)	92.3	2.5	2.2	Present	D1	Irregular with flower on main stem	Narrow elliptical	DG	Green

LG: light green; DG: dark green; LP: light purple; DP: dark purple. * indicates backcross progenies.
Growth habit: erect growth habit indicates that main stem is erect; D1 (Decumbent-1), complete spreading, i.e., primary branches at 90° angle to the main stem; D2 (Decumbent-2), semi-spreading, i.e., primary branches at 60° to the main stem; D3 (Decumbent-3), semi erect, i.e., primary branches at 45° to the main stem.
Branching pattern: sequential, flowers on main stem and primary branches but not on secondary branches; irregular with flower on main stem, flowers on main stem, primary branches and secondary branches; irregular without flower on main stem, no flowers on main stems, but present on primary branches and secondary branches.
Leaf shape: wide elliptical, broad and round; oblong elliptical, medium; narrow elliptical-small, obovate-wide elliptical, small but round.

3.2. *Evaluation and identification of introgression lines with foliar disease resistance*

Hybrids in different generations (F_1, BC_1F_1, and BC_2F_1) were scored for rust and LLS response and those possessing resistance for components of response compared to the respective susceptible parents were selected (Fig. 2). After each backcross, the plants were selfed to obtain segregating backcross F_2s (BC_1F_2, BC_2F_2), which were selfed twice to obtain BC_1F_4 and BC_2F_4 backcross progenies. These were then subjected to phenotyping and several lines with high levels of resistance to rust and LLS compared to the susceptible parents were selected. The numbers of resistant plants in each cross, generation, and range of disease scores were recorded (Table 3). Among the BC_2F_4 introgression lines, very high frequencies of resistant lines (90 of 164) were selected from the cross ICGS 76 × ISATGR 278-18 followed by 18 lines (out of 52) from DH 86 × ISATGR 278-18. No resistant plants were detected in JL 24 × ISATGR 5B and ICGV 91114 × ISATGR 5B.

A few morphological variants that were phenotypically similar to the amphidiploid parents for traits such as growth habit, plant height, leaf morphology (shape and size) and color, flowers on main stem, flower color, peg pattern, stem pubescence, stem pigmentation, testa color, number of primary and secondary branches, and pod constriction/reticulation were recovered in the selected backcross lines (Tables 4, 5, Figs. 2, and 3). Line AB-ICGS76-25-3 showed dense stem pubescence and a high number of secondary branches. Line AB-ICGS76-73-6 produced broad leaves, AB-ICGS76-1-4 had narrow leaves, AB-ICGS76-10-1 had deep constrictions and reticulations in pods, and AB-ICGS76-7-1 showed high resistance to both diseases along with erect growth habit (Tables 5, 6, and Fig. 2).

Fig. 2 – Variation in morphological characteristics and disease resistance in the susceptible female parent and resistant BC_2F_4 lines from the cross ICGS 76 × ISATGR 278-18. This figure shows variability between cultivated parents and introgression lines for stem pigmentation, pubescence, growth habit, and branching pattern.

Table 5 – Morphological trait features of introgression lines and their parental genotypes.

Introgression line	Morphological feature	Phenotype	Phenotype of recurrent parent	Phenotype of amphidiploid
ICGS 76 × (ICGS 76 × ISATGR 278-18)^2 BC_2F_4 generation			*ICGS 76*	*ISATGR 278-18*
AB-ICGS76-13-1	Stem pigmentation	Deep violet	Light violet	Green
AB-ICGS76 14-1	Stem pigmentation	Green	Light violet	Green
AB-ICGS76-25-3	Stem pubescence	Present	Absent	Present
AB-ICGS76 -52-2	Growth habit	Decumbent-2	Decumbent-3	Decumbent-1
AB-ICGS76 -101-1	Growth habit	Erect	Decumbent-3	Decumbent-1
AB-ICGS76-92-2	Branching pattern	Irregular with flower on main stem	Irregular without flower on main stem	Irregular with flower on main stem
AB-ICGS76 15-7	Flower color	Yellow	Orange	Yellow
AB-ICGS76 8-1	Main stem flowering	Present	Absent	Present
AB-ICGS76-92-5	Peg pattern	Multiple pegging	Double pegging	Multiple pegging
AB-ICGS76-10-1	Pod constriction/ reticulation	None to deep-segregating	Slight	None
AB-ICGS76 -100-1	Leaf color	Dark green	Green	Dark green
AB-ICGS76-64-1	Plant height	33.0 cm	13.1 cm	93.0 cm
AB-ICGS76-73-6	Leaf shape	broad leaves (Wide elliptical)	Oblong elliptical	Narrow elliptical
AB-ICGS76-1-4	Leaf size	3.51 cm	4.90 cm	3.3 cm
AB-ICGS76-39-3	No. of secondary branches	24 secondary branches	15 secondary branches	30 secondary branches
JL 24 × (JL 24 × ISATGR 278-18)^2 BC_2F_4 generation			*JL 24*	ISATGR 278-18
AB-JL24 2-1	Plant height	45.0–50.0 cm	26.0 cm	93.0 cm
AB-JL24 5-4	Leaf size	2.6 cm	5.8 cm	3.3 cm
AB-JL24 10-3	Leaf shape	Round leave	Wide elliptical	Narrow-elliptical
AB-JL24-6-3	Growth habit	Decumbent-2	Erect	Decumbent-1
DH 86 × (DH 86 × ISATGR 278-18)^2 BC_2F_4 generation			*DH 86*	*ISATGR 278-18*
AB-DH 86-36-1	Growth habit	Decumbennt-1 (spreading)	Erect	Decumbennt-1
ICGV 91114 × ISATGR 278-18 F_3 generation			*ICGV 91114*	ISATGR 278-18
F_3-91114-1-2	Testa color	Red	Off-white	Off-white

4. Discussion

Enriching the primary gene pool is necessary for groundnut, which has a very narrow genetic base. Only limited success has been achieved by using wild relative species for improving cultivated groundnut germplasm. GPBD 4 is a good example of an improved variety that was developed as a second cycle derivative of an interspecific cross. Synthetics may be another effective way for bringing useful genes from wild relatives. In this direction, several synthetics are now available by using different diploid species and these need to be utilized for improving the cultivated gene pool [14–17]. Thus, in this study, highly diverse synthetics were used to introgress disease resistance in five cultivars. As a result, foliar disease (leaf rust and LLS) resistance was introgressed into one or more of the genetic backgrounds of ICGV 91114, ICGS 76, ICGV 91278, JL 24, and DH 86 using two synthetic resistance sources namely ISATGR 278-18 and ISATGR 5B (Table 3). Seed setting percentage improved with repeated backcrossing. The presence of phenotypic traits from the donor synthetics enabled confirmation of hybrids as crossing in groundnut can be very difficult. In later generations, the presence of one or more of these traits still enabled confirmation of backcross hybrids.

Backcrossed introgression lines in different generations were scored for rust and LLS response and lines possessing disease resistance were identified. Of the 10 attempted combinations, resistant derivatives were obtained in high frequencies for ICGS 76 × ISATGR 5B and DH 86 × ISATGR 278-18. Unfortunately, no resistant plant could be recovered from JL 24 × ISATGR 5B and ICGV 91114 × ISATGR 5B. It is clearly evident that the frequency and level of resistance to both diseases were higher among crosses involving ISATGR 278-18 compared to ISATGR 5B. Thus, ISATGR 278-18 appears to be a potentially better source of disease resistance and other agronomic traits for further diversifying the primary gene pool of groundnut. Besides disease resistance, the synthetic derivatives also showed a high level of variation in morphological traits and several backcross lines were selected for those traits (Tables 5 and 6). Due to abnormal pairing during meiotic division in synthetic amphidiploids, arising of different types of allelic combinations in the segregating backcrossed populations was reported [20]. Thus, the introgression lines are of importance and need further evaluation, as they might harbor currently undetected genes useful for the improvement of groundnut. Seeds of the introgression lines are available on request from the University of Agricultural Sciences (UAS), Dharwad (Ramesh S. Bhat).

Several wild species from the section *Arachis* had been successfully crossed with *A. hypogaea* and fertile hybrids [14–16] and various backcross introgression lines were obtained [21]. Earlier *Arachis glabrata* Benth. from section Rhizomatosae was crossed with *A. hypogaea* by using in vitro techniques [22] and traits of interest such as resistance to late

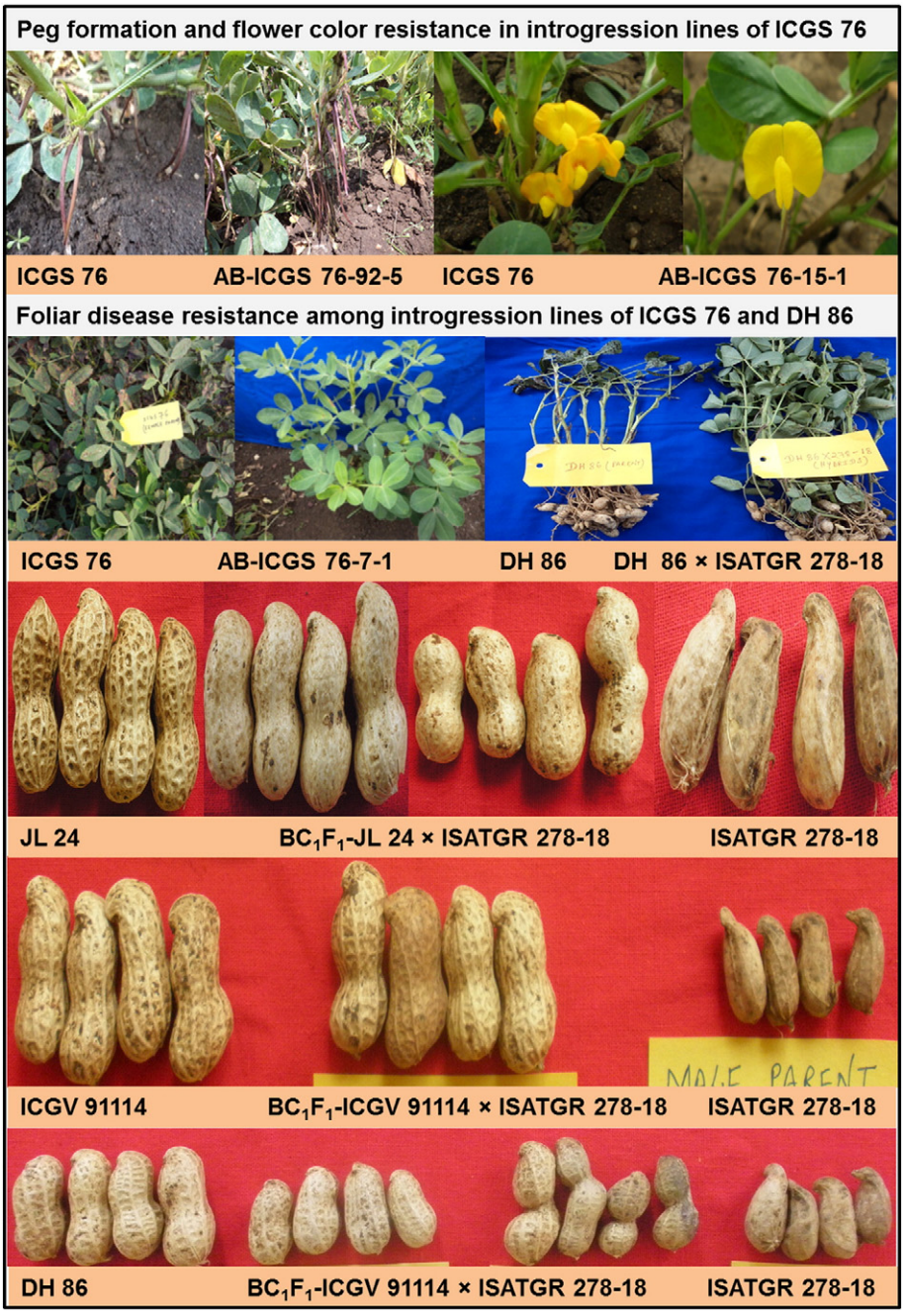

Fig. 3 – Variation in morphology, disease reaction and pod features in introgression lines derived from synthetic amphidiploids. This figure shows variability between cultivated parents and introgression lines for peg formation, flower color, foliar disease resistance and pod features.

leaf spot and groundnut viral diseases such as peanut mottle virus (PMV), peanut stripe virus (PSTV), and peanut bud necrosis virus (PBNV) were transferred to elite genotype [23]. Simpson et al. [24] developed TxAG-6, an amphidiploid [*A. batizocoi* K9484 × (*A. cardenasii* GKP10017 × *Arachis diogoi* GKP10602)] with resistance to early and late leaf spot (caused by *Cerospora arachidicola* S. Hori and *Phaeoisariopsis personata* Berk. & M.A. Curtis, respectively). With an objective of improving resistance, TxAG-6 was then used to generate a backcross population (78 progeny) and used to create a linkage map of RFLP markers [25]. A similar study reported development of amphidiploid AiAd (*A. ipaensis* × *A. duranensis*) [26]. This amphidiploid was extensively used for developing backcross populations by using cultivated tetraploid cultivar Fleur 11 as the recurrent parent and analyzed in different generations (BC_1F_1, BC_2F_1, BC_3F_1, BC_2F_2, and BC_4F_3) for linkage mapping [27] and QTL analysis [28,29] of various agronomic and yield traits.

In summary, several introgression lines possessing disease resistance and other important traits were developed by backcross breeding using two synthetic amphidiploids (ISATGR 5B and ISATGR 278-18) and five cultivs (ICGV 91114,

Table 6 – Foliar disease resistance among a synthetic amphidiploid parent, second backcross introgression lines and controls.

Parental genotype and introgression lines	Pedigree	Type of line	Mean rust score	Mean LLS score	Growth habit
ISATGR 278-18	(*A. duranesis* ICG 8138 × *A. batizocoi* ICG 13160)	Resistant donor parent	2.1	2.9	Spreading
ICGS 76	TMV 10 × Chico	Susceptible recipient parent	8.0	8.0	Semi-erect
AB-ICGS76-7-1	ICGS 76^{2*} × ISATGR 278-18	Introgression lines	3.1	3.2	Erect
AB-ICGS76-16-1	ICGS 76^{2*} × ISATGR 278-18	Introgression lines	3.1	3.1	Erect
AB-ICGS76-18-4	ICGS 76^{2*} × ISATGR 278-18	Introgression lines	3.3	3.0	Erect
AB-ICGS76-26-4	ICGS 76^{2*} × ISATGR 278-18	Introgression lines	3.2	3.1	Erect
AB-ICGS76-40-6	ICGS 76^{2*} × ISATGR 278-18	Introgression lines	3.1	3.1	Erect
DH 86	DH 40 × DH 8	Susceptible recipient parent	8.8	8.9	Erect
AB-DH 86-47-1	DH 86^{2*} × ISATGR 278-18	Introgression lines	3.1	3.1	Erect
AB-DH 86-8-2	DH 86^{2*} × ISATGR 278-18	Introgression lines	3.1	3.3	Erect
AB-DH 86-8-4	DH 86^{2*} × ISATGR 278-18	Introgression lines	3.1	3.1	Erect
JL 24	Selection from EC 94943	Susceptible check	6.4	7.2	Erect
TMV 2	Mass selection from Gudhiatham bunch	Susceptible check	5.4	7.3	Erect
GPBD 4	KRG 1 × CS 16 (ICGV 86855)	Resistant check	3.0	3.1	Erect

ICGS 76, DH 86, GPBD 4, JL 24, TMV 2: cultivated lines; ISATGR 278-18: synthetic amphidiploid. Disease scores: 1–3, resistant; 4–5, moderately resistant; 6–7, moderately susceptible; 8–9, susceptible. ^{2*} indicates two backcrosses.

ICGS 76, ICGV 91278, JL 24, and DH 86). In order to assess and harness the full potential of these lines for other important traits, further phenotyping of the lines for a range of traits is required. Thus, these introgression lines possess disease resistance and several other traits useful for future genetic enhancement of groundnut such as improved pod yield, superior oil quality and resistance to biotic and abiotic constraints.

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