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Foliar fungal disease-resistant introgression lines of groundnut (*Arachis hypogaea* L.) record higher pod and haulm yield in multilocation testing

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Abstract

Introgression lines (ILs) of groundnut with enhanced resistance to rust and late leaf spot (LLS) recorded increased pod and haulm yield in multilocation testing. Marker-assisted backcrossing (MABC) approach was used to introgress a genomic region containing a major QTL that explains >80% of phenotypic variance (PV) for rust resistance and 67.98% PV for LLS resistance. ILs in the genetic background of TAG 24, ICGV 91114 and JL 24 were evaluated for two seasons to select 20 best ILs based on resistance, productivity parameters and maturity duration. Multilocation evaluation of the selected ILs was conducted in three locations including disease hot spots. Background genotype, environment and genotype × environment interactions are important for expression of resistance governed by the QTL region. Six best ILs namely ICGV 13192, ICGV 13193, ICGV 13200, ICGV 13206, ICGV 13228 and ICGV 13229 were selected with 39–79% higher mean pod yield and 25–89% higher mean haulm yield over their respective recurrent parents. Pod yield increase was contributed by increase in seed mass and number of pods per plant.

Key words: Marker-assisted backcrossing (MABC) — leaf rust — late leaf spot — groundnut — introgression line — pod yield

Groundnut (*Arachis hypogaea* L.) is an important oil, food and feed legume crop grown on 25.44 million ha area worldwide with a total production of 45.22 million tons of pods in 2013 (FAOSTAT 2014). China and India are the leading groundnut producers followed by USA and Nigeria. Groundnuts are rich in protein, oil and several micronutrients and hence are important for combating protein, energy and micronutrient malnutrition. Groundnuts are high in energy (564 K cal from 100 g) as they contain 48–50% oil and also contain about 20–25% easily digestible high-quality protein (USDA nutrient database, http://ndb.nal.usda.gov). Groundnut protein products are being tested in peanut milk in Senegal, to add nutritional value to the diets of children. Groundnut skin contains phenolics with high antioxidant properties. Groundnut-based ready-to-use therapeutic products are popularly used by various agencies for treating acute malnutrition among children, women and patients across Africa (UNICEF 2007).

Among biotic constraints that limit groundnut yield, foliar fungal diseases are important globally. Late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk. & M.A. Curtis) van Arx, early leaf spot (ELS) caused by *Cercospora arachidicola* (Hori) and leaf rust caused by *Puccinia arachidis* (Spegazzini) are important foliar fungal diseases of groundnut. Depending on genotype, time and stage of occurrence, losses in pod and fodder yield to the extent of 50–70% and also adversely affect the quality of the produce (Subrahmanyam et al. 1984, Waliyar 1991). Foliar fungal diseases are prevalent during the rainy season in all groundnut-growing areas of Africa and Asia. They also affect the seed quality and fodder value of the plants. Due to these diseases over 50% loss in pod and fodder yield has been estimated when fungicides are not used and production costs are increased by 10% when fungicides are used. An estimated global yield loss of 600 million US$ annually due to LLS alone has been reported (Dwivedi et al. 2003). Host resistance is an economically viable option to the resource poor groundnut farmers as adoption of resistant cultivars by the farmers minimizes losses at farm level and maintains good product quality which inturn contributes to the better economic gains for the farmers.

Sources of resistance to both early and late leaf spot and rust were identified in cultivated groundnut, *A. hypogaea* (Anderson et al. 1993, Waliyar et al. 1993), mini core collections at ICRISAT (Upadhyaya et al. 2014, Sudini et al. 2015), and used to develop breeding lines with resistance (Gorbet et al. 1982, Melouk et al. 1984, Wells et al. 1994, Xue and Holbrook 1998, 1999a,b, Branch and Fletcher 2001). 'Southern Runner' was the first moderate LLS-resistant cultivar to be released in the USA (Gorbet et al. 1987). At ICRISAT, breeding for economically important foliar fungal diseases has resulted in the development of several genotypes with significant levels of resistance to LLS and rust (Singh et al. 2003), which are either released for cultivation in several countries of Asia or have been used as parents in national breeding programmes. Groundnut improvement programmes have relied largely on phenotyping tools that include screening under field and controlled environments to breed foliar fungal disease-resistant varieties (Janila et al. 2013). As compared to the cultivated *A. hypogaea*, very high levels of resistance to LLS and rust were reported in related wild species of groundnut (Stalker and Moss 1987, Stalker and Simpson 1995) and these were utilized to derive interspecific derivatives such as, GPBD 4 (Gowda et al. 2002). ICGV 86855, one of the parents of GPBD 4, is an interspecific derivative between *A. hypogaea* × *A. cardenasii* and shows resistance to both LLS and rust. However, the use of resistance from wild species is limited as a consequence of associated linkage drag resulting in delayed maturity, and undesirable pod and kernel features.

Genomic tools were deployed successfully in groundnut improvement programme with identification of QTL regions governing economically important traits that include leaf rust and late leaf spot resistance (Khedikar et al. 2010, Sujay et al. 2012), drought tolerance (Varshney et al. 2009, Ravi et al. 2011, Gautami et al. 2012), nematode resistance (Church et al. 2000),

peanut bud necrosis disease (PBND) resistance (Bera et al. 2014), seed quality traits (Sarvamangala et al. 2011, Pandey et al. 2014, Wang et al. 2015) and seed mass (Selvaraj et al. 2009). Markers that are closely linked or associated with genes/QTLs for some important target traits have been identified in groundnut and are being utilized to transfer genes/QTLs to elite cultivars. 'NemaTAM', the first root-knot nematode-resistant groundnut variety was bred using marker-assisted breeding (MAB), and it was released for cultivation in the USA (Simpson et al. 2003). Following the identification of markers linked to *FAD* gene alleles conferring the high oleate trait in groundnut (Chu et al. 2007, 2009), efforts were made to pyramid nematode resistance with the high oleate trait. The nematode-resistant cultivar 'Tifguard' was used as recurrent female parent while Georgia-02C and Florida-07 served as donor parents for the high oleate trait to develop a variety, 'Tifguard High O/L' that has resistance to nematode and high oleate trait (Chu et al. 2011). For the first time at ICRISAT, MABC approach was used to improve three popular cultivated groundnut varieties for foliar fungal disease resistance. A major QTL explaining >80% of phenotypic variance for rust resistance was targeted when the MABC was initiated in 2007–08. However, later it was found that the same QTL region explained 67.98% phenotypic variance for LLS resistance (Sujay et al. 2012). In this paper, we report pod and haulm yield performance of LLS and rust-resistant introgression lines of groundnut from multilocation testing that include disease hot spots.

Materials and Methods

Deriving introgression lines (ILs): Three popular varieties cultivated in India, TAG 24, ICGV 91114 and JL 24, were selected as recurrent parents. They are susceptible to foliar fungal diseases. ICGV 91114 is a drought-tolerant variety, and JL 24 is a widely adapted variety that was released in India and several countries in Africa. TAG 24 is a semi-dwarf variety suitable for rainy and postrainy season cultivation. GPBD 4 was used as donor parent for transferring a major QTL governing resistance to rust, and it was reported later that same QTL also governed resistance to LLS (Sujay et al. 2012). The detailed information on the development of rust-resistant ILs using MABC approach is given in Varshney et al. (2014). In brief, three crosses were made with recurrent parents as ovule, and donor as pollen parent. The F_1's hybridity was confirmed by markers to select true hybrids that are used as pollen parent and crossed with recurrent parent to derive BC_1F_1 generation. Backcrossing was repeated to derive BC_2F_1 generation plants. True BC_2F_1 plants, identified using markers, were selfed to obtain BC_2F_2 generation. Leaf samples from BC_2F_2 plants from all three backcrosses were collected and genotyped to identify homozygotes for markers linked to leaf rust and LLS. Homozygous BC_2F_2 selected plants were advanced to BC_2F_6 by selfing. During generation advancement, selections were made for plant morphology, and number, size and shape of pods and kernels from BC_2F_4 generation onwards.

Plant material and field design: Fifty-four ILs were selected from MABC programme for foliar fungal diseases (LLS and rust) resistance to evaluate pod yield, disease resistance and other quality parameters. Evaluations were carried out along with their recurrent parents, TAG 24, ICGV 91114 and JL 24, and donor parent, GPBD 4. The entries were grouped into three trials based on the recurrent parent from which they were derived; groundnut varietal trial (GVT) 1–3. Seventeen ILs in the genetic background of TAG 24 and four checks namely TAG 24, GPBD4, ICGV 86590 and ICGV 91114 constituted GVT-1, 15 ILs in the background of ICGV 91114 and three checks namely GPBD4, ICGV 86590 and ICGV 91114 constituted GVT-2, and 22 ILs in the background of JL 24 and three checks namely JL 24, GPBD4, ICGV 86 590 constituted GVT-3.

The trials were conducted in two seasons, 2013 rainy and 2013/14 postrainy seasons in a Randomized Complete Block Design with two replications in Alfisols (Alfisol-Patancheru Soil Series; Udic Rhodustolf) precision field at ICRISAT, Patancheru. The plot size was four rows of 4 m long on a broad-bed-furrow system of planting. Bed to bed distance was 60 cm, distance between rows of the bed is 30 cm, and plant to plant distance within a row was 10 cm. Standard package of practices, 60 kg P_2O_5 as basal application, seed treatment with Mancozeb @ 2 g per kg of seed and Imidacloprid @ 2 ml per kg of seed, pre-emergence application of Pendimethalin @ 1 kg active ingredient per ha, irrigation soon after planting and subsequently as and when needed in the rainy season, gypsum @ 400 kg per ha at the peak flowering stage, protection against insect pests were followed to raise a healthy crop. The experimental design remained same in 2013/14 postrainy season; however, only mild natural infection of rust was observed in this season.

Following two seasons of evaluations at ICRISAT, 20 best test ILs were selected based on pod yield, disease score, plant morphology, and pod and kernel features. The selected lines were evaluated in multilocation evaluation trials during 2014 rainy season. Two hot spot locations for foliar fungal diseases in India, Dharwad in Karnataka and Aliyarnagar in Tamil Nadu, were identified besides ICRISAT for multilocation evaluation. This trial was referred as advanced groundnut varietal trial (AGVT-1) that has 20 test entries, three recurrent parents, one donor parent and one local control. ALG-06-320 at Aliyarnagar, G-2-52 at Dharwad and ICGV 86590 at ICRISAT were used as local controls.

Disease-screening nursery: In 2013 and 2014 rainy seasons, the trials were conducted in disease-screening nursery that has infector beds after every four broad beds, and along the four borders of the nursery. In 2013, the disease nursery was for rust, while in 2014 the disease nursery was for both rust and LLS. In rust disease nursery, at 45 days after sowing (DAS), glasshouse potted plants of susceptible cultivar, 'TMV 2', inoculated with urediniospores of *Puccinia arachidis,* were placed randomly throughout infector row beds of the experimental field. At 50 DAS, test plants were inoculated by spraying with urediniospores of rust pathogen to ensure uniform heavy disease pressure in the experiment. In rust nursery, LLS was managed by spraying Carbendazim 50% WP (Bavistin) at 1.5 g per litre of water as a preventative measure from 50 DAS at 15 days interval to prevent its incidence in order to avoid interference with rust. In disease nursery for rust and LLS, potted plants of TMV inoculated with conidial suspension of *Phaeoisariopsis personata* and urediniospores of *Puccinia arachidis* were placed followed by spraying of urediniospores and conidial suspension of both rust and LLS pathogens, respectively. In both the disease nurseries, perfo-irrigation was provided daily for 30 min in the evening hours for 30 consecutive days after inoculation, to promote disease development and spread. Infector rows were sown at all the three locations.

Phenotypic observations: Field observations included, days to 75% flowering and maturity, pod and haulm yield, shelling outturn, hundred kernel weight, oil content, number of primary branches, plant height and leaf size. Pod yield was estimated as the weight of dry pod/plot and converted to kg per ha. To obtain shelling outturn, a random sample of 200 g pods were shelled, kernels were weighed and expressed as percentage. Oil content of whole kernels was measured using near-infrared reflectance spectroscopy (NIRSystems model XDS monochromator, FOSS Analytical AB, Sweden, Denmark) which was earlier calibrated. The calibration equation for oil content has an R^2 value of 0.83. Disease score for rust and LLS diseases were recorded at 75 and 90 DAS in each plot on a modified 1–9 scale, as given by Subrahmanyam et al. (1995). The haulm yield was estimated by allowing the harvested plants to dry in the field for two days and subsequently taking the weight of the haulms that included stems and leaves on per-plot basis and converted to kg/ha.

Number of primary branches, plant height and leaf size were measured on 10 random plants in each plot, and average value was taken as observation for each plot. Plant height was measured on 60-day-old plants,

wherein ten randomly selected plants were tagged and plant height (in cm) was measured from the point of emergence of plant from soil surface to tip of main axis. Number of primary branches on the main axis was recorded on the tagged plants at about 60 days by counting. Leaf area of fully expanded leaves of the selected ten tagged plants was measured at 60 days. Second or third node leaf of main axis from each tagged plant was detached, and leaf area of the four leaflets was measured using leaf area meter (LI-COR area meter, Model 3100C, LI-COR Inc., Lincoln, NE, USA) and recorded in square centimetres (cm^2).

Statistical analysis: The data obtained were subjected to analysis of variance (ANOVA) to test the significance of genotypes using *F*-test. Combined analysis of variance (ANOVA) was performed to assess variation attributed to different sources for which mixed model procedure was used to model individual environment error variance. All statistical analysis was carried out using GenStat 15th edition for windows (VSN International, 2012).

Results

Preliminary trials conducted during 2013–14

The ANOVA of three preliminary trials, GVT 1, 2 and 3, showed significant genotypic differences for pod yield, shelling outturn, and hundred-seed weight (HSW) in rainy and postrainy seasons (Tables 1 and 2). Genotypic differences were also significant for leaf area, number of primary branches on main axis and plant height. Combined ANOVA showed significant effect of seasons and G × E interactions for pod yield, shelling outturn and HSW (data not shown). The genotypic differences for rust disease score at 75 and 90 days after sowing (DAS) were significant in all the three trials conducted in 2013 rainy season.

Groundnut varietal trial 1, 2 and 3 trials were scored for rust disease in rainy season in rust nursery, and all the tested ILs had a mean disease score of 2.0–2.5 at 90 DAS except ICGV 13224 in the background of JL 24 that recorded a disease score of 3.0 (Table 3). In GVT 1, 17 ILs recorded a mean rust disease score of 2.0, while their recurrent parent, TAG 24, had a disease score of 4.0, and donor parent GPBD 4 recorded 2.5 at 90 DAS. In GVT 2, of the total 15 ILs tested, 11 ILs recorded a mean disease score of 2.0 and 4 had 2.5. The recurrent parent ICGV 91114 recorded a mean disease score of 6.5 and GPBD 4 recorded 2.0 at 90 DAS. In GVT 3, 18 of the 22 tested ILs recorded a mean disease score of 2.0, 3 ILs had 2.5 and 1 IL recorded 3.0. Mean disease score of the recurrent parent JL 24 in this trial is 7.0 and that of GPBD 4 is 2.5.

Table 1: Individual ANOVA of GVT 1, 2 and 3 trials conducted at ICRISAT-Patancheru in rust disease nursery during 2013 rainy season

Source of variation	DF	HSW	PYH	SHP	DS-Rust75	DS-Rust90
GVT 1						
Replication	1	4.7^{NS}	20593^{NS}	15.5^{NS}	0.1^{NS}	0.2^{NS}
Genotype	20	13.7^{1}	649177^{1}	19.0^{1}	2.6^{1}	3.7^{1}
Error	20	4.2	28976	4.5	0.4	0.1
GVT 2						
Replication	1	49.2^{NS}	14400^{NS}	4.7^{NS}	1.1^{NS}	0.6^{NS}
Genotype	17	42.4^{1}	307711^{1}	21.7^{1}	2.4^{1}	4.9^{1}
Error	17	2.6	16504	4.7	0.4	0.4
GVT 3						
Replication	1	6.5^{NS}	42535^{NS}	8.2^{NS}	2.5^{NS}	0.1^{NS}
Genotype	24	82.1^{1}	510539^{1}	42.4^{1}	2.7^{1}	7.4^{1}
Error	24	5.2	77623	4.5	0.7	0.3

[1]Significant at 5%.
NS, non-significant; DF, degree of freedom; HSW, 100-seed weight (g); PYH, pod yield (kg/ha); SHP, shelling outturn (%); DS-Rust75, rust disease score at 75 DAS; DS-Rust90, rust disease score at 90 DAS.

Yield observations of GVT 1, 2 and 3 were recorded in both rainy and postrainy seasons. Limited irrigation during postrainy season resulted in lower pod yields as compared to rainy season. In rainy season trials, 9 ILs of TAG 24, all 15 ILs of ICGV 91114 and 14 ILs of JL 24 recorded higher pod yield than respective recurrent parent (Table 4). The ILs of TAG 24 recorded pod yield ranging from 1844 to 3089 kg/ha compared to 1750 kg/ha of TAG 24. Similarly, ILs of ICGV 91114 recorded higher pod yield varying from 1503 to 2641 kg/ha as compared to ICGV 91114 with 1438 kg/ha pod yield. The pod yield of ILs of JL 24 varied from 2420 to 3096 kg/ha, while that of JL 24 is 2392 kg/ha (Table 4). Despite poor yields in 2013/14 postrainy season, the pod yield and other parameters of ILs were superior compared to their respective recurrent parents (Table 5). Ten ILs of TAG 24 recorded higher pod yield of 1802–2092 kg/ha than TAG 24 (1783 kg/ha). Similarly, 10 ILs of ICGV 91114 recorded higher pod yield of 1368–1693 kg/ha than ICGV 91114 (1292 kg/ha). The haulm yield, shelling outturn, hundred-seed weight and oil content are given in Table 5.

Based on performance of ILs in GVT 1, 2 and 3 conducted during rainy and postrainy seasons, 20 ILs were selected and advanced to multilocation trial. The selected ILs are as follows: ICGVs 13199, 13200, 13203, 13206, 13207, 13208 and 13209 in the background of TAG 24, ICGVs 13185, 13186, 13189, 13191, 13192 and 13193 in the background of ICGV 91114, and ICGVs 13219, 13220, 13221, 13227, 13228, 13229 and 13230 in the background of JL 24. They recorded a disease score of 2.0 at 90 DAS at ICRISAT during 2013 rainy season as well as higher pod yield, shelling outturn, and other desirable plant, pod and kernel features.

Multilocation trials (MLT) conducted during 2014

Disease incidence at these two locations is by natural infection wherein, both late leaf spot and rust occur together. The incidence of rust is severe at Aliyarnagar during the season, while LLS is moderate. In all the locations, infector rows of susceptible variety around the experimental plot and in between test entries ensured uniform spread of disease. Only the disease scores at ICRISAT, Patancheru and Aliyarnagar, Tamil Nadu were considered for ANOVA as the scoring at Dharwad-Karnataka was recorded on single replication. However, for mean disease score of genotypes, the disease score from Dharwad, Karnataka is also used Individual ANOVA showed significant genotypic differences for LLS and rust disease scores at 90 DAS (Table 6) in both locations. Besides, genotypes were significantly different for pod and haulm yield, shelling outturn, and HSW (Table 6). Combined ANOVA over three environments showed significant differences between genotypes, environments as well as significant genotype × environment interactions for yield and disease traits, except for a non-significant genotype and genotype × environment interaction for LLS at 75 DAS (Table 7). The range and mean of various parameters in individual locations were given in Table 8. The pod yield of test entries showed a huge variation across the three locations; it varied from 1998 to 4774 kg/ha at ICRISAT, Patancheru, from 916 to 2851 kg/ha in Aliyarnagar and from 2384 to 4732 kg/ha at Dharwad. The mean pod yield was low at Aliyarnagar (1982 kg/ha) and high at Dharwad (3837 kg/ha). Higher shelling outturn with a mean of 74% was observed at Dharwad, while at other two locations the mean shelling outturn was 67–69%. For HSW, the trial mean at

Table 2: Individual ANOVA of GVT 1, 2 and 3 trials conducted at ICRISAT-Patancheru under irrigated conditions during 2013/14 postrainy season

Source of Variation	DF	HSW	HYH	LA	OC	OYH	PB	PH	PYH	SHP
GVT 1										
Replication	1	7.8^{NS}	60^{NS}	23.5^{NS}	1.0^{NS}	8861^{NS}	0.1^{NS}	6.4^{NS}	81048^{NS}	4.5^{NS}
Genotype	20	39.4[1]	845877[1]	34.1[1]	11.5[1]	19567[1]	1.7[1]	10.6[1]	101820[1]	20.9[1]
Error	20	2.5	47393	4.016	1.9	2212	0.3	0.8	11263	3.6
GVT 2										
Replication	1	0.3^{NS}	3086^{NS}	14.1^{NS}	2.1^{NS}	4620^{NS}	0.1^{NS}	0.4^{NS}	41322^{NS}	3.8^{NS}
Genotype	17	30.5[1]	465686[1]	26.1^{NS}	4.9[1]	18460[1]	0.8[1]	5.1[1]	99635[1]	17.4^{NS}
Error	17	3.1	146877	17.1	1.2	2340	0.4	1.3	10408	10.4
GVT 3										
Replication	1	0.7^{NS}	116806^{NS}	3.2^{NS}	0.3^{NS}	565^{NS}	1.5^{NS}	0.8^{NS}	8115^{NS}	0.7^{NS}
Genotype	24	35.9[1]	1281829[1]	142.0[1]	1.6[1]	20398[1]	1.6^{NS}	7.4[1]	147746[1]	20.7[1]
Error	24	2.6	699331	9.8	0.7	1268	0.7	1.4	7750	5.0

[1]Significant at 5%; NS, nonsignificant; DF, degree of freedom; HSW, 100-seed weight (g); PYH, pod yield (kg/ha); SHP, shelling outturn (%); OC, oil content (%); HYH, haulm yield (kg/ha); LA, leaf area (cm^2); OYH, oil yield (kg/ha); PB, number of primary branches per plant; PH, plant height (cm).

Table 3: Disease score of introgression lines and their parents evaluated in preliminary GVT 1, 2 and 3 trials conducted at ICRISAT-Patancheru during 2013 rainy season

Genotypes (parents/introgression lines)	Number of Introgression lines	Rust disease score (90 DAS)
ICGVs 13199, 13200, 13201, 13202, 13203, 13204, 13205, 13206, 13207, 13208, 13209, 13210, 13211, 13212, 13213, 13214, 13215 of TAG 24 background, 13186, 13187, 13188, 13189, 13190, 13191, 13192, 13194, 13196, 13197, 13198 of ICGV 91114 background, 13216, 13217, 13218, 13219, 13220, 13221, 13222, 13223, 13227, 13228, 13229, 13231, 13232, 13233, 13234, 13235, 13236, and 13237 of JL 24 background	46	2.0
ICGVs 13184, 13185, 13193 and 13195 of ICGV 91114 background, and ICGVs 13225, 13226 and 13230 in the background of JL 24	7	2.5
ICGV 13224 in the back ground of JL 24	1	3.0
GPBD 4 (donor parent)		2.0–2.5
TAG 24		4.0
ICGV 91114		6.5
JL 24		7.0

Table 4: Mean performance of introgression lines during 2013 rainy season under disease pressure at ICRISAT-Patancheru

Genotype (s)	Pod yield (kg/ha)	Shelling outturn (%)	Hundred-seed weight (g)
TAG 24 (RP)	1750	69	34
9 ILs of TAG 24[1]	1844–3089	67–73	32–37
ICGV 91114 (RP)	1438	73	31
All 15 ILs of ICGV 91114	1503–2641	62–73	27–46
JL 24 (RP)	2392	70	33
14 ILs of JL 24[1]	2420–3096	66–73	34–48
GPBD 4 (DP)	3095	71	37

[1]ILs selected based on higher pod yield than respective recurrent parent; RP, recurrent parent; DP, donor parent.

Aliyarnagar was 35 g, while it was 39 g at ICRISAT and 40 g at Dharwad. The oil content of the entries varied from 43 to 46% at Dharwad, while at ICRISAT it varied from 49 to 56%.

The disease scores on susceptible parent and infector rows indicate severe incidence of rust in tested environments. TMV 2, a susceptible variety used as infector row, recorded a disease score of 9.0 for rust and LLS at 90 DAS at ICRISAT. The disease scores at 90 DAS at different locations in susceptible recurrent parents, their respective ILs and resistant donor parent given in Table 9 indicated that the disease environment clearly distinguished the resistant and susceptible lines. The disease score of GPBD 4 for rust at 90 DAS varied between 1.0 and 2.0 across three locations, while LLS disease score at 90 DAS on GPBD 4 varied between 1 and 3. The rust disease score of TAG 24 was 6 across three environments, while the ILs had a disease score of 1–4. Similarly, rust disease score of ICGV 91114 and JL 24 varied from 4 to 6, whereas their ILs score was between 1–3 and 2–4, respectively. With one exception of an IL, ICGV 13219 (in the background of JL 24) that recorded a rust disease score of 7.0. Besides rust, the ILs also showed enhanced resistance to LLS. Incidence of LLS at Aliyarnagar, Tamil Nadu was low; therefore, LLS scores from this location are not used for making comparisons. The LLS disease score of TAG 24 was 6 at ICRISAT and 7 at Dharwad, while the ILs has a score of 2–5 at ICRISAT and 3–7 at Dharwad. Similarly, some ILs of ICGV 91114 has a LLS disease score of 2 at ICRISAT and 3 at Dharwad, while ICGV 91114 has a score of 5 at ICRISAT and 7 at Dharwad. JL 24 had a LLS disease score of 6 at both ICRISAT and Dharwad, and some ILs of JL 24 recorded score of 3 at ICRISAT and 4 at Dharwad. Thus, some of the ILs recorded a disease score of 2 and 3 similar to donor parent at ICRISAT and Dharwad. The disease reaction of recurrent parents, TAG 24, ICGV 91114, JL24 and some selected introgression lines at harvest in disease-screening nursery at ICRISAT can be seen in Fig. 1.

Mean performance of introgression lines over three locations

Mean performance of ILs and their respective RPs for yield and related traits over three environments are given in Table 10. The

Table 5: Mean performance of introgression lines during 2013/14 postrainy season at ICRISAT-Patancheru

Genotype (s)	Pod yield (kg/ha)	Shelling outturn (%)	Hundred-seed weight (g)	Oil content (%)	Haulm yield (kg/ha)
TAG 24 (RP)	1783	72	36	51	1083
10 ILs of TAG 24[1]	1802–2092	65–77	32–47	50–56	1250–1842
ICGV 91114 (RP)	1292	66	34	49	2500
10 ILs of ICGV 91114[1]	1368–1693	62–71	34–44	51–55	1150–3000
JL 24 (RP)	1211	71	34	50	2663
10 ILs of JL 24[1]	1302–1534	65–77	32–47	49–53	1542–3525
GPBD 4 (DP)	1434	72	33	53	2495

[1]ILs selected based on higher pod yield than respective recurrent parent; RP, recurrent parent; DP, donor parent.

Table 6: Individual ANOVA of multilocation trial conducted at three locations during 2014 rainy season

Source of variation	DF	HSW	HYH	PYH	SHP	DS-LLS 75	DS-LLS 90	DS-Rust75	DS-Rust90
ICRISAT, Patancheru (Location 1)									
Replication	1	4.8^{NS}	–	753583^{NS}	8.4^{NS}	0.2^{NS}	0.1^{NS}	0.6^{NS}	1.1^{NS}
Genotype	24	51.1^{1}	–	777908^{1}	18.8^{1}	1.7^{NS}	6.1^{1}	3.2^{NS}	5.9^{1}
Error	24	10.6	–	34700	9.3	1.1	0.8	1.4	0.9
Aliyarnagar, Tamil Nadu (Location 2)									
Replication	1	10.8^{NS}	3865^{NS}	6540^{NS}	15.5^{NS}	1.4^{NS}	1.1^{NS}	0.5^{NS}	0.6^{NS}
Genotype	24	43.5^{1}	774997^{1}	530901^{1}	31.1^{1}	0.2^{NS}	6.8^{1}	3.0^{1}	12.0^{1}
Error	24	3.2	99787	62321	2.2	0.2	1.7	0.6	0.9
Dharwad, Karnataka (Location 3)[2]									
Replication	1	2.1^{NS}	37919^{NS}	221230^{NS}	1.3^{NS}	na	na	na	na
Genotype	23	44.7^{1}	937368^{1}	654177^{1}	24.2^{1}	na	na	na	na
Error	23	4.3	167978	133201	4.3	na	na	na	na

[1]Significant at 5%; NS, nonsignificant; DF, degree of freedom; HSW, 100-seed weight (g); HYH, haulm yield (kg/ha); PYH, pod yield (kg/ha); SHP, shelling outturn (%); DS-Rust75, rust disease score at 75 days after sowing; DS-Rust90, rust disease score at 90 DAS; DS-LLS75, late leaf spot disease score at 75 DAS; DS-LLS90, late leaf spot disease score at 90 DAS.
[2]ICGV 91114 data not recorded at Dharwad, Karnataka.
na, not available as disease score at Dharwad, Karnataka was recorded on single replication.

Table 7: Combined ANOVA of yield and disease parameters of groundnut introgression lines over three environments during 2014 rainy season

Source of variation	DF	HSW	PYH	SHP	DS-LLS75	DS-LLS90	DS-Rust75	DS-Rust90
Genotype	24	64.2^{1}	1163597.0^{1}	83.9^{1}	0.9^{NS}	9.0^{1}	5.9^{1}	12.3^{1}
Environment	2	349.2^{1}	45647089.0^{1}	33.3^{1}	203.7^{1}	260.7^{1}	88.4^{1}	52.4^{1}
Genotype × Environment	47	28.4^{1}	413459.0^{1}	613.5^{1}	0.9^{NS}	3.1^{1}	2.7^{1}	2.9^{1}
Residual	70	5.7	86180.0	13.2	0.6	1.0	0.9	1.0

[1]Significant at 5%.
NS, nonsignificant; DF, degree of freedom; HSW, 100-seed weight (g); PYH, pod yield (kg/ha); SHP, shelling outturn (%); DS-Rust75, rust disease score at 75 DAS; DS-Rust90, rust disease score at 90 DAS; DS-LLS75, late leaf spot disease score at 75 DAS; DS-LLS90, late leaf spot disease score at 90 DAS.

Table 8: Range and mean of various yield and quality parameters observed in the multilocation trials conducted in three disease environments during 2014 rainy season

Trait	Parameters	ICRISAT, Patancheru, Telangana	Aliyarnagar, Tamil Nadu	Dharwad, Karnataka
Pod yield (kg/ha)	Range	1998–4774	916–2851	2384–4732
	Mean	3442	1982	3837
Shelling outturn (%)	Range	64–75	58–74	67–78
	Mean	69	67	74
100-seed weight (g)	Range	26–47	25–49	31–48
	Mean	39	35	40
Oil content (%)	Range	49–56	–	43–46
	Mean	52	–	45
Haulm yield (kg/ha)	Range	–	1921–4580	1419–4324
	Mean	–	2972	2759

pod yield of 7 ILs of TAG 24 ranged from 2770 to 3811 kg/ha, which is higher in comparison with their recurrent parent, TAG 24 with a pod yield of 2388 kg/ha. One of the ILs of TAG 24, ICGV 13203, recorded a mean pod yield of 2023 kg/ha. Similarly, 6 ILs of ICGV 91114 recorded a mean pod yield ranging from 3053 to 3546 kg/ha, in comparison to their recurrent parent, ICGV 91114 with a pod yield of 1846 kg/ha. All the seven ILs of JL 24 recorded higher pod yield, it varied from 3034 to

Table 9: Disease score of groundnut introgression lines and recurrent and donor parents in three locations during 2014 rainy season evaluations in disease nursery

	DS-Rust90			DS-LLS90		
Location	ICRISAT, Patancheru	Aliyarnagar, Tamil Nadu	Dharwad, Karnataka[1]	ICRISAT, Patancheru	Aliyarnagar, Tamil Nadu	Dharwad, Karnataka[1]
TAG 24	6	6	6	6	3	7
7 ILs of TAG 24	2–3	1	3–4	2–5	1–3	3–7
ICGV 91114	5	6	4	5	3	7
6 ILs of ICGV 91114	2–3	1–3	2–3	2–4	1–4	3–6
JL 24	6	6	4	6	3	6
7 ILs of JL 24	2–4	2–3	2–4[2]	3–5	1–2	4–7
GPBD 4	2	1	2	2	1	3

[1]Observation on single replication; DS-Rust 90, rust disease score at 90 DAS; DS-LLS90, LLS disease score at 90 DAS. Disease score is on a 1–9 scale.
[2]One IL, ICGV 13219 recorded a disease score of 7 for rust (not included).

3303 kg/ha compared to their recurrent parent, JL 24 with a pod yield of 2075 kg/ha. The mean shelling outturn over three environments is 69% for TAG 24 and its ILs recorded a shelling outturn varying from 68 to 74%. The shelling outturn of ICGV 91114 is 71% while the ILs of ICGV 91114 recorded 69 to 70%. The ILs of JL 24 recorded shelling outturn of 63 to 72% compared to JL 24, which had a shelling outturn of 70%. The mean HSW of TAG 24 is 36 g and its ILs recorded a HSW varying from 32 to 41 g. The HSW of ICGV 91114 is 31 g and its ILs recorded HSW varying from 35 to 43 g. Similarly, JL 24 had a HSW of 38 g and its ILs recorded HSW varying from 34 to 47 g. The haulm yield of TAG 24 is 1670 kg/ha and its ILs recorded a haulm yield of 2381–3079 kg/ha. ICGV 91114 had a haulm yield of 2003 kg/ha, and its ILs recorded higher haulm yields of 2816–3785 kg/ha. The ILs of JL 24 recorded a haulm yield of 2301–3160 kg/ha, while JL 24 had a haulm yield of 2336 kg/ha. The oil content of ILs and their respective recurrent parent were similar. The ILs and recurrent parents were harvested at 103 days after sowing at ICRISAT, and between 105 and 110 days at Aliyarnagar, while, GPBD 4, the donor parent was harvested late, 115 days at ICRISAT and 120 days at Aliyarnagar.

Discussion

Foliar fungal diseases, early leaf spot, late leaf spot and rust are important biotic constraints to groundnut production across the growing regions world over. Late leaf spot and rust are economically important in South Asia and Africa. Both of them usually appear together and can substantially reduce the pod and haulm yield by 50–70% depending on the genotypes and time of occurrence. Phenotyping tools such as field and controlled environment disease-screening protocols (Janila et al. 2013) were used to screen for rust and LLS-resistant/LLS-tolerant lines among the cultivated and wild species and to develop resistant breeding lines. Wild species were used in crossing programme to derive resistant interspecific derivatives (Company et al. 1982, Gardner and Stalker 1983) and second-cycle breeding lines. However, such interspecific derivatives and breeding lines often lacked desirable pod and kernel features, and consequently, they were not preferred by the farmers and traders. Identification of resistant lines followed by intense selection for desirable pod and kernel features among segregating populations was used to overcome the limitations imposed by hybridizing resistant derivatives with promising cultivars. This subsequently led to development of disease-resistant groundnut varieties with desirable pod and kernel features, such as GPBD 4 (Gowda et al. 2002). Another limitation that remained a challenge to achieve in groundnut improvement programmes is combining foliar fungal disease resistance with early maturity. Early maturing varieties are preferred in several rainfed agroecologies of Africa and Asia where the length of the crop growing season is short and end-off season drought is a major production limiting factor.

As compared to conventional selection, DNA markers offer significant advantages and so several efforts have been made to identify genes and map QTLs for LLS and rust resistance in groundnut. Leal-Bertioli et al. (2009) using an F_2 mapping population derived from a cross between *A. duranensis* accession K7988 and *A. stenosperma* V10309 have reported 34 sequence confirmed disease resistance genes and five QTLs linked to the A-genome of *Arachis* for LLS resistance. Further, it is not an easy task to move QTL(s) from wild diploid to cultivated tetraploid genotypes. Therefore, it is always beneficial to identify resistance genes in cultivated or intermediate lines with whom there is no problem of crossability. The most significant breakthrough came when a major QTL explaining over 80% of phenotypic variance for rust resistance was reported in groundnut (Sujay et al. 2012). Markers linked to this QTL were utilized at ICRISAT to improve rust resistance of three popular varieties TAG 24, ICGV 91114 and JL 24 by marker-assisted backcross breeding programme (Varshney et al. 2014). Later Sujay et al. (2012) reported that the same QTL region also contributes to LLS resistance and explained about 67.98% of phenotypic variance. QTLs conferring multiple disease resistance have also been reported in maize (Lübberstedt et al. 2006, Belcher 2009, Ali et al. 2013), ryegrass (Jo et al. 2008), etc. Earlier, MABC approach was also used to improve rust resistance in wheat (Randhawa et al. 2009) and sunflower (Bulos et al. 2013).

Marker-assisted backcrossing approach was previously used in groundnut to improve nematode resistance and oil quality in the USA, and varieties were released for cultivation (Simpson et al. 2003, Chu et al. 2011). The first MABC groundnut variety for nematode resistance was released in 2003 (Simpson et al. 2003). For foliar fungal disease resistance, we report for first time the development of superior performing groundnut ILs using MABC approach. The most significant outcome of the MABC programme was combining LLS and rust resistance with early maturity in groundnut (Varshney et al. 2014). One dominant (IPAHM103) and three codominant (GM2079, GM1536, GM2301) linked markers (Sujay et al. 2012) were employed for foreground selection of QTL region in the backcross population while for recurrent parent phenotype, visual selection for morphological, pod and kernel features was carried out. This approach is a combination of conventional breeding for back-

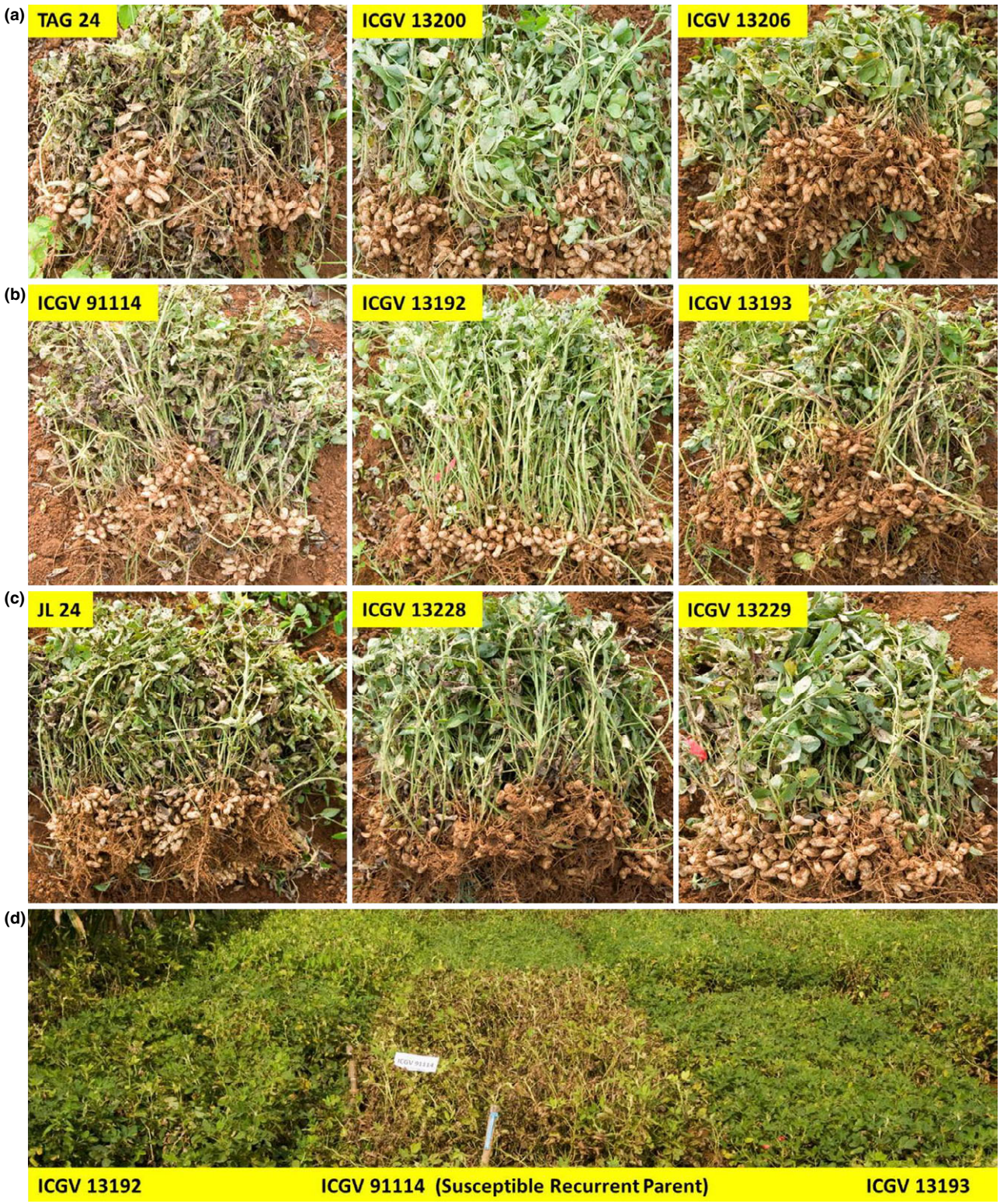

Fig. 1: Disease reaction of recurrent parent, TAG 24, ICGV 91114 and JL 24, and some selected introgression lines (a, b, c) at the time of harvest (d) in the filed before harvest in disease-screening nursery at ICRISAT, Patancheru, during 2014 rainy season

ground selection, and MABC for foreground selection of QTL region for resistance. Hybridity confirmation of backcross plants in BC_1F_1 and BC_2F_1 was carried out based on foreground selection. BC_2F_1 plants were derived by making cross between recurrent parent as female and BC_1F_1 as pollen parent. The BC_1F_1 plants are heterozygous for all the four makers in the QTL region. From screening of 194 BC_2F_1 plants with four markers of the QTL regions, 56 plants, including 13 from recurrent parent, ICGV 91114, 21 from JL 24 and 22 from TAG 24 were heterozygotes for all four SSR markers (Fig 2). The BC_2F2 seed was collected from 56 BC_2F_1 hybridity-confirmed plants. A total of 498 BC_2F_2 plants were tested for marker homozygotes to

Table 10: Mean performance of groundnut introgression lines and their respective recurrent parents in a multilocation trial conducted during 2014 rainy season in disease nurseries

	Mean over environment					
Trait	TAG 24	Seven ILs of TAG 24	ICGV 91114	Six ILs of ICGV 91114	JL 24	Seven ILs of JL 24
Pod yield (kg/ha)	2388	2770–3811[1]	1846	3053–3546	2075	3034–3303
Shelling outturn (%)	69	68–74	71	69–70	70	63–72
100-seed weight (g)	36	32–41	31	35–43	38	34–47
Haulm yield (kg/ha)[2]	1670	2381–3079	2003[3]	2816–3785	2336	2301–3160
Oil content (%)[3]	48	48–49	49[3]	47–49	47	47–50

[1]ICGV 13203 is not included, it recorded mean pod yield of 2023 kg/ha, which was lower than TAG 24.
[2]Mean of two environment, Aliyarnagar, Tamil Nadu and Dharwad, Karnataka.
[3]Mean of two environments, ICRISAT-rust nursery and Dharwad-Karnataka.

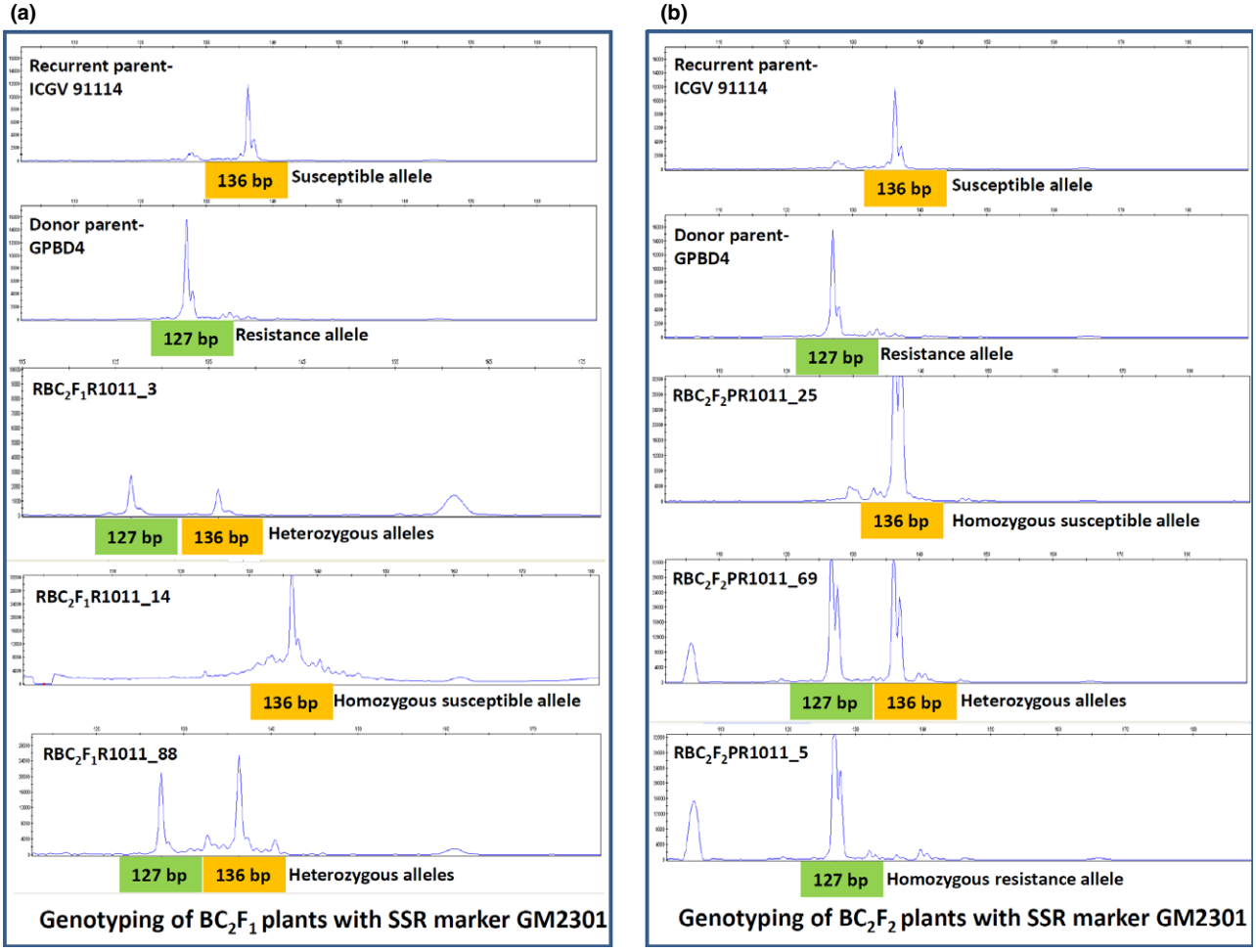

Fig. 2: Foreground selection for resistance alleles in BC_2F_1 and BC_2F_2 generations using linked marker, GM2301 in a cross, ICGV 91114 X GPBD 4. (a) Selection of heterozygous plants in BC_2F_1 (b) Selection of homozygous resistance allele in BC_2F_2 generation. The resistant parent GPBD 4 produced 127-bp amplicon while susceptible parent produced 136-bp amplicon

identify 87 BC_2F_2 plants with homozygous resistant alleles for all the four SSR loci (Fig 2). Rejecting 80% of population in BC_2F_2 generation based on markers reduced the burden and resources of handling large populations. In conventional breeding, all BC_2F_2s will be advanced to $BC_2F_{4/5}$ generations, and phenotypic selections for disease resistance are made in these advanced generation progenies. Further generation advancement of 20% selected BC_2F_2 plants was performed by selfing and by phenotypic selection for plant morphology and pod shape, size and number. The selected 117 progenies of BC_2F_5 were screened for rust disease and marker confirmation was also performed using four linked markers (IPAHM103, GM2079, GM1536 and GM2301) of the QTL region. All the selected BC_2F_6 progenies confirmed homozygosity for all the four markers, but only 62 progenies recorded a disease score of 2.0 for rust similar to donor parent GBPD 4. The observation suggests probable role of background genotypes on expression of the QTL. The homozygous marker combination of four markers in the QTL region,

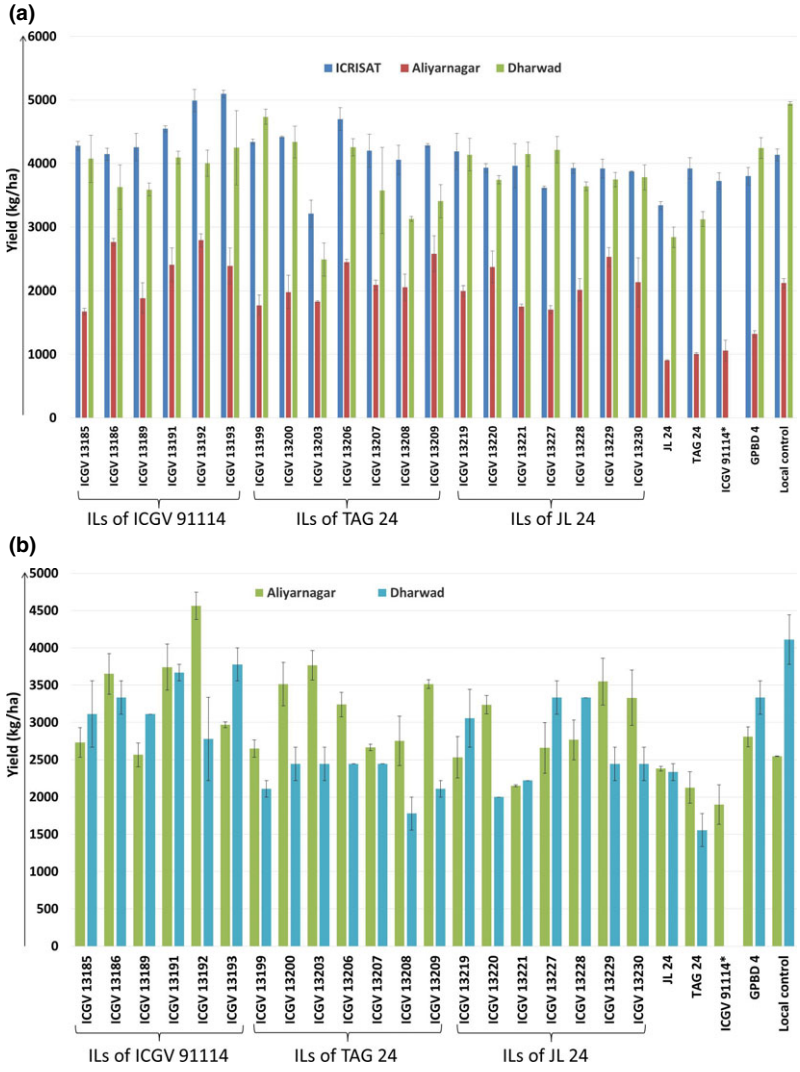

Fig. 3: (a) Pod yield of introgression lines, recurrent (JL 24, TAG 24 and ICGV 91114) and donor parents (GBPD 4), and local control at ICRISAT, Aliyarnagar and Dharwad locations during 2014 rainy season under foliar fungal disease conditions. (b) Haulm yield of introgression lines, recurrent (JL 24, TAG 24 and ICGV 91114) and donor parents (GBPD 4), and local control at Aliyarnagar and Dharwad locations during 2014 rainy season under foliar fungal disease conditions

selected in BC_2F_2, remained stable in BC_2F_6. Selections were made for pod shape, size and yield to select 54 progenies in BC_2F_6.

A set of 54 ILs in the background of three popular varieties of groundnut, TAG 24 ICGV 91114 and JL 24, were selected from the MABC programme to evaluate for rust disease, yield and quality parameters, and morphological features. ILs were evaluated in three separate trials, GVT 1, 2 and 3 in rust disease nursery during rainy season, as well as in disease-free postrainy season at ICRISAT during 2013–14. The rust disease score at 90 DAS of ILs was comparable with that of resistant donor, GPBD 4 which has a disease score of 2.0–2.5 across three trials (Table 3). Of 54 tested ILs, 46 recorded a mean disease score of 2.0 and 7 ILs recorded 2.5. This again suggests probable role of background genotype on the level of resistance manifested by the QTL.

The ILs recorded 1–84% pod yield increase over their respective recurrent parent in rainy season and 1–31% in postrainy season (Table 4, 5). The 9 ILs of TAG 24 recorded 5–77% higher pod yield than TAG 24 in rainy season, and 10 ILs of TAG 24 recorded 1–17% pod yield increase over TAG 24 in postrainy season. Similarly, the 15 ILs of ICGV 91114 recorded 5–84% pod yield increase in rainy season, while 10 ILs of ICGV 91114 recorded 6–31% pod yield increase in postrainy season. The 14 ILs of JL 24 recorded 1–29% higher pod yield than JL 24 in rainy season, while 10 ILs of JL 24 recorded 8–27% higher pod yield in postrainy season. The significant increase in pod yield in rust nursery in rainy season indicates protection offered by resistance QTL to pod yield loss caused by diseases. However, the rust disease score of all the ILs of TAG 24 recorded was 2.0, but the pod yield of ILs varied from 1844 to 3089 kg/ha, suggesting the importance of selection for yield and yield parameters along breeding cycles and yield evaluation trials in MABC programmes to identify superior performing progenies. MABC approach greatly reduces the burden on time and resources for selection of target trait, nonetheless, it needs strong phenotyping both for the target trait, foliar fungal disease resistance in this case, and other yield-related parameters. Even under disease-free environment of postrainy season, it is interesting to note that the ILs recorded higher pod yield than their respective recurrent parents, suggesting in part the possible contribution of the QTL region to pod yield besides, resistance to rust and LLS (data not shown). Number of pods per plant and seed mass are two important contributing factors for enhanced pod yield in groundnut (Ratnakumar et al. 2012). However in the present study, increase in number of pods per plant of ILs may have contributed to the observed significant increase in pod yield of ILs compared to their respective recurrent parent as the association of seed mass and pod yield among ILs was very low (data not shown). Significant differences were observed among the genotypes for measured morphological traits such as, leaf area, plant height and

Table 11: Best-performing introgression lines based on mean performance over the environment from multilocation trials conducted in 2014 rainy season

Groundnut genotype	Pod yield (kg/ha)	Haulm yield (kg/ha)[1]	HSW (g)	Oil content (%)[2]	Shelling outturn (%)	Rust score at 90 DAS[1]	LLS score at 90 DAS[2]
ICGV 13200 (IL)	3309	2956	40	49	70	2.3	2.0
ICGV 13206 (IL)	3811	2818	36	49	72	2.1	2.0
TAG 24 (RP)	2388	1670	36	48	69	5.6	5.0
ICGV 13192 (IL)	3241	3785	35	47	69	2.2	3.5
ICGV 13193 (IL)	3310	3275	37	48	70	2.7	4.7
ICGV 91114 (RP)	1846	2003[3]	31	49[3]	71	5.0	5.2
ICGV 13228 (IL)	3152	2951	42	47	65	2.5	3.9
ICGV 13229 (IL)	3248	2900	47	49	67	2.4	4.0
JL 24 (RP)	2075	2326	38	47	70	4.8	4.5

[1]Mean of two environments, Aliyarnagar, Tamil Nadu and Dharwad, Karnataka.
[2]Mean of two environments, ICRISAT and Dharwad, Karnataka.
[3]Mean of two environments ICRISAT and Aliyarnagar, Tamil Nadu.
IL, Introgression line; RP, recurrent parent; HSW, 100-seed weight (g); Rust and LLS scores are on a scale of 1–9.

number of primary branches, as well as observed morphological traits, such as leaf colour and shape, pod shape, reticulation, constriction and beak, and kernel colour, shape and size suggesting need for genotype-based background selection. However, additional backcrosses may not be desirable as the level of resistance for LLS and rust was low in the lines derived from third and backcrossing (data not shown).

Selections were made based on disease reaction, pod yield and yield parameters, and on relatedness of ILs to recurrent parent phenotype for the above morphological features during 2013 and 2013/14 trials. Twenty superior ILs were selected for multilocation trial (MLT). In the meanwhile, it was reported that the same QTL region also confers resistance to LLS and explains about 67.98% of phenotypic variance (Sujay et al. 2012). In field conditions, incidence of LLS and rust occurs together. Considering these two, MLT was planned in disease hot spot locations, Aliyarnagar, Tamil Nadu and Dharwad, Karnataka where both LLS and rust occur together. The third location was ICRISAT disease nursery for LLS and rust. Significant genotypic differences were observed for pod and haulm yield, shelling outturn, HSW and rust and LLS disease scores at 90 DAS at the locations. Combined ANOVA over three location showed significant genotype × environment interactions for pod yield, HSW and shelling outturn, and rust and LLS disease score at 90 DAS. The superiority of introgression lines over recurrent parents for pod and haulm yield at three testing locations is shown in Fig. 3(a, b). The genotypes performed differently for pod and haulm yield at three locations, pod yield was low for all genotypes at Aliyarnagar location. However, the uniformity of disease score of ILs across three locations suggests stability of the resistance transferred to the susceptible recurrent parents. The role of background genotype, environment and their interactions were profound on QTL expression. Genotypes responded differently in different environment (Fig 3a, b) so we proceeded to select best genotypes for each of the tested environment. Best genotypes across the environments were identified based on their mean performance over three locations.

Best-performing ILs for each of the three locations were identified based on their performance at the respective locations. Among the ILs of TAG 24, ICGV 13206 with pod yield of 4777 kg/ha is best at ICRISAT, ICGV 13199 with pod yield of 4656 kg/ha is best at Dharwad and ICGV 13209 with pod yield of 2549 kg/ha is best at Aliyarnagar. Among ILs of ICGV 91114, ICGV 13185 with pod yield of 4690 kg/ha at ICRISAT and 4172 kg/ha at Dharwad was the best performer at both these locations, while ICGV 13192 with pod yield of 2800 kg/ha was the best performer at Aliyarnagar. Similarly among 7 ILs of JL 24, ICGV 13227 is best at ICRISAT as well as Dharwad with pod yield of 3866 kg/ha and 4276 kg/ha, respectively at these locations. ICGV 13229 with pod yield of 2553 kg/ha is best at Aliyarnagar. The ILs had similar maturity duration like their recurrent parents at the tested locations.

Two best ILs in the background of each recurrent parent were identified based on their mean performance over three environments (Table 11). Among the ILs of TAG 24, ICGV 13200 and ICGV 13206 were best performers across three locations. The mean disease score for rust is 2.3 for ICGV 13200 and 2.1 for ICGV 13206, similar to donor parent score of 2.0, while TAG 24 recorded a mean score of 5.6. For LLS, the disease score at 90 DAS is 2.0 for both ICGV 13200 and ICGV 13206, while it is 3.3 for donor-resistant parent. The LLS score of TAG 24 is 5.0. The selected ILs, ICGV 13192 and ICGV 13193 in the background of ICGV 91114, recorded a mean pod and haulm yield of 3241 kg/ha and 3785 kg/ha, and 3310 kg/ha and 3275 kg/ha, respectively, which was an increase of 76–79% in pod yield and 64–89% in haulm yield over ICGV 91114. The mean rust score at 90 DAS of ICGV 13192 is 2.2 similar to its donor parent GPBD 4 and that of ICGV 13193 is 2.7 as compared to disease score of 5.0 of ICGV 91114. The LLS score for ICGV 13192 is 3.5 which was close to that of donor parent score of 3.3 while ICGV 13193 and ICGV 91114 had scores of 4.7 and 5.2, respectively. Among the ILs of JL 24, ICGV 13228 had a mean pod yield of 3152 kg/ha and haulm yield of 2951 kg/ha, and ICGV 13229 recorded a mean pod yield of 3248 kg/ha and haulm yield of 2900 kg/ha. The pod and haulm yield increase are 52–57% and 25–27% compared to their recurrent parent, JL 24, respectively. The rust disease score was 2.5 for ICGV 13228 and 2.4 for ICGV 13229, similar to donor parent score of 2.0, while score of JL 24 is 4.8. The LLS score was 3.9 for ICGV 13228 and 4.0 for ICGV 13229, which is closer to JL 24 with a score of 4.5. Shelling outturn, HSW and oil content of ILs and recurrent parent are given in Table 11.

The ILs of groundnut used in this study combined resistance to LLS and rust diseases with early maturity. The improvement in level of resistance for LLS is low compared to rust among the ILs, and it may in part be attributed to absence of phenotypic selection for LLS resistance in advance generations as well as low PV explained for LLS resistance. In case of rust, the PV explained was >80% and rigorous phenotyping for rust resistance in advance generations may have resulted in background genotype selection to produce ILs with rust resistance same as GPBD 4. The target QTL in our study explains about 68% PV

for LLS resistance and might have therefore showed weak correlation with molecular marker selection compared to rust resistance. Moreover, the selected progenies in $BC_2F_{5/6}$ generations were screened in rust disease nursery that might have enabled background selection. However, in case of resistance to LLS, only QTL homozygote selection was made, and phenotypic selection was performed later in advance trials; consequently, there was no opportunity for background genotype selection. The selected ILs recorded 39–79% higher mean pod yield and 25–89% higher mean haulm yield than their respective recurrent parents in multilocation testing over three locations. The HSW of selected ILs is slightly higher or comparable to their recurrent parents, while shelling outturn and oil content of ILs is at par with the recurrent parents. The ILs showed resistance to rust and LLS similar to the donor parent, GPBD 4 but maturity duration was similar to recurrent parent. The best-performing lines can be released as variety after completion of evaluations at state/national level.

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