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Validation of markers linked to late leaf spot and rust resistance, and selection of superior genotypes among diverse recombinant inbred lines and backcross lines in peanut (*Arachis hypogaea* L.)

M. Sukruth · S. A. Paratwagh · V. Sujay · V. Kumari · M. V. C. Gowda · H. L. Nadaf · B. N. Motagi · S. Lingaraju · M. K. Pandey · R. K. Varshney · R. S. Bhat

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Abstract Recombinant inbred lines (RILs) from four populations involving cultivated varieties, and backcross lines from three populations involving cultivated varieties and synthetic tetraploids (developed from wild diploids) were employed for validating late leaf spot (LLS) and rust resistance-linked markers and identifying superior genotypes in peanut. GM2009, GM2301, GM2079, GM1536, GM1954 and IPAHM103 markers showed significant association with rust resistance. They were successfully validated in a new RIL (TG 19 × GPBD 4) and two backcross (DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5) populations. GM1954, GM1009 and GM1573 markers showed significant association with LLS resistance. TAG 19 × GPBD 4 and ICGS 76 × ISATGR 278-18 populations showed strong co-segregation of LLS-linked markers with the phenotype. From these genetic resources, six superior genotypes were identified. RIL 78-1 was resistant to LLS and rust, and recorded 30 % more pod yield than GPBD 4 (control). It also had higher kernel yield and oil yield along with higher oleate and linoleate content over GPBD 4. These genetic and genomic resources could be useful in breeding for LLS and rust resistance in peanut.

Keywords Recombinant inbred lines · Backcross lines with synthetic tetraploids · Late leaf spot and rust resistance · Marker validation · Productivity · Superior genotypes

M. Sukruth · S. A. Paratwagh · R. S. Bhat (✉)
Department of Biotechnology, University of Agricultural Sciences, Dharwad 580 005, India
e-mail: bhatramesh12@gmail.com

V. Sujay · V. Kumari · M. V. C. Gowda · H. L. Nadaf · B. N. Motagi
Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad 580 005, India

S. Lingaraju
Department of Plant Pathology, University of Agricultural Sciences, Dharwad 580 005, India

M. K. Pandey · R. K. Varshney
Center of Excellence in Genomics (CEG), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad 502 324, India

Introduction

Peanut (*Arachis hypogaea* L.) is a legume crop mainly grown for its seed which contains 25–28 % protein and 48–50 % oil. In India, Spanish types are most widely cultivated, and they are highly susceptible to foliar fungal diseases like rust (*Puccinia arachidis* Speg,), early leaf spot (*Cercospora arachidicola* Hori) and late leaf spot (LLS) (*Phaeoisariopsis personata* [(Berk. and Curt) Deighton)]. These diseases cause severe yield losses (up to 70 %) and reduce the quality of the pod and fodder (McDonald et al. 1985).

Breeding for resistant varieties is a preferred means of managing the foliar diseases over chemical control

considering the additional cost and biological safety. But, the success of breeding for disease resistance is influenced by the availability and identification of resistance sources, and combining resistance with high productivity and desirable pod features. Valencia landraces and wild species of peanut possess high level of resistance to foliar diseases, but the resistance is generally linked to low productivity, late maturity, poor adaptability and undesirable pod features (Wynne et al. 1991; Singh et al. 1997). Complex inheritance pattern of foliar disease resistance (Bromfield & Bailey 1972; Tiwari et al. 1984; Paramasivam et al. 1990) and interference among these diseases make phenotypic selection less effective.

Integration of genomic tools like markers and marker assisted selection (MAS) with conventional breeding approaches might enhance the precision and speed of developing peanut cultivars with late LLS and rust resistance. In this direction, several recombinant inbred line (RIL) and backcross line (BCL) mapping populations were developed at UAS, Dharwad, India (Bhat et al. 2012). BCLs were developed (Varshakumari et al. 2014) using LLS and rust susceptible varieties (ICGS 76 and DH 86) and LLS resistant synthetic tetraploids (ISATGR 278-18 and ISATGR 5B) developed at ICRISAT. The RILs were derived from susceptible varieties (TAG 24, TG 26, TG 19 and TG 49) and GPBD 4, an improved Spanish type with disease resistance and superior productivity. These RILs were extensively phenotyped over the years (2004-2011) for foliar disease resistance. QTL analysis in two RIL populations (TAG 24 × GPBD 4 and TG 26 × GPBD 4) has led to identification of two major genomic regions governing resistance to LLS and rust (Khedikar et al. 2010; Sujay et al. 2012). One QTL region present on linkage group (LG) XV showed 67.98 % and 82.96 % phenotypic variance explained (PVE) towards resistance to LLS and rust, respectively. The other QTL region on LG XII showed PVE of 62.34 % towards LLS resistance. Rust resistance-linked markers were identified and successfully validated (Khedikar et al. 2010; Yeri et al. 2014). The former QTL region was introgressed to develop resistant types in the elite and popular varieties of peanut (Varshney et al. 2014).

A continued validation of the markers and QTL in new backgrounds is always useful. In this study, diverse RIL populations involving GPBD 4, and backcross populations developed from synthetic tetraploids were used to validate LLS and rust resistance-linked markers mapped on both LG XV and XII. The validated markers would be of great practical value in selecting disease resistant genotypes in peanut breeding programs. The diverse RIL and BCLs were also employed to select superior genotypes for disease resistance and productivity.

Materials and methods

Field evaluation of RILs and backcross lines

Four RIL populations (TAG 24 × GPBD 4, TG 26 × GPBD 4, TG 49 × GPBD 4 and TG 19 × GPBD 4) and three backcross populations (ICGS 76 × ISATGR 278-18, DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5B) (Bhat et al. 2012) were considered for this study. Previously, the RILs were developed by crossing LLS and rust susceptible varieties (TAG 24, TG 26, TG 49 and TG 19) with a resistant variety, GPBD 4, and advancing the generations by single seed decent (SSD). BCLs were developed by crossing LLS and rust susceptible varieties (ICGS 76 and DH 86) with disease resistant synthetic tetraploids (ISATGR 278-18 and ISATGR 5B), and backcrossing the progenies twice with respective recurrent parent (ICGS 76 or DH 86) (Varshakumari et al. 2014). Based on the performance in the previous generations, a total of 47 RILs (F_{12}) with resistance to LLS and rust, and acceptable pod features were selected from four RIL populations. Similarly, 26 BCLs (BC_2F_5) from three populations were selected. They were grown at IABT Garden of the Department of Biotechnology, UAS, Dharwad, India during the rainy season of 2012 and 2013 in randomized block design with two replications. Each replication consisted of two rows of 2.5 m length with a spacing of 45 × 10 cm.

Genotypes were evaluated for plant height, pod yield, hundred seed weight, shelling percentage, protein content, oil content, oleic acid and linoleic acid content using "Groundnut descriptors" (IBPGR/ICRISAT 1992). The genotypes were subjected to field screening for rust and LLS reaction using spreader row technique (Subrahmanyam et al. 1995) in which the disease spreader plants (TMV 2 and mutant 28-2) were planted at regular interval of 10 rows. Disease scoring for both rust and LLS was done

at 90 days after sowing (DAS) according to modified 9-point scale (Subbarao et al. 1990).

Genotyping of RILs and backcross lines

Genomic DNA was isolated from the young leaves of RILs, BCLs and their parents by following CTAB method with minor modifications (Cuc et al. 2008). DNA yield was quantified using Nano Drop (UV technologies, USA). Touch-down PCR was carried out in a final volume of 20 µl containing 50 ng genomic DNA, 10X PCR buffer, 2 mM dNTPs, 10 pmol of each primer and 1 U of *Taq* DNA Polymerase (New England Biolabs, Ipswich, MA, USA) for the rust and LLS resistance-linked markers. Amplification was carried out in a mastercycler (Eppendorf, Hamburg, Germany) by setting the conditions for one cycle of pre-denaturation (94 °C for 5 min), 35 cycles of denaturation (94 °C for 30 s), annealing (starting from 65 °C for 30 s with a decrease of 1 °C/cycle for the first five cycles) and extension (72 °C for 30 s). One cycle of final elongation (72 °C for 10 min) was included before the product was held at 4 °C for 30 min. PCR products were resolved by 4 % polyacrylamide gel electrophoresis (PAGE) using Sequi-Gen (BIO RAD, Hercules, California, USA) followed by silver staining. The PCR product resembling that of disease resistant parents (GPBD 4, ISATGR 278-18 and ISATGR 5B) was scored as resistance allele, while the product resembling that of disease susceptible parents (TAG 24, TG 16, TG 26, TG 49, ICGS 76 and DH 86) was scored as susceptible allele.

Statistical analysis

Phenotypic data analyses like analysis of variance (ANOVA), estimation of phenotypic and genotypic coefficients of variation (GCV and PCV), heritability (h^2_{bs}), phenotypic correlation and genetic advance as percent of mean (GAM) were carried out for all the traits using Windostat version 8 by pooling the data of the two seasons (rainy season of 2012 and 2013). Molecular marker data were analyzed for polymorphic information content (PIC), and the association of the markers with LLS and rust resistance was tested by Single marker analysis (SMA) using WinQTL Cartographer version 2.5 (Wang et al. 2007), and locus-by-locus AMOVA using *Arlequin* Ver 3.1 (Excoffier et al. 2005). Since the disease reaction was scored with ordinal scale (0-9), a non-parametric Kruskal–Wallis ANOVA was also used for testing the association using PAST (Paleontological Statistics), Version 2.17 (Zar 2003). For marker validation in different RIL and backcross populations, each genotype was tested for co-segregation by looking at the type of allele and the phenotype. Genotype showing resistance allele at linked marker loci and disease resistance (score less than 5.0) was considered positive for co-segregation. In each population, the proportion of the genotypes showing co-segregation was compared with that of genotypes not showing co-segregation using z test (standard normal deviate test for proportion) (Rao 2007), where the z value was compared with the critical value of 1.96 at 5 % level of significance (irrespective degrees of freedom). High proportion of individuals showing co-segregation and a significant z value was considered as a good case of marker validation in a population.

Results and discussion

Forty seven RILs (11 from TAG 24 × GPBD 4, 18 from TG 26 × GPBD 4, 17 from TG 49 × GPBD 4 and 1 from TG 19 × GPBD 4) and 26 BCLs (11 from ICGS 76 × ISATGR 278-18, 10 from DH 86 × ISATGR 278-18 and 5 from DH 86 × ISATGR 5B) were evaluated for productivity and quality traits in addition to resistance to LLS and rust during the rainy season of 2012 and 2013. Analysis of variance for the pooled data revealed significant genotypic differences for resistance to LLS and rust, and for productivity and quality traits. In general, rust resistance and LLS resistance were positively and significantly correlated (0.498).

RILs and BCLs along with their parents were genotyped with previously identified (Sujay et al. 2012) LLS (GM1009, GM1573, pPGPseq 8D09, GM2009, GM2301, GM2079, GM1536, GM1954 and IPAHM103) and rust (GM2009, GM2301, GM2079, GM1536, GM1954 and IPAHM103) resistance-linked markers. All the nine markers revealed polymorphism between the parents of four RIL and three backcross populations (Fig. 1). The markers also showed high polymorphism information content (PIC) value with an average of 0.47. All the RIL and backcross populations consisted of lines carrying

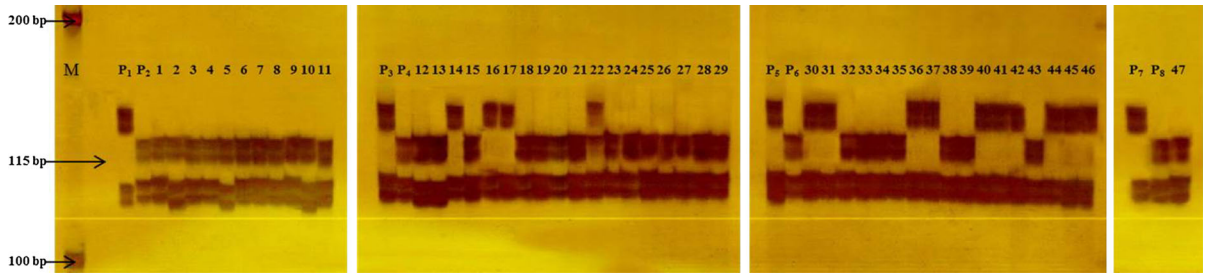

Fig. 1 Contrasting alleles at GM1954 locus among the RILs and parents of TAG 24 × GPBD 4, TG 26 × GPBD 4, TG 49 × GPBD 4 and TG 19 × GPBD 4. (*M* 100 bp DNA ladder, P_1 TAG 24; P_2 GPBD 4, P_3 TG 26; P_4 GPBD 4, P_5 TG 49; P_6 GPBD 4, P_7 TG 19; P_8 GPBD 4, 1: 14-1a, 2: 83-1, 3: 97, 4: I29-1, 5: I39-3, 6: II7-Ib, 7: 51, 8: 86, 9: 95-1, 10: 100, 11: 23-1, 12: 25, 13: 26, 14: 32-2, 15: 44-2, 16: 48, 17: 60-1, 18: 78-1, 19: 79-1b, 20: 87-2, 21: 89-1, 22: 98-2, 23: 103-3, 24: 105, 25: 106-1, 26: 109-1, 27:111-1, 28: 133, 29: I64-1, 30: 1-9, 31: 1-10, 32: 1-26, 33: 1-27, 34: 2-27, 35: 2-32, 36: 2-34, 37: 3-3, 38: 3-6, 39: 3-8, 40: 3-10, 41: 3-11, 42: 3-12, 43: 3-26, 44: 4-9, 45: 4-12, 46:4-21, 47: 6-10)

either resistance or susceptible allele, but with varying frequencies (data not shown). Marker validation was attempted at two levels; first by single marker analysis, Kruskal–Wallis ANOVA and locus-by-locus AMOVA over all the RILs and BCLs and then by analyzing each population for the extent of co-segregation between the marker and the phenotype. Single marker analysis across 47 RILs and 26 BCLs revealed significant association of all six SSR markers with rust resistance, where GM2009 (49.89 %) followed by IPAHM103 (49.33 %) and GM2079 (47.01 %) showed the highest R^2 (Table 1). Significance of marker-trait association was also confirmed by Kruskal–Wallis ANOVA and locus-by-locus AMOVA where the former showed that the genotypes differing for the alleles at GM2079 and GM2009 also varied significantly for the phenotype (because the *Hc* values were high and significant) and the latter estimated the contribution (F_{ST}) of GM2009 (51.4 %) and GM2079 (48.4 %) towards the differentiation between the rust-response types. For LLS resistance, all the nine markers showed significant association with the trait upon SMA, while Kruskal–Wallis ANOVA showed significance of all the markers except GM1009. GM1954 recorded the highest R^2 (18.72 %) and *Hc* (13.83). But locus-by-locus AMOVA indicated significant contribution of GM1009 followed by GM1954 and GM1573 towards differentiation between the LLS-response types. These results clearly indicated the strong association of previously identified markers with LLS and rust resistance among the RILs and BCLs.

The second level of marker validation employed testing the significance of co-segregation between the marker allele and the phenotype using the z test. Within each cross, the proportions of the lines showing co-segregation between the marker and the phenotype was compared statistically (z test) with the proportion of the lines not showing such a co-segregation. For rust resistance, the proportion of the lines showing co-segregation of the resistance allele (similar to those of disease resistant parents) at GM1536, GM2009, GM2301, GM2079 and IPAHM103 with the resistant phenotype was significantly (z value more than 1.96) higher than the proportion of the lines not showing co-segregation in a new population, TG 19 × GPBD 4. GM2079 and IPAHM103 showed validation in TG 49 × GPBD4 as well. Interestingly, all the six markers showed significant co-segregation with rust resistance in two backcross populations, DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5B.

Significantly high proportion of lines showing co-segregation between LLS resistance and the allele at GM1573, GM1536, GM2009, GM2301, GM2079 and IPAHM103 was observed among the RILs of TG 19 × GPBD 4. In addition to these markers, PPGPseq 8D09 and GM1954 also showed significant co-segregation among the BCLs of ICGS 76 × ISATGR 278-18. Thus, LLS and rust resistance-linked markers could be validated not only among the RILs of new populations, but also among the BCLs of populations involving synthetic tetraploids. This will have a greater impact in introgressing disease resistance from wild relatives into cultivated peanut.

Table 1 Association of SSR markers with LLS and rust resistance

Markers	LLS						Rust					
	Single marker analysis		Kruskal–Wallis ANOVA		Locus-by-locus AMOVA		Single marker analysis		Kruskal–Wallis ANOVA		Locus-by-locus AMOVA	
	F value	R^2 (%)	*Hc* value	*F* value	*F*ST	*P* value	*F* value	R^2 (%)	*Hc* value	*F* value	*F*ST	*P*-value
GM1536	9.964 (0.002)**	10.20	5.053 (0.024)*	5.31*	0.015	0.271	96.89 (0.000)**	44.13	34.25 (0.000)**	56.69**	0.444	0.000**
GM1954	18.72 (0.000)**	20.76	13.83 (0.000)**	16.32**	0.085	0.047*	55.26 (0.000)**	37.10	30.75 (0.000)**	47.61**	0.379	0.000**
GM2009	14.72 (0.000)**	14.40	8.246 (0.004)**	9.02**	0.041	0.129	70.06 (0.000)**	49.89	39.50 (0.000)**	72.92**	0.514	0.000**
GM2301	12.26 (0.000)**	12.10	6.973 (0.008)**	7.51**	0.056	0.084	68.99 (0.000)**	41.93	33.24 (0.000)**	53.94**	0.420	0.000**
GM2079	12.26 (0.000)**	9.76	8.204 (0.004)**	8.97**	0.056	0.085	68.99 (0.000)**	47.01	40.27 (0.000)**	75.62**	0.484	0.000**
IPAHM103	11.27 (0.000)**	11.68	6.478 (0.010)**	6.93*	0.041	0.097	77.59 (0.000)**	49.33	38.95 (0.000)**	71.05**	0.449	0.000**
GM1009[a]	5.246 (0.024)*	1.15	1.321 (0.250)	1.33	0.168	0.002**	NA					
GM1573[a]	8.957 (0.003)**	14.08	8.654 (0.003)**	9.52**	0.072	0.050*						
pPGPseq 8D09[a]	9.125 (0.003)**	9.30	7.451 (0.006)**	8.07**	0.028	0.173						

Chi square value (*Hc*): * Significance at $P < 5$ % (α: 0.05, k − 1 df) and ** Significance at $P < 1$ % (α: 0.01, k − 1 df)

F Value: * Significance at $P < 5$ % (α: 0.05, k − 1 & N − K − 1 df) and ** Significance at $P < 1$ % (α: 0.01, k − 1 & N − K − 1 df)

% R^2 Percent of the phenotypic variation, *Hc* test statistic value (Chi square value), *NA* not applicable

Numbers in parenthesis indicate probability, *, ** significance at 5 % and 1 %, respectively

[a] Markers tested for association with only LLS (90 DAS)

Development of GPBD 4 (Gowda et al. 2002), an improved variety, from KRG 1 × ICGV 86855 exemplifies the importance of trait introgression from wild diploids. KRG 1, a selection from Argentine, is susceptible to foliar diseases, and ICGV 86855 is a foliar disease resistant Virginia bunch (*A. hypogaea* subsp. *hypogaea* var. *hypogaea*) interspecific derivative involving cultivated peanut and *A. cardenasii*, a diploid wild species with A genome contributing resistance to LLS and rust.

Since the RILs and backcross populations used in this study were derived from diverse parents (cultivated varieties and synthetic tetraploids from diploid wild species) differing greatly for productivity and disease resistance, they were also used to select superior genotypes. RILs and BCLs were evaluated for productivity and quality traits along with reaction to LLS and rust. Overall, phenotypic and genotypic coefficients of variations were high for majority of the traits. In general, higher variability (both PCV and GCV) was observed among 26 BCLs when compared to 47 RILs for all the productivity and quality traits (Table 2). This could be due to the use of wild diploid species of peanut through an amphidiploid (ISATGR 278-18, *A. duranensis* × *A. batizocoi*) and an autotetraploid (ISATGR 5B, *A. magna* × *A. batizocoi*) in developing the BCLs. These results confirm the diversification (Varshakumari et al. 2014) and broadening of the genetic base in these genetic resources which can be used for selecting the superior lines combining high productivity and disease resistance. In fact, ISATGR 278-18 and ISATGR 5B were shown to be highly resistant to LLS and rust (Mallikarjuna et al. 2012; Shilpa et al. 2013; Varshakumari 2013).

By and large, all the traits showed high heritability and genetic advance over mean, indicating a great scope for selection. In general, both LLS and rust had a negative association with the productivity traits, while the various productivity traits were positively correlated. When selection was exercised, six RILs with significant or marginal superiority over GPBD 4 (a released superior check variety) for pod yield (kg/ha) could be identified (Table 3). They were assessed for other productivity and quality traits, and resistance to LLS and rust. Of the six lines, RIL 78-1 from TG 26 × GPBD 4, RIL 44-2 from TG 26 × GPBD 4 and RIL 100 from TAG 24 × GPBD 4 had significantly higher pod yield (kg/ha) (31, 27 and 22 %, respectively) over GPBD 4. In addition, they exhibited either significant or marginal superiority over GPBD 4 for several other desirable traits as well. RIL 78-1 possessed significantly higher kernel yield (kg/ha) (30 %) and oil yield (kg/ha) (34 %) when compared to GPBD 4. It also exhibited marginal superiority for hundred seed weight (g) (Fig. 2), oil content (%) and

Table 2 Estimates of genetic parameters for productivity and nutritional traits, and resistance to LLS and rust among RILs and backcross lines

Traits	RILs				BCLs				Pooled			
	PCV	GCV	h^2bs	GAM	PCV	GCV	h^2bs	GAM	PCV	GCV	h^2bs	GAM
Pod yield (kg/ha)	40.78	39.74	95.0	79.80	47.50	46.61	96.3	94.21	44.86	43.85	95.5	88.29
Hundred seed weight (g)	11.96	11.76	96.2	23.84	15.20	11.91	96.7	30.13	14.11	13.83	96.1	27.94
Shelling percentage (%)	6.649	6.288	82.9	11.85	6.937	6.317	89.4	12.24	6.796	6.235	84.2	11.784
Protein (%)	6.612	6.487	96.3	13.11	33.05	33.02	99.8	67.97	20.26	20.21	99.6	41.54
Oil (%)	4.047	3.940	94.8	7.903	32.97	32.96	99.9	67.87	19.46	19.44	99.8	39.99
Oleic acid (%)	6.604	6.132	86.2	11.72	33.47	33.35	99.3	68.45	20.23	20.06	98.3	40.98
Linoleic acid (%)	9.459	8.515	81.0	15.79	33.69	33.49	98.8	68.56	21.34	20.95	96.3	42.35
Oleic/Linoleic acid	16.76	16.47	96.5	33.33	36.28	36.08	98.9	73.92	25.46	25.23	98.2	51.49
LLS	18.47	16.77	82.4	31.37	23.16	22.84	97.3	46.40	20.49	19.30	88.7	37.45
Rust	26.69	22.13	91.9	44.91	26.72	25.58	97.1	50.51	27.25	26.22	92.5	51.95

RILs Recombinant inbred lines, *BCLs* Backcross lines, *GCV* Genotypic coefficient of variation, *PCV* Phenotypic coefficient of variation, h^2bs Heritability in broad sense, *GAM* Genetic advance as percent of mean

Table 3 Superior genotypes identified for productivity and quality traits, and resistance to LLS and rust

Crosses	Genotypes	PY (kg/ha)	SP (%)	KY (kg/ha)	OY (kg/ha)	HSW (gm)	Protein (%)	Oil (%)	OLE (%)	LIN (%)	LLS	Rust
TAG 24 × GPBD 4	100	2,729	64.3	1,759	865	40.8	35.1	49.1	44.9	33.7	5.5	4.5
TG 26 × GPBD 4	44-2	2,824	59.9	1,690	872	39.5	33.0	48.1	51.0	30.2	5.0	4.5
	78-1	2,909	68.5	1,992	1,063	37.4	31.2	50.1	49.2	31.3	5.0	4.3
	87-2	2,234	61.4	1,369	752	39.9	33.1	46.4	47.6	32.8	5.8	4.3
	89-1	2,343	62.1	1,458	767	40.1	31.7	47.0	46.2	34.8	4.8	4.5
	109-1	2,591	63.5	1,646	830	33.8	30.8	48.3	43.0	34.9	5.3	6.3
	GPBD 4	2,229	68.6	1,527	796	36.3	33.3	49.7	47.1	32.4	3.1	3.1
	TAG 24	836	61.1	511	264	32.0	30.6	47.2	42.3	38.9	8.8	7.8
	TG 26	889	63.0	564	330	33.5	26.0	46.1	42.5	38.0	6.5	8.0
	CD at 5 %	385	5.0	299	183	3.2	1.2	1.2	3.4	3.6	1.2	1.0

PY Pod yield (kg/ha), *SP* Shelling percentage (%), *KY* Kernel yield (kg/ha), *OY*: Oil yield (kg/ha), *HSW* Hundred seed weight (g), *OLE* Oleic acid (%), *LIN* Linoleic acid (%)

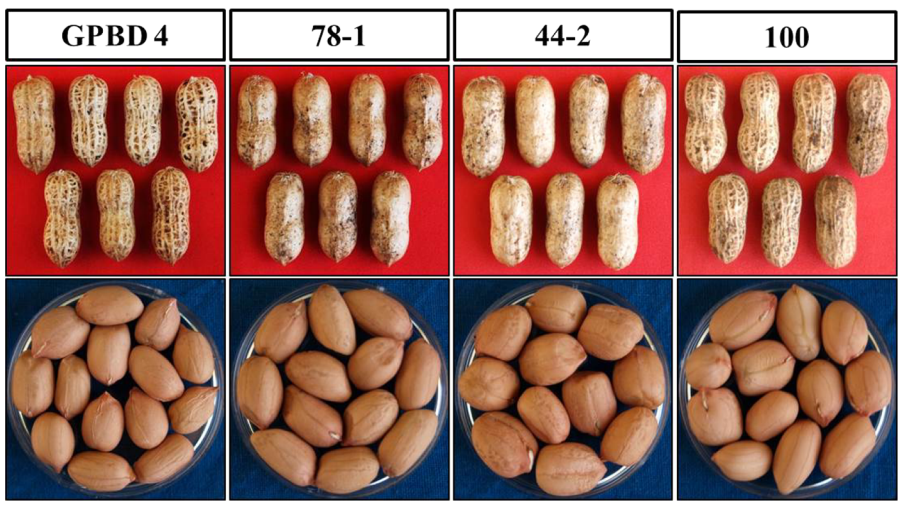

Fig. 2 Pod and kernel features of superior RILs

oleic acid (%) over GPBD 4. It was resistant (≤ 5.0 score) to both LLS and rust, but had marginally lower linoleate content than GPBD 4. RIL 100 exhibited significantly higher hundred seed weight (g) and protein content over GPBD 4. RIL 44-2 recorded significantly higher hundred seed weight (g) and oleate content than GPBD 4. Both RIL 44-2 and 100 showed a score of 4.5 for rust and 5.5 and 5.0 for LLS, respectively. These six lines also carried resistance allele at all the afore-mentioned nine markers, in addition to a rust resistance linked marker, GO340445.

It was interesting to note that the superior lines originated from RILs, but not from backcross populations. In contrast, analysis of a few BCLs (40-6, 85-1 and 17-5) showed high resistance to LLS and rust, but failed to show any superiority over GPBD 4 for productivity and quality traits, indicating that the lines with desirable combination of productivity and quality traits were more frequent among RILs developed from cultivated varieties than among the BCLs involving wild diploids. In general, disease resistance in wild diploid species is linked to less preferred pod features like pod constriction and pod reticulation (Wynne et al. 1991). Such an undesirable linkage was evident among the three BCLs (40-6, 85-1 and 17-5), which showed pod constriction score of 5.0, while the

RILs had a score of 4.0. These observations clearly indicated the need for additional cycles of backcrossing with the recurrent parent in order to improve the recovery of background genome.

In conclusion, the study reported the validation of the markers linked to rust resistance and LLS resistance using diverse RIL and backcross populations of peanut, and identification of superior recombinants. RILs 78-1, 44-2 and 100 that are superior for productivity traits and *on par* for disease resistance when compared to GPBD 4 are being included into variety release trials for their evaluation in larger plots in multi-locations. The markers validated in this study are being used for marker assisted backcross breeding in peanut to improve LLS and rust resistance.

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