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Development of late leaf spot and rust resistant backcross lines in Jl 24 variety of groundnut (*Arachis hypogaea* L.)

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Abstract
A marker assisted backcross breeding (MABC) programme was undertaken in an elite but foliar disease susceptible variety of groundnut, JL 24 for developing backcross lines using the donor, GPDB 4 by employing late leaf spot (LLS) and rust resistance-linked markers. Backcrossing and selfing resulted in the development of BC_1F_4, BC_2F_3 and BC_3F_3 generations. Evaluation of the backcross lines could identify JG4_81 and JG4_43 from BC_1F_4, and JG2-3_14 from BC_2F_3 as superior lines for disease resistance and productivity. A BC_3F_2 plant (JG_18) was homozygous at LLS and rust resistance-linked marker loci (IPAHM103, GM2301 and pPGPseq8D09) and 30 background markers loci. JG_18 had 87% background genome similarity with JL 24. When advanced to BC_3F_3, JG_18 showed resistance to both LLS and rust when compared to JL 24, while it was on par with JL 24 for the productivity traits. Currently, JG4_81, JG4_43, JG2-3_14 and JG_18 are under field evaluation for variety development.

Key words: Groundnut, marker assisted backcross breeding, late leaf spot and rust resistance, productivity, superior backcross lines

Introduction:

Groundnut is an important oilseed, food and forage legume crop. A yield loss of 70% could be caused by the co-occurrence of late leaf spot (LLS) and rust diseases (Subrahmanyam *et al.*, 1983). Hence, breeding for disease resistant varieties is the major objective in the groundnut breeding programmes, especially in India. But, the success in breeding for LLS and rust resistant genotypes is influenced by the efficient selection of the genotypes which recombine resistance with high productivity, early maturity and desirable pod features. Also, complex inheritance pattern of foliar disease resistance (Bromfield and Bailey, 1972; Tiwari *et al.*, 1984; Paramasivam *et al.*, 1990) and interference among these diseases make phenotypic selection less effective.

Integration of genomic resources with conventional breeding approaches might enhance the precision and speed of developing groundnut cultivars with LLS and rust resistance. In this direction, extensive genomic resources have been developed in groundnut. They include various mapping populations, linkage maps, QTL maps, markers linked to LLS and rust resistance etc. A QTL region on the 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. The markers linked to these QTL were successfully validated (Khedikar *et al.*, 2010; Yeri *et al.*, 2014; Sukruth *et al.*, 2015). The markers linked to former QTL region were employed for marker assisted backcross breeding (MABC) in groundnut (Varshney *et al.*, 2014).

JL 24 is an elite groundnut variety released in 1979 for Maharashtra state (Patil *et al.*, 1980). It is very popular among the farmers of even other states because of its bold seeds, early maturity and wide adaptation under adverse environments. However, it is highly susceptible to LLS and rust. In this study, an effort was made to develop LLS and rust resistant backcross lines in JL 24 by adopting MABC where GPBD 4, a disease resistant variety (Gowda *et al.*, 2002) was used as the donor, and the markers linked LLS and rust resistance were employed for selection

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Materials and Methods

JL 24 was used as the recurrent parent, while GPBD 4 was used as the donor parent in the MABC (Fig. 1). A large number of backcross lines (BC_3F_3) were developed by following MABC (Fig. 1). IPAHM103 and GM2301 linked to genomic region on LG XV, and pPGPseq8D09 linked to QTL on LG XII were employed for foreground

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selection. A large number of *A. hypogaea* transposable element (AhTE) markers (Shirasawa *et al.*, 2012a; Shirasawa *et al.*, 2012b) was used for background selection.

DNA was isolated from the young leaves using the modified cetyltrimethyl ammonium bromide (CTAB) extraction method (Cuc *et al.*, 2008). The polymerase chain reaction (PCR) conditions and analysis of PCR products for LLS and rust resistance-linked SSR markers were followed as described by Sujay *et al.* (2012). PCR and amplicon analysis for AhTE markers was taken up as described by Shirasawa *et al.* (2012a). The amplicons were scored as either resistant-type or susceptible-type based on the resemblance with those of parents.

Genotypes developed under MABC were evaluated for resistance to LLS and rust, and productivity traits like yield, shelling percentage, test weight and sound mature kernel percentage. An artificial epiphytotic condition for LLS and rust was created using 'Spreader Row Technique' (Subrahmanyam *et al.*, 1995). The genotypes were scored for response to LLS and rust at 70, 80 and 90 days after sowing (DAS) using a modified 9 point scale (1-9 score) (Subbarao *et al.*, 1990). Data were analyzed for treatment significance, variability and heritability parameters. Mean values were used to identify the superior lines combining disease resistance and productivity.

Results and Discussion

In total, 106 and 20 BC_1F_4 and BC_2F_3 lines, respectively and 11 BC_3F_2 plants were developed by marker assisted backcross breeding in JL 24 using GPBD 4 as the donor. All the three markers (IPAHM103, GM2301 and pPGPseq8D09) linked to LLS and rust resistance were polymorphic between JL 24 and GPBD 4, hence they were used for the foreground selection of the QTL regions on LG XII and XV. Field evaluation of 106 BC_1F_4 lines during the rainy season of 2013 showed significant differences among the lines for all the productivity traits and response to LLS and rust. Pod yield per plant (PYPP) and response to LLS and rust showed higher phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) than productivity traits (Table 1). Lines JG4_81 and JG4_43 were resistant to LLS as compared to JL 24 (Table 2). Incidence of rust disease was low during the season; JG4_81 and JG4_43 recorded lower scores than that of JL 24. The backcross lines were also significantly superior for PYPP, test weight (TW), shelling percentage (SP) and sound mature kernel percentage (SMKP).

Similarly, 20 BC_2F_3 lines were evaluated for disease resistance and a few productivity traits during the rainy season of 2013. BC_2F_3 lines significantly differed for all the productivity traits and response to LLS and rust. They also revealed high variability (both PCV and GCV) for response to LLS and rust and all the productivity traits except SMKP (Table 1). Based on the performance, lines JG2-3_13 and JG2-3_14 were found to be resistant to LLS as compared to JL 24 (Table 2). They also recorded significantly higher PYPP than JL 24. Identification of superior lines like JG4_81 and JG4_43 from BC_1F_4, and JG2-3_14 from BC_2F_3 proved that promising backcross lines can be recovered among the selfed generations of early backcross lines provided a large number of genotypes are generated (Neeraja *et al.*, 2007; Septiningsih *et al.*, 2009). These lines need to be evaluated for rust resistance under epiphytotic conditions.

A detailed marker analysis for identifying the homozygous lines was attempted in BC_3F_2 generation. Of the 11 plants screened, one plant (JG_18) was found to be homozygous at marker loci (IPAHM103, GM2301 and pPGPseq8D09). This plant was analyzed for the recovery of the background genome from the recurrent parent. For this purpose, 294 AhTE markers were screened for polymorphism between JL 24 and GPBD 2. In total, 30 markers were polymorphic, and the alleles at these loci were in homozygous condition. Of the 30 markers, only 19 were mapped on the groundnut consensus map (Gautami *et al.*, 2012). These 30 markers were used to analyze JG_18 BC_3F_2 plant. Based on the allele pattern, it was observed that 26 marker loci were similar to those of JL 24 and four were similar to GPBD 4 with a background recovery of 87%. The donor being an improved cultivated variety with many desirable traits, a thorough background evaluation may not be very critical (Young and Tanksley, 1989; Ribaut and Ragot, 2007; Varshney *et al.*, 2014). JG_18 was advanced to BC_3F_3, and evaluated for disease resistance and a few productivity traits during the post-rainy season of 2013. JG_18 was resistant to both LLS and rust when compared to JL 24, while it was on par with JL 24 for the productivity traits (Table 2 and Fig. 2). Currently, JG4_81, JG4_43, JG2-3_14 and JG_18 are under field evaluation for variety development.

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References

Bromfield, K. and Bailey, W. 1972. Inheritance of resistance to *Puccinia arachidis* in peanut. Phytopathology, 62: 748.

Cuc, L. M., Mace, E. S., Crouch, J. H., Quang, V. D., Long, T. D. and Varshney, R. K. 2008. Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (*Arachis hypogaea*). BMC Plant Biol., 8: 55.

Gautami, B., Fonceka, D., Pandey, M. K., Moretzsohn, M. C., Sujay, V., Qin, H., Hong, Y., Faye, I., Chen, X., BhanuPrakash, A., Shah, T. M., Gowda, M. V. C., Nigam, S. N., Liang, X., Hoisington, D. A., Guo, B., Bertioli, D. J., Rami, J. F. and Varshney, R. K. 2012. An international reference consensus genetic map with 897 marker loci based on 11 mapping populations for tetraploid groundnut (*Arachis hypogaea* L.). PLoS One, 7: e41213.

Gowda, M. V. C., Motagi, B. N., Naidu, G. K., Diddimani, S. B. and Sheshagiri, R. 2002. GPBD 4: a spanish bunch groundnut genotype resistant to rust and late leaf spot. Int. Arachis Newslet., 22: 29-32.

Khedikar, Y., Gowda, M. V. C., Sarvamangala, C., Patgar, K., Upadhyaya, H. and Varshney, R. 2010. A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.). Theor. Appl. Genet., 121: 971-984.

Neeraja, C. N., Maghirang-Rodriguez, R., Pamplona, A., Heuer, S., Collard, B. C. Y., Septiningsih, E. M., Vergara, G., Sanchez, D., Xu, K. and Ismail, A. M. 2007. A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. Theor. Appl. Genet., 115: 767-776.

Paramasivam, K., Jayasekhar, M., Rajasekharan, R. and Veerabadhiran, P. 1990. Inheritance of rust resistance in groundnut (*Arachis hypogaea* L.). Madras Agric. J., 77: 50-52.

Patil, G., Desale, S., Patil, P. and Patil, S. 1980. 'Phule-Pragati', a high yielding early bunch groundnut for Maharashtra. Journal of Maharashtra Agricultural Universities, 5: 47-52.

Ribaut, J. M. and Ragot, M. 2007. Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives. J. Exp. Bot., 58: 351.

Septiningsih, E. M., Pamplona, A. M., Sanchez, D. L., Neeraja, C. N., Vergara, G. V., Heuer, S., Ismail, A. M. and Mackill, D. J. 2009. Development of submergence-tolerant rice cultivars: the Sub1 locus and beyond. Ann. Bot., 103: 151-160.

Shirasawa, K., Hirakawa, H., Tabata, S., Hasegawa, M., Kiyoshima, H., Suzuki, S., Sasamoto, S., Watanabe, A., Fujishiro, T. and Isobe, S. 2012a. Characterization of active miniature inverted-repeat transposable elements in the peanut genome. Theor. Appl. Genet., 124: 1429-1438.

Shirasawa, K., Koilkonda, P., Aoki, K., Hirakawa, H., Tabata, S., Watanabe, M., Hasegawa, M., Kiyoshima, H., Suzuki, S., Kuwata, C., Naito, Y., Kuboyama, T., Nakaya, A., Sasamoto, S., Watanabe, A., Kato, M., Kawashima, K., Kishida, Y., Kohara, M., Kurabayashi, A., Takahashi, C., Tsuruoka, H., Wada, T. and Isobe, S. 2012b. *In silico* polymorphism analysis for the development of simple sequence repeat and transposon markers and construction of linkage map in cultivated peanut. BMC Plant Biol., 12: 80.

Subbarao, P. V., Subramanyam, P. and Reddy, P. M., 1990, A modified nine points diseases scale for assessment of rust and late leaf spot of groundnut. *Proc. Second International Congress of French Phytopathological Society*, 28-30 November 1990, Montpellier, France, p 25.

Subrahmanyam, P., Hammons, R. O., Nigam, S. N., McDonald, D., Gibbons, R. W., Fan, M.-Y. and Yeh, W.-L. 1983. International cooperative screening for resistance of peanut to rust and late leaf spot. Plant Dis., 67: 1108-1111.

Subrahmanyam, P., McDonald, D., Waliar, F., Reddy, L. J., Nigam, S. N., Gibbons, R. W., Rao, V. R., Singh, A. K., Pande, S., Reddy, P. M. and Rao, P. V. S., 1995, Screening methods and sources of resistance to rust and late leaf spot of groundnut. Information Bulletin, 47, ICRISAT, Patancheru, Andhra Pradesh.

Sujay, V., Gowda, M. V. C., Pandey, M. K., Bhat, R. S., Khedikar, Y. P., Nadaf, H. L., Gautami, B., Sarvamangala, C., Lingaraju, S., Radhakrishan, T., Knapp, S. J. and Varshney, R. K. 2012. QTL analysis and construction of consensus genetic map for foliar disease resistance based on two RIL populations in cultivated groundnut (*Arachis hypogaea* L.). Mol. Breed., 30: 773-788.

Sukruth, M., Paratwagh, S. A., Sujay, V., Varshakumari, Gowda, M. V. C., Nadaf, H. L., Motagi, B. N., Lingaraju, S., Pandey, M. K., Varshney, R. K. and Bhat, R. S. 2015. 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.). Euphytica, 204: 343-351.

Tiwari, S., Ghewande, M. and Misra, D. 1984. Inheritance of resistance to rust and late leaf spot in groundnut (*Arachis hypogaea* L.). J. Cytol. Genet, 19: 97-101.

Varshney, R. K., Pandey, M. K., Pasupuleti, J., Nigam, S. N., Sudini, H., Gowda, M. V. C., Sriswathi, M., Radhakrishan, T., Manohar, S. S. and Patne, N. 2014. Marker-assisted introgression of a QTL region to improve rust resistance in three elite and popular varieties of peanut (*Arachis hypogaea* L.). Theor. Appl. Genet., 127: 1771-1781.

Yeri, S. B., Shirasawa, K., Pandey, M. K., Gowda, M. V. C., Sujay, V., Shriswathi, M., Nadaf, H. L., Motagi, B. N., Lingaraju, S., Bhat, A. R. S., Varshney, R. K., Krishnaraj, P. U. and Bhat, R. S. 2014. Development of NILs from heterogeneous inbred families for validating the rust resistance QTLs in peanut (*Arachis hypogaea* L.). Plant Breed., 133: 80-85.

Young, N. and Tanksley, S. 1989. RFLP analysis of the size of chromosomal segments retained around the Tm-2 locus of tomato during backcross breeding. Theor. Appl. Genet., 77: 353-359.

Table 1. Estimates of genetic components among the backcross generations in groundnut

Traits	PCV	GCV	H^2	GA	GAM
			BC_1F_4		
PYPP	16.7	15.0	80.6	3.6	27.8
TW	7.9	7.4	87.6	7.5	14.3
SP	5.6	5.2	85.3	7.1	9.8
SMKP	2.6	2.4	85.9	4.5	4.7
Rust	13.3	13.0	95.1	0.9	26.0
LLS	21.5	20.9	94.9	2.3	41.9
			BC_2F_3		
PYPP	14.1	11.9	71.6	2.7	20.8
TW	11.9	10.5	78.4	7.4	19.2
SP	23.8	20.2	72.0	19.4	35.3
SMKP	6.3	4.8	59.7	7.1	7.7
Rust	12.4	12.0	93.0	0.8	23.8
LLS	24.8	24.5	97.0	2.8	49.6

Rust: rust score, LLS: late leaf spot score, PYPP: Pod yield per plant (gm), TW: Test weight (gm), SP: Shelling percentage (%), SMKP: Sound mature kernel percentage (%), PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, H^2: Heritability, GA: Genetic advance, GAM: Genetic advance over mean

Table 2. Performance of superior lines from three backcross generations for LLS and rust resistance and productivity traits in groundnut

Lines	Rust	LLS	PYPP	TW	SP	SMKP
			BC_1F_4			
JG4_81	3.0	4.0	17.3	60.0	77.5	98.9
JG4_43	3.0	3.0	15.5	53.0	75.0	100.0
JL 24	4.0	6.8	12.5	52.5	72.5	93.5
GPBD 4	3.0	3.0	12.8	42.5	76.5	100.0
CD (5%)	0.3	0.8	1.4	4.1	4.3	2.7
			BC_2F_3			
JG2-3_13	4.0	4.0	16.2	36.3	69.5	93.1
JG2-3_14	3.0	4.0	16.3	42.2	59.2	93.0
JL 24	3.5	6.5	12.6	52.5	73.0	98.2
GPBD 4	3.0	3.0	12.2	42.5	68.8	98.7
CD (5%)	0.3	0.7	2.0	4.3	5.3	2.5
			BC_3F_3			
J8_18	4.0	4.0	11.6	30.4	60.4	80.4
JL 24	6.5	6.0	12.9	36.7	70.4	95.6
GPBD 4	3.0	3.0	11.3	29.2	74.5	93.5
CD (5%)	0.7	0.5	2.8	8.7	19.6	8.0

Rust: rust score, LLS: late leaf spot score, PYPP: Pod yield per plant (gm), TW: Test weight (gm), SP: Shelling percentage (%), SMKP: Sound mature kernel percentage (%)

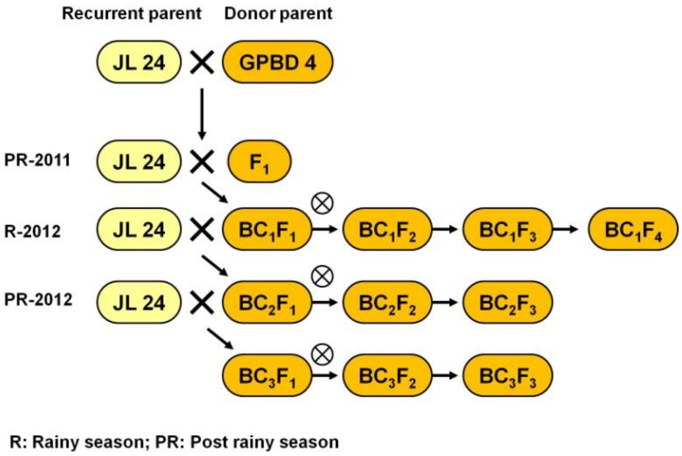

Fig. 1. Marker assisted backcross breeding scheme followed in JL 24

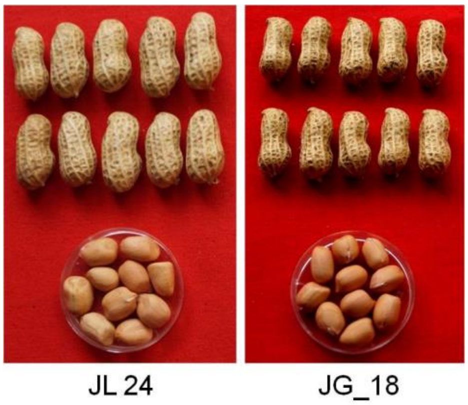

Fig. 2. Pod and kernel features of JG_18 and JL 24