Int.J.Curr.Microbiol.App.Sci (2019) *8*(1): 2956-2965

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 01 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.801.314

Evaluation of a New Recombinant Inbred Line Mapping Population for Genetic Mapping in Groundnut (*Arachis hypogaea* L.)

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Keywords

Groundnut, Recombinant inbred lines, Late leaf spot and rust diseases, Productivity traits, Variability, Parental polymorphism

Article Info

Accepted:
20 December 2018
Available Online:
10 January 2019

A B S T R A C T

A new recombinant inbred line (RIL) population was developed from a late leaf spot (LLS) susceptible mutant (VL 1) and its secondary mutant (110) which was resistant to LLS. The RILs (114) were evaluated for yield, yield components, nutritional and oil quality traits, and response to LLS and rust diseases during the rainy season of 2015 to assess the suitability of the mapping population for mapping these traits. The RILs differed significantly for all the traits studied. Phenotypic coefficient of variation and genotypic coefficient of variation were moderate to high for pod yield, number of pods per plant, pod weight per plant, shelling percentage, test weight, protein, oleic to linoleic acid ratio, kernel yield, oil yield, and LLS and rust score at 70, 80 and 90 days after sowing (DAS). The RILs exhibited normal distribution for all the studied traits except for rust score at 80 and 90 DAS, and shelling percentage. VL 1 and 110 despite being the primary and secondary mutants, showed polymorphism in terms of SNP, CNV and transposable element insertion. Therefore, this RIL population could be of importance for mapping the agronomic and productivity traits.

Introduction

The cultivated allotetraploid (2n = 4x = 40) groundnut (*Arachis hypogaea* L.) is an important oilseed, food and legume crop with a global production of 42.29 mt from 25.46 mha area. India has the largest groundnut-growing area of 5.50 mha with 6.30 mt production and 1,150 kg/ha productivity (FAO, 2017). Groundnut is regarded as "king of oilseed crops" on account of its diversified uses. Groundnut is an excellent source of plant protein (25–28%), oil (48–50%), calcium, iron and vitamin B complex like thiamine, riboflavin, niacin and vitamin A. The haulms are used as livestock feed. Groundnut offers many health benefits like weight gain control (Alper and Mattes, 2002), prevention of cardiovascular diseases, protection against Alzheimer disease and cancer inhibition (Awad *et al.*, 2000).

Groundnut is affected by various diseases and pests which limit its productivity. Conventional breeding had less impact on delivering disease/pest resistant/tolerant cultivars to the farmers because of complex inheritance of the gene controlling the trait, narrow genetic diversity (Pandey *et al.*, 2012) and more over it is highly dependent on

phenotypic selection. So, with the aid of molecular markers, n number of genotypes can be screened and best genotype/line can be selected based on genotype of the material rather than phenotype, which further enhances the breeding efficacy in identifying promising progeny/line for the trait of interest.

Genomics-assisted breeding (GAB) has accelerated crop improvement programs for development of improved cultivars. Likewise, LLS (*Phaeoisariopsis personata* [(Berk. and Curt) Deighton)] and rust (*Puccinia arachidis* Speg.) is a highly devastating disease among all cultivable areas. Many conventional and molecular breeding strategies were utilised in developing several mapping populations (RILs, NILs, MABCs) to identify significant and major QTL controlling the trait. Many molecular marker systems had been validated using RFLP, AFLP, DAF, SSR, DArT, AhTE and SNPs. In groundnut, GAB has been successful for rust resistance.

QTL and markers were identified (Khedikar *et al.,* 2010; Sujay *et al.,* 2012; Varshney *et al.,* 2014; Kolekar *et al.,* 2016; Zhou *et al.,* 2016, Yeri and Bhat, 2016), validated (Khedikar *et al.,* 2010; Yeri *et al.,* 2014; Sukruth *et al.,* 2015) and used for marker-assisted backcrossing (MABC) (Varshney *et al.,* 2014; Yeri *et al.,* 2016; Pasupuleti *et al.,* 2016; Kolekar *et al.,* 2017). Recently, MABC was also attempted to develop LLS resistant genotypes. However, genomic dissection of LLS resistance is expected to enhance the efficiency of MABC further.

This could be achieved with the use of appropriate mapping populations. In this regard, VL 1, a Valencia type rust resistant mutant was obtained from Dharwad Early Runner (DER), a cross between two *fastigiata* cultivars, viz. Dh 3-20 and CGC-1 (Gowda *et al.,* 1989). Further EMS mutagenesis in VL 1 gave rise to a Spanish type LLS resistant mutant (110) (Gowda *et al.,* 2010). VL 1 and 110 also differed for main stem length, primary and secondary branches, leaves, pods, kernels, and response to late leaf spot and rust disease.

Considering these phenotypic differences, a RIL population was developed by crossing VL 1 with 110 at UAS, Dharwad, India. The RILs derived from the closely related parents have been shown to be useful in mapping the traits (Hake *et al.,* 2017). Therefore, an effort was made in this study to assess the extent of polymorphism between VL 1 and 110, and to evaluate their RILs for suitability to map the traits in groundnut.

Materials and Methods

The present study employed a RIL mapping population (MP) derived from VL 1 × 110. The field evaluation of 114 RILs along with the parents (VL 1 and 110) was carried out during the rainy season of 2015 (R–15) at IABT Garden (E115) of Main Agricultural Research Station, UAS, Dharwad. The experiment was laid out in randomized block design (RBD) with two replications where the plants were spaced at 30 × 10 cm. All recommended package of practices was followed to raise good crop.

Observations were recorded on the productivity and nutritional traits. Pod yield (PY), number of pods per plant (NPPP), pod weight per plant (PWPP), shelling percentage (SP), test weight (TW) and sound mature kernel weight (SMKW) were recorded as per the groundnut descriptor (IBPGR\ICRISAT, 1992). Nutritional traits such as percent protein and oil content of each genotype was estimated by near infrared spectroscopy (NIRS) using FOSS NIR System, 6500 Composite (FOSS Analytical A/S, Denmark) at Seed Quality Testing and Research Laboratory, Seed Unit, UAS, Dharwad.

Response to LLS and rust were recorded at 70, 80 and 90 days after sowing (DAS) using the modified 9-point scale (1–9 score) (Subbarao *et al.,* 1990) on randomly selected five plants from each genotype. The phenotypic data were analysed for ANOVA, variability and association using Windostat Version 9.1. Frequency distribution of the RILs checked using SPSS Version 16.0. VL 1 and 110 were subjected for whole genome re-sequencing (WGRS) to identify the single nucleotide polymorphism (SNP) and copy number variation (CNV) (Shirasawa *et al.,* 2016).

Results and Discussion

Groundnut improvement through application of genomic tools requires identification of gene/QTL linked to trait of interest. Development of mapping populations, marker discovery and screening with DNA/molecular markers and identification of QTL associated with economically important target traits are the most important steps in marker assisted selection. Contrasting parents differing for rust and LLS disease could help in dissecting the QTL (Pandey *et al.,* 2017). VL 1 being rust resistant and LLS susceptible and 110 being LLS resistant and rust susceptible allow dissection of rust and LLS resistance. Therefore, the RILs derived from these parents were evaluated for various traits. The RILs differed significantly for all productivity and nutritional traits and response to LLS and rust disease at 70, 80 and 90 DAS (Table 2).

VL 1 recorded a score of 8 for LLS at 90 DAS, whereas 110 recorded a score of 3.5. However, not much difference was observed between the parents for the score of rust. The parents also differed significantly for pod yield, number of pods per plant, pod weight per plant, shelling percentage, test weight, sound mature kernel weight, protein, oil, oleic to linoleic acid ratio, kernel and oil yield (presented in table 2 along with CV and CD). Considerably wide range was observed among the RILs for all productivity, nutritional and, LLS and rust disease reaction traits.

High PCV and GCV were observed for number of pods per plant, pod weight per plant, oleic to linoleic acid ratio. Traits such as test weight, protein and LLS disease reaction at 90 DAS exhibited moderate PCV and GCV, whereas low PCV and GCV was observed for sound mature kernel weight and oil content (Table 4). Pod yield, kernel yield, oil yield, LLS disease response at 70 and 80 DAS, and rust disease response at 70, 80 and 90 DAS recorded high PCV and moderate GCV. Shelling percentage exhibited moderate PCV with low GCV.

The distribution of the RILs of VL 1 × 110 for quantitative characters (productivity, nutrition and disease reaction) was studied by working out the Skewness and kurtosis (Zhang *et al.,* 2014) using SPSS version 16.0 software. Skewness ranging from -2 to +2 suggested a normal distribution, where 0 skewness indicated a perfect symmetric distribution. Skewness below or above the range (-2 to +2) indicated a negatively and positively skewed distribution, respectively (Lomax and Hahs-Vaughn, 2013). Kurtosis ranging from -3 to +3 indicated a normal distribution. RILs showed normal distribution for all the traits studied except for shelling percentage. Rust disease score at 80 and 90 DAS showed skewed kurtosis (Table 1 and Fig. 1).

Knowledge on the trait association would help in trait mapping. Pod yield had positive and significant association with pod weight per plant, shelling percentage, test weight, sound mature kernel weight, kernel and oil yield. Number of pods per plant was positively and significantly associated with test weight, LLS score at 90 DAS, and rust score at 80 and 90 DAS. Pod weight per plant, shelling percentage, test weight and sound mature

kernel weight had positive and significant association with kernel and oil yield. Similarly, kernel and oil yield, LLS and rust disease response at 70, 80 and 90 DAS are positively and significantly associated with each other. But, LLS and rust disease reaction was observed to be negatively associated with each other (Table 3).

Fig.1 Frequency distribution of the RILs of VL 1 × 110 population for LLS and rust reaction

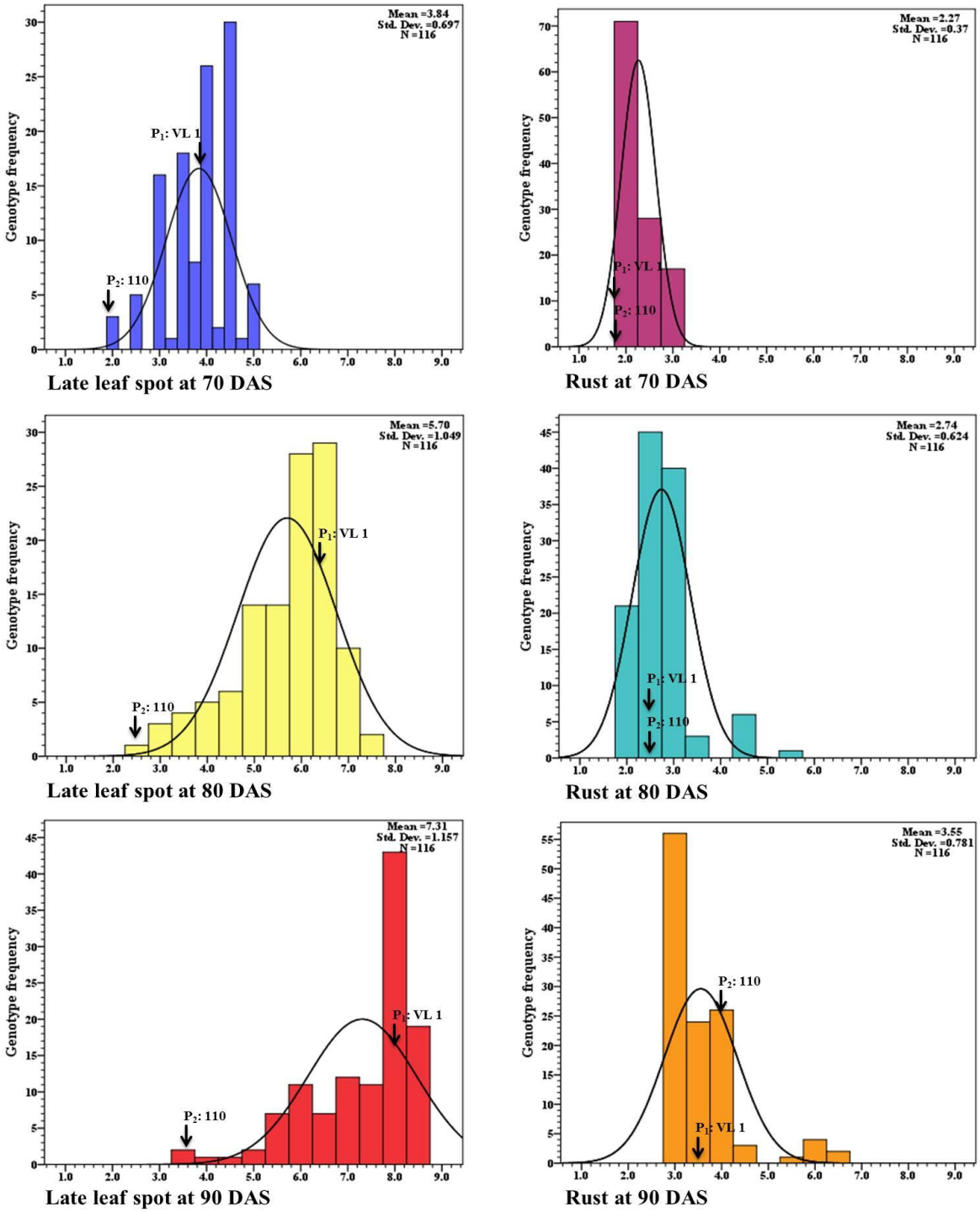

Fig.2 Copy number variation in 110 when compared to VL 1

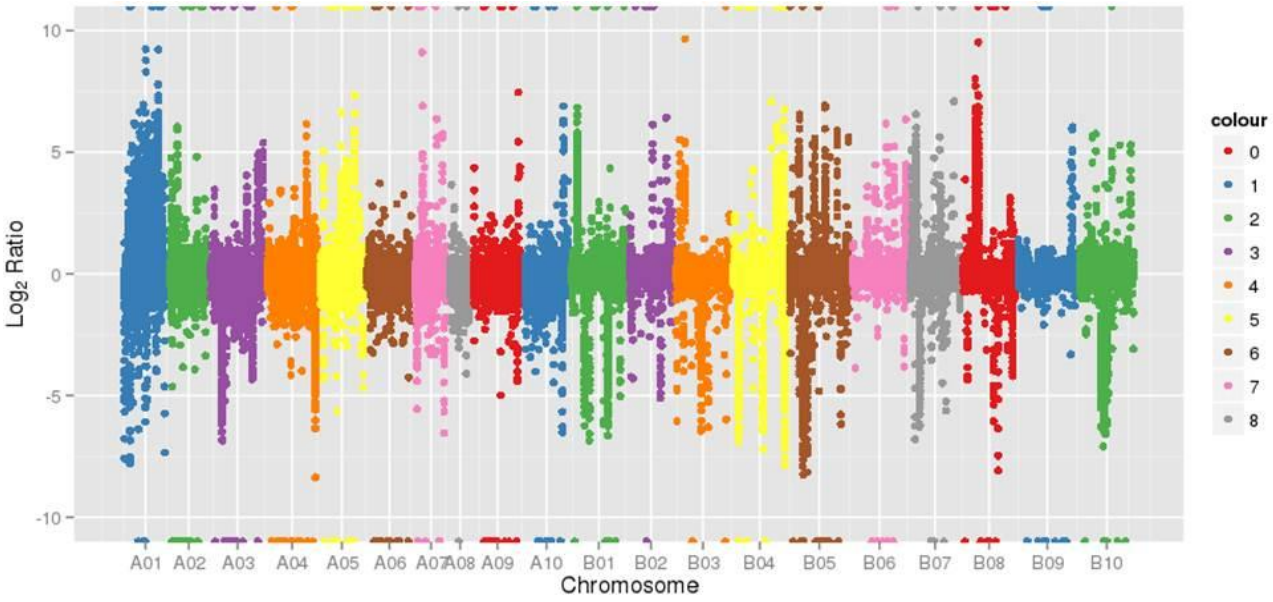

Table.1 Frequency distribution of the RILS of VL 1 × 110 for productivity, nutritional and disease reaction traits

Traits	Skewness	Kurtosis	Distribution
PY	-0.27	-0.192	Normal
NPPP	0.402	-0.45	Normal
PWPP	1.269	2.335	Normal
SP	-3.923	23.169	Skewed kurtosis
TW	-0.032	-0.397	Normal
SMKW	-1.141	1.484	Normal
PROTEIN	-0.846	1.231	Normal
OIL	-0.316	0.959	Normal
O/L	1.495	2.231	Normal
KY	-0.564	0.956	Normal
OY	-0.433	0.742	Normal
LLS_70	-0.561	-0.139	Normal
LLS_80	-0.954	0.53	Normal
LLS_90	-1.235	1.094	Normal
RUST_70	0.996	-0.458	Normal
RUST_80	2.015	4.415	Skewed kurtosis
RUST_90	2.094	4.738	Skewed kurtosis

PY: Pod yield (kg/ha); NPPP: Number of pods per plant; PWPP: Pod weight per plant (g); SP: Shelling percentage (%); TW: Test weight (g); SMKW: Sound mature kernel weight (%);O/L: Oleic to linoleic acid ratio; KY: Kernel yield (kg/ha); OY: Oil yield (kg/ha); LLS_70: Late leaf spot score at 70 days after sowing (DAS); LLS_80: Late leaf spot score at 80 DAS; LLS_90: Late leaf spot score at 90 DAS; RUST_70: Rust score at 70 DAS; RUST_80: Rust score at 80 DAS; RUST_90: Rust score at 90 DAS

Table.2 ANOVA for productivity, nutritional and disease reaction traits in the RIL population of VL 1 × 110

Source of variation	df	PY	NPPP	PWPP	SP	TW	SMKW	PROTEIN	OIL	O/L	KY	OY	LLS_70	LLS_80	LLS_90	RUST_70	RUST_80	RUST_90
Replication MSS	1	68.2E03	1.46	0.21	11.65	44.31	0.34	0.03	1.70	0.22	33.4E02	33.3E02	1.24	2.08	5.28	0.15	0.52	0.06
Genotype MSS	115	80.1E04**	31.55**	105.95**	85.85**	158.63**	10.06**	20.18**	11.58**	0.59**	53.5E04**	13.4E04**	0.97**	2.19**	2.67**	0.27**	0.77**	1.22**
Error MSS	115	34.6E04	1.87	1.00	41.02	28.79	6.00	0.98	0.79	0.11	22.3E04	52.0E03	0.51	1.09	1.54	0.17	0.25	0.23
CV		14.95	7.85	10.47	9.50	8.15	2.47	3.30	1.71	18.93	18.42	18.75	19.23	17.59	16.91	11.47	11.47	8.43
CD at 5%		641.09	1.97	2.43	7.93	5.90	3.19	1.20	1.01	0.42	507.95	245.69	0.87	1.23	1.55	0.42	0.50	0.48
SEm±		433.05	1.37	1.65	5.64	4.05	2.29	0.97	0.82	0.33	339.45	164.48	0.62	0.87	1.11	0.25	0.30	0.28

*, **: Significant at 5% and 1%, respectively; df: degrees of freedom; CV: Coefficient of variation; CD: Critical difference; SEm±: Standard error of mean; MSS: Mean sum of square: PY: Pod yield (kg/ha); NPPP: Number of pods per plant; PWPP: Pod weight per plant (g); SP: Shelling percentage (%); TW: Test weight (g); SMKW: Sound mature kernel weight (%);O/L: Oleic to linoleic acid ratio; KY: Kernel yield (kg/ha); OY: Oil yield (kg/ha); LLS_70: Late leaf spot score at 70 days after sowing (DAS); LLS_80: Late leaf spot score at 80 DAS; LLS_90: Late leaf spot score at 90 DAS; RUST_70: Rust score at 70 DAS; RUST_80: Rust score at 80 DAS; RUST_90: Rust score at 90 DAS

Table.3 Phenotypic correlation coefficients for productivity, nutritional and disease reaction traits in the RILs of VL1 × 110 population

Traits	PY	NPPP	PWPP	SP	TW	SMKW	PROTEIN	OIL	O/L	KY	OY	LLS_70	LLS_80	LLS_90	RUST_70	RUST_80	RUST_90
PY	1																
NPPP	0.146	1															
PWPP	0.204*	0.082	1														
SP	0.728**	0.077	0.159	1													
TW	0.250**	0.194*	0.132	0.250**	1												
SMKW	0.236*	-0.069	-0.007	0.338**	-0.034	1											
PROTEIN	-0.018	-0.036	-0.038	-0.105	0.019	-0.027	1										
OIL	0.069	-0.022	0.086	-0.038	-0.031	0.007	-0.078	1									
OLR	0.084	0.002	0.107	0.112	0.048	0.118	-0.207*	-0.017	1								
KY	0.967**	0.113	0.201*	0.848**	0.282**	0.281**	-0.055	0.054	0.091	1							
OY	0.954**	0.113	0.211*	0.819**	0.261**	0.272**	-0.068	0.229*	0.081	0.983**	1						
LLS_70	0.005	0.134	-0.032	-0.034	-0.002	0.001	-0.067	0.111	0.137	0.005	0.028	1					
LLS_80	-0.044	0.096	-0.101	-0.013	0.003	-0.048	-0.043	0.002	-0.017	-0.025	-0.029	0.568**	1				
LLS_90	0.138	0.183*	-0.092	0.182*	0.089	0.098	-0.133	0.028	0.065	0.178	0.176	0.451**	0.766**	1			
RUST_70	0.032	0.172	0.038	-0.004	-0.024	-0.041	-0.052	0.118	0.203*	0.015	0.041	0.069	0.113	0.131	1		
RUST_80	0.095	0.214*	0.126	0.001	0.270**	0.004	-0.015	-0.064	0.176	0.063	0.044	-0.068	-0.066	0.082	0.458**	1	
RUST_90	0.134	0.216*	0.016	0.066	0.163	0.081	-0.022	-0.015	0.082	0.108	0.111	-0.096	-0.166	-0.051	0.268**	0.630**	1

PY: Pod yield (kg/ha); NPPP: Number of pods per plant; PWPP: Pod weight per plant (g); SP: Shelling percentage (%); TW: Test weight (g); SMKW: Sound mature kernel weight (%); O/L: Oleic to linoleic acid ratio; KY: Kernel yield (kg/ha); OY: Oil yield (kg/ha); LLS_70: Late leaf spot score at 70 days after sowing (DAS); LLS_80: Late leaf spot score at 80 DAS; LLS_90: Late leaf spot score at 90 DAS; RUST_70: Rust score at 70 DAS; RUST_80: Rust score at 80 DAS; RUST_90: Rust score at 90 DAS

Table.4 Mean, range and genetic variability components for productivity, nutritional and disease resistance traits among the RILs of VL1 × 110

Traits	Mean	Minimum	Maximum	GCV (%)	PCV (%)	h^2bs	GAM
PY	2944.44	1322.22	4566.67	14.51	23.05	39.63	18.82
NPPP	18.50	9.83	27.17	22.03	23.37	88.81	42.76
PWPP	24.50	4.37	44.62	46.59	47.03	98.13	95.08
SP	50.83	23.50	78.15	6.74	11.34	35.34	8.25
TW	52.38	30.95	73.80	15.47	18.59	69.28	26.53
SMKW	91.75	86.00	97.50	1.51	3.01	25.28	7.57
PROTEIN	27.76	19.11	36.42	10.48	11.00	90.74	20.56
OIL	46.22	38.98	53.46	4.87	5.21	87.23	9.37
O/L	2.21	0.82	3.60	28.06	33.88	68.57	47.86
KY	1875.03	357.39	3392.67	16.92	26.38	41.12	22.35
OY	902.80	165.45	1640.15	18.19	27.36	44.17	24.90
LLS_70	3.50	2.00	5.00	12.50	22.42	31.08	14.36
LLS_80	5.00	2.50	7.50	13.01	22.47	33.54	15.53
LLS_90	6.00	3.50	8.50	10.29	19.86	26.84	10.98
RUST_70	2.50	2.00	3.00	9.86	20.69	22.73	9.69
RUST_80	3.75	2.00	5.50	18.63	26.09	50.98	27.40
RUST_90	4.75	3.00	6.50	19.81	23.97	68.28	33.72

Vg: Genotypic variance; Vp: Phenotypic variance; GCV: Genotypic coefficient of variation (%); PCV: Phenotypic coefficient of variation (%); h^2bs: Heritability in broad sense (%); GAM: Genetic advance as percent of mean; PY: Pod yield (kg/ha); NPPP: Number of pods per plant; PWPP: Pod weight per plant (g); SP: Shelling percentage (%); TW: Test weight (g); SMKW: Sound mature kernel weight (%); O/L: Oleic to linoleic acid ratio; KY: Kernel yield (kg/ha); OY: Oil yield (kg/ha); LLS_70: Late leaf spot score at 70 days after sowing (DAS); LLS_80: Late leaf spot score at 80 DAS; LLS_90: Late leaf spot score at 90 DAS; RUST_70: Rust score at 70 DAS; RUST_80: Rust score at 80 DAS; RUST_90: Rust score at 90 DAS

Table.5 Total number of SNPs between VL 1 and 110

Sl. No.	A chromosome	No. of SNPs	B chromosome	No. of SNPs
1	Aradu.A01	2,54,108	Araip.B01	13,661
2	Aradu.A02	8,083	Araip.B02	11,064
3	Aradu.A03	6,659	Araip.B03	9,065
4	Aradu.A04	4,032	Araip.B04	9,263
5	Aradu.A05	7,315	Araip.B05	15,964
6	Aradu.A06	6,554	Araip.B06	14,351
7	Aradu.A07	4,289	Araip.B07	14,065
8	Aradu.A08	2,180	Araip.B08	8,837
9	Aradu.A09	8,289	Araip.B09	1,514
10	Aradu.A10	6,536	Araip.B10	15,046
Total		3,08,045		1,12,830

Aradu: *Arachis duranensis*; Araip: *Arachis ipaensis*

Apart from the presence of significant variability among the RILs, genetic relatedness/similarity between the parents would also contribute for efficient detection of QTL by avoiding background noise (Chen *et al.,* 2008). With this objective, VL 1 and 110 were compared using the WGRS data for SNP and CNV.

The WGRS reads of VL 1 and 110 were compared with those of the two groundnut progenitors i.e., *A. duranensis* (A genome) and *A. ipaensis* (B genome). A total of 4,20,875 SNPs (3,08,045 from A sub-genome and 1,12,830 from B sub-genome) were detected (Table 5; Fig. 3). The number of SNPs ranged from 2,180 (A08 chromosome) to 2,54,108 (A01 chromosome). In B sub-genome SNPs ranged from 1,514 (B09 chromosome) to 15,964 (B05 chromosome).

CNVs are genomic rearrangements resulting from gains or losses of DNA segments. This type of polymorphism has recently been shown to be a key contributor to intra-species genetic variation, along with single-nucleotide polymorphisms and short insertion-deletion polymorphisms. In many of the cases, CNVs of specific genes have been linked to important traits such as flowering time, plant height and resistance to biotic and abiotic stress. Hence, an effort was made to check the copy number variations (CNVs) between VL 1 and 110 mutant genotypes. A total of 600 genomic regions showed significant CNVs across 18 chromosomes (Fig 2). A and B chromosome consists of 163 and 437 significant CNVs.

VL 1 and 110 also showed polymorphism of 2.7 to 66.1 % with AhTE markers (Hake *et al.,* 2017). The genetic differences between VL 1 and 110 in terms of SNPs and CNVs could be useful in mapping the traits which showed considerable variability among the RILs. The QTL and the markers identified from the marker-trait association studies will be useful for molecular breeding in groundnut.

Acknowledgement

Funds received from BRNS project (no. 2013/35/12/BRNS), and the DST-JSPS Bilateral Program is gratefully acknowledged.

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How to cite this article:

Sukruth, M., K. Shirasawa and Bhat, R.S. 2019. Evaluation of a New Recombinant Inbred Line Mapping Population for Genetic Mapping in Groundnut (*Arachis hypogaea* L.). *Int.J.Curr.Microbiol.App.Sci.* 7(08): 2956-2965. doi: https://doi.org/10.20546/ijcmas.2019.801.314