PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.3282

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RESEARCH ARTICLE

Genetic variability, correlation, and path analysis in the BC_2F_2 population of groundnut

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OPEN ACCESS

ARTICLE HISTORY

Received: 18 January 2024
Accepted: 15 April 2024

Available online
Version 1.0 : 12 May 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
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CITE THIS ARTICLE

Vargheese RL, Saravanan S, Hepziba SJ, Kumari SMP, Pushpam AK, Pillai MA. Genetic variability, correlation, and path analysis in the BC_2F_2 population of groundnut . Plant Science Today (Early Access). https://doi.org/10.14719/pst.3282

Abstract

The present study was carried out in the backcross population of groundnut involving TMV 7 and ICG 15419. Allele-specific primers were used to screen the population for high oleic acid and a total of 11 yield-contributing traits were included in this study. The number of primary and secondary branches had higher estimates of PCV and GCV whereas pod yield per plant had moderate PCV but low GCV. Along with the variability parameters, plant height, number of primary and secondary branches, pod width, hundred pod weight, oleic acid content and linoleic acid content had good estimates of heritability and genetic advance as a percent of the mean, whereas pod yield per plant had moderate and low, heritability and GAM respectively, with a negatively significant skewed distribution. Association analysis exhibited a positive correlation between the number of primary branches, number of secondary branches, pod length, and hundred pod weight with pod yield per plant and it was evident that oleic acid was indirectly proportional to linoleic acid content. The trait, hundred pod weight had the highest direct effect on pod yield per plant. Selection based on traits with a better relationship with pod yield per plant and moderate to high estimates of PCV, GCV, heritability and genetic advancement would help in accelerating the groundnut improvement program. High oleic, low linolenic lines of BC_2F_2 with better pod yield would be forwarded to the next generation.

Keywords

groundnut; backcross; allele specific primers; variability and association

Introduction

Groundnut is a versatile legume crop belonging to the family of Fabaceae. It is primarily used for oil but it is also consumed in its raw form, boiled and fried. India is the second largest producer and consumer of groundnuts, next to China. Being a tropical crop, it requires a warm climate with an average temperature of 30–35 °C. It is mainly used for oil extraction and hence enhancing the quality of the oil by improving its stability, flavor and shelf life becomes a priority for plant breeders. In groundnut oil, monounsaturated fatty acids occupy the majority of the percentage, with saturated fatty acids occupying just 20 percentage. Among the UFA, monounsaturated fatty acids and polyunsaturated fatty acids are almost equal

and among them, oleic acid and linoleic acid are predominant respectively (1). PUFA are beneficial to health but the higher the occurrence of double bonds in the fatty acids, the higher the extent of oxidation. Therefore, increasing the percentage of mono-unsaturated fatty acids would effortlessly bring down the action of oxidation.

Developing improved genotypes with increased oleic acid content either by conventional hybrid breeding or marker-assisted backcross breeding has drastically helped in improving the overall quality of the groundnut oil. Fatty acid desaturase enzymes controlled by FAD genes are responsible for the conversion of oleic acid to linoleic acid in oilseeds. In groundnuts, a naturally occurring FAD gene mutant was found to have an oleic acid content of around 80 %, compared to less than 45 % in the wild type (2). Two mutations such as substitution in the A genome and insertion in the B genome regulate the activity of the *ahFAD* gene and prevent the conversion of oleic acid to linoleic acid. Improving a particular variety cannot comprise its grain yield. It is therefore the most important trait of any plant breeding program and is also one of the most influential traits. Being governed by several genes it is highly dependent on other factors such as environment, soil type, plant-to-plant interactions, and gene-to-gene interactions. The present study was done to estimate the genetic parameters like heritability, coefficient of variation, skewness, kurtosis, and association studies in the BC_2F_2 population of groundnuts.

Materials and Methods

The current investigation was conducted at V.O.C Agricultural College, Killikulam. A medium oleic-rich ICRISAT germplasm line ICG 15419 and TMV 7 a bunch type genotype, a selection from Tennessee were used as the donor and recurrent parent respectively. ICG 15419 was taken as the donor parent and TMV 7 as the recurrent parent. Hybridization was done at the crossing block between the donor and recurrent parent and the F_1 generation was developed. The F_1 plants were genotyped with allele-specific primers and true F_1s were identified and tagged. Confirmed F_1 genotypes were backcrossed with the recurrent parent TMV 7 to develop the BC_1F_1 generation. The BC_1F_1 genotypes were further screened for oleic acid and the true BC_1F_1 plants were tagged and crossed with the recurrent parent TMV 7 to develop the BC_2F_1 generation. Positive plants in the BC_2F_1 generation were allowed to self to develop the BC_2F_2 population.

Molecular analysis

Leaf samples from approximately 2 week-old seedlings were collected from the donor parent, recurrent parent, F_1, BC_1F_1, BC_2F_1, and BC_2F_2 populations and DNA was isolated based on the protocol (3). Agarose gel of 0.8 % and a nanodrop spectrophotometer were used for DNA quantification and the concentration of DNA was adjusted to 40 ng/µL with sterile distilled water or TE buffer (Tris-Ethylenediaminetetraacetic acid). To detect the substitution mutation in the A genome, allele-specific primers which included a forward primer F435-F and a reverse primer F435SUB-R were employed (4). The cocktail mixture for PCR comprised 2 µL of diluted DNA, 1 µL of forward primer, 1 µL of reverse primer, 3 µL of master mix, and 3 µL of PCR-grade water. The PCR profile started with 4 min of initial denaturation at 94 °C followed by 35 cycles of denaturation, annealing, and extension for 30 sec at 94 °C, for 45 sec at 55 °C and for 1 min at 72 °C respectively and a final extension at 72 °C for 20 min. The products were separated in 3 % agarose gel and documented.

Biometrical observations and statistical analysis

Data was recorded on individual plants for plant height, number of primary branches, number of secondary branches, pod length, pod width, hundred pod weight, hundred seed weight, oil content, oleic acid content, linoleic acid content, and pod yield per plant. All parameters of continuous variation including mean, range, standard deviation, genotypic and phenotypic variances (4), genotypic and phenotypic coefficient of variation (5), heritability (4, 6), genetic advance (4, 7), skewness and kurtosis were calculated using SPSS statistics version 22 (8). GRAPES, an online R-based tool was used to calculate the correlation coefficients (4, 9, 10), and the path analysis which was analyzed by partitioning the correlation coefficients into direct and indirect effects were computed using PB Perfect, an online tool (11, 12).

Results and Discussion

Molecular marker analysis

Hybridization was performed between the donor and recurrent parent in Rabi 2021, to develop F_1, BC_1F_1 and BC_2F_1, and BC_2F_2 generations. A total of eight plants positive for the target allele in the BC_2F_1 (Fig.1) generation was obtained by screening the plants with allele-specific primers and were allowed to self-develop the BC_2F_2 population (Fig. 2) of 123 plants. Similar studies to improve the oleic acid content in GJG 9, GG 20 and GJGHPS 1 using the same primers were reported by (13). Two Spanish bunch cultivars GPBD 4 and G 2-52 were also improved using allele-specific primers by marker-assisted back cross breeding (14).

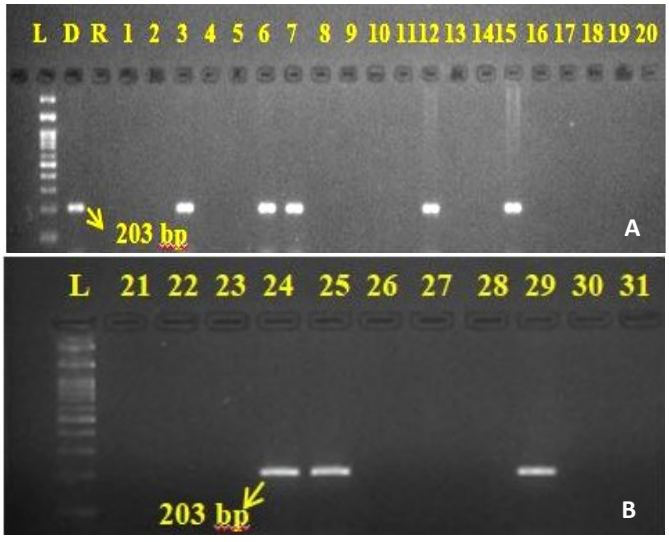

Fig. 1. Genotyping of BC_2F_1 for *ahFAD2A* allele: **L** – 100 bp ladder, **D** – ICG 15419 (donor), **R** – TMV 7 (recurrent), **1 – 31** BC_2F_1.

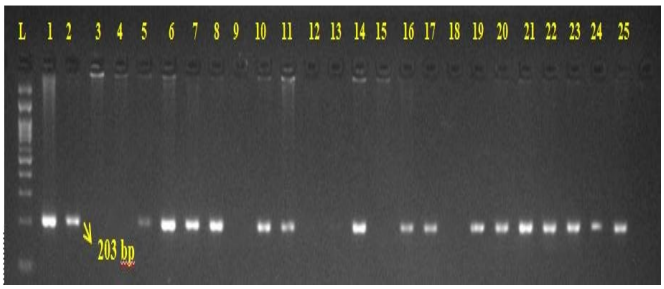

Fig. 2. Genotyping of BC_2F_2 population for *ahFAD2A* allele: **L** – 100 bp ladder.

Estimation of variability parameters

Estimating various components of variations is a prerequisite in any crop breeding program out of which the variation arising due to genetic components is more important as they are the ones that get transmitted to the next generation. Instead of identifying alternative sources of variability to cater to the emerging needs, it would be an easy way to improve an agronomically superior variety by crossing it with a donor possessing the desirable trait and further carrying out the process by the backcross breeding method. Variation in the improved inbred lines can be assessed based on the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) of the traits under study (Table 1) (Fig. 3). The highest PCV and GCV were found in the number of primary and secondary branches. Hundred pod weight, plant height, linoleic acid, and oleic acid had moderate PCV and GCV whereas pod yield per plant had moderate PCV but low GCV depicting that it is highly influenced by the environment. Pod length, hundred seed weight and oil content had low GCV and PCV which indicated that these traits contributed relatively very little to the total variation of the population. Similar findings for high GCV for the number of primary branches (15, 16) and low GCV for oil content were reported by (17, 18). It was also evident from the increased differences between the PCV and GCV estimates of hundred seed weight, oil content, and pod yield per plant that these traits were comparatively under the influence of the environment rather than being genetically controlled.

Heritability and genetic advance

Heritability denotes the amount of variation that is passed on from parents to their offspring which is statistically assessed by calculating the genotypic and phenotypic variance. But genetic gains can be accurately assessed only by coupling the estimates of heritability with genetic advance as percentage of the mean as it includes both additive and epistatic effects which would make the selection more effective. Traits such as plant height, number of primary and secondary branches, pod width, hundred pod weight, oleic acid, and linoleic acid content had higher estimates of both heritability and genetic advance as percentage of the mean (Table 1) (Fig. 3). Pod yield per plant had moderate estimates of heritability and genetic advance as percentage of the mean. It was found that both the estimates were low for oil content indicating that the environment plays a huge part and therefore selection would be futile. Identical findings were reported for plant height, number of primary and secondary branches, and hundred pod weight (19); for oil content (20); for hundred pod weight (15, 20) and high GCV, PCV, heritability and GAM for linoleic acid (21).

Table 1. Variability parameters, skewness and kurtosis in the BC_2F_2 population of TMV 7 x ICG 15419.

Traits	Min	Max	Phen. var	Env. var	Gen. var	PCV	GCV	h^2	GAM	Skewness	Kurtosis
PH	24.00	78.00	63.15	6.09	57.05	16.05	15.25	90.35	29.86	-0.31	1.90
PB	2.00	19.00	10.19	0.33	9.86	34.90	34.33	96.73	69.55	0.47	0.05
SB	2.00	31.00	48.53	1.00	47.53	44.56	44.10	97.94	89.90	0.28	-0.49
PL	2.10	2.90	0.04	0.00	0.03	7.62	7.25	90.49	14.20	0.14	-0.79
PW	0.80	1.50	0.02	0.00	0.02	11.88	10.92	84.44	20.66	-0.77	-0.06
HPW	22.22	111.11	143.01	1.59	141.42	17.39	17.29	98.89	35.43	0.05	2.48
HSW	21.57	33.33	4.29	2.25	2.04	7.84	5.40	47.50	7.67	0.02	0.28
Oil	44.26	54.34	3.20	2.29	0.91	3.60	1.92	28.36	2.10	0.01	0.44
OA	38.26	57.72	37.72	0.72	37.00	12.77	12.65	98.08	25.80	-0.43	-1.56
LA	24.21	39.86	23.38	0.94	22.44	15.31	15.00	95.97	30.26	0.38	-1.46
PYP	13.36	27.89	7.46	4.35	3.11	12.80	8.27	41.75	11.00	-0.60	0.37

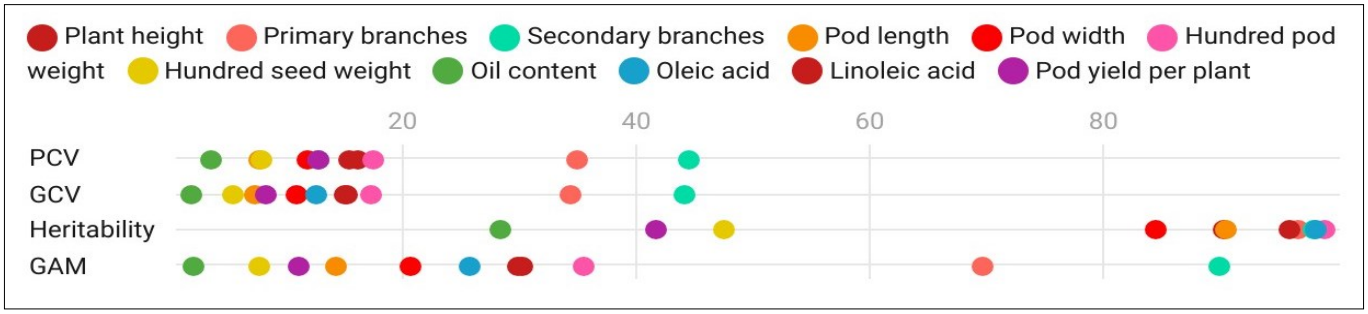

Fig. 3. Dot plot of PCV,GCV, Heritability and Genetic advance as per cent of mean of eleven biometrical traits.

Skewness and kurtosis

A normally distributed population is always preferred in most of the natural phenomena or properties in nature. The asymmetry of a normal distribution is measured by a statistical component termed skewness. If the given values are greater than one and concentrated on the right side it denotes positive skewness and if the values are lesser than one and concentrated on the left side, it denotes negative skewness. Kurtosis on the other hand explains the tailedness of a population. Tailedness depends on the occurrence of the outliers. Kurtosis has three categories, mesokurtic, platykurtic, and leptokurtic. Mesokurtic is a population following a normal distribution with a kurtosis value of zero. Positive kurtosis or leptokurtic with a kurtosis value greater than 3 has flatter peaks and fat tails whereas negative kurtosis or platykurtic with a kurtosis value lesser than 3 has sharper peaks and light tails. Overall, both skewness and kurtosis help us to get a clear view of the distribution of the data and peakness of the data respectively. The frequency distribution of all the 11 traits is given in Fig. 4.

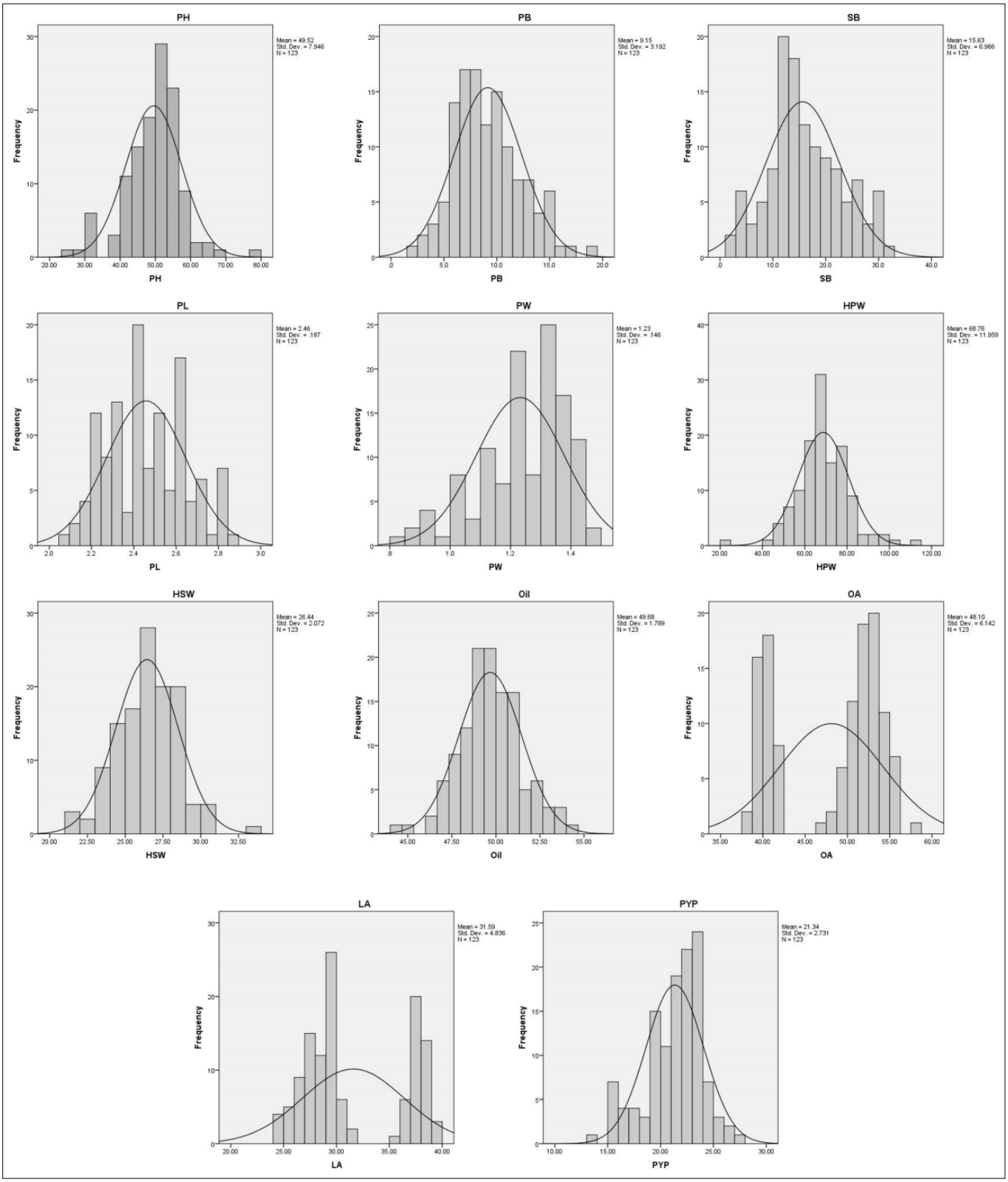

Fig. 4. Frequency distribution of the eleven biometrical traits in the BC_2F_2 population involving TMV 7 and ICG 15419.

A significant positive skewness was observed in the number of primary branches per plant which indicated the presence of complementary gene action. Parallel findings for primary branches were reported (22). Pod yield per plant and pod width exhibited a significant negative skewness indicating that most of the lines had values greater than the mean value (Fig. 4). This can be due to duplicate gene action. Regarding kurtosis, plant height, and hundred pod weight had a positive significant leptokurtic distribution, whereas oleic acid and linoleic acid content followed a significantly negative platykurtic distribution indicating the presence of fewer genes in the former and polygenes in the latter. Similar findings for leptokurtic distribution in plant height and platykurtic distribution in oleic acid content and linoleic acid content were reported (23).

Correlation

The association between any trait pair needs to be estimated for the overall improvement of the production and productivity of a crop. Statistically, the strength of a linear relationship between any 2 traits is measured by estimating the correlation coefficients that range from -1 to +1. Studying the relationship between any trait pair is an indirect way of improving the yield of the plant. A trait pair with a correlation coefficient of +1 denotes a positive greatest correlation depicting that improving one of the traits would parallelly improve the other. A correlation coefficient of -1 explains a negative correlation depicting that if one trait is improved the other one has an antagonistic effect and if the correlation coefficient is zero, it depicts no relationship between the trait pair under study.

In the present study, a positive significant correlation was observed between the following traits viz., hundred pod weight (0.386), number of primary branches (0.283), number of secondary branches (0.276), and pod length (0.206) with pod yield per plant (Fig. 5). Plant height exhibited a significant positive correlation with number of secondary branches (0.201), pod length (0.199), hundred pod weight (0.262) and hundred seed weight (0.194). A significant positive correlation was observed between the number of primary branches and 2 traits namely, number of secondary branches (0.273) and pod length (0.179). The number of secondary branches, pod length, and hundred pod weight exhibited a positive significant correlation with pod length (0.271), pod weight (0.474), and hundred seed weight (0.261) respectively. A study by (24) also reported a positive correlation between the number of secondary branches and pod length with pod yield per plant. A negatively significant correlation was found between oleic acid

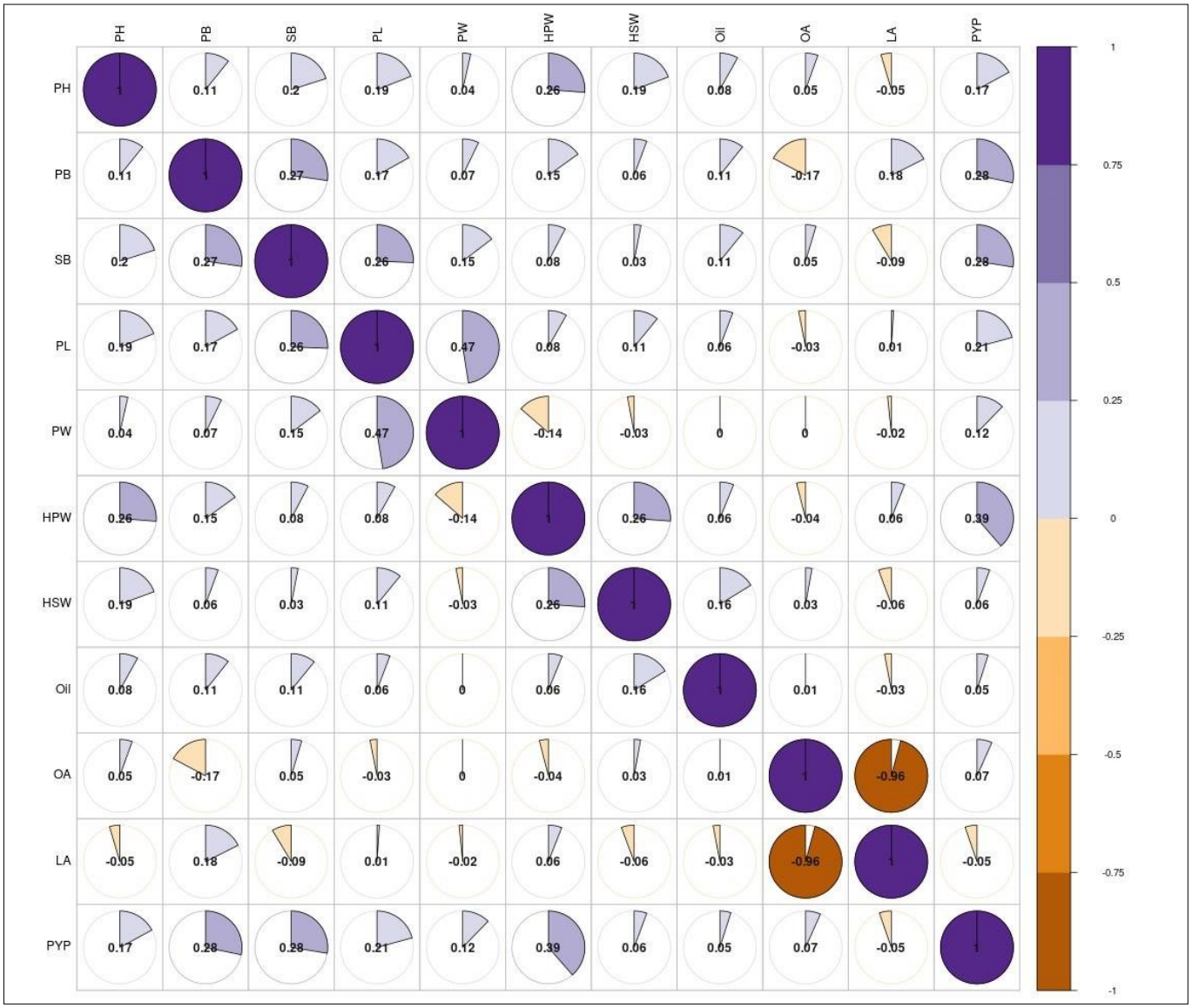

Fig. 5. Correlogram depicting the correlation coefficients of eleven traits.

and linoleic acid content (-0.958) (Fig. 6A, Fig. 6B). Similar results of negative correlation between monounsaturated and polyunsaturated fatty acids were reported by (25, 26). Therefore, the traits positively correlating with pod yield per plant can be emphasized in the groundnut breeding program. Additionally, it is obvious from the study that the BC_2F_2 lines that had increased oleic acid had lower amounts of linoleic acid and vice versa and hence, the lines with increased oleic acid can be taken for further breeding program.

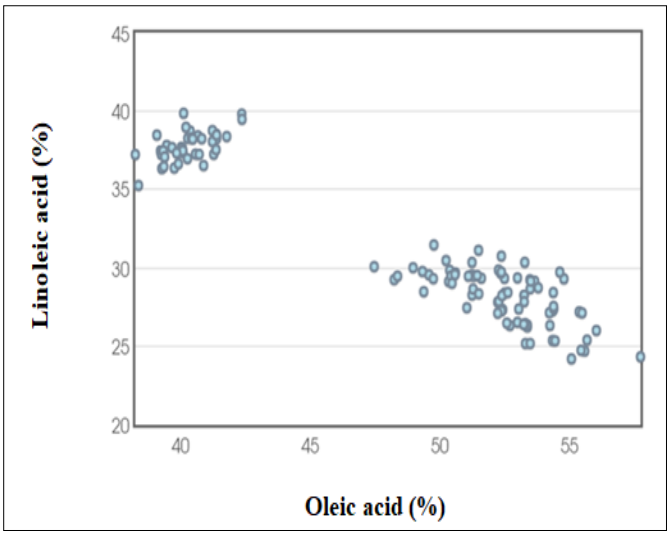

Fig. 6A. Scatter plot of oleic acid vs linoleic acid.

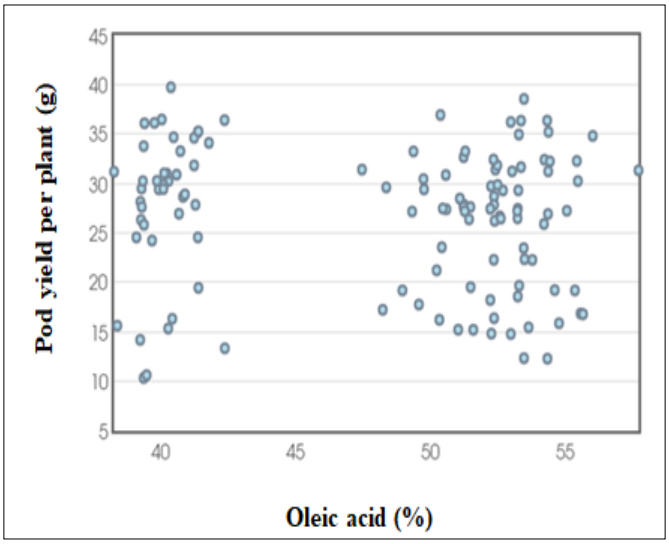

Fig. 6B. Scatter plot of oleic acid vs pod yield per plant.

Path analysis

Path analysis is a statistical tool to describe the cause and effect of the relationship by partitioning the correlation coefficients into direct and indirect effects. In plant breeding, it is used to estimate the contribution of independent traits both direct and indirect, on a dependent trait, the yield. If the contribution of an independent trait on the dependent trait is devoid of any mediators it is termed a direct effect and if the contribution is through a mediator, it is termed an indirect effect. The direct and indirect effects of the ten independent traits over the pod yield per plant are given in Table 2. Hundred pod weight (0.370) and oleic acid content (0.218) had the highest and moderate direct effect on pod yield. Low direct effects were observed in pod length, number of secondary branches (25), and linoleic acid content. The residual effect was found to be 0.363 and the total variability contributed by both dependent and independent traits in the BC_2F_2 population was 63.7 %.

Conclusion

Stringent selection of inbred lines in a segregating population is a very crucial step. From this study, selection based on plant height, number of primary branches, hundred pod weight, oleic acid content, and pod yield per plant would yield compromising effects because of their better estimates of PCV, GCV, heritability, and GAM. This indicates the presence of additive gene action and crop improvement programs involving selection based on such traits would increase the genetic gain in a population.

Acknowledgements

Our heartfelt thanks to the Science and Engineering Research Board, Department of Science and Technology for the financial support extended to conduct this research.

Authors' contributions

RLV conducted the research experiments and wrote the manuscript. SJ and SM helped in conducting the experiments. MAP and SS designed the study and supervised it. AK helped in the statistical analysis and interpretation. MAP helped in genotype collection, and corrected and revised the manuscript.

Table 2. Direct and indirect effects on pod yield per plant in the BC_2F_2 population of TMV7 x ICG 15419.

Traits	PH	PB	SB	PL	PW	HPW	HSW	Oil	OA	LA	PYP
PH	0.010	0.020	0.033	0.012	0.004	0.097	-0.012	0.000	0.012	-0.005	0.171
PB	0.001	0.183	0.045	0.011	0.008	0.056	-0.003	0.000	-0.038	0.020	0.283**
SB	0.002	0.050	0.165	0.017	0.015	0.028	-0.002	0.000	0.010	-0.010	0.276**
PL	0.002	0.031	0.042	0.065	0.050	0.030	-0.007	0.000	-0.007	0.001	0.206*
PW	0.000	0.013	0.024	0.031	0.105	-0.050	0.002	0.000	0.000	-0.002	0.14
HPW	0.003	0.028	0.013	0.005	-0.014	**0.370**	-0.016	0.000	-0.009	0.007	0.386**
HSW	0.002	0.010	0.005	0.007	-0.003	0.097	-0.061	-0.001	0.006	-0.006	0.056
Oil	0.001	0.020	0.018	0.004	0.000	0.023	-0.010	-0.003	0.001	-0.003	0.049
OA	0.001	-0.032	0.008	-0.002	0.000	-0.015	-0.002	0.000	0.218	-0.109	0.067
LA	0.000	0.032	-0.014	0.001	-0.002	0.022	0.003	0.000	-0.208	0.113	-0.053

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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