

RESEARCH ARTICLE

Evaluation of variability and principal component analysis in segregating populations of groundnut (*Arachis hypogaea* **L.)**

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Abstract

Groundnut is a favourable and profitable crop for resource-poor farmers in Africa and Asia, both for edible oil production and direct consumption. There is significant potential to breed high-yielding, better-quality groundnut cultivars by generating new variations through artificial techniques. In this study, the F_2 generations of the crosses CO 7 × Chico and ICGV 07222 × Chico was analysed to assess the variability created through artificial hybridization in groundnut. The various yield and yield-related traits were analysed to estimate genetic parameters, skewness, kurtosis and subjected to principal component analysis (PCA). The variability study of the F_2 population from both crosses revealed significant variations for the traits under study. The traits "days to accumulation of 25 flowers" and "shelling %" showed low GCV (genotypic coefficient of variation) and PCV (phenotypic coefficient of variation) in both populations. Most traits exhibited moderate to high heritability and genetic advance, whereas "days to accumulation of 25 flowers" and "maturity duration" had moderate heritability and low genetic advance. Only shelling % had low estimates of heritability and genetic advances. Tests for skewness and kurtosis revealed that both F_2 population did not follow a normal distribution. The traits "days to maturity", "shelling %", "kernel yield," and "hundred kernel weight" displayed significant positive skewness. The traits "days to accumulation of 25 flowers", "number of matured pods", "height of main axis", "shelling %", "hundred pods" and "hundred kernel weight" and "pod yield" showed platy- kurtosis, while "Kernel yield" displayed lepto-kurtosis in both populations. The first principal component explained 37 % and 32 % of the total variance in the two F_2 populations respectively, with a focus on yieldrelated traits. The PCA biplot effectively clustered the genotypes based on the 10 different traits studied and clearly, grouped the population based on maturity duration. Thus, hybridization created significant variation in groundnut for all yield-related traits and yield, except for "days to maturity". The traits require further enhancement using additional sources and could be improved through intense selection.

Keywords

genetic advance; heritability; kurtosis; PCA biplot; skewness; variation

Introduction

Groundnut (*Arachis hypogaea* L.), a member of the Fabaceae family, is the second most important legume globally (1). Native of South America, it is cultivated in tropical, sub-tropical and warm temperate regions of the world. The

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seeds are widely consumed by human, both as confectionaries and as rich source of oil and protein. Additionally, groundnut plant residues are commonly used as fodder for cattle in many regions. The seeds are particularly rich in palmitic, linoleic and oleic acid and are also a valuable source of vitamins B (thiamine and niacin), K and E and essential mineral (2, 3). Groundnut kernels contain between 10.5 % to 20 % of carbohydrates, 16 % to 36 % of protein and 36 % to 54 % oil, making these constituents the primary constituents (4). They also contain functional components such as arginine, polysterols (5) and coenzyme Q10 (6), which contribute to its status as a functional food.

Due to its wide range of benefits, groundnut is often referred to as the "wonder nut" and "poor man's cashew nut. Locally, it is known by various names such as earthnuts, peanuts, goober peas, monkey nuts, pygmy nuts and pig nuts. Despite its name and appearance, groundnut is a legume, not a true nut. Like other legumes, it enriches the soil by fixing nitrogen through bacteria, making it particularly valuable as a soil-improving crop. Groundnut has a rapid growth cycle and is photoperiod-insensitive, allowing breeders to cultivate 2 crops per year, thereby shortening the crop improvement cycle. The crop also displays a wide spectrum of variability in yield, yield components and quality traits, offering great potential for breeding cultivars with improved yields and enhanced quality.

One effective solution to increase yield and economic returns is the development of high-yielding cultivars with short crop duration or early-maturing trait. Short-duration varieties reduce the risk of crop failure by shortening the time the crop is exposed to unfavourable conditions and offer greatest flexibility in planting within a given growing season. Additionally, they help to lower irrigation costs. This is particularly beneficial in areas with short rainy seasons or where water availability from irrigation canals is limited (7).

In general, groundnut varieties that mature in less than 100 days are classified as short- duration varieties. Earlymaturing cultivars are especially well-suited for regions with short crop cycles, where end-of-season droughts or frost may pose risks to crop growth (7). However, groundnut germplasm exhibits relatively low variability due to the crop's highly selfpollinating nature. To increase genetic variability and improved breeding outcomes, artificial hybridization has proven to be a valuable technique.

The observed variability in a population is a result of both genetic inheritance and environmental influence. This variability can be divided into heritable and non-heritable components, such as the genotypic coefficient of variation (GCV) and the phenotypic coefficient of variation (PCV). Broadsense heritability refers to the proportion of genotypic variance relative to phenotypic variance and its magnitude is essential for designing breeding programmes and evaluating experimental results (8). Heritability, when combined with high genetic advance, serves as an effective predictor for selecting superior genotypes for yield and its contributing traits (9).

Third and fourth-order statistics *viz.,* skewness and kurtosis, provides insights into the distribution of quantitative traits within a population (10). These estimates also offer clues about the nature and number of genes controlling these traits. Principal Component Analysis (PCA) is a valuable tool for assessing the genetic relationships among genotypes to aid in crop improvement. It groups genetically similar genotypes and generates scatter plot to represent their genetic distances with minimum distortion (11).

Given the utility of these statistical tools, this study aimed to generate variation through artificial hybridization using distantly related parent plants. The segregating F_2 generation was analysed for genetic variation by estimating genetic parameters, including skewness and kurtosis and by applying principal component analysis to assess biometric traits.

Materials and Methods

The experimental material consisted of three groundnut varieties: CO 7, ICGV07222 and Chico, each displaying distinct agronomic and morphological traits. CO 7 and ICGV07222, released by Tamil Nadu Agricultural University (TNAU) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) respectively, are highly valued by farmers for their high yield potential, with maturity periods exceeding 110 days. In contrast, Chico, a registered variety from the USA, matures in just 75 days and was used as the male parent in this study.

The parental varieties were cultivated during the 2018 *rabi* season in the fields of the Department of Oilseeds at the Centre for Plant Breeding and Genetics at TNAU, Coimbatore, with a spacing of 30×10 cm. Standard agronomic practices were followed throughout the cultivation. Crosses were made using hand emasculation and dusting techniques, resulting in the combinations CO 7 × Chico and ICGV07222 × Chico.

The successful F_1 hybrids were identified in the following season by comparing their traits with those of the parental varieties. This F_1 seeds were sown alongside both parents in 3-meter rows, maintaining a spacing of 30×10 cm during the *kharif* season of 2019. True F₁ plants were tagged and allowed to self-pollinate to produce the F_2 population. The harvested F_2 seeds from these crosses were then cultivated during the 2019 *rabi* season at the Department of Oilseeds, Centre for Plant Breeding and Genetics, TNAU, Coimbatore.

A range of morphological traits were evaluated for 200 F² plants from each cross, including days to accumulation of 25 flowers, shelling %, number of branches, height of the main axis, number of mature pods, days to maturity, kernel yield per plant, hundred-kernel weight, hundred-pod weight and pod yield per plant.

The F_2 data from each cross were subjected to the following statistical analysis.

- Genetic parameters such as heritability, genetic advance variability, GCV, and PCV as a % of the mean were calculated using the formulae provided (12) in the TNAUSTAT-Statistical package (13).
- The estimates of GCV and PCV were categorized according

to the scale given by (14). Heritability and genetic advance were categorized (12).

- The normality of the F_2 populations was tested using the 'Shapiro-Wilks' W test (15) with the RStudio Statistical package (16).
- PCA analysis and biplot construction were also carried out using RStudio package (16).

Results and Discussion

An essential requirement for any crop breeding program is the availability of sufficient variation, which enables selection based on specific objectives. The analysis of variance (Table 1.) for the F_2 population in this study, focusing on 10 biometric traits, revealed highly significant differences. This suggest that hybridization in groundnut has generated extensive variation within the population, making it well-suited for selection. These findings are consistent with expectations from hybridization involving highly diverse parents such as CO 7, ICGV 07222 and Chico. Table 2 depicts the estimates the genetic variability parameters.

The traits examined, including yield and early maturity, are influenced by a large number of genes and are significantly affected by environmental factors. Since PCV includes environmental effect, it is more appropriate to assess characters based on GCV, which represents the heritable portion of total variability (17). However, genotypic coefficient of variation alone is insufficient for selection. As reported, GCV combined with heritability estimates provides a clearer understanding of the potential genetic gains from selection (18). Thus, genetic advance depends on genetic variability, heritability and selection intensity.

The experiment material, consisting of the F_2 generation of the crosses CO 7 × Chico and ICGV 07222 × Chico, revealed a broad range of phenotypic and genotypic variation for all the traits under study. The trait "days to accumulation of 25 flowers" exhibited low GCV and PCV in both crosses, indicating that this trait is not suitable for selection. This finding aligns with the results reported earlier in groundnut variability, where it was also found that low GCV and PCV was low for days to 50 % flowering, suggesting a narrow genetic base for this trait (19, 20).

In both crosses, the traits "height of main axis", "days to maturity", "shelling %" and "number of matured pods" exhibited moderated PCV, while "height of main axis", "shelling $\%$ " and "pod yield" reported low GCV. The F₂ generation of the cross ICGV 07222 × Chico showed high PCV for the traits "number of branches", "kernel yield", "hundred pod" and kernel weight.

Table 1. ANOVA of 200 F₂ population of the crosses CO 7 × Chico and ICGV 07222 × Chico for the quantitative traits

*Significant at 5 %

DTF - Days to accumulation of 25 flowers, HMA - Height of the main axis (cm), NB - No. of branches, DM – Days to maturity, NMP- No. of matured pods, PY/P - Pod yield per plant (g), KY/P - Kernel yield per plant (g), HPW - Hundred pods weight (g), HYW - Hundred kernels weight (g), SP - Shelling percentage (%).

Table 2. Estimates of genetic variability parameters in F₂ generations of the crosses CO 7 × Chico and ICGV 07222 × Chico

Abbreviations for the traits are the same as in Table 1.

Similar findings were reported earlier who observed moderate PCV and GCV for the number of branches and plant height in groundnut (20-22). For 'days to maturity," reports are on low coefficients of variations, consistent with the present study (23, 24).

The moderate variation in "days to maturity" and high variation in "pod yield" in this study is likely due to the deliberate inclusion of parent plants with significant differences in maturity and yield. This suggests that the current material is suitable for selecting genotypes with varying maturity duration and yield potential.

Heritability and genetic advance as per cent of mean: Heritability estimates indicate the degree of trait inheritance, while genetic advance helps to formulate effective selection strategies. In both F_2 generation, the traits "days to accumulation of 25 flowers" and "maturity duration" exhibited moderate heritability and low genetic advance, suggesting non -additive gene action. Consequently, phenotypic selection for these traits would not be highly effective, as also noted (25).

Reports are on the moderate heritability with low genetic advance for days to 50% flowering in earlier studies on genetic variability in groundnut (26), while there are observations on high heritability with low genetic advance for days to first flowering (27). Similarly, stated that the trait "days to 50 % flowering" is governed by moderate heritability combined with low genetic advance (24, 28).

The trait "height of the main axis" exhibited high heritability coupled with moderate genetic advance, while "hundred-pod weight," "kernel weight," "number of branches" and "pod yield" showed moderate heritability and genetic advance. This indicates that these traits are primarily under additive gene control, with reasonable genetic gain and minimal environmental influence. Similar findings on additive gene action for the number of matured pods per plant were reported (28, 29), for plant height (19, 30) and for the number of branches (31).

Only "shelling %" was found to be governed by nonadditive genes, as reflected by its low heritability and genetic advance estimates. This suggests that shelling % is influenced by both additive and non-additive gene action, implying that more variability needs to be generated before effective selection for this trait can be practiced. The findings for shelling % in groundnut are in line with the present study (32, 33).

A notable distinction between the F_2 generations of the crosses is that "kernel yield" and "number of matured pods" recorded low heritability for CO $7 \times$ Chico, while they showed moderate heritability for ICGV 07222 × Chico. Additionally, "pod yield" exhibited moderate heritability in the F_2 generation of CO 7 \times Chico, but low heritability in ICGV 07222 \times Chico. This variation is likely attributable to the differences among the selected parent plants.

Skewness and Kurtosis

The Shapiro-Wilks W test of normality is a powerful tool for assessing the distribution of a population. If the W statistic is significant, the null hypothesis, that the distribution is normal, should be rejected. According to the results of the "Shapiro-Wilks W test" the F_2 populations of the crosses CO 7 \times Chico and ICGV 07222 \times Chico did not exhibit normal distribution (Table 3). The frequency distributions of the F_2 population can be better interpreted through third- and fourth-degree order statistics, specifically Skewness and Kurtosis.

In general, the variation in skewness and kurtosis values for the traits studied in each population can be attributed to differences in crossing over and linkage, leading to varying degrees of parental genomes in the offspring. A population with negative skewness and platykurtosis suggests that the traits are controlled by a large number of genes and influenced by dominant duplicate epistasis. This allows mild selection to achieve rapid genetic gain for the traits in question. Conversely, positive skewness combined with platykurtosis in a population indicates that the traits are governed by dominant complementary epistasis. As a result, the population requires more intense selection to achieve significant genetic progress for these traits (34).

The estimates of Skewness and kurtosis of different traits in the F_2 populations examined in this study are presented in Table 4 and their frequency distribution are shown as histogram in Fig. 1 and 2. In the F_2 population of the cross CO 7 × Chico, traits such as the number of branches and kernel yield exhibited significantly positive skewness. Similarly, positive skewness was observed for days to maturity, shelling %, kernel yield and hundred kernel weight in the F_2 population of ICGV 07222 \times Chico. This indicates that these traits cluster around lower mean values within the population.

Abbreviations for the traits are the same as in Table 1.

Table 4. Test of skewness and peak of the distribution curve of the 10 traits recorded in F₂ population of the crosses CO 7 × Chico and ICGV 07222 × Chico

| | CO 7 \times Chico | | | | | | ICGV 07222 × Chico | | | | | |
|------------|-----------------------|---------|---|---------|-------|------------------------------|--------------------|---------|-------------|----------|---------|-------------|
| Trait | | | Skewness T-value Probability Kurtosis T-value | | | Probability Skewness T-value | | | Probability | Kurtosis | T-value | Probability |
| DTF | -0.19 | -1.10 | 1.73 | $1.87*$ | 5.39 | 0.00 | -0.19 | 1.09 | 1.72 | $2.25*$ | 6.50 | 0.00 |
| HMA | -0.15 | -0.88 | 1.62 | $1.74*$ | 5.01 | 0.00 | 0.24 | 1.37 | 0.17 | $2.12*$ | 6.13 | 0.00 |
| NB. | $1.74*$ | 10.02 | 0.00 | $5.30*$ | 15.29 | 0.00 | 0.19 | 1.12 | 0.26 | $1.66*$ | 4.79 | 0.00 |
| DM | -0.62 | -3.57 | 2.00 | $2.52*$ | 7.27 | 0.00 | $0.87*$ | 5.00 | 0.00 | $11.75*$ | 33.93 | 0.00 |
| NMP | -0.13 | -0.76 | 1.55 | $2.39*$ | 6.89 | 0.00 | -0.31 | -1.82 | 1.93 | $2.77*$ | 8.01 | 0.00 |
| SP | 0.03 | 0.15 | 0.88 | $2.09*$ | 6.04 | 0.00 | $0.42*$ | 2.42 | 0.02 | $2.75*$ | 7.95 | 0.00 |
| KY | $1.95*$ | 11.26 | 0.00 | $5.34*$ | 15.43 | 0.00 | $1.66*$ | 9.61 | 0.00 | $4.23*$ | 12.21 | 0.00 |
| HPW | -0.62 | -3.57 | 2.00 | $2.33*$ | 6.73 | 0.00 | -0.58 | -3.36 | 2.00 | $2.25*$ | 6.51 | 0.00 |
| HKW | 0.25 | 1.46 | 0.15 | $2.22*$ | 6.40 | 0.00 | $0.59*$ | 3.38 | 0.00 | $2.93*$ | 9.03 | 0.00 |
| PY | -0.22 | -1.26 | 1.79 | $2.24*$ | 6.45 | 0.00 | -0.86 | -4.98 | 2.00 | $2.87*$ | 8.29 | 0.00 |

*Significant at 5 %, Abbreviations for the traits are the same as in Table 1.

Fig. 1. Histogram for quantitative traits of F_2 population of the groundnut cross CO 7 \times Chico.

Fig. 2. Histogram for quantitative traits of F_2 population of the groundnut cross ICGV 07222 \times Chico.

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The kurtosis values were significant for all the traits in both the populations, suggesting that the distributions are peaked and feature heavy tails. In both populations, the traits height of the main axis, days to accumulation of 25 flowers, number of matured pods, shelling %, hundred pods weight, hundred-kernel weight and pod yield exhibited platy kurtosis, implying these traits are controlled by numerous genes. Additionally, days to maturity in the F_2 population of CO 7 \times Chico and the number of branches in the $F₂$ population of ICGV 07222 × Chico also displayed platy kurtosis.

In contrast, kernel yield demonstrated leptokurtosis in both populations. A key difference between the two populations is that the number of branches in the F_2 population of CO 7 \times Chico and days to maturity in the F₂ population of ICGV 07222 × Chico also showed leptokurtosis. This suggests that these traits are controlled by a smaller number of genes.

The results of skewness and kurtosis suggest that the trait days to maturity is governed by dominant and complementary gene action involving fewer genes in the F_2 population of CO 7 × Chico, but a larger number of genes in the F_2 population of ICGV 07222 \times Chico. The contrasting findings for the days to maturity trait indicate that greater variability

must be generated using different genetic sources and more intensive selection is required to improve this trait in any population.

Principal Component Analysis (PCA) is a multivariate technique used to extract principal components and visually represent the similarity patterns of observations in a graphical format. PCA identifies the minimum number of components that explain the maximum variability from the total dataset, ranking the genotypes based on their PC scores (35, 36). Recognizing the utility of PCA, the populations in this study were analyzed to dissect the component traits related to yield and rank the genotypes accordingly.

The eigenvalues of the different vectors, their contribution to total variance and the cumulative variation are presented in Table 5. Principal components with eigenvalues greater than one (i.e., PC 1, PC 2 and PC 3) were selected to assess variability among the F_2 populations of the crosses CO 7 \times Chico and ICGV 07222 \times Chico (37). The first 3 principal components accounted for the maximum variation observed among the 10 quantitative traits. These results are illustrated in a scree plot (Fig. 3), which shows the relationship between the variation explained and the individual principal components for both populations.

Table 5. Eigen values total variance, % of variance and cumulative variance of 200 F₂ plants of the crosses CO 7 × Chico and ICGV 07222 × Chico studied for 10 traits

Fig. 3. Scree plot of principal component analysis for F₂ population of the groundnut crosses between the % of variances explained and principal components.

In the F₂ population of the CO $7 \times$ Chico cross, the first 3 principal components explained 74.5 % of the total variation, while in the F_2 population of the ICGV 07222 \times Chico cross, they explained 73.27 % (Table 6).

In the F_2 populations of both crosses under study, the first 3 principal components recorded eigenvalues greater than one. In the F₂ population of CO $7 \times$ Chico, PC 1 contributed 36.65 % to the total variation, while PC 2 and PC 3 contributed 23.76 % and 14.09 % respectively.

The variation explained by PC 1 was primarily associated with the traits number of branches, kernel yield and hundred-kernel weight, while PC 2 was influenced by the trait days to accumulation of 25 flowers, days to maturity and 100 pod weight. The traits number of matured pods and shelling % contributed to the variance explained by PC 3.

Similarly, in the F_2 population of ICGV 07222 \times Chico, PC 1 accounted for 31.58 % of the total variance, with the traits shelling %, kernel yield, hundred-pod weight and hundredkernel weight having the largest influence. PC 2 and PC 3 explained 27.56 % and 14.13 % of the total variance respectively. The variation explained by PC 2 was associated with days to accumulation of 25 flowers, number of branches, height of the main axis and days to maturity, while PC 3 was influenced by the traits number of matured pods and shelling %.

Comparable differentiation and grouping of groundnut genotypes through PCA have been reported in a multivariate analysis of groundnut accessions for yield and yieldassociated traits (38).

The traits kernel yield and hundred kernel weight are common in PC 1 of both populations, indicating that the first principal component focuses on direct yield-contributing traits for discriminating the population. Similar findings were reported, where it was observed that the first principal component in groundnut was in correlation with yield-related traits (39).

Principal component analysis biplot: The biplot and grouped elliptical biplot (Fig. 4 and 5) shows the relationship between different traits and genotypes of the F_2 population, with the first 2 principal components explaining most of the

Abbreviations for the traits are the same as in Table 1.

Fig. 4. Biplot diagram and grouping of F₂ population of the groundnut cross CO 7 × Chico based on the first two principal components.

Fig. 5. Biplot diagram and grouping of F₂ population of the groundnut cross ICGV 07222 × Chico based on the first two principal components.

variation. These results align with those reported in groundnut (40). The dimension vectors, which represents traits with smaller angles between them in the same direction, indicates strong correlations among those traits in discriminating the genotypes. Genetic distances among genotypes are represented by the geometrical distances between them. Genotypes with higher values for a particular trait are plotted closer to and in the direction of the relevant vectors.

In the F_2 population of the cross CO 2 \times Chico, the traits days to accumulation of 25 flowers, height of main axis, days to maturity and 100 kernel weight form a vector with minimum angles between them, indicating that they are associated with one another in grouping the population. Similarly, the traits 100 pods weight and shelling %, along with number of matured pods, kernel yield and number of branches, also show close associations.

Most genotypes are plotted near the vectors for shelling % and hundred-pod weight, suggesting they exhibit high values for these 2 traits. A considerable number of genotypes are also closer to the vectors for number of branches and kernel yield, indicating higher estimates for these traits.

In the F_2 population of the cross ICGV 07222 \times Chico, the traits days to accumulation of 25 flowers, number of matured pods, number of branches, shelling %, days to maturity and hundred-pod weight are closely related, helping to discriminate and group the population. The traits kernel yield, 100-kernel weight and height of main axis form another vector that discriminates the population in a different direction.

Both populations were grouped in the biplot according to their maturity durations, early, medium and late, based on the vectors. Both populations exhibited a higher number of genotypes with medium and late maturity, with a significant proportion also being early maturing. Comparatively the F² population of CO 2 × Chico exhibited a higher number of earlymaturing genotypes. Similar observations, regarding the diversity of maturity duration and number of flowers were already reported (41-43).

Conclusion

The study aimed to investigate the genetic variability induced through artificial hybridization in groundnut. The F_2 population generated by crossing the widely diverse parents CO 2, ICGV 07222 and Chico, using discrete pedigree methods, exhibited a significant number of genetic variations. Yield and yield-related traits showed moderate to high variability. The traits demonstrated both dominant and complementary gene action, with varying numbers of genes influencing them, as inferred from skewness and kurtosis estimates. Principal component analysis biplot effectively highlights the genetic distances among the F₂ genotypes and identified the key yieldrelated traits that contributed to population differentiation. The trait days to maturity exhibited mixed variation across the 2 populations studied. To enhance maturity duration in groundnut, further hybridization with additional sources, followed by selection, should be perused. In conclusion, artificial hybridization can be effectively utilised to enrich genetic variability in groundnut, ultimately aiding the development of elite varieties.

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Authors' contributions

RSVP conducted the experiment, recorded the biometric data for all the generations and carried out the analysis. The author also interpreted the analysis and drafted the manuscript. PLV formulated the idea of the research, monitored the conduct of the experiment. SM evaluated the results and corrected the manuscript. LR and TS suggested corrections in the manuscript and fine-tuned the writing. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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