Genetic improvement of peanut (*Arachis hypogea* L.) genotypes by developing short duration hybrids

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**Abstract**

Peanut, the only cash crop of rainfed areas of Pakistan, is facing immense challenges due to global warming. Climatic factors particularly the temperature fluctuations and rain pattern shift significantly impact the production and yield of peanut and unavailability of resilient varieties exacerbate this impact. To deal with the cropping pattern change and yield losses, due to climate vagaries, a study was conducted to develop early maturing hybrids using line into tester mating design. The F1 hybrids from the parental lines were produced in the year 2018 using Line × Tester mating design and then grown in the field in the year 2019 for further evaluation. The hybrids were evaluated based on the early maturity and yield-related attributes in comparison with the parental lines. Based on the general combining ability estimate, line V-3 (Golden), was found as best parent with highly significant values for plant height, days to peg formation, days to maturity, number of pegs per plant, number of pods per plants, number of seeds per plant, 100 pod weight 100 seed weight. Similarly, tester V-7 (PI 635006 01 SD) showed highly significant results of GCA for days to germination, day to 50% flowering, plant height, days to peg formation, days to maturity, number of pegs per plant, number of pods per plants, number of seeds per plant, 100 kernel weight, shelling percentage. All the combinations were evaluated for specific combining ability and significant results were observed for V-3 × V-4 (Golden × PI 619175 01 SD) and V-1 × V-6 (BARI-2000 × PI 564846 01 SD) by developing or maturity and yield-related attributes. The hybrid combinations V-3 × V-5 (Golden × PI 635006 01 SD) followed by V-3 × V-6 showed highly significant results for mid parent heterosis and better parent heterosis for days to 50% flowering, plant height, days to peg formation, number of pegs, days to maturity, number of mature seeds per plant, shelling ratio, 100 pod weight and 100 kernel weight. These parents and hybrid combinations with early maturity genes and high yield attributes can further be used for the development of short duration variety.

1. Introduction

Peanut (*Arachis hypogaea* L.) is an important global food and oil-seed crop with immense economic importance in the rainfed areas of Pakistan (Dasmae et al., 2017; Ali et al., 2002; Ali et al., 2001). Its production and cropping pattern is highly influenced due to climatic changes including temperature and rain pattern fluctuations. Early maturing peanut cultivars with enhanced yield are prerequisites for many agro-ecological regions of the world to meet changing climatic conditions (Hamidou et al., 2013; Upadhyaya, 2005).

Globally, peanut is an important crop which is cultivated in 108 countries of the world and China, India, Nigeria, the USA and Myanmar are reported as its major producers (FAO, 2018). It contains an invaluable source of protein, calories, essential fatty acids, vitamins, and minerals for human nutrition (Arya et al., 2016). It belongs to the family Fabaceae and is an allotetraploid (AABB-type genome; 2n = 4x = 40) which arose from the event of
hybridization between two diploid species, *Arachis duranensis* and *Arachis ipaensis* and then genome polyploidization (Moretzsohn et al., 2013). Its self-pollinated flower is comprised of one large yellow color standard petal and two lateral wings with a white keel petal. The keel enclosed eight anthers which surrounds a club shaped stigma and style which run through a hallow stalk, hypan-thium (Chu et al., 2016). Geocarpy is an exceptional characteristics of pod development in peanut in which flowering and fertilization occur above the ground, whilst the pod formation is under the ground (Zhang et al., 2018). The high temperature and drought spells highly influence its delicate flowers, pollen viability, peg formation and penetration in the soil for pods development. Whereas early maturity enables escape from these stresses, which reduces yield loss (Janila et al., 2013).

Pakistan is among the top most countries affected due to climate change and global warming, which has robust influence on agricultural productivity (Ali et al., 2021). The average per hectare yield of the peanut crop in Pakistan is very low as compared to its yield potential throughout the world. This yield gap is due to the unavailability of high-yielding, short-duration varieties, unprecen-ded environmental conditions, less rainfall and low inputs by the farmers (Khan et al., 1993). The world is producing an average yield of 1630 kg/ha, even Israel is producing 6325 kg/ha as compared to Pakistan with an average yield of 921 kg/ha (FAO, 2018). Yield losses in Pakistan are highly affected due to early, mid and end season drought stress which compelled farmers to shift its planting season from mid-April to the beginning of July. This adopted cropping pattern helps to avoid mid-season drought which causes flower shedding, peg formation and penetration problems. On the other hand, short growing season due to late sowing, prevent peanut from maturing properly and results in belated wheat planting (Ijaz, 2011). Whilst immature pods also abortion in the soil for pods development. Whereas early maturity enables escape from these stresses, which reduces yield loss (Janila et al., 2013).

Breeding of short duration and high yielding peanut varieties is an important objective to improve peanut yield and quality particularly in the rain fed area like Pothohar belt (Upadhyaya et al., 2006; Ijaz, 2011). Maturity is an indeterminate trait, controlled by different sets of genes, and is highly influenced by environmental factors including light and temperature along with the latitude of a growing region. However, it varies in different peanut varieties and commonly ranges from 100–120 days and 130–150 days (Nigam and Aruna, 2008; Carter et al., 2017). Early maturing cultivars also form an important component of high intensity multiple cropping systems in South and Southeast Asia. Since peanut is a self-pollinating plant, a cross breeding procedure is useful for producing new varieties of this crop (Chu et al., 2016). Genetic studies concerning inheritance, genetic variability and heritability, combining ability and trait correlations have provided a better understanding of the crop’s genetics to develop appropriate breeding strategies for target traits (Desmae et al., 2019). The current research is mainly aimed to develop an early maturing breeding population by crossing selected genotypes using line × tester mating design for subsequent crop improvement.

### 2. Materials and methods

Total seven peanut genotypes, including three adaptable and higher yielding local varieties along with four short duration accessions with better yield, from USDA (United States Department of Agriculture), were selected after screening in the field for hybridization experiment (Table 1). The field experiments were conducted in Rawalpindi region with latitude 32° 10' to 34° 9' N and longitude 71° 10' to 71° 55' E in the peanut growing season from mid-April to mid-October.

| No. | Selected genotypes | Names | General characters for parental selection |
|-----|--------------------|-------|------------------------------------------|
| 01  | Female Parent (Lines) | BARI-2000 | High yielding, adaptable to local environment |
| 02  | V-2 | BARI-2011 | High yielding, adaptable to local environment |
| 03  | V-3 | Golden | High yielding, adaptable to local environment |
| 04  | Male Parent (Tester) | USDA lines | Bunch type, Short duration (Matures in 118–125 days), Better yield |
| 05  | V-4 | PI 594,923 01 SD | Bunch type, Medium duration, Better yield |
| 06  | V-6 | PI 478,787 01 SD | Bunch type, Short duration (maturity 110–120 days), Better yield |
| 07  | V-7 | PI 542,961 01 SD | Variable growth habit, Short duration, Better yield |

#### 2.1. Hybridization

The parental seeds of lines and testers were sown in replication of three, into the pots (30 cm depth and 60 cm width) in the soil prepared with 3:1:1 ratio soil: sand: manure in year 2018. Hybridization experiment was started after 4–5 days of initiation of the flowers. Line × tester analysis was applied to conduct conventional breeding studies (Kampthorne, 1957). Crossing between the selected three lines (female parents) including V-1, V-2 and V-3 and four testers (male parents) V-4, V-5, V-6, V-7 (3 × 4 line into tester) was performed to get F1 generation as shown in Fig. 1.

#### 2.2. Emasculation and pollination

For peanut hybridization, to produce artificial hybrids, the female flowers were emasculated well before anther dehiscence in the late evening before bud’s opening. The mature pollen from the male testers plants were collected and applied on the stigma of the female flower of the lines in the next morning. The flowers were pollinated in different timings to achieve the successful crosses with total number of crosses made (Table 2). Since humidity is one of the very important factors regarding successful pollination, the moisture level was increased by water spray before pollination. To avoid the mixing of the hybrids, extra flowers on the female plants after crossing were removed.

#### 2.3. Evaluation of F1 hybrids

For evaluation of F1 hybrids total nineteen genotypes including 12 hybrid combinations and 7 parental genotypes (Table 1) were sown in the field in RCB Design with standard agronomical practices in year 2019. The parents and the hybrids seeds were sown with plant to plant distance of 12 cm and row to row distance of 45 cm in three replications in three different blocks.

#### 2.4. Agronomic attributes

The F1 obtained after hybridization and their parental lines were evaluated to get F2 based on the morphological attributes associated with early maturity and high yield. Five plants from each replication were randomly selected to collect the data for each attributes. Days to germination were calculated by observing
50 percent germination of plants by counting the days after sowing. Days to first flower was counted from the sowing to the commencement of the first flower. For 50% flowering date, days were counted when about 50% of the flowers bloom in each row in each replication. Peanut plant height was measured with a meter rule on 5 tagged plants of each row in each plot. Height was measured from ground level to the top most leaf axil of the main stem of each peanut plant and the mean height was expressed in centimeters. The days to the first peg formation after appearance of the first flower from each row in the field were noted by closely observing the plants of each row in each replication. Days to maturity was calculated by counting days from sowing to harvesting. The number of pegs per plant was counted from the harvested plants. Total number of mature pods were counted from selected harvested plant. The peanut pods were bulked and a hundred pod weight was measured in grams using weighing balance. The kernels were deshelled and counted for weighing 100 seed weight. Shelling percentage was measured using the formula:

\[
\text{Shelling}\% = \frac{\text{Number of deshelled kernel}}{\text{Number of shelled pods}} \times 100
\]

2.5. Statistical analysis

To analyze the hybrid’s performance heterosis and combining ability was evaluated. On the basis of studied traits heterosis including mid parent heterosis and better parent heterosis percentage, general and specific combining ability of the parents was analyzed using software R-Studio version 3.5.1.

3. Results

3.1. Hybridization condition optimization

Peanut artificial hybridization is a very critical process due to very small and delicate flowers. Total 220 flowers were emasculated and pollinated for obtaining the crosses (Table 3). The per-

| Time of pollination (Morning) | Number of crosses made |
|------------------------------|------------------------|
| 6am – 7 am                   | 160                    |
| 6:45am to 8am                | 60                     |

**Table 3**

Percentage of successful crosses with different pollinations times during hybridization experiment.

| Number of crosses with pollination time 6am to 7 am | Number of crosses with pollination time 6:45am to 8 am | Total |
|-----------------------------------------------------|--------------------------------------------------------|-------|
| 160                                                 | 60                                                     | 220   |
| 132                                                 | 7                                                      | 139   |
| 82.48%                                              | 11.67%                                                 | 63.18%|
centage of successful crosses pollinated during 6am to 7am and 6:45am to 8am was found 82.48% and 11.67% respectively. It is evident from the results that the time which suits best for the pollina-

3.2. Estimation of general combining ability (GCA)

The estimate of general combining ability of three lines and four testers of peanut are shown in Table 4. Male parent V-7 showed significant general combining ability i.e. −0.69 for day to germination, rest of the varieties showed non-significant GCA for this trait as shown in the table. General combining ability was found significant with the negative value −2.36 for days to the first flower among seven varieties. General combining ability calculated for days to fifty percent flowering was highly significant for V-5 and V-7 with the values 3.64 and −3.47 respectively. However significant GCA was observed for V-1 i.e. −1.61.

Highly significant general combining ability was observed in V-1, V-2, V-3, V-4 and V-5 with the values −0.54, 2.80, −2.26, −0.93 and 1.17 respectively for plant height. Days to pegs formation also showed highly significant GCA values for V-1, V-2, V-3, V-4 and V-5 i.e. −1.67, 0.75, 0.92, 1.36 and −1.75. The GCA for days to peg pen-

3.3. Estimation of specific combining ability (SCA)

Specific combining ability of all 12 hybrid combinations for studied morphological attributes as shown in the Table 5. For days to 50% flowering, only V-1 × V-4 showed negative significant results with the value −3.3. For plant height, all combinations showed highly significant results except V-1 × V-5 and V-2 × V-4. Highly significant results were observed for plant height in V-2 × V-7 with value 2.24. The hybrid combination V-3 × V-5 with the value 2.29 was found with maximum positive and highly significant specific combining ability for day to peg formation. For

3.4. Estimation of mid parent heterosis and better parent heterobeltiosis

Heterosis over mid and better parent was calculated for different agronomic attributes associated with the early maturity and high yield (Tables 6 and 7). Hybrid combination V-3 × V-7 was found the best hybrid, with maximum significance level, among twelve combinations based on the mid and better parent heterosis. Total eleven attributes, associated with the earliness and high yield in peanut were studied for the hybrid evaluation and V-3 × V-7 followed by V-3 × V-6 showed significant results of its performance over mid parent and better parent in maximum studied attributes.

In terms of evaluation for earliness in peanut, for days to germi-

Table 4

| Parents     | Days to germination | Days to first flower | Days to 50% flowering | Plant height | Days to maturation | Days to peg formation | Days to 50% flowering | 100 pods weight | 100 kernel weight | Shelling % |
|-------------|---------------------|----------------------|-----------------------|-------------|-------------------|-----------------------|-----------------------|-------------------|-------------------|------------|
| V-1         | −0.33**             | −2.36                | −1.61                 | −0.54**      | −1.67**           | 1.22**                | −0.50**               | 3.57**           | −2.28**           | 31.25      |
| V-2         | 0.00**              | 1.56**               | 0.97**                | 2.80**       | 0.75**            | 0.47**                | 19.75**              | 15.94**          | 0.70**            | 2.11**     |
| V-3         | 0.33**              | 0.81**               | 0.64**                | −2.26**      | 0.92**            | −1.69**               | −19.25**             | −5.97**           | 4.77**            | 6.19**     |
| V-4         | −0.03**             | −0.83**              | −0.03**               | −0.27**      | 1.36**            | 0.61**                | 17.22**              | 11.58**          | 1.32**            | 1.61**     |
| V-5         | 0.19**              | 0.06**               | 0.64**                | 0.07**       | 0.25**            | 6.06**                | 23.22**              | 9.52**            | 1.30**            | 5.14**     |
| V-6         | 0.53**              | 1.17**               | −0.14**               | −0.93**      | 0.14**            | −2.28**               | −12.56**             | −4.97**           | 4.88**            | −5.79**    |
| V-7         | −0.69*              | −0.39**              | −3.47**               | 1.17**       | −1.75**           | −4.39**               | −27.89**             | −16.53**          | −7.50**           | −0.96**    |

For the total number of pegs, V-1 × V-7 followed by V-3 × V-4 (55.39, 37.36) showed maximum positive significant specific com-

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4. Discussion

During the current hybridization experiment, it was assessed that the personal training, environmental conditions and timing of emasculation, and pollination are of significant importance for successful breeding. Personal training involves the handling of the flower during emasculation and pollination whereas low temperature and moisture are prerequisites for pollination success. Some observations were reported by Chu et al., 2016 in the previous studies and they evaluated the effect of multiple factors on the success rate of artificial hybridization. Their research indicated that operator, pollination time and environment significantly affected the success rate of peanut hybridization. Prasad et al. (2001) also reported that temperature is a very important factor for pollen

Table 5
Specific combining ability for Days to germination, Days to first flower, Days to 50% flowering, Plant height, Days to peg formation, Days to maturity, Number of pegs per plant, Number of pods per plant, Number of seeds per plant, 100 pod weight, 100 seed weight and shelling percentage of combination from line × tester mating design.

| Crosses | Days to germination | Days to first flower | Days to 50% flowering | Plant height | Days to peg formation | Days to maturity | Number of pegs per plant | Number of pods per plant | Number of seeds per plant | 100 pod weight | 100 seed weight | Shelling % |
|---------|---------------------|---------------------|-----------------------|--------------|-----------------------|-----------------|------------------------|--------------------------|--------------------------|----------------|----------------|-----------|
| V-1 × V-4 | -0.22** | 2.25** | 1.61** | -1.15** | -1.11** | -6.78** | -55.06** | -40.92** | 2.30** | -1.32** | -4.25** | -10.41** |
| V-1 × V-5 | 0.22** | -0.31** | -1.39** | -0.05** | 1.00** | -3.89** | -28.72** | -8.58** | -3.42** | -7.70** | 2.28** | 2.69** |
| V-1 × V-6 | -0.11** | 1.08** | -0.28** | 1.75** | -0.22** | 3.11** | 28.39** | 9.64** | 7.56** | 6.38** | 1.20** | 1.47** |
| V-1 × V-7 | 0.11** | -0.86** | 0.06** | -0.55** | 0.33** | 7.56** | 55.39** | 39.86** | -6.43** | 2.65** | 0.77** | 6.25** |
| V-2 × V-4 | 0.11** | -2.67** | 1.69** | 0.01** | 1.14* | -1.03* | 17.69** | -1.17** | -11.47** | -5.93** | 4.66** | 4.64** |
| V-2 × V-5 | -0.11** | -0.56** | 0.30** | 1.21** | 1.25** | 3.53** | 21.73** | 7.83** | -1.90** | -2.08** | 0.03** | -0.62** |
| V-2 × V-6 | 0.22** | 1.67** | 0.14** | 3.46** | -1.97** | 0.19* | 23.81** | 23.72** | 0.54** | -2.64** | -1.55** | 1.83** |
| V-3 × V-7 | -0.22** | 1.56** | -2.19** | 2.24** | -0.42** | -2.69** | -62.53** | -30.39** | 12.83** | 10.65** | -3.15** | -5.85** |

Table 6
Mid parent (MPH), and better parent (BPH) heterosis for Days to germination, Days to first flower, Day to 50% flowering, Plant height, Days to peg formation, Number of pegs for the F1 hybrid combinations.

| Cross | Days to germination | Days to first flower | Days to 50% flowering | Plant height | Days to peg formation | Number of pegs |
|-------|---------------------|---------------------|-----------------------|--------------|-----------------------|---------------|
| V-1 × V-4 | -14.81** | -28.13** | -5.45** | -15.22** | 2.98** | -1.63** |
| V-1 × V-5 | -5.09** | -21.88** | -1.15** | -20.65** | 6.49** | 0.00** |
| V-1 × V-6 | -16.67** | -21.88** | -10.30** | -19.57** | -6.50** | -6.50** |
| V-1 × V-7 | -16.98** | -31.25** | -13.58** | -23.91** | -12.03** | -13.82** |
| V-2 × V-4 | -1.96** | -13.79** | -10.71** | -21.05** | 1.57** | -9.15** |
| V-2 × V-5 | -3.83** | -13.79** | 0.01** | -11.58** | 8.80** | -4.23** |
| V-2 × V-6 | -5.26** | -6.90** | 11.90** | -1.05** | -6.42** | -12.68** |
| V-2 × V-7 | -12.00** | -24.14** | 7.88** | -6.32** | -17.69** | -24.65** |
| V-3 × V-4 | -1.89** | -16.13** | -3.53** | -15.46** | -12.06** | -22.07** |
| V-3 × V-5 | -3.70** | -16.13** | 1.18** | -11.34** | 8.30** | -5.52** |
| V-3 × V-6 | -8.47** | -12.90** | -0.01** | -12.37** | -8.21** | -15.17** |
| V-3 × V-7 | -7.69** | -22.58** | -4.19** | -17.53** | -9.51** | -17.93** |

Table 7
Mid parent (MPH), and better parent (BPH) heterosis for Days to maturity, Number of pods per plant, Number of mature seeds per plant, 100 pod weight, 100 kernel weight and Shelling percentage for the F1 hybrid combinations.

| Cross | Days to Maturity | Number of mature pods per plant | Number of mature seeds per plant | 100 pod weight | 100 kernel weight | Shelling ratio |
|-------|-----------------|---------------------------------|---------------------------------|----------------|-----------------|---------------|
| V-1 × V-4 | -38.82** | -50.94** | -61.26** | -61.68** | 7.24** | -6.49** |
| V-1 × V-5 | -9.41** | -27.36** | 7.50** | -31.53** | -12.29** | -14.11** |
| V-1 × V-6 | -17.98** | -31.13** | -27.42** | -28.34** | 0.28** | -4.22** |
| V-1 × V-7 | -5.33** | -24.53** | 2.09** | -10.51** | 36.13** | -41.43** |
| V-2 × V-4 | -15.19** | -28.72** | 9.59** | -0.31** | 4.46** | -2.24** |
| V-2 × V-5 | 22.78** | 3.19** | 95.99** | 30.04** | 6.09** | 0.29** |
| V-2 × V-6 | -25.30** | -34.04** | 21.27** | 12.75** | 6.66** | -5.23** |
| V-2 × V-7 | -40.13** | -50.00** | -43.40** | -43.73** | 1.32** | -13.28** |
| V-3 × V-4 | 20.83** | 8.75** | 23.08** | 19.63** | 36.07** | 20.53** |
| V-3 × V-5 | 12.50** | 1.25** | 31.11** | 15.84** | 15.57** | 15.21** |
| V-3 × V-6 | -40.79** | -43.75** | -64.53** | -64.71** | -4.94** | -10.74** |
| V-3 × V-7 | -52.45** | -57.50** | -48.49** | -52.15** | -22.47** | -30.05** |

1Highly significant, **Significant, ***Non significant.
viability during pollination which is best suitable before dawn as observed in current experiments (Peltonen-Sainio et al., 2016).

Banks (1976) and Prasad et al. (2001) observed time of pollination as one of the keys factors for hybrid formation.

Line into tester analysis is considered as most powerful tools for predicting gene action and provides information about genetic mechanism controlling yield and other components (Vishnuprabha et al., 2021). Evaluating the combining ability of candidate lines is an important aspect to identify superior and good combiner parents and progenies. It helps to comprehend the type of gene action involved in trait inheritance and to determine suitable selection method (Daudi et al., 2021). Usman et al. 2015 utilized the estimate of combining ability as an important tool for the identification of superior parents which can be used for the development of new cultivars and hybrids. In current study most of the crosses with desirable specific combining ability involved parents with high and low general combining ability, which indicates the influence of non-additive gene interactions in these crosses. Such parents were recommended for bi parental crossing or reciprocal recurrent selection program for developing superior varieties with high kernel yield in previous studies (Waghmode et al., 2017). However, the crosses with higher general and specific combining ability in both parents could be further used as the yield transgressive segregants.

Since the identification of early maturing parental lines and their combination is the key objective of the current study for short duration varietal development under changing climate, which has also an augmented role in seed yield improvement by extrication from disease and insect attack on the crop. Savithramma et al. (2010) found some parental lines with significant negative GCA for days to maturity which helps develop early maturing genotypes to escape groundnut pests and diseases. Such crosses are considered to be of prime importance for a breeding program.

Based on the heterosis/hybrid vigor over the mid and better parent, the hybrid combinations showed variable results in all studies traits. Days to flowering was very important parameter associated with the early maturity. The significant negative mid parent and better parent heterosis were observed which was correlated with the significant and positive mid parent heterosis and better parent heterosis of the combinations to select the best hybrid combination (Boriaiah et al., 2012). Waghmode et al. (2017) also reported that for selection of the desirable segregants from the line into tester crossing, significant heterosis is exhibited in the crosses for yield related traits in peanut. In the present results, the prevalence of the mid and better parent heterosis in majority of hybrid’s combinations, indicated the influence of both additive and non-additive gene action for mature kernels as reported in a previous study (Khanala and Mishra, 2017). Similarly, the data revealed by Upadhaya and Nigam (1994), suggested that the days to first flower in the crosses is governed by a single gene with additive gene action which is highly associated with the earliness in the peanut crosses.

5. Conclusion

Present investigations provided a considerable information for parental selection, their performance in F₁ and selection of best possible hybrid combinations. Genotype V-3 was good female general combiner and V-7 was a good male general combiner. Hybrids V-3 × V4 and V-1 × V6 were good specific combiners whereas the hybrid V-3 × V-5 was good F1 hybrid on the basis of mid parent heterosis. It is further suggested that these parents and hybrid combinations may be utilized for development of early maturing/short duration peanut variety to improve peanut yield in the rainfed areas.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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