

## DEVELOPMENT OF INTERSPECIFIC HYBRID LINES OF *ARACHIS* AND CHARACTERISATION FOR VARIOUS MORPHO-METRIC TRAITS USING PRINCIPAL COMPONENT ANALYSIS

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Groundnut (*Arachis hypogaea* L.), a segmental allopolyploid species ( $2n = 4x = 40$ ), exhibits a unique subterranean fruiting habit through the production of geocarpic pegs. Although native to South America, India and China are the leading contributors to global groundnut production, collectively accounting for approximately 51% of the world's output in 2018-19 (Agriculture Market Intelligence Centre, 2021). This crop is susceptible to a range of diseases, with foliar and fungal diseases, such as Late Leaf Spot (LLS), being particularly prevalent in groundnut-growing regions worldwide. LLS is known to cause yield reductions of up to 50% (Subrahmanyam *et al.*, 1980; Ghuge *et al.*, 1981), with potential losses escalating to as much as 70% when accompanied by rust disease (Vidyasekaran, 1981). Developing resistant or tolerant lines represents an environmentally sustainable strategy to mitigate economic losses for farmers. One of the most effective approaches to combat these diseases is the creation of pre-breeding lines utilizing resistant wild species of groundnut, as these species offer substantial genetic potential for disease resistance. Various pathways, including hexaploidy and diploid/tetraploid approaches, have been proposed for the introgression of resistance genes from wild species into cultivated groundnut (Simpson, 1991; 2001). The current study focuses on adopting the diploid/tetraploid pathway to introgress genes for resistance to foliar and fungal diseases from induced autotetraploid forms into well-adapted yet highly susceptible cultivars of *A. hypogaea* L. As an initial step in resistance breeding, interspecific hybrid lines were produced and evaluated for various morphological traits to assess the degree of variability between them.

### Plant materials

High-yielding cultivars of *Arachis hypogaea* ssp. *fastigiata* var. *vulgaris* (Spanish Bunch), including TMV 12, K 6, VRI 2, and JL 24 ( $2n = 4x = 40$ ), were selected as female parents. Two induced autotetraploids of *Arachis villosa* Benth. and *Arachis duranensis* Krapov. & W.C. Gregory ( $2n = 4x = 40$ ) were used as male parents for

the development of interspecific hybrid lines.

### Production of induced autotetraploids

Two diploid wild species, *Arachis villosa* Benth. ( $2n = 20$ ) and *Arachis duranensis* Krapov. & W.C. Gregory ( $2n = 20$ ), were cultivated in Field A7 of the New Farm at the Regional Research Station, Vridhachalam, during the *kharif* season of 2020. Prior to flower initiation, the terminal shoot buds of selected plants were treated by covering them with cotton soaked in a 0.25% colchicine solution for two consecutive days. The apical buds were subsequently washed with sterile distilled water. To confirm chromosome doubling, flower buds produced from the treated apical buds were fixed in Carnoy's fluid (6:3:1 ethanol:chloroform:acetic acid) and stained with 2.0% acetocarmine for chromosome counting. To enhance pod formation, pegs were treated with 176 ppm gibberellic acid ( $GA_3$ ) following the method suggested by Stalker *et al.* (1987). Mature pods from the treated plants were harvested separately, properly dried, and stored for sowing in the subsequent season.

### Interspecific hybridization

The pods collected from induced autotetraploid strains of *Arachis villosa* (4x) and *Arachis duranensis* (4x) were manually shelled, and the kernels were stored in paper envelopes. Likewise, pods of *Arachis hypogaea* cultivars, including TMV 12, K 6, VRI 2, and JL 24, were hand-shelled and stored in paper envelopes. A dedicated crossing block was established in Field B1 of the New Farm during the *rabi*/summer season of 2020-21. Female parental plants were arranged in two rows, while male parental plants were planted in a single row, with each row measuring 4 meters in length. This planting configuration was designed to promote synchronous flowering between male and female plants.

Unopened flower buds from the female parents were carefully selected and emasculated in the late afternoon (4:00 PM) on the day before pollination. The following morning, fully opened flowers from the induced autotetraploids were collected, and pollen grains were applied to the stigmatic surfaces of the emasculated

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flowers at 8:00 AM. To mark the crossed flowers, coloured threads were loosely tied around the hypanthium. Daily records were maintained, documenting the number of flowers emasculated and pollinated. The success rate of each cross combination was calculated as the ratio of the total number of pollinated flowers to the total number of pods successfully obtained. The crossed pods were then harvested, dried, and stored for subsequent sowing.

## Hybrid identification

During the *kharif* season of 2021, the crossed pods were shelled, and the kernels were planted alongside their respective parental lines in Field A7 at New Farm. Out of the eight cross combinations planted, six exhibited successful field emergence, while the remaining two did not germinate. True hybrid lines were identified using cytological techniques, which were also employed to confirm the successful induction of autotetraploids.

## Morphological characterisation

Twenty plants were randomly selected from each of the cultivars, namely TMV 12, K 6, VRI 2, and JL 24, as well as six plants from each of the two induced autotetraploids and viable interspecific hybrid lines. Observations were recorded on a range of morphological traits, including plant height (cm), number of primary branches, number of secondary branches, number of tertiary branches, length of primary branches (cm), length of secondary branches (cm), length of tertiary branches (cm), petiole length (cm), leaflet length (cm),

leaflet width (cm), stipule length (cm), sepal length (joined) (cm), sepal length (single) (cm), hypanthium length (cm), standard petal length (cm), standard petal width (cm), wing petal length (cm), wing petal width (cm), keel petal length (cm), and keel petal width (cm).

## Statistical analysis

The mean, standard deviation, coefficient of variation, and standard error were calculated for all 20 traits of the parental lines and interspecific hybrid lines. Principal component analysis (PCA) was conducted using the XLSTAT software. The parents and interspecific hybrid lines were subsequently grouped using the clustering method proposed by Ward (1963).

## Pod setting percentage of interspecific hybridization

The following table (Table 1) presents the number of flowers pollinated, the number of pods obtained, the pod setting percentage, and the number of viable F<sub>1</sub> hybrid lines identified.

In general, the success rate was relatively low across all crosses made during the *rabi*/summer 2020-21 season. Among the eight crosses, the highest seed set percentage was observed in *Arachis hypogaea* L. cv. VRI 2 × autotetraploid of *Arachis duranensis* (4x) (20.1%), closely followed by *Arachis hypogaea* L. cv. VRI 2 × autotetraploid of *Arachis villosa* (4x) (13.0%). The cross *Arachis hypogaea* L. cv. TMV 12 × autotetraploid of *Arachis villosa* (4x) exhibited the lowest seed set percentage at 2.0%.

**Table 1. Success rates of pollination and pod setting in eight interspecific *Arachis* crosses**

| Sr. No. | Crosses                                                         | No. of pollinations done | No. of pods obtained | Pod setting % | Number of viable hybrid lines identified |
|---------|-----------------------------------------------------------------|--------------------------|----------------------|---------------|------------------------------------------|
| 1       | <i>A. hypogaea</i> L. cv. TMV 12 X<br><i>A. villosa</i> (4x)    | 759                      | 15                   | 2.0           | 2                                        |
| 2       | <i>A. hypogaea</i> L. cv. K 6 X<br><i>A. villosa</i> (4x)       | 695                      | 22                   | 3.2           | 6                                        |
| 3       | <i>A. hypogaea</i> L. cv. VRI 2 X<br><i>A. villosa</i> (4x)     | 728                      | 95                   | 13.0          | 0                                        |
| 4       | <i>A. hypogaea</i> L. cv. JL 24 X<br><i>A. villosa</i> (4x)     | 643                      | 40                   | 6.2           | 0                                        |
| 5       | <i>A. hypogaea</i> L. cv. TMV 12 X<br><i>A. duranensis</i> (4x) | 746                      | 14                   | 1.9           | 2                                        |
| 6       | <i>A. hypogaea</i> L. cv. K 6 X<br><i>A. duranensis</i> (4x)    | 559                      | 50                   | 8.9           | 5                                        |
| 7       | <i>A. hypogaea</i> L. cv. VRI 2 X<br><i>A. duranensis</i> (4x)  | 670                      | 135                  | 20.1          | 7                                        |
| 8       | <i>A. hypogaea</i> L. cv. JL 24 X<br><i>A. duranensis</i> (4x)  | 663                      | 42                   | 6.3           | 1                                        |

## Per se performance of parents and interspecific hybrid lines

Among the parents evaluated, the cultivar *TMV 12* exhibited significantly higher mean values for plant height, petiole length, leaflet length, stipule length, sepal length (joined), and standard petal length. Similarly, the cultivar *JL 24* showed significantly higher mean values for plant height, leaflet length, leaflet width, stipule length, and sepal length (single). The induced autotetraploid *A. duranensis* (4x) recorded significantly higher mean values for plant height, sepal length (joined), sepal length (single), hypanthium length, standard petal width, and wing petal width (Tables 2a and 2b). These results indicate that the cultivars are superior in terms of vegetative traits, whereas the induced autotetraploids are notable for their flowering traits. These findings are consistent with those of Mothilal *et al.* (2017).

Among the six interspecific hybrid lines examined, hybrid HY 1 recorded significantly higher mean values for a greater number of traits (13 traits), including the number of primary branches, secondary branches, length of primary branches, petiole length, leaflet length, leaflet width, stipule length, sepal length (joined), hypanthium length, standard petal width, wing petal length, wing petal width, and keel petal width. These traits involved parents with either non-significant mean

values or where only one parent exhibited significant mean values for the respective trait (Supplementary Figures 1 to 6). Hybrid HY 6 also showed significant superiority for a larger number of traits (9 traits), including the number of primary branches, secondary branches, tertiary branches, length of primary branches, secondary branches, and tertiary branches, sepal length (single), wing petal length, and keel petal length, where the parents displayed non-significant mean values. Hybrid HY 4 similarly exhibited significantly higher mean values for various vegetative and flowering traits, involving parents with non-significant values.

## Principal component analysis (PCA)

Principal component analysis (PCA) is widely used to evaluate the relationships among various biometrical traits. In this study, the PCA resulted in 11 principal components, with the first five components having eigenvalues  $\geq 1$ , collectively contributing 87.92% of the total variation across 20 quantitative traits (Fig. 1). The first principal component (PC1), with an eigenvalue of 7.614, accounted for 38.072% of the total variation. PC1 showed a positive correlation with traits such as the number of primary branches, number of secondary branches, number of tertiary branches, length of primary branches, length of secondary branches, length of tertiary branches, wing petal length, and keel

**Table 2a. Per se performance of parents and interspecific hybrid lines for various traits**

| Parents / Hybrid lines    | Traits  |         |          |          |         |         |         |        |        |        |
|---------------------------|---------|---------|----------|----------|---------|---------|---------|--------|--------|--------|
|                           | PH      | NPB     | NSB      | NTB      | LPB     | LSB     | LTB     | LP     | LL     | LW     |
| TMV 12                    | 32.37** | 3.50    | 1.75     | 0.00     | 29.40   | 10.05   | 0.00    | 6.80** | 4.64** | 2.15   |
| K 6                       | 25.50   | 4.00    | 4.00     | 0.00     | 26.10   | 17.12   | 0.00    | 5.75** | 4.86** | 2.62** |
| VRI 2                     | 26.00   | 3.50    | 0.00     | 0.00     | 12.88   | 0.00    | 0.00    | 6.10** | 4.19   | 2.35** |
| JL 24                     | 33.37** | 4.25    | 6.50     | 0.00     | 31.43   | 11.57   | 0.00    | 4.95   | 4.91** | 2.54** |
| <i>Arachis villosa</i>    | 23.00   | 11.00   | 11.00    | 8.00     | 66.40** | 30.10   | 11.40   | 5.00   | 2.94   | 1.25   |
| <i>Arachis duranensis</i> | 31.73** | 14.60   | 10.00    | 0.33     | 22.50   | 15.80   | 2.50    | 2.73   | 1.77   | 1.00   |
| HY 1                      | 22.50   | 24.00** | 138.00** | 100.50   | 53.30** | 24.41   | 11.03   | 5.82** | 4.62** | 2.37** |
| HY 2                      | 23.00   | 33.50** | 117.00** | 89.50    | 40.62   | 25.51   | 14.07   | 3.91   | 3.14   | 1.81   |
| HY 3                      | 23.80   | 15.16   | 76.00    | 53.00    | 47.17   | 30.40   | 15.04   | 4.61   | 3.60   | 2.26   |
| HY 4                      | 27.80   | 18.80   | 129.00** | 251.00** | 70.93** | 42.73** | 25.97** | 4.34   | 3.38   | 2.11   |
| HY 5                      | 26.35   | 14.71   | 46.85    | 5.42     | 29.45   | 13.92   | 9.62    | 3.43   | 2.59   | 1.53   |
| HY 6                      | 20.00   | 23.00** | 130.00** | 185.00** | 60.23** | 46.74** | 27.24** | 3.66   | 3.00   | 1.94   |
| Mean                      | 26.3    | 14.2    | 55.8     | 57.7     | 40.9    | 22.4    | 9.7     | 4.8    | 3.6    | 2.0    |
| SD                        | 4.28    | 9.59    | 58.08    | 84.34    | 18.62   | 13.68   | 9.78    | 1.21   | 1.01   | 0.51   |
| CV (%)                    | 16.3    | 67.7    | 104.0    | 146.1    | 45.6    | 61.2    | 100.5   | 25.4   | 27.7   | 25.6   |
| LSD ( $p \leq 0.05$ )     | 2.58    | 5.77    | 34.98    | 50.79    | 11.21   | 8.24    | 5.89    | 0.73   | 0.61   | 0.31   |

PH-plant height, NPB-number of primary branches, NSB-number of secondary branches, NTB-number of tertiary branches, LPB-length of primary branches, LSB-length of secondary branches, LTB-length of tertiary branches, LP-length of petiole, LL-leaflet length, LW-leaflet width

HY 1- *A. hypogaea* L. cv. TMV 12 (4X) X *A. villosa* (4X); HY 2- *A. hypogaea* L. cv. TMV 12 (4X) X *A. duranensis* (4X); HY 3- *A. hypogaea* L. cv. K 6 X *A. villosa* (4X); HY 4- *A. hypogaea* L. cv. K 6 X *A. duranensis* (4X); HY 5- *A. hypogaea* L. cv. VRI 2 X *A. duranensis* (4X); HY 6- *A. hypogaea* L. cv. JL 24 X *A. duranensis* (4X)

Table 2b. *Per se* performance of parents and interspecific hybrid lines for various traits

| Parents /<br>Hybrid lines | Traits |        |        |        |        |        |        |        |        |        |
|---------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|                           | SL     | SJ     | SS     | HPL    | SPL    | SPW    | WPL    | WPW    | KPL    | KPW    |
| TMV 12                    | 3.15** | 0.77** | 0.67   | 3.75   | 2.35** | 1.52   | 0.76   | 0.57   | 0.65   | 0.27   |
| K 6                       | 3.65** | 0.75** | 0.65   | 3.80   | 1.32   | 1.45   | 0.91   | 0.65   | 0.61   | 0.30   |
| VRI 2                     | 2.65   | 0.57   | 0.75   | 4.37   | 1.25   | 1.47   | 0.90   | 0.65   | 0.60   | 0.27   |
| JL 24                     | 3.22** | 0.67   | 0.82** | 4.70   | 1.32   | 1.67   | 0.91   | 0.66   | 0.67   | 0.30   |
| <i>Arachis villosa</i>    | 2.13   | 0.66   | 0.80** | 5.13** | 1.73   | 1.70   | 0.91   | 0.71   | 0.66   | 0.20   |
| <i>Arachis duranensis</i> | 1.61   | 0.75** | 0.80** | 5.18** | 1.55   | 1.90** | 1.01   | 0.79** | 0.63   | 0.25   |
| HY 1                      | 3.35** | 0.78** | 0.58   | 5.42** | 1.63   | 2.06** | 1.15** | 0.80** | 0.76   | 0.50** |
| HY 2                      | 2.46   | 0.61   | 0.63   | 5.80** | 1.48   | 1.71   | 1.13** | 0.69   | 0.72   | 0.23   |
| HY 3                      | 2.52   | 0.61   | 0.66   | 4.87   | 1.60   | 1.63   | 1.23** | 0.63   | 0.76   | 0.23   |
| HY 4                      | 2.56   | 0.56   | 0.62   | 4.48   | 1.45   | 1.28   | 1.21** | 0.66   | 0.89** | 0.24   |
| HY 5                      | 2.21   | 0.62   | 0.68   | 4.35   | 1.53   | 1.54   | 1.02   | 0.71   | 0.83** | 0.21   |
| HY 6                      | 2.36   | 0.66   | 0.76** | 4.26   | 1.56   | 1.50   | 1.13** | 0.58   | 0.88** | 0.24   |
| Mean                      | 2.7    | 0.7    | 0.7    | 4.7    | 1.6    | 1.6    | 1.0    | 0.7    | 0.7    | 0.3    |
| SD                        | 0.58   | 0.08   | 0.08   | 0.63   | 0.28   | 0.21   | 0.15   | 0.07   | 0.10   | 0.08   |
| CV (%)                    | 22.0   | 11.7   | 11.5   | 13.5   | 18.2   | 13.0   | 14.4   | 10.5   | 14.2   | 29.2   |
| LSD ( $p \leq 0.05$ )     | 0.35   | 0.05   | 0.05   | 0.38   | 0.17   | 0.13   | 0.09   | 0.04   | 0.06   | 0.05   |

SL-stipule length, SJ-sepal joined, SS-sepal single, HPL-hypanthium length, SPL-standard petal length, SPW-standard petal width, WPL-wing petal length, WPW-wing petal width, KPL-keel petal length and KPW-keel petal width.

HY 1- *A. hypogaea* L. cv. TMV 12 (4X) x *A. villosa* (4X); HY 2- *A. hypogaea* L. cv. TMV 12 (4X) x *A. duranensis* (4X); HY 3- *A. hypogaea* L. cv. K 6 x *A. villosa* (4X); HY 4- *A. hypogaea* L. cv. K 6 x *A. duranensis* (4X); HY 5- *A. hypogaea* L. cv. VRI 2 x *A. duranensis* (4X); HY 6- *A. hypogaea* L. cv. JL 24 x *A. duranensis* (4X).

petal length. A high positive load for the number of branches per plant in PC1 has also been reported by Kumar *et al.* (2010). However, contrary to these results, Mubai *et al.* (2020), Habite (2023), and Eswari *et al.* (2022) reported a high positive effect of the number of primary branches in PC2. Similarly, Sukrutha *et al.* (2023) observed a high positive load for the number of

secondary branches per plant in PC4, while Rajput *et al.* (2024) noted a high positive effect of the number of branches in PC3. In the current study, PC1 highlighted the relative importance of branching and flowering traits (Table 3). The second principal component (PC2), which explained 19.447% of the total variation with an eigenvalue of 3.889, had the highest loadings for leaf traits, including leaflet length, leaflet width, and stipule length. The third principal component (PC3), with an eigenvalue of 3.315, accounted for 16.575% of the total variation and showed a high load for floral traits, such as sepal length (joined), standard petal width, wing petal width, and keel petal width. The fourth (PC4) and fifth (PC5) components accounted for 8.103% and 5.75% of the total variation, respectively. PC4 emphasized the importance of flowering traits (sepal length: joined, standard petal length), while PC5 highlighted the significance of vegetative traits (plant height and petiole length).

### Principal component analysis biplot

Figure 2 presents the principal component analysis (PCA) biplot, which includes the first two principal components (PC1 and PC2). In this biplot, variables such as wing petal width, hypanthium length, number of primary branches, and wing petal length are positioned on the positive side of PC1 (x-axis) and the negative

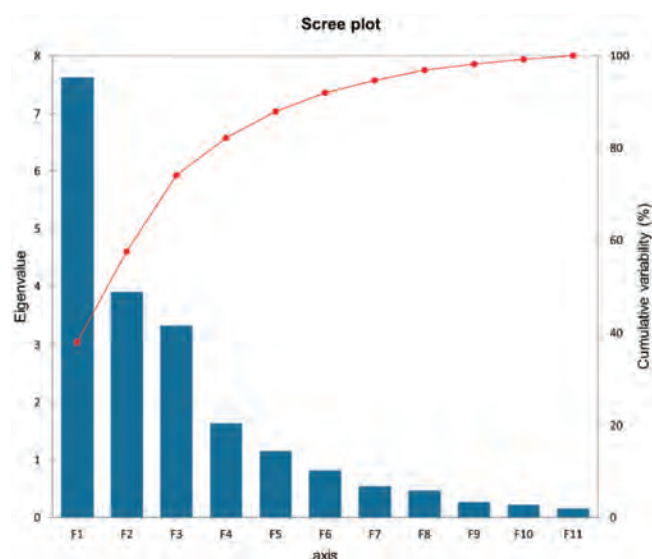
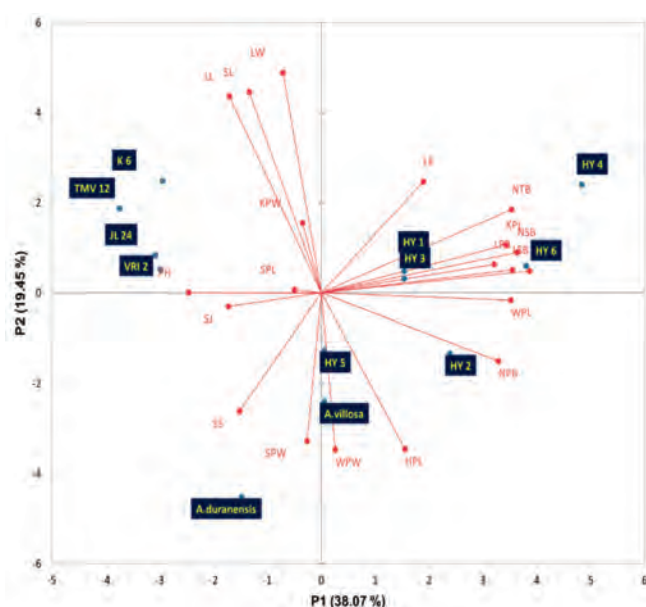


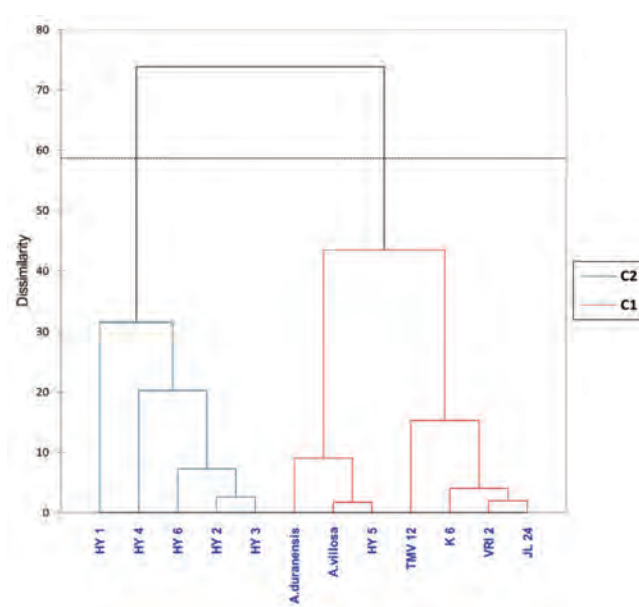
Fig. 1. Scree plot showing variances for principal components under PCA analysis

**Table 3. Component matrix showing latent vectors associated with first five principal components**

| Sr. No. | Trait                             | Principal components |        |        |        |        |
|---------|-----------------------------------|----------------------|--------|--------|--------|--------|
|         |                                   | PC1                  | PC2    | PC3    | PC4    | PC5    |
| 1       | Plant Height (cm)                 | -0.617               | 0.000  | -0.189 | 0.091  | 0.647  |
| 2       | Number of primary branches        | 0.823                | -0.268 | 0.326  | -0.016 | -0.110 |
| 3       | Number of secondary branches      | 0.910                | 0.161  | 0.319  | 0.005  | -0.036 |
| 4       | Number of tertiary branches       | 0.885                | 0.331  | -0.042 | 0.039  | 0.176  |
| 5       | Length of primary branches (cm)   | 0.803                | 0.113  | 0.005  | 0.309  | 0.034  |
| 6       | Length of secondary branches (cm) | 0.888                | 0.091  | -0.091 | 0.247  | -0.092 |
| 7       | Length of tertiary branches (cm)  | 0.968                | 0.087  | -0.165 | 0.104  | -0.085 |
| 8       | Length of petioles (cm)           | 0.474                | 0.442  | -0.267 | -0.061 | 0.675  |
| 9       | Leaflet length (cm)               | -0.427               | 0.780  | 0.369  | -0.060 | -0.070 |
| 10      | Leaflet width (cm)                | -0.177               | 0.875  | 0.276  | -0.275 | -0.122 |
| 11      | Stipule length (cm)               | -0.334               | 0.798  | 0.457  | -0.013 | -0.063 |
| 12      | Sepal joined (cm)                 | -0.432               | -0.054 | 0.570  | 0.577  | 0.037  |
| 13      | Sepal single (cm)                 | -0.381               | -0.468 | -0.453 | -0.044 | -0.111 |
| 14      | Hypanthium length (cm)            | 0.388                | -0.618 | 0.479  | -0.230 | 0.040  |
| 15      | Standard petal length (cm)        | -0.124               | 0.013  | 0.008  | 0.919  | 0.023  |
| 16      | Standard petal width (cm)         | -0.065               | -0.586 | 0.772  | 0.107  | -0.013 |
| 17      | Wing petal length (cm)            | 0.881                | -0.029 | 0.197  | -0.249 | 0.018  |
| 18      | Wing petal width (cm)             | 0.065                | -0.621 | 0.573  | -0.212 | 0.384  |
| 19      | Keel petal length (cm)            | 0.858                | 0.190  | -0.127 | 0.102  | 0.050  |
| 20      | Keel petal width (cm)             | -0.088               | 0.278  | 0.896  | 0.003  | 0.140  |
|         | Eigenvalue                        | 7.614                | 3.889  | 3.315  | 1.621  | 1.145  |
|         | Variability (%)                   | 38.072               | 19.447 | 16.575 | 8.103  | 5.725  |
|         | Cumulative (%)                    | 38.072               | 57.519 | 74.094 | 82.197 | 87.922 |



**Fig. 2. PCA biplot showing distribution of various traits of six parents and six interspecific hybrid lines across two principal components**



**Fig. 3. Dendrogram showing six parents and six interspecific hybrid lines based on the scores of first five principal components**



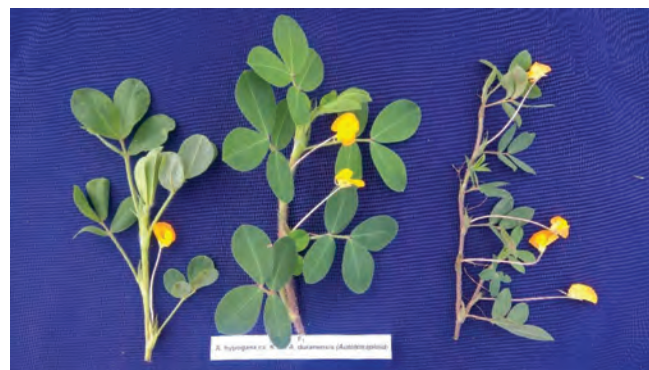
Supplementary Fig. 1. Left: *Arachis hypogaea* cv. TMV 12 (Female); Middle:  $F_1$ ; Right: Autotetraploid of *A. villosa* (Male)



Supplementary Fig. 2. Left: *Arachis hypogaea* cv. TMV 12 (Female); Middle:  $F_1$ ; Right: Autotetraploid of *A. duranensis* (Male)



Supplementary Fig. 3. Left: *Arachis hypogaea* cv. K 6 (Female); Middle:  $F_1$ ; Right: Autotetraploid of *A. villosa* (Male)



Supplementary Fig. 4. Left: *Arachis hypogaea* cv. K 6 (Female); Middle:  $F_1$ ; Right: Autotetraploid of *A. duranensis* (Male)



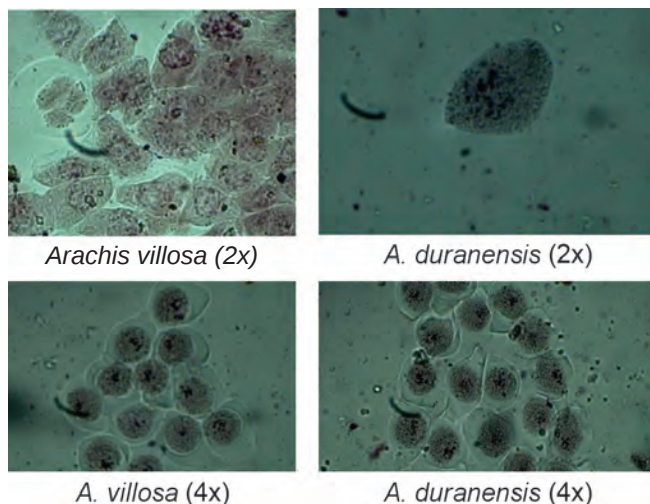
Supplementary Fig. 5. Left: *Arachis hypogaea* cv. VRI 2 (Female); Middle:  $F_1$ ; Right: Autotetraploid of *A. duranensis* (Male)



Supplementary Fig. 6. Left: *Arachis hypogaea* cv. JL 24 (Female); Middle:  $F_1$ ; Right: Autotetraploid of *A. duranensis* (Male)

side of PC2 (y-axis). On the other hand, traits such as petiole length, number of tertiary branches, keel petal length, number of secondary branches, length of primary branches, length of secondary branches, and length of tertiary branches are located on the positive side of both PC1 and PC2. Leaflet width, stipule length, leaflet length, keel petal width, and standard petal length are projected to the negative side of PC1 and the positive side of PC2. Additionally, the traits stipule joined, stipule single, and standard petal width are clustered on the

negative side of both PC1 and PC2. The x-axis of the biplot effectively distinguishes between parents and interspecific hybrid lines. The cultivars (TMV 12, K 6, VRI 2, JL 24) and the induced autotetraploid wild species *A. duranensis* are located on the left side of the biplot, while all six interspecific hybrid lines and the induced autotetraploid wild species *A. villosa* are clustered on the right side. The traits that exhibit the greatest variation along the first principal component vector include petiole length, number of tertiary branches, keel



**Supplementary Fig. 7. Meiotic analysis of diploid (2x) and tetraploid Arachis (4x)**

petal length, number of secondary branches, length of primary branches, length of secondary branches, wing petal length, number of primary branches, hypanthium length, and wing petal width. From the PCA biplot, it is evident that particular emphasis should be placed on the number of secondary branches, number of tertiary branches, and the lengths of primary, secondary, and tertiary branches for differentiating true  $F_1$  hybrid lines. Additionally, hypanthium length, wing petal length, and wing petal width can also be considered important traits for distinguishing interspecific hybrid lines.

### Cluster analysis

Ward's hierarchical cluster analysis, based on the first five principal component scores accounting for more than 73% of the total variation, resulted in the formation of two distinct clusters (Fig. 3). The first cluster comprises five interspecific hybrid lines (HY1, HY4, HY6, HY2, and HY3), which are characterized by both vegetative and flowering traits. The second cluster includes the parental lines involved in the hybridization program (TMV 12, K 6, VRI 2, JL 24), the two induced autotetraploids (*A. duranensis* and *A. villosa*), and the lone interspecific hybrid HY5. This second cluster is predominantly characterized by vegetative traits, indicating a greater degree of genetic diversity between the parents and the interspecific hybrid lines.

In conclusion, the *per se* performance of the interspecific hybrid lines for various traits indicates the involvement of parental lines with non-significant values for most of the traits observed, highlighting the contribution of hybrid vigour. Sufficient variability was observed for several morphological traits, including the number of primary branches, secondary branches, tertiary branches, and the lengths of these branches, as well as wing petal length and keel petal length,

which were all associated with the maximum total variation in the first principal component (PC1). Among the 20 traits analyzed, the number of secondary branches, number of tertiary branches, and the lengths of primary, secondary, and tertiary branches were identified as key discriminative traits for distinguishing the interspecific hybrid lines. Cluster analysis further supported these findings, grouping the parents involved in the hybridization program into one cluster, while the interspecific hybrid lines formed a distinct cluster, thus confirming the presence of significant genetic diversity between the parental lines and the hybrid lines. These findings provide valuable insights into the genetic variability and potential for future improvement in the interspecific hybridization program.

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### Conflicts of interest

The authors declare that they have no conflicts of interest.

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