



# Genetic Improvement of *Arachis hypogaea* var TMV(Gn) 13 Through Induced Mutagenesis

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## ABSTRACT

**Background:** Groundnut, a reputed cash crop govern the national economy integrating biometrical and nutritional superiority. The role of oleic and linoleic acids determining the oil quality were controlled by alleles a09 (*ahFAD2A*) and b09 (*ahFAD2B*) respectively. Groundnut exhibiting significant levels of oleic acid can supplement variety of nutritional and physiological benefits. The present study involved the investigation of major biometrical and nutritional traits on the putative mutants developed from the groundnut variety TMV(Gn)13.

**Methods:** Genetic material used for the present study involved M<sub>3</sub> populations (200Gy, 250Gy and 30 EMS, 50 EMS) developed involving a pureline from TMV (Gn) 13. The seeds of TMV(Gn)13 were exposed to gamma irradiation with an acute dose ranged between 100 and 700 Gy and EMS during Kar 2024. Based upon the GR 50 values, the progenies of 200 gy and 250 gy besides chemical EMS dose of 30 EMS and 50 EMS dosage were evaluated in M<sub>1</sub> during Rabi 2022 and selected putative mutants was forwarded to M<sub>2</sub> during Kharif 2023. Phenotypic traits including oil quality traits along with LLS were investigated.

**Result:** Biometrical traits viz., pod length, kernel width and other yield contributing traits had exhibited minimum coefficient of variation while the greater CV was registered for the oil quality traits. Putative mutants PM 8, PM 24 and PM 32 construed as superior mutants among the thirty-two putative mutants expressing greater value for most of the yield and oil quality traits. Principal component analysis construed that the first five principal components explained about 86.99 per cent of the total genetic variability. Cluster analysis grouped 32 putative mutants and three checks under three clusters indicating the maximum accommodation of putative mutants in cluster I. The putative mutants were genotyped with allele specific marker along with the primers specific to LLS and oleic acid trait implicated that Girnar 4(71.27%), PM 8(43.23%), PM 24(44.34%) and PM 32(43.47%) were found to have *ahFAD2A* mutation with high oleic acid content when compared with the control TMV (Gn) 13 (35-39%).

**Key words:** Allele specific PCR, Genetic diversity, Multivariate analysis, Putative mutants.

## INTRODUCTION

Groundnut (*Arachis hypogaea* L.), the 'King of Oilseeds' belong to the Fabaceae family often called as 'Wonder legume' for its flowering, pegging and pod production pattern. Groundnut often considered as a reputed cash crop governing significantly to the economy attributing a crop coverage of 4.8 million ha besides the yield of 9.9 million tonnes in India. Groundnut always known for its oil superiority in China and India while it has been exploited for confectionery and culinary purposes in US and Europe continents (Bithal *et al.*, 2010). The proportionate of oleic and linoleic acids determining the oil quality were controlled by alleles a09 (*ahFAD2A*) and b09 (*ahFAD2B*) respectively (Jung *et al.*, 2000). Groundnut exhibiting significant levels of oleic acid can supplement variety of nutritional and physiological benefits.

Exhaustive reports on mutagenic treatments could help the breeder to adopt appropriate mutagen for broadening the genetic variation in crops (Nurmansyah *et al.*, 2020). Though the physical radiation often reported advantageous, it is always challenging to optimize the dose for safer exposure ensuring DNA deletions delivering significant variation in crops (Oladosu *et al.*, 2016). Cluster analysis help to group the putative mutants based on their genetic

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identity while the chromosomal rearrangement and linkage disequilibrium interpreted by genetic divergence denoting the extent of genetic variation exhibited by the putative mutants among themselves and with the parent entity. Principal

Component Analysis aids in determining the extent of population variance persists among the group of progenies (Das *et al.*, 2017). Mutation of the wild alleles of *ahFAD2A* and *ahFAD2B* could reflect in alteration in oleic acid. The current study have been conducted to categorize the putative mutants on biometrical ethics besides behaving in phenotypic superiority than the base parent TMV (Gn)13.

## MATERIALS AND METHODS

### Materials and Treatments

Genetic material used for the study involved M<sub>3</sub> populations (200 gy, 250 gy and 30 EMS, 50 EMS) developed involving a pureline from Pollachi local, TMV (Gn) 13. The seeds of TMV(Gn)13 were exposed to gamma irradiation at BARC with an acute dose ranged between 100 and 700 Gy and EMS during Kar 2022. Based upon the GR 50 values,

the progenies of 200 gy and 250 gy besides chemical EMS dose of 30 EMS and 50 EMS dosage were evaluated in M<sub>1</sub> during *Rabi* 2022 and selected putative mutants was forwarded to M<sub>2</sub> during *Kharif* 2023. Based upon the higher values of O/L ratio and LLS score the desirable mutants are forwarded to M<sub>3</sub> during Summer 2024.

### Experimental plan

Putative mutants of TMV(Gn)13 along with three checks (TMV (Gn) 13, Girnar 4, GPBD 4) were raised at Experimental farm of Department of Genetics and Plant Breeding at VOC Agricultural College and Research Institute, Killikulam, Tamil Nadu during *Rabi* 2022. Selected thirty-two putative mutants along with checks were validated through molecular and biometrical assessment for isolating genetically superior putative mutant progenies during Kar 2023 (Table 1 and Fig 1). Sixteen standard biometrical

**Table 1:** List of putative mutants and checks used in this study.

| PM / Checks | Source                                                               |
|-------------|----------------------------------------------------------------------|
| TMV (Gn) 13 | ORS, Tindivanam                                                      |
| Girnar4     | DGR, Junagadh                                                        |
| GPBD 4      | UAS, Dharwad                                                         |
| PM1         | Early flowering PM from 200gy M <sub>3</sub> of TMV (Gn) 13          |
| PM2         | Small leaf PM from 200gy M <sub>3</sub> of TMV (Gn) 13               |
| PM3         | Broad leaf PM from 200gy M <sub>3</sub> of TMV (Gn) 13               |
| PM4         | Dwarf PM from 200gy M <sub>3</sub> of TMV (Gn) 13                    |
| PM5         | Late flowering PM from 200gy M <sub>3</sub> of TMV (Gn) 13           |
| PM6         | Spreading type PM from 200gy M <sub>3</sub> of TMV (Gn) 13           |
| PM7         | LLS resistant PM from 200gy M <sub>3</sub> of TMV (Gn) 13            |
| PM8         | More Secondary Branche PM from 200gy M <sub>3</sub> of TMV (Gn) 13   |
| PM9         | Early flowering PM from 250gy M <sub>3</sub> of TMV (Gn) 13          |
| PM10        | Small leaf PM from 250gy M <sub>3</sub> of TMV (Gn) 13               |
| PM11        | Broad leaf PM from 250gy M <sub>3</sub> of TMV (Gn) 13               |
| PM12        | Dwarf PM from 250gy M <sub>3</sub> of TMV (Gn) 13                    |
| PM13        | Late flowering PM from 250gy M <sub>3</sub> of TMV (Gn) 13           |
| PM14        | Spreading type PM from 250gy M <sub>3</sub> of TMV (Gn) 13           |
| PM15        | LLS resistant PM from 250gy M <sub>3</sub> of TMV (Gn) 13            |
| PM16        | More secondary branch PM from 250 gy M <sub>3</sub> of TMV (Gn) 13   |
| PM17        | Early flowering PM from 30 EMS M <sub>3</sub> of TMV (Gn) 13         |
| PM18        | Small leaf PM from 30 EMS M <sub>3</sub> of TMV (Gn) 13              |
| PM19        | Broad leaf PM from 30 EMS M <sub>3</sub> of TMV (Gn) 13              |
| PM20        | Dwarf PM from 30 EMS M <sub>3</sub> of TMV (Gn) 13                   |
| PM21        | Late flowering PM from 30 EMS M <sub>3</sub> of TMV (Gn) 13          |
| PM22        | Spreading type PM from 30 EMS M <sub>3</sub> of TMV (Gn) 13          |
| PM23        | LLS resistant PM from 30 EMS M <sub>3</sub> of TMV (Gn) 13           |
| PM24        | More secondary branches PM from 30 EMS M <sub>3</sub> of TMV (Gn) 13 |
| PM25        | Early flowering PM from 50 EMS M <sub>3</sub> of TMV (Gn) 13         |
| PM26        | Small leaf PM from 50 EMS M <sub>3</sub> of TMV (Gn) 13              |
| PM27        | Broad leaf PM from 50 EMS M <sub>3</sub> of TMV (Gn) 13              |
| PM28        | Dwarf PM from 50 EMS M <sub>3</sub> of TMV (Gn) 13                   |
| PM29        | Late flowering PM from 50 EMS M <sub>3</sub> of TMV (Gn) 13          |
| PM30        | Spreading type PM from 50 EMS M <sub>3</sub> of TMV (Gn) 13          |
| PM31        | LLS resistant PM from 50 EMS M <sub>3</sub> of TMV (Gn) 13           |
| PM32        | More Secondary branch PM from 50 EMS M <sub>3</sub> of TMV (Gn) 13   |

traits including LLS score and Oleic content were measured. Late leaf spot (LLS) score done on 1 to 9 scale, 90 days after sowing (Subbarao *et al.*, 1990). These biometrical data obtained were analyzed for cluster analysis with STAR (ver 2.0.1) (IRRI, 2014) for descriptive statistics and principal component analysis (PCA).

#### Molecular interpretation of putative mutants

Molecular diversity of putative mutants along with checks were done with allele-specific markers suggested by Chen *et al.* (2010) associated with LLS and oleic acid traits (Table 2). Fresh leaf samples from 10 to 15 days old plants were collected for DNA extraction by modified CetylTrimethyl Ammonium Bromide (CTAB) method (Mace *et al.*, 2003). The quality of DNA was checked on 0.8 percent Agarose gel and its concentration was estimated on ND100 Spectrophotometer, adjusting the working concentration to 20 ng per litre. The amplified DNA fragments along with 100 bp DNA marker were separated on a 3 per cent horizontal Agarose gel. Gel electrophoresis was carried out in 1X TBE buffer at 100 V current for 1 to 2 hours. Ethidium bromide was used for staining the DNA fragments and the gel was documented using Bio Rad gel documentation unit. Molecular clustering was done with DARwin (ver. 6.0.21). Oil content and oleic-linoleic

composition were estimated with Near-Infrared Spectrometer (NIR) (Make: M/s ZEUTECH, Germany; Model: SPA1.0).

## RESULTS AND DISCUSSION

#### Studies on genetic variability for biometrical traits

Higher coefficient of variation (CV) was observed for the number of secondary branches (34.81%) as opined by Ajith *et al.* (2023) while the moderate CV were observed for days to fifty per cent flowering, pod width, 100-kernel weight and oleic acid as reported by Patidar and Nadaf (2017) in the backcross derived high oleic advanced breeding lines.

#### Mean performance for pod and kernel traits

Among the putative mutants validated for various biometric traits, PM 24 and PM 32 construed as the superior progenies exhibiting significantly higher mean values for major yield related traits viz., number of pods per plant, 100 kernel weight and shelling percent besides oil quality. PM 22 and PM 23 had excelled with maximum kernel length while PM 23 and PM 24 had shown the superiority for higher kernel breadth. Further, high oleic acid content was registered by PM 8 (48 per cent), PM 24 (50 per cent) and PM 32 (49 per cent) (Table 3).

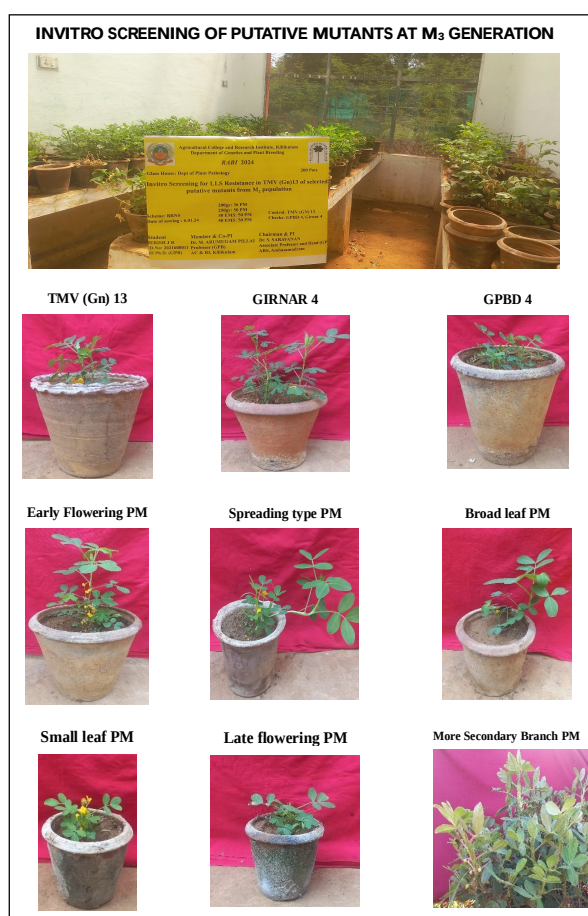


Fig 1: Phenotype of PMs and Checks.

**Table 2:** Polymorphic primers used for molecular diversity in this study.

| Name of the primer/Gene | Sequence                                       | Trait      | Annealing temp. | Reference                     |
|-------------------------|------------------------------------------------|------------|-----------------|-------------------------------|
| ah FAD2A                | GATTACTGATTATTGACTT<br>CCAACCCAAACCTTTTCAGAG   | Oleic acid | 48°C            | Chu <i>et al.</i> (2011)      |
| ah FAD2B                | CAGAACCATTAGCTTTG<br>CTCTGACTATGCATCAG         | Oleic acid | 48°C            | Chu <i>et al.</i> (2011)      |
| GM1009                  | TTTCCTTCTTTCCCTTCTTCTTC<br>CGTTGTTGCCGTTAACTGA | LLS        | 57°C            | Prabhu <i>et al.</i> , (2016) |
| GM1536                  | AAAGCCCTGAAAAGAAAGCAG<br>CAACCAGCTCCTTCTCTACCC | LLS        | 59°C            | Prabhu <i>et al.</i> , (2016) |
| GM1573                  | GAGACCGGAGACGGAGAGTAT<br>ACGCCCATAGATTAACCCAGT | LLS        | 60°C            | Prabhu <i>et al.</i> , (2016) |
| GM2009                  | CAAACGCATACACCCCATAAC<br>TTTGGTTCTCGTTTGTGTTTT | LLS        | 55°C            | Prabhu <i>et al.</i> , (2016) |
| GM2079                  | GGCCAAGGAGAAGAAGAAAGA<br>GAAGGAGTAGTGGTGCTGCTG | LLS        | 60°C            | Prabhu <i>et al.</i> , (2016) |
| GM2301                  | GTAACCACAGCTGGCATGAAC<br>TCTTCAAGAACCCACCAACAC | LLS        | 59°C            | Prabhu <i>et al.</i> , (2016) |
| IPAHM103                | GCATTCACCACCATAGTCCA<br>TCCTCTGACTTTCTCCATCA   | LLS        | 58°C            | Prabhu <i>et al.</i> , (2016) |
| seq8D09                 | TGAGTTTCCCCAAAAGGAGA<br>CAACAACAATACGGCCAACA   | LLS        | 55°C            | Prabhu <i>et al.</i> (2016)   |
| F435                    | ATCCAAGGCTGCATTCTCAC                           | Oleic acid | 55°C            | Chen <i>et al.</i> (2010)     |
| F435SUB                 | TGGGACAAACACTTCGTT                             |            |                 |                               |
| F435                    | ATCCAAGGCTGCATTCTCAC                           | Oleic acid | 55°C            | Chen <i>et al.</i> (2010)     |
| F435INS-R               | AACACTTCGTCGCGGTCT                             |            |                 |                               |

**Table 3:** Descriptive statistics of putative mutants and checks for pod and kernel traits.

| Trait                        | Mean  | SD   | CV (%) | Minimum        | Maximum                                                 |
|------------------------------|-------|------|--------|----------------|---------------------------------------------------------|
| Plant height (cm)            | 30.00 | 5.34 | 17.80  | 20 (TMV 13)    | 41 (PM 11)                                              |
| Days to 50% flowering        | 24.8  | 4.11 | 16.60  | 17 (TMV 13)    | 35 (PM 29)                                              |
| Number of primary branches   | 7.20  | 1.39 | 19.30  | 4 (TMV 13)     | 12 (PM 24)                                              |
| Number of becondary branches | 4.05  | 1.41 | 34.81  | 2 (TMV 13)     | 7 (PM 32)                                               |
| No of pods per plant         | 30.65 | 9.56 | 31.19  | 11 (TMV 13)    | 38 (PM 24),<br>40 (PM 32)                               |
| Pod length (cm)              | 2.25  | 0.17 | 7.5    | 1.90 (TMV 13)  | 2.1 (PM 22),<br>2.3 (PM 23)                             |
| Pod width (cm)               | 0.87  | 0.09 | 10.92  | 0.76 (TMV 13)  | 0.80 (PM 23),<br>0.82 (PM 24),<br>0.79 (PM 11)          |
| 100 kernal weight (g)        | 36.91 | 3.96 | 10.73  | 40.00(TMV 13)  | 42.23 (PM 24)                                           |
| Kernal length (cm)           | 1.04  | 0.06 | 5.96   | 1.03 (TMV 13)  | 1.06 (PM 22),<br>1.04 (PM 23)                           |
| Kernal width (cm)            | 0.51  | 0.03 | 6.67   | 0.39 (TMV 13)  | 0.41 (PM 23),<br>0.40 (PM 24)                           |
| Shelling percentage (%)      | 71.09 | 1.12 | 1.57   | 73.40 (TMV 13) | 75.6 (PM 24),<br>74.8 (PM 32)                           |
| LLS scoring                  | 4.94  | 1.25 | 25.29  | 2 (PM24)       | 7 (TMV 13)                                              |
| Oil content (%)              | 51.64 | 1.09 | 2.28   | 46.17 (TMV 13) | 53.8 (PM 32)                                            |
| Oleic acid content (%)       | 51.50 | 5.60 | 13.49  | 37.43 (TMV 13) | 48 (PM 8)<br>50 (PM 24),<br>49 (PM 32)<br>54 (Girnar 4) |
| Linoleic acid content (%)    | 33.72 | 5.31 | 15.29  | 37.89 (PM 27)  | 58.37 (TMV 13)                                          |
| Pod yield per plant (g)      | 17.11 | 4.50 | 26.30  | 32 (TMV 13)    | 40 (PM 32)<br>39 (PM 24)<br>35 (GPBD 4)                 |

### Principal component analysis

The principal component with an eigenvalue more than 1 i.e., PC1 (5.23), PC2 (4.09), PC3 (2.22), PC4 (1.32) and PC5 (1.03) had contributed maximum to the genetic variability (Fig 2). The components with eigenvalue less than 1 i.e., PC6 to PC10 are less informative and accounted for lesser variance and hence not retained. The results from the PCA indicated that 86.99 per cent of the total genetic variance was contributed by the first five principal components. The first principal component (PC1) with an eigenvalue of 5.23 explained 32.71 per cent of the total variability with higher positive weight to linoleic acid (0.2996) and shelling percentage (0.1788). The second principal component (PC2) with an eigenvalue of 4.09 contributed 25.60 per cent of the total variability. PC2 is positively correlated with number of primary branches (0.3819), number of secondary branches (0.3810), number of pods per plant (0.3211), oleic acid content (0.2085) and pod length (0.0539). The third principal component (PC3) with an eigenvalue of 2.22 is responsible for 13.94 per cent of the total variability. Pod length (0.2378), number of secondary branches (0.2054), days to fifty percent flowering (0.1646) and number of primary branches (0.1418) contributed positively towards PC3. The fourth principal component with an eigenvalue of 1.32 contributed 8.29 per cent of the total genetic variability. Linoleic acid content, plant height, days to fifty percent flowering, number of secondary branches and 100 kernel weight are positively contributed to PC4. Pod width and oleic acid content contributed the maximum for the variability in PC1. In PC2, traits such as number of primary branches and number of secondary branches contributed to most of the variability (Table 4). Studies by Sukrutha *et al.* (2023) had shown that

first five PCs contributed 73.24 per cent genetic variation with eigenvalue more than 1. The present study substantiated that PC1 accounted for a maximum of 37.29 per cent of the total variation than PC2 (20.49 per cent). Similar results were obtained by Khan *et al.* (2021); Rohit Sharma *et al.* (2023) and Mythili *et al.* (2023). When the two vectors are close, forming a small angle, the variables are positively correlated. As such, pod width, pod length, oil content, number of pods per plants and 100 kernel weight are positively correlated similar to that of correlation analysis (Table 5). When the angle between the vectors is at 90°, linoleic acid is not correlated with pod width, pod length, kernel width, kernel length and 100 kernel weight (Fig 3). When the vectors are greatly diverged at a larger angle, the variables are negatively correlated as revealed

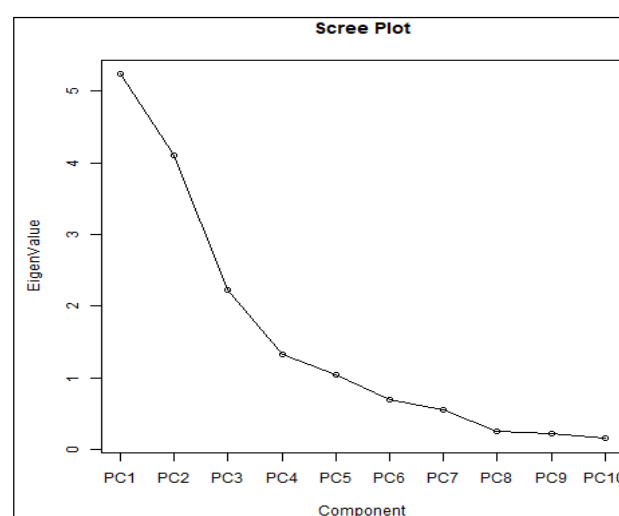


Fig 2: Scree plot showing Eigen value variation.

Table 4: Principal component analysis (PCA) of five components.

|                              | PC1                 | PC2     | PC3     | PC4     | PC5     |
|------------------------------|---------------------|---------|---------|---------|---------|
| Eigen value                  | 5.23                | 4.09    | 2.22    | 1.32    | 1.03    |
| Variability %                | 32.71               | 25.60   | 13.94   | 8.29    | 6.46    |
| Cumulative %                 | 32.71               | 58.31   | 72.24   | 80.53   | 86.99   |
| <b>Variable</b>              | <b>Eigen vector</b> |         |         |         |         |
| Plant height (cm)            | -0.1556             | 0.2678  | -0.4013 | 0.2869  | -0.0641 |
| Days to 50% flowering        | -0.0336             | -0.1867 | 0.1646  | 0.6546  | -0.3620 |
| Number of primary branches   | -0.0986             | 0.3819  | 0.1418  | -0.1632 | -0.4140 |
| Number of secondary branches | -0.1314             | 0.3810  | 0.2054  | 0.0284  | -0.3971 |
| Number of pods per plant     | -0.2304             | 0.3211  | -0.2818 | 0.1204  | 0.2947  |
| Pod length (cm)              | -0.3259             | 0.0539  | 0.2378  | -0.0753 | -0.2239 |
| Pod width (cm)               | -0.3906             | -0.0405 | 0.1012  | -0.1212 | -0.0126 |
| 100 Kernel weight (g)        | -0.1077             | -0.3903 | -0.0444 | 0.3800  | 0.0304  |
| Kernal length (cm)           | -0.3773             | -0.2287 | -0.0499 | -0.0136 | -0.0235 |
| Kernal width (cm)            | -0.3079             | -0.2380 | -0.1578 | -0.3110 | -0.1370 |
| Shelling percentage (%)      | 0.1788              | -0.1047 | -0.3968 | -0.3523 | -0.2376 |
| LLS Scoring                  | 0.0011              | -0.0271 | -0.5616 | 0.0773  | -0.4417 |
| Oil content (%)              | -0.2560             | 0.2085  | 0.1710  | 0.0157  | 0.1641  |
| Oleic acid content (%)       | -0.3808             | -0.1853 | -0.0495 | -0.0125 | -0.0137 |
| Linoleic acid content (%)    | 0.2996              | 0.2311  | -0.0042 | 0.1687  | -0.0707 |
| Pod yield per plant (g)      | -0.2341             | 0.3010  | -0.2637 | 0.1634  | 0.3077  |

**Table5:** Correlation among yield and oil quality traits.

|     | PH     | DFF    | NSB     | NPB     | NP     | PL     | PW      | HKW    | KL      | KW      | SH     | OL     | OA      | LA    | LLS  | PY   |
|-----|--------|--------|---------|---------|--------|--------|---------|--------|---------|---------|--------|--------|---------|-------|------|------|
| PH  | 1.00   |        |         |         |        |        |         |        |         |         |        |        |         |       |      |      |
| DFF | -0.05  | 1.00   |         |         |        |        |         |        |         |         |        |        |         |       |      |      |
| NSB | 0.31   | -0.23  | 1.00    |         |        |        |         |        |         |         |        |        |         |       |      |      |
| NPB | 0.37*  | -0.05  | 0.87**  | 1.00    |        |        |         |        |         |         |        |        |         |       |      |      |
| NP  | 0.80** | -0.31  | 0.38*   | 0.40*   | 1.00   |        |         |        |         |         |        |        |         |       |      |      |
| PL  | 0.11   | 0.12   | 0.37*   | 0.47**  | 0.26   | 1.00   |         |        |         |         |        |        |         |       |      |      |
| PW  | 0.17   | -0.01  | 0.18    | 0.25    | 0.34*  | 0.79** | 1.00    |        |         |         |        |        |         |       |      |      |
| HKW | -0.16  | 0.56** | -0.61** | -0.56** | -0.28  | 0.07   | 0.26    | 1.00   |         |         |        |        |         |       |      |      |
| KL  | 0.08   | 0.18   | -0.14   | -0.10   | 0.18   | 0.56** | 0.83**  | 0.60** | 1.00    |         |        |        |         |       |      |      |
| KW  | 0.04   | 0.00   | -0.18   | -0.20   | 0.06   | 0.44** | 0.66**  | 0.34*  | 0.85**  | 1.00    |        |        |         |       |      |      |
| SH  | 0.01   | -0.24  | -0.21   | -0.46** | -0.22  | -0.36* | -0.37*  | -0.05  | -0.25   | 0.13    | 1.00   |        |         |       |      |      |
| OL  | 0.28   | 0.03   | 0.48**  | 0.45**  | 0.51** | 0.41*  | 0.38*   | -0.23  | 0.24    | 0.17    | -0.38* | 1.00   |         |       |      |      |
| OA  | 0.15   | 0.21   | -0.08   | -0.05   | 0.22   | 0.51** | 0.76**  | 0.48** | 0.92**  | 0.82**  | -0.20  | 0.42*  | 1.00    |       |      |      |
| LA  | 0.11   | -0.07  | 0.17    | 0.13    | -0.03  | -0.35* | -0.55** | -0.42* | -0.78** | -0.74** | 0.20   | -0.27  | -0.77** | 1.00  |      |      |
| LLS | 0.46** | 0.02   | -0.03   | -0.08   | 0.19   | -0.28  | -0.17   | 0.11   | 0.12    | 0.24    | 0.48** | -0.34* | 0.07    | -0.05 | 1.00 |      |
| PY  | 0.44** | -0.26  | 0.33    | 0.39*   | 0.48** | 0.30   | 0.34*   | 0.22   | 0.20    | 0.25    | -0.27  | 0.46** | 0.24    | -0.06 | 0.19 | 1.00 |

Significance levels: p &lt; .01 \*\*\*, p &lt; .05 \*\*.

PH: Plant height, DFF: Days to 50 % flowering, NSB: Number of secondary branches, NP: Number of pods, PL: Pod length, PW: Pod width, HKW: Hundred kernel weight, KL: Kernel length, KW: Kernel width, SH: Shelling Percentage, OL: Oil content, OA: Oleic Acid content, LO: linoleic acid content, LLS: Late leaf spot score and PY: Pod yield.

by linoleic acid been negatively correlated with oleic acid content (Mythili *et al.*, 2023).

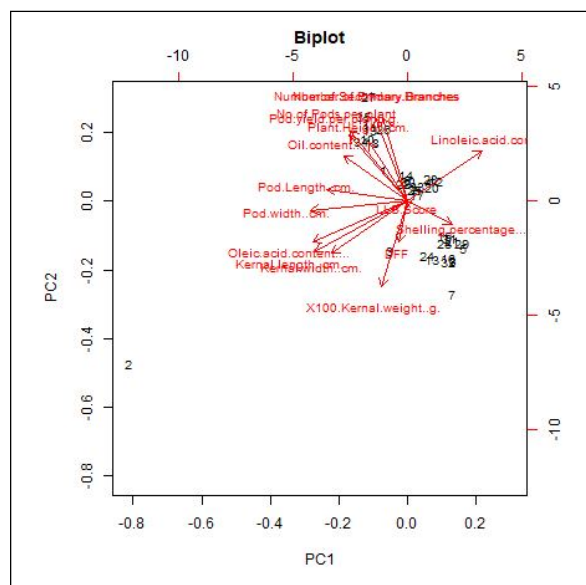
### Cluster analysis

Thirty two putative mutants along with the check entries have been accommodated in three clusters with cluster I had the greater occupancy of 19 cultures (TMV(Gn)13, PM1, PM3, PM6, PM7, PM8, PM9, PM11, PM14, PM15, PM16, PM17, PM19, PM22, PM23, PM24, PM27, PM30 and PM31) while the cluster III had 13 cultures (PM2, PM4, PM5, PM10, PM12, PM13, PM18, PM20, PM21, PM26, PM28, PM29 and GPBD 4) besides the cluster II had three entries (PM32, PM 25 and Girnar 4) (Table 6). PM 25 located at the maximum genetic distance (13.9772) whereas PM 8 and PM 16 are located at a minimum genetic distance (0.3826). The results were in agreement with the reports of Khan *et al.*, (2021).

### Molecular assessment of putative mutants for genetic divergence

Molecular investigation of groundnut putative mutants for high oleic acid content was interpreted based on the accurate allele-specific PCR assay to identify the mutant allele in ahFAD2A (substitution) and ahFAD2B (insertion) genes as suggested by Chen *et al.* (2010), Madhurjit Singh Rathore *et al.* (2023) and Hasan Talha Gulien *et al.*, (2023). The target band appears at 230bp for substitution (Fig 4). Girnar 4, PM8, PM9 and PM23 have ahFAD2A (substitution) mutation. Molecular diversity was done with eight polymorphic primers specific to Late Leaf Spot and two for oleic acid along with AS-PCR. It was inferred that most of the markers exhibited PIC value greater than 0.5 and ranged

from GM2009 (0.51) to F435SUB (0.94). A total of 27 alleles were observed and allele size ranged from 110 bp in GM2009 to ah FAD2B in 1230bp (Table 7). The genetic relationship between 32 putative mutants and three checks for LLS resistance was shown in the SSR-based UPGMA tree constructed by the hierarchical clustering method. A total of six clusters were formed and desired higher oleic acid superior lines had accommodated in I (PM24), III (PM 8) and IV (PM 32) clusters (Fig 5).



**Fig 3:** Distribution of PMs and checks under two major principal component axis.

**Table 6:** Clustering of the PMs, Checks and the size of each cluster.

| Cluster | Size | % of PM/Checks | PM/Checks                                                                                                       |
|---------|------|----------------|-----------------------------------------------------------------------------------------------------------------|
| I       | 19   | 54.29          | TMV(Gn)13, PM1, PM3, PM6, PM7, PM8, PM9, PM11, PM14, PM15, PM16, PM17, PM19, PM22, PM23, PM24, PM27, PM30, PM31 |
| II      | 3    | 9.09           | Girnar 4, PM32, PM 25                                                                                           |
| III     | 13   | 36.36          | PM2, PM4, PM5, PM10, PM12, PM13, PM18, PM20, PM21, PM26, PM28, PM29 and GPBD4                                   |

**Table 7:** Polymorphic information content of SSR Markers.

| Primer   | Allele size | No. of alleles | PIC  |
|----------|-------------|----------------|------|
| ah FAD2A | 790-830     | 3              | 0.89 |
| ah FAD2B | 1190-1230   | 3              | 0.93 |
| GM1009   | 290-310     | 2              | 0.51 |
| GM1536   | 390-410     | 3              | 0.92 |
| GM1573   | 270-290     | 3              | 0.82 |
| GM2009   | 110-130     | 2              | 0.51 |
| GM2079   | 390-420     | 3              | 0.71 |
| GM2301   | 230-250     | 2              | 0.54 |
| IPAHM103 | 150-170     | 2              | 0.53 |
| seq8D09  | 100-130     | 3              | 0.73 |
| F435SUB  | 203         | 1              | 0.94 |



Fig 4: Molecular profile of SSR markers.

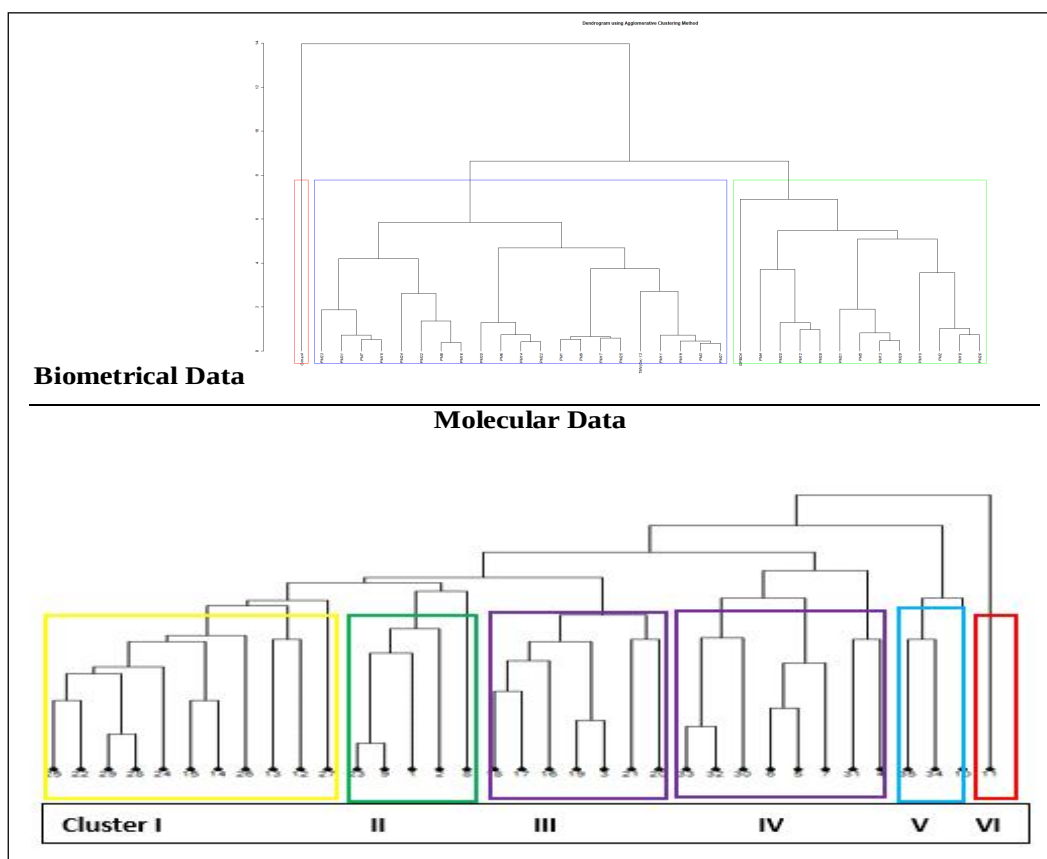


Fig 5: Dendrogram based on cluster analysis for the biometrical traits and molecular data of the PMS and checks.

## CONCLUSION

The foregoing discussion had depicted that the minimum CV was recorded for pod length, kernel width and other yield contributing traits but any greater CV was registered for oil quality traits. Further, the PM 24 and PM 32 construed as superior mutants among the thirty two putative mutants expressing greater value for most of the yield and oil quality traits. In PCA analysis, the first five principal components explained about 86.99% of the total variability. Cluster analysis grouped 32 putative mutants and three checks into three clusters indicating the maximum accommodation of putative mutants in cluster I. The putative mutants were genotyped with allele specific marker along with the primers specific to LLS and oleic acid trait. The molecular analysis revealed that genotypes Girnar 4 (71.27%), PM 8(43.23%), PM 24(44.34%) and PM32(43.47%) were found to have ahFAD2A mutation with high oleic acid content when compared with the control TMV (Gn) 13 (35-39%).

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## Conflict of interest

All authors declare that they have no conflict of interest.

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