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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_3 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_1 during *Rabi* 2022 and selected putative mutants was forwarded to M_2 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 (Birthal *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 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

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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_3 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 M1 during *Rabi* 2022 and selected putative mutants was forwarded to M_2 during *Kharif* 2023. Based upon the higher values of O/L ratio and LLS score the desirable mutants are forwarded to M_3 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_3 of TMV (Gn) 13
PM2	Small leaf PM from 200gy M_3 of TMV (Gn) 13
PM3	Broad leaf PM from 200gy M_3 of TMV (Gn) 13
PM4	Dwarf PM from 200gy M_3 of TMV (Gn) 13
PM5	Late flowering PM from 200gy M_3 of TMV (Gn) 13
PM6	Spreading type PM from 200gy M_3 of TMV (Gn) 13
PM7	LLS resistant PM from 200gy M_3 of TMV (Gn) 13
PM8	More Secondary Branche PM from 200gy M_3 of TMV (Gn) 13
PM9	Early flowering PM from 250gy M_3 of TMV (Gn) 13
PM10	Small leaf PM from 250gy M_3 of TMV (Gn) 13
PM11	Broad leaf PM from 250gy M_3 of TMV (Gn) 13
PM12	Dwarf PM from 250gy M_3 of TMV (Gn) 13
PM13	Late flowering PM from 250gy M_3 of TMV (Gn) 13
PM14	Spreading type PM from 250gy M_3 of TMV (Gn) 13
PM15	LLS resistant PM from 250gy M_3 of TMV (Gn) 13
PM16	More secondary branch PM from 250 gy M_3 of TMV (Gn) 13
PM17	Early flowering PM from 30 EMS M_3 of TMV (Gn) 13
PM18	Small leaf PM from 30 EMS M_3 of TMV (Gn) 13
PM19	Broad leaf PM from 30 EMS M_3 of TMV (Gn) 13
PM20	Dwarf PM from 30 EMS M_3 of TMV (Gn) 13
PM21	Late flowering PM from 30 EMS M_3 of TMV (Gn) 13
PM22	Spreading type PM from 30 EMS M_3 of TMV (Gn) 13
PM23	LLS resistant PM from 30 EMS M_3 of TMV (Gn) 13
PM24	More secondary branches PM from 30 EMS M_3 of TMV (Gn) 13
PM25	Early flowering PM from 50 EMS M_3 of TMV (Gn) 13
PM26	Small leaf PM from 50 EMS M_3 of TMV (Gn) 13
PM27	Broad leaf PM from 50 EMS M_3 of TMV (Gn) 13
PM28	Dwarf PM from 50 EMS M_3 of TMV (Gn) 13
PM29	Late flowering PM from 50 EMS M_3 of TMV (Gn) 13
PM30	Spreading type PM from 50 EMS M_3 of TMV (Gn) 13
PM31	LLS resistant PM from 50 EMS M_3 of TMV (Gn) 13
PM32	More Secondary branch PM from 50 EMS M_3 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 ofputative mutants along with checks weredone 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 per cent 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 ZEUTEC, Germany; Model: SPA 1.0).

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 kernal 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 CCAACCCAAACCTTTCAGAG	Oleic acid	48°C	Chu *et al*. (2011)
ah FAD2B	CAGAACCATTAGCTTTG CTCTGACTATGCATCAG	Oleic acid	48°C	Chu *et al*. (2011)
GM1009	TTTCCTTCTTTCCCTTCTTCTTC CGTTGTTGCCGTTAAACTGA	LLS	57°C	Prabhu *et al*. (2016)
GM1536	AAAGCCCTGAAAAGAAAGCAG CAACCAGCTCCTTCTCTACCC	LLS	59°C	Prabhu *et al*. (2016)
GM1573	GAGACCGGAGACGGAGAGTAT ACGCCCATAGATTAACCCAGT	LLS	60°C	Prabhu *et al*. (2016)
GM2009	CAAACGCATACACCCCATAAC TTTGGTTCTCGTTTGTGTTTT	LLS	55°C	Prabhu *et al*. (2016)
GM2079	GGCCAAGGAGAAGAAGAAAGA GAAGGAGTAGTGGTGCTGCTG	LLS	60°C	Prabhu *et al*. (2016)
GM2301	GTAACCACAGCTGGCATGAAC TCTTCAAGAACCCACCAACAC	LLS	59°C	Prabhu *et al*. (2016)
IPAHM103	GCATTCACCACCATAGTCCA TCCTCTGACTTTCCTCCATCA	LLS	58°C	Prabhu *et al*. (2016)
seq8D09	TGAGTTTCCCCAAAAGGAGA CAACAACAATACGGCCAACA	LLS	55°C	Prabhu *et al*. (2016)
F435 F435SUB	ATCCAAGGCTGCATTCTCAC TGGGACAAACACTTCGTT	Oleic acid	55°C	Chen *et al*. (2010)
F435 F435INS-R	ATCCAAGGCTGCATTCTCAC AACACTTCGTCGCGGTCT	Oleic acid	55°C	Chen *et al*. (2010)

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), 40 (PM 32)
Pod length (cm)	2.25	0.17	7.5	1.90 (TMV 13)	2.1 (PM 22), 2.3 (PM 23)
Pod width (cm)	0.87	0.09	10.92	0.76 (TMV 13)	0.80 (PM 23), 0.82 (PM 24), 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), 1.04 (PM 23)
Kernal width (cm)	0.51	0.03	6.67	0.39 (TMV 13)	0.41 (PM 23), 0.40 (PM 24)
Shelling percentage (%)	71.09	1.12	1.57	73.40 (TMV 13)	75.6 (PM 24), 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) 50 (PM 24), 49 (PM 32) 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) 39 (PM 24) 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 kernal 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

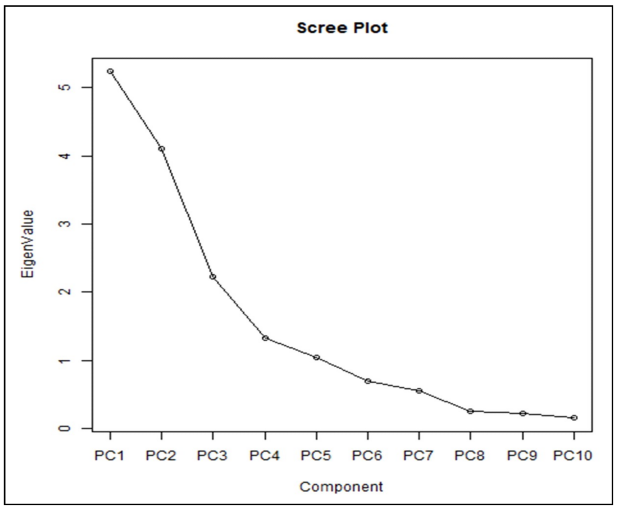


Fig 2: Scree plot showing Eigen value variation.

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
Variable			**Eigen vector**		
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 Kernal 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: $p < .01$ '**', $p < .05$ '*'.

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: Kernal length, KW: Kernal 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 *et al*. (2023) and Hasan *et al*. (2023). The target band appears at 230 bp 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 1230 bp (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).

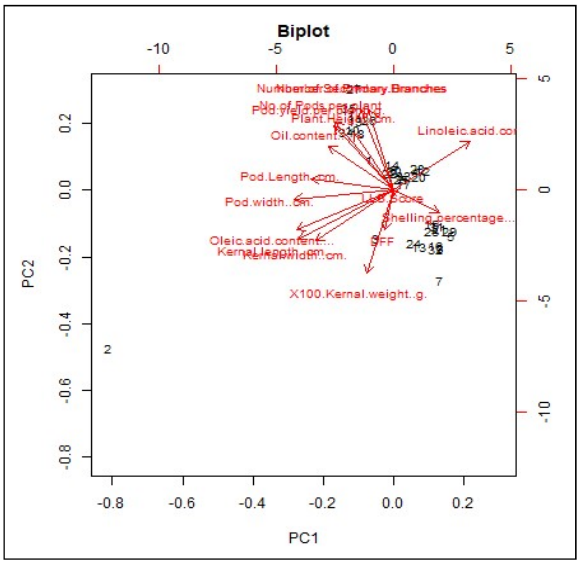


Fig 3: Distribution of PMs and checks under two major principal component axis.

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

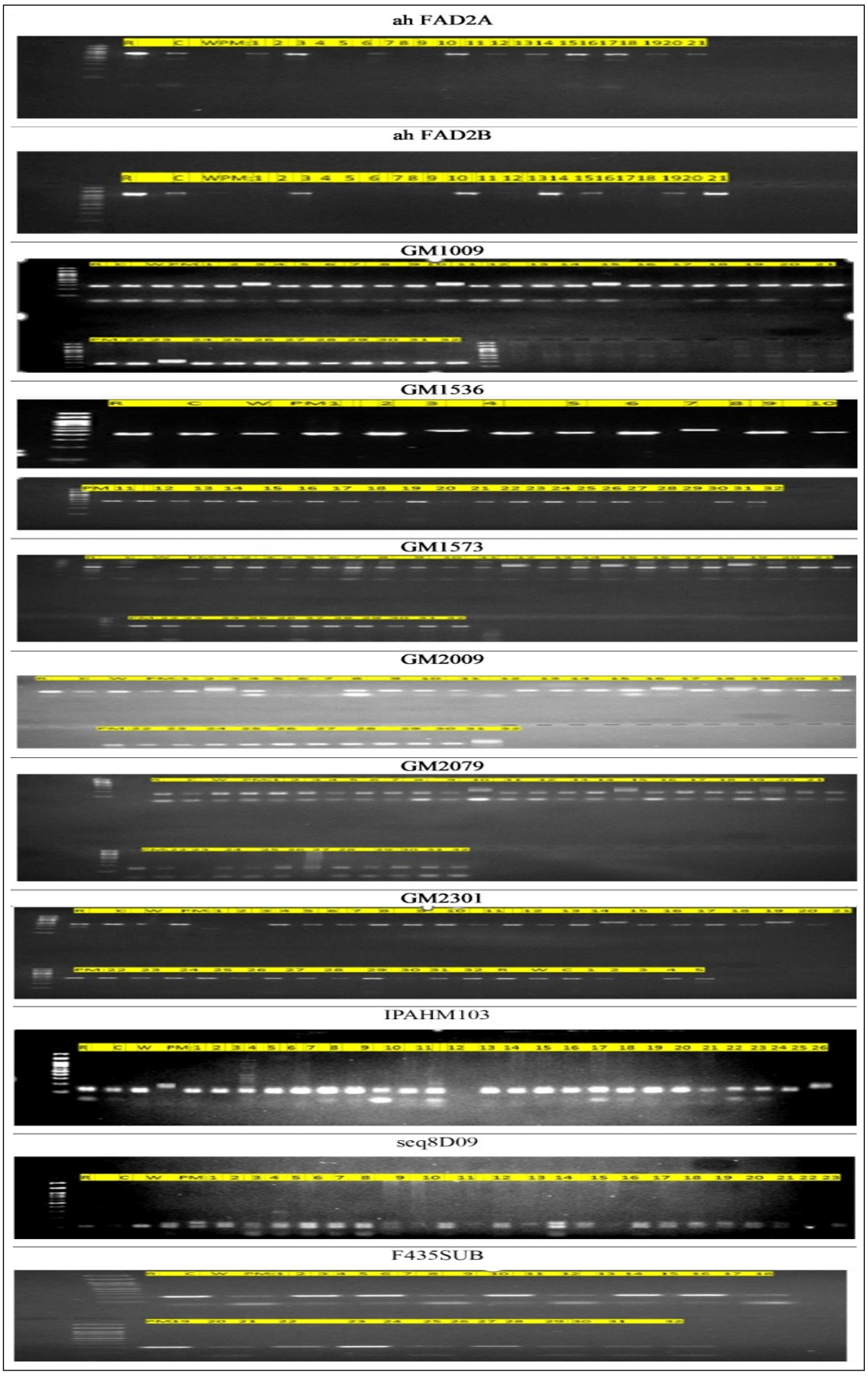


Fig 4: Molecular profile of SSR markers.

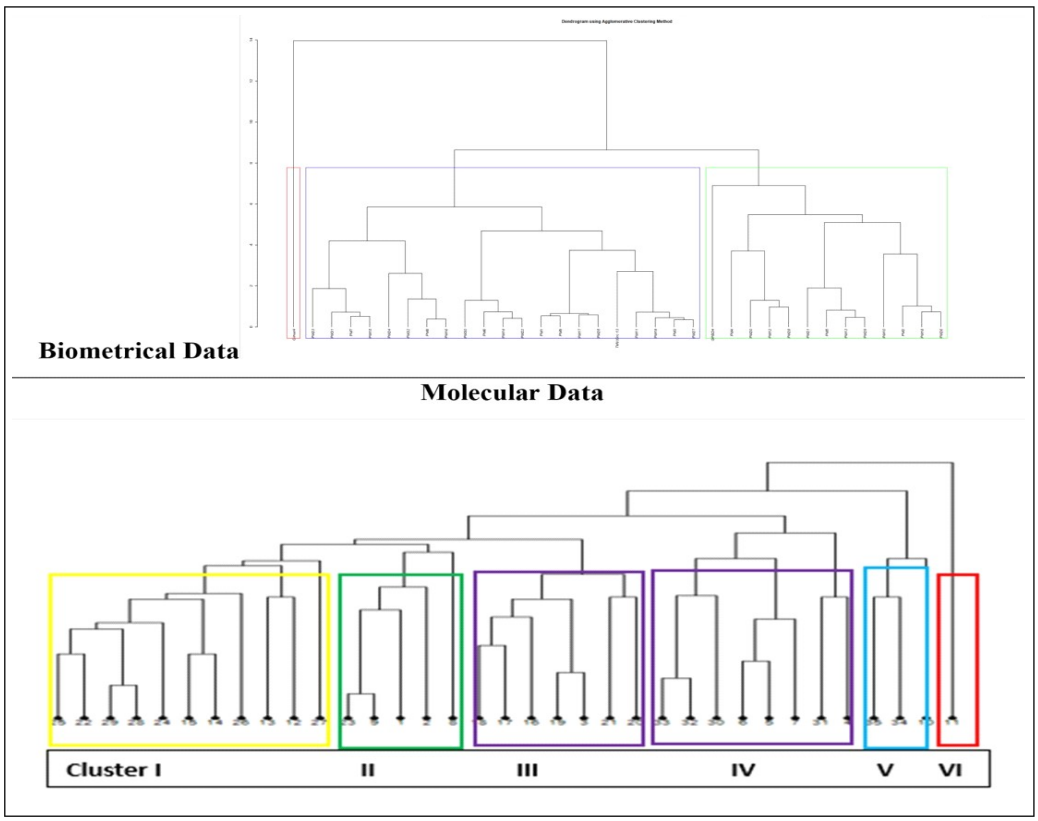


Fig 5: Dendrogram based on cluster analysis for the biometrical traits and molecular data of the PMs and checks.

CONCLUSION

The foregone 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 mutantsand 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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