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Genetic Variability Analysis of Gamma Irradiated M_2 Populations for Higher Oleic Acid Content in TMV (Gn) 13 (*Arachis hypogaea* L.)

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ABSTRACT

Background: Groundnut (*Arachis hypogaea* L.) is an important commercial crop augmenting protein and oil industry. Breeding for improved peanut crop with higher oleic acid content through induced mutagenesis could the best cooking oil in terms of health benefits. Further, the knowledge of the variability parameters and frequency distribution of desired traits could benefit plant breeders while developing selection criteria to maximising the genetic gain.

Methods: The present study aimed to determine the mean lethal dose (LD 50) of gamma irradiation on groundnut TMV (Gn) 13) to create the genetic variability and to screen the putative mutants with allele specific primers based on phenotypic and agronomic traits.

Result: Five traits *viz*., plant height, number of pods per plant, pod length, pod width and pod yield per plant had expressed significantly higher heritability coupled with high Genetic advance per cent of Mean substantiating the role of additive gene action. Also, the frequency distribution pointed out the positively skewed traits in both the populations. Further, molecular validation through AS-PCR assay revealed the presence of ahFAD2A mutant allele in 4 out of 155 putative mutants. As a result, these mutants have 43-45% oleic acid than the control TMV (Gn) 13, which has 35-39%.

Key words: AS-PCR, Genetic variability, Heritability, Kurtosis, Probit analysis, Skewness.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop with higher amount of protein (25 to 30 per cent) and oil (42 to 52 per cent) significantly contributing to human and animal nutrition (Juhaimi *et al*., 2018 and FAOSTAT, 2020). Groundnut often is a multipurpose legume grown worldwide with a production of 50.35 million metric tonnes (USDAFAS, 2022). Peanut been a self-pollinated crop possesses a narrow genetic variability and therefore creation of genetic variability remains vital in action. In Tamil Nadu, the red coloured groundnuts are found to be popular among farmers of Pollachi and Thiruvannamalai districts. TMV (Gn)13, a red kernel groundnut variety was a pureline selection from local genotype “Pollachi red” and matures in 100-105 days. The variety has recorded an overall mean dry pod yield of 1613 and 2580 kg ha^{-1} under rainfed and irrigated situations respectively. The red coloured variety is preferred by oil mills owing to higher oil (content) recovery. Though the pod yield of TMV (Gn)13 is significantly higher, it often succumbs to foliar diseases that eventually obstruct the crop expression (Ramakrishnan, 2017). As such, TMV(Gn)13 groundnut variety with low oleic acid (35-39%) was triggered for the genetic variation through induced mutagenesis could enable to isolate the improved version of the target variety (Karkwal, 2012).

Induced mutagenesis employed to alter oligogenic and polygenic traits favouring heritable changes in the phenotype besides improvement in target traits on popular variety of crops (Sangle and Lad, 2020). Genetic modifications that use different physical and chemical mutagen treatments have been successfully reported for many crops including groundnut (Nurmansyah *et al*., 2020). Physical mutations bring about large-scale deletions of DNA, when gamma rays are used for irradiation (Oladosu

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et al., 2016 and Spencer-Lopes *et al*., 2018). Thus, the first step in mutation constitutes radiation optimization, besides ideal dose depends on plant materials and the desired outcome (Oladosu *et al*., 2016). According to Bharathi *et al*., 2005 there was an increase in genotypic variability for pod number, pod yield and seed yield per plant in M_2 generation of groundnut. Nkuna *et al*. (2021) identified high-oleic content of seeds, size and shape of pods in the virginia mutants through screening with 15 mmol/L sodium nitrate mutagenized M_2 population.

In normal cultivated groundnut O/L ratio ranges from 1.0 to 4.0 whereas in high oleate mutant O/L ratio ranges from 30 to 40. In general, high amount of linolate in the oil is not good for cooking purposes as it is vulnerable to oxidative rancidity and becomes thermodynamically unstable when heated at high temperature. Therefore, High oleate content oil is considered most preferred cooking oil in terms of health benefits (Kamdhar *et al*., 2017).

Thus, breeding groundnut for increased oleic acid but reduced linoleic and palmitic acid content are ultimate traits. The two mutant alleles, ahFAD2A and ahFAD2B control the composition of oleic, linoleic and palmitic acid in groundnut (Kamdar *et al*., 2021). Assessment on LD 50 of gamma irradiated TMV(Gn)13 groundnut and forwarding it to M2 and screening the genetic diverseness using allele specific markers (Chen *et al*., 2010) is the significance of this study.

MATERIALS AND METHODS

Plant materials and Mutation strategy

The present investigation was carried out at experimental farm of Department of Genetics and Plant Breeding, VOC Agricultural College and Research Institute, Killikulam. Genetic material used for the study involved M_2 populations (200 gy and 250 gy) of the groundnut cultivar TMV (Gn) 13. During Rabi 2022-23, seeds of the groundnut variety TMV(Gn)13 were treated with gamma radiation at Bhaba Atomic Research Centre, Mumbai and sown in field condition for critical study on LD 50 wherein 200Gy and 250 Gy have identified ideal. Selected plants from each treatment were further evaluated at M_2 level during Kharif 2023 against the control TMV(Gn)13 along with higher oleic acid content GIRNAR 4 (71.27%) adopting a spacing of 30 × 15 cm with a row length of 5m. Biometrical observations on thirteen characters *viz*., plant height, days to 50 per cent flowering, primary branches per plant, secondary branches per plant, hundred seed weight, number of pods per plant, number of mature pods per plant, seed length, seed breadth, shelling per cent, late leaf spot (LLS) scoring, oil content and pod yield per plant were observed on 234 and 321 plants in the M_2 populations of 200gy and 250gy treatments respectively.

Statistical analysis

LD 50 of different treatments was obtained using Probit Analysis (Regupathy and Dhamu, 1991) method. Genetic variability parameters *viz*., phenotypic and genotypic coefficient of variation (PCV and GCV) for all thirteen characters were estimated based on formulae of Burton, (1952) and Sivasubramanian and Madhavamenon (1973). While broad sense heritability referred as per Lush (1940), the ratio of genotypic variance to total variance and other genetic advance parameters were computed as per Johnson *et al*. (1955). To understand the distribution pattern of all thirteen traits in M_2 population, skewness and kurtosis were computed based on the reports of Snedecor and Cochran (1989). Software used for this analysis was SPSS 2021.

Molecular analysis

Fresh leaf samples from 10 to 15 days old plants of M_2 progenies from 200 Gy and 250 Gy were collected for DNA extraction by modified Cetyl Trimethyl 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. Progenies from both treatments were genotyped using AS-PCR markers (Chen *et al*. 2010) (Table 1) in C1000 Thermal cycler with a reaction volume of 25 per litre (Bera *et al*., 2018). The amplified DNA fragments along with 100 bp DNA marker were separated on a 3 percent 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.

LLS scoring and oil content

All the M_2 population was scored for Late Leaf spot (LLS) on 1 to 9 scale, 90 days after sowing (Subbarao *et al*., 1990). Oil and oleic acid content was estimated by NIR Spectroscopy utilizing 5 g of grain sample (Deshmukh *et al*., 2021).

RESULTS AND DISCUSSION

LD 50 studies

Anent to probit analysis the LD 50 was adjudged at 217.88 Gy and as such the 200 and 250 Gy of gamma radiant mutants were forwarded to M_2 generation during Kharif 2023. Seventy one desired plants were selected under 200 Gy in M_2 generation while eighty four plants from 250 Gy treatment.

ANOVA and mean

Analysis of variance between the groups (200 gy and 250 gy) were performed. F statistic value (4.85) is greater than F critical value (3.98) shows significant difference exists between the groups. Mean data shows significant variations among the groups (Table 2).

Genetic variability assessment

The phenotypic coefficient of variation (PCV) was observed to be higher in magnitude when compared with genotypic

Table 1: Probit analysis for calculating LD 50 in TMV (Gn) 13.

Treatment	Control	100.00 Gy	150.00 Gy	200.00 Gy	250.00 Gy	300.00 Gy	350.00 Gy	400.00 Gy	450.00 Gy	500.00 Gy	600.00 Gy
L	-	5.00	5.18	5.30	5.40	5.48	5.54	5.60	5.65	5.70	5.78
OMP	24	32	40	48	64	68	72	76	84	88	96
CMP	-	10.53	21.05	31.58	52.63	57.89	63.16	68.42	78.95	84.21	94.74
EPU	-	3.75	4.20	4.52	5.07	5.20	5.34	5.48	5.80	6.00	6.62
LD_{50} value						217.88					

Where, L= Lethality; OMP= Observed mortality per cent; CMP= Corrected mortality per cent; EPU= Empirical probit unit.

Table 2: Mean, range, variability parameters, skewness and kurtosis for twelve biometrical traits in the M_2 populations of two treatments (200 gy and 250 gy).

Traits	Mean		Range		PCV %		GCV %		h^2		GAM (%)		Skewness		Kurtosis	
	T I	T II	T I	T II	T I	T II	T I	T II	T I	T II	T I	T II	T I	T II	T I	T II
Plant height (cm)	31.49	39.86	20-41	22-52	18.91	18.40	15.85	16.50	70.29	80.41	27.35	30.46	-0.336	-0.95	-0.075	0.315
Days to 50% flowering	26.27	26.56	22-30	21-34	9.30	10.89	8.03	9.85	74.6	81.92	14.27	25.49	-0.254	0.153	-1.332**	-0.54
Primary branches per plant (nos)	5.07	5.16	1-9	2-9	33.22	30.08	20.78	17.32	40.01	31.36	27.36	19.55	-0.058	0.088	-0.036	-0.404
Secondary branches per plant (nos)	7.01	9.6	3-14	1-15	56.46	43.98	34.87	34.01	38.15	59.79	44.39	53.46	1.343**	1.474**	1.503**	1.574**
Pods per plant (nos)	41.07	41.73	32-58	32-57	19.02	19.30	16.88	17.26	78.77	80.02	30.87	31.81	0.687**	0.513**	-0.797**	-1.181**
Pod length (cm)	2.42	2.5	1.5-3.2	1.5-3.5	16.68	17.57	13.64	14.91	66.87	72.02	22.93	26.05	0.163	-0.220	0.285	-0.057
Pod width (cm)	0.93	0.92	0.4-1.6	0.5-1.6	33.22	33.33	27.55	27.49	68.42	68.08	46.91	46.63	0.431*	0.437*	-0.449	-0.498
Seed Length (cm)	1.05	1.06	0.5-1.5	0.5-1.4	21.21	21.69	13.86	14.29	42.30	43.39	18.35	19.37	-0.363*	-0.340*	-0.040	-0.071
Seed width (cm)	0.54	0.56	0.4-0.9	0.5-0.9	24.8	26.48	18.51	21.12	55.55	63.63	28.11	34.29	1.07**	0.989**	0.693	0.145
LLS Scoring	5.028	5.45	2-9	3-9	35.09	27.52	29.44	21.12	70.41	60.08	50.87	34.01	0.261	0.215	-0.587	-0.481
Oil content	47.67	47.50	40.01-52.43	42.03-53.45	7.14	7.19	6.13	6.15	73.13	73.31	19.87	19.79	-0.219	-0.173	-0.039	-0.076
Pod yield per plant (g)	23.59	22.14	12.31-38.28	8.14-43.23	27.75	34.73	25.20	32.45	82.47	87.29	47.09	62.44	0.068	0.459**	-0.668	-0.109

**Significance at 1%. TI- 200 Gy; TII- 250 Gy.

coefficient of variation (GCV) for twelve biometric traits studied in both the M_2 populations (Table 2, Fig 1) signifying the environmental influence on those traits. Further, the difference of PCV and GCV was less than 3.00 percent for six traits *viz*., plant height, days to fifty percent flowering, number of pods per plant, pod width and oil content in both the treatments specifying lesser environment influence and hence mere phenotypic selection can be duly considered (Ali *et al*., 2010; Patil *et al*., 2014 and Chen *et al*., 2020).

Maximum coefficients of variation observed were 56.46 (PCV), 34.87 per cent (GCV) and 43.98 (PCV), 34.01 per cent (GCV) for number of secondary branches at 200 Gy and 250 Gy respectively. While, the minimum values were observed for oil content with 7.14 (PCV) and 6.13 per cent (GCV) at 200 Gy and 7.19 (PCV), 6.15 per cent (GCV) at 250 Gy. Traits *viz*., pod yield per plant, LLS scoring and pod width recorded higher PCV and GCV (>20%) at both the treatments. Magnitude of variability was found to be higher for these traits with limited environmental influence and hence efficient selection can be exercised Yadlapalli (2014); Patidar *et al.* (2014) and Cui *et al*. (2020).

Heritability and genetic advance

Heritability, being a predictive function, reveal the extent to which a particular trait could be passed to successive generation. To practice selection, the prime criterion to be considered is the heritable variation that responds to selection, along with genetic progress as a percentage of mean, improves the precision of determining heritable variation (Mehandi *et al*., 2013).

Broad sense heritability at 200 Gy (200 gy) ranged from 38.15 (number of secondary branches) to 82.47 percent (pod yield per plant) and at 250 Gy from 31.36 (number of primary branches) to 87.29 per cent (pod yield per plant). GAM estimates at 200 Gy ranged from 14.27 (days to 50% flowering) to 50.87 per cent (LLS scoring) whereas at 250 Gy from 19.37 (seed length) to 62.44 per cent (pod yield per plant). Five traits *viz*., plant height, number of pods, pod length, pod width and pod yield per plant expressed high heritability coupled with higher GAM substantiating the role of additive gene action and efficiency of selection (Shashikumara *et al*., 2016). However, seed length expressed low to moderate heritability and GAM confronting non-additive gene action and hence selection may not be rewarding.

Skewness and kurtosis

It is evident that kurtosis enumerate the number of genes controlling a trait while skewness points out the nature of gene action involved. The genetic behavior of traits were

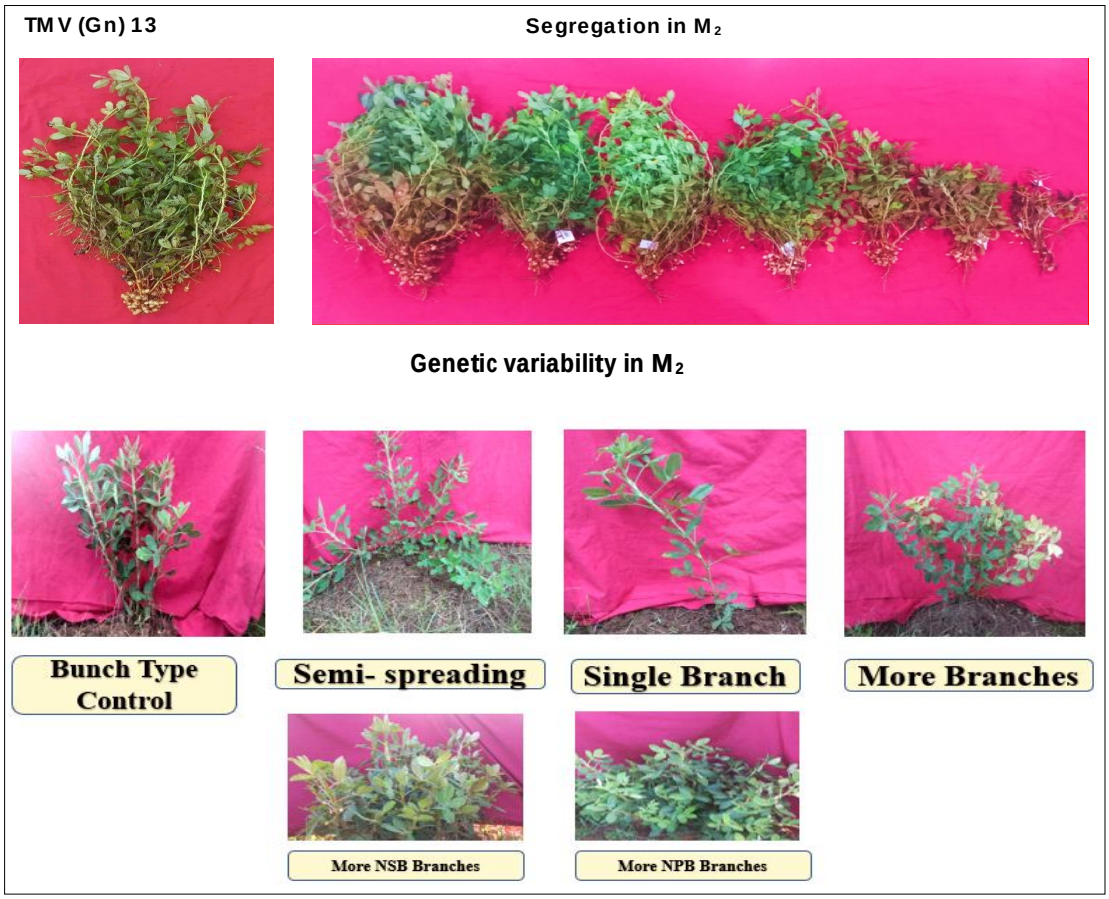


Fig 1: Morphological variation in M_2 population of TMV (Gn) 13.

depicted on a frequency distribution graph for the two population and is given in Fig 2 and 3. Skewness estimates at 200 Gy ranged from -0.058 (number of primary branches) to 1.343 (number of secondary branches per plant) and at 250 Gy from -0.1734 (oil content) to 1.474 (number of secondary branches per plant). Significant and positive skewness was observed for traits *viz*., number of secondary branches per plant, number of pods per plant,

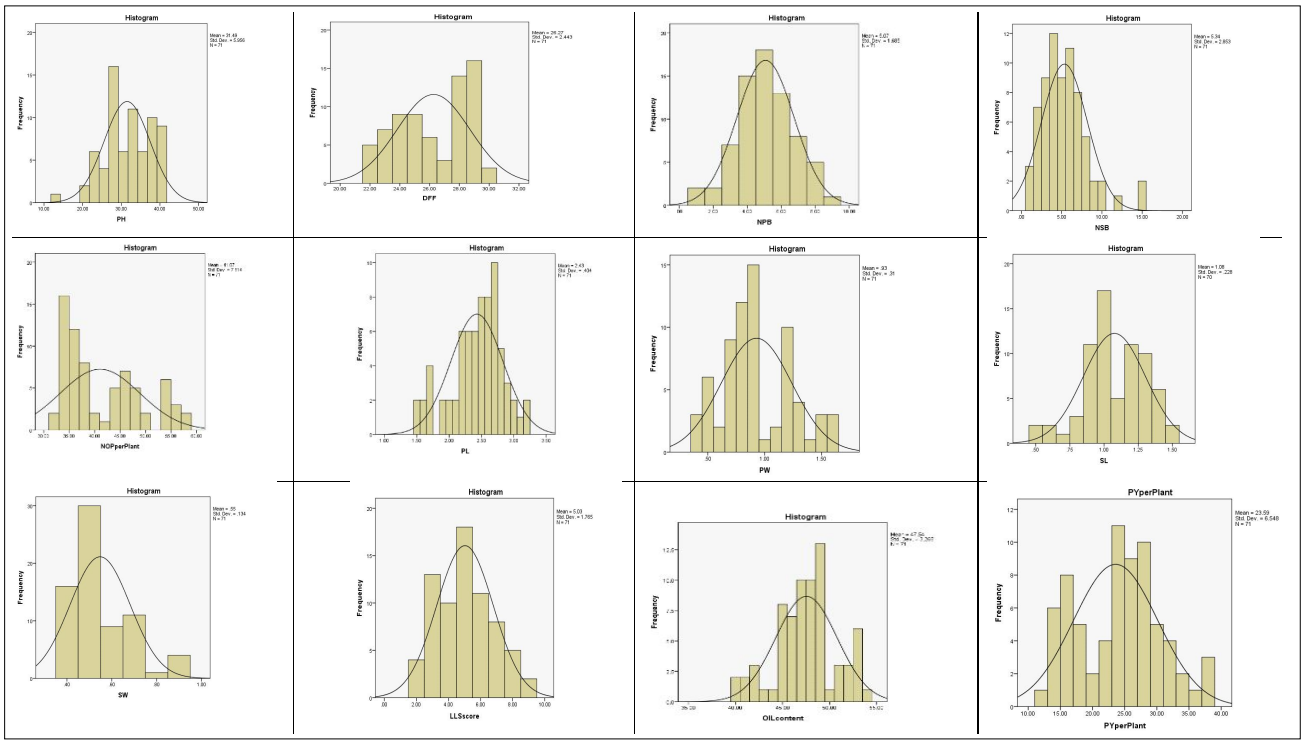


Fig 2: Frequency distribution of twelve biometrical traits in M_2 population of 200 Gy.

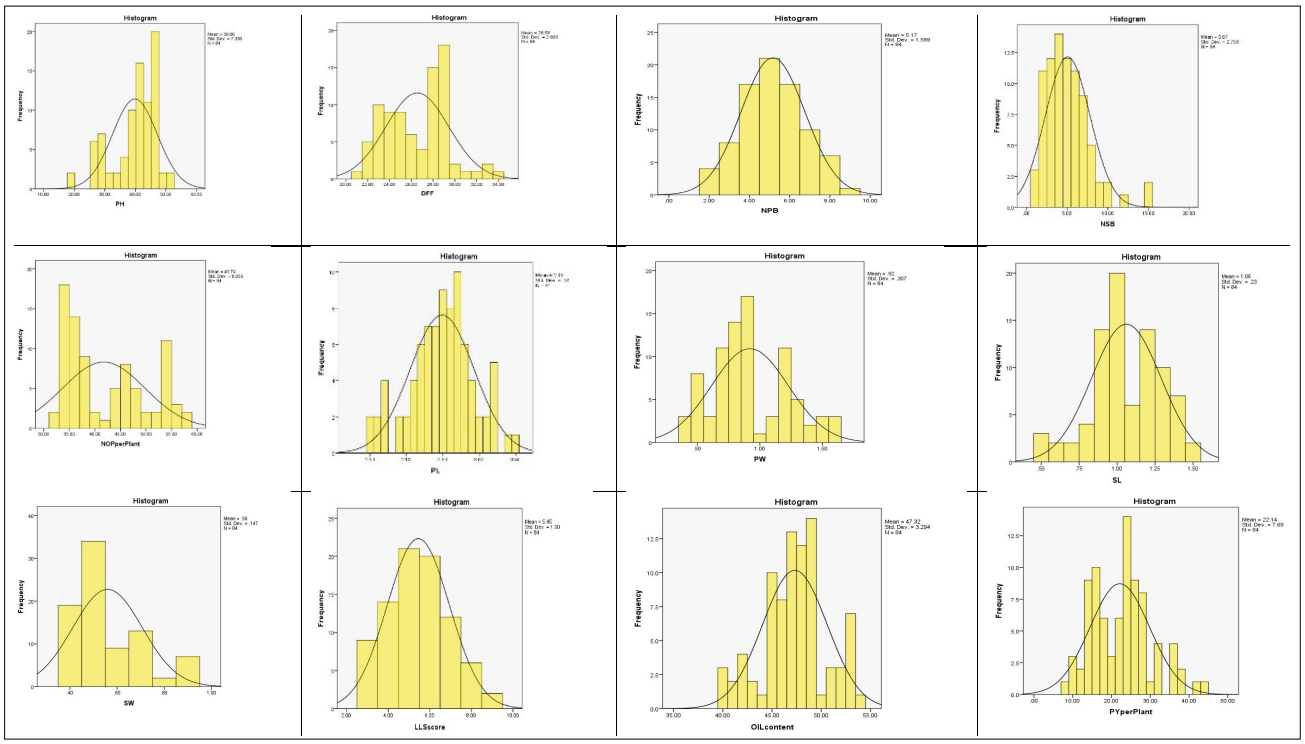
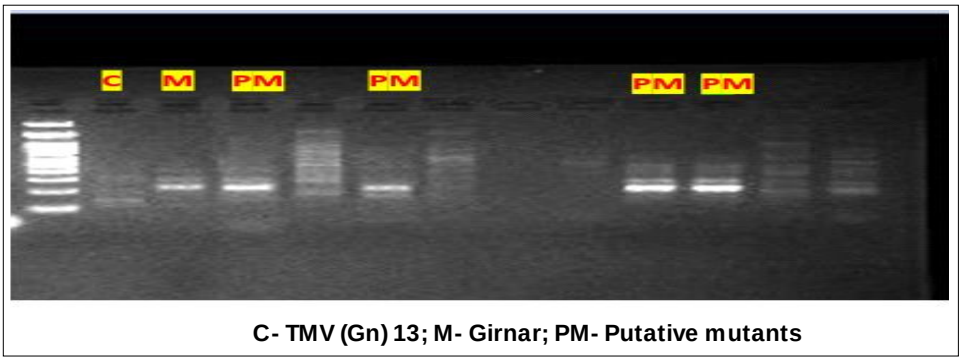


Fig 3: Frequency distribution of twelve biometrical traits in M_2 of 250 Gy.



C- TMV (Gn) 13; M- Girnar; PM- Putative mutants

Fig 4: Validation of putative mutants in M_2 population for ahFAD2A mutant alleles of TMV (Gn)13.

Table 3: Allele-specific polymorphic chain reaction markers for selecting plants with ahFAD2A and ahFAD2B mutant alleles.

Allele	Markers	Sequence (5′ to 3′)	Wild allele	Size of mutant allele	Reference
ahFAD2A	F435-F	ATCCAAGGCTGCATTCTCAC	Null allele	203 bp	Chen *et al.* (2010)
	F435SUB-R	TGGGACAAACACTTCGTT			
ahFAD2B	F435-F	ATCCAAGGCTGCATTCTCAC	Null allele	195 bp	
	F435INS-R	AACACTTCGTCGCGGTCT			

pod width and seed width at both treatment levels but for pod yield at 250Gy only signifying the role of dominant and complementary gene action. The frequency distribution graph of those traits depicted that the higher frequency of segregants recorded values lesser than the mean value of the particular trait**.** However, in both the M_2 populations, among the positively skewed traits only four traits *viz*., number of pods per plant, pod length, pod width, plant height and pod yield per plant recorded moderate to high GCV and high estimates of heritability and GAM (Fig 2 and 3). Further, rigorous selection of segregants for the above four traits will facilitate the population improvement. Kurtosis estimates at 200 Gy ranged from -0.036 (number of primary branchs per plant) to 1.503 (number of secondary branches per plant) and 250 gy from -0.057 (pod length) to 1.574 (number of secondary branches per plant) (Prabhu *et al*., 2020).

Molecular analysis

Total of 155 plants from both 200 and 250Gy were subjected to AS-PCR assay along with Girnar (mutant allele) using diagnostic AS-PCR markers associated with ahFAD2A and ahFAD2B mutant alleles (Table 3) (Fig 4). AS-PCR assay revealed the presence of ahFAD2A mutant allele in 4 out of 155 putative mutants but no putative mutant was positive for ahFAD2B mutant allele (Kamdar, *et al*., 2021). As a result, these mutants have 43-45% oleic acid than the control TMV (Gn) 13, which has 35-39%.

CONCLUSION

The best cooking oil in terms of health benefits is said to be one with a high oleate content. Phenotypic traits *viz*., number of pods per plant, pod length, pod width and pod yield per plant had interpreted to govern by additive gene effects with discernible amount of genetic variability. Therefore, selection of these traits will be effective to boost genetic gain and yield improvement. Further molecular validation of AS-PCR markers associated with ahFAD2A and ahFAD2B mutant alleles through AS-PCR assay revealed the presence of ahFAD2A mutant allele in 4 out of 155 putative mutants. These mutants thus contain 43-45% of oleic acid content compared with control 35-39%. Stringent selection of these mutants with desirable traits will be rewarding since the populations are positively skewed.

Conflict of interest

All authors declared that there is no conflict of interest.

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