

Genetic improvement for oil quality through induced mutagenesis in groundnut (*Arachis hypogaea* L.)

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Abstract

The present research was aimed towards oil quality improvement in two groundnut cultivars (GPBD-4 and TPG-41) through induced mutagenesis using EMS and gamma rays. Six hundred true breeding mutants were isolated in M₃ and were characterized for fatty acid profile at M₄/M₅ generations. Twenty high oleate mutants showing consistent fatty acid profile across the generations were further evaluated for economic traits. The induced variability was skewed towards high levels of oleic acid and low level of linoleic acid in GPBD-4 and it was reverse in TPG-41. Greater magnitude of induced variability was found for oleic acid (37.40-75.16%), linoleic acid (9.01-40.30%) and oleic to linoleic acid ratio (O/L) (0.95-8.34) in mutant populations at M₅. Mutant, GE-113 recorded the highest increase in oleic acid (74.48%), lowest reduction in linoleic acid (9.17%) and highest increase in O/L ratio (8.12) compared to parent GPBD-4. High oleate mutants were having reduced levels of palmitic acid, long chain saturated fatty acids and iodine value and were comparable to parents for economic traits. Mutant GE-113 had pod yield of 25.33 qt/ha comparable to GPBD-4 (27.06 qt/ha).

Key words: Groundnut, fatty acids, ethyl methane sulphonate, gamma rays, induced variability, high oleate, gas chromatography, calibration.

Introduction

Oilseed research has primarily emphasized on increased crop yields and higher oil content in India. Until recently, there has been little interest for research on improved oil quality for consumers and industries/traders. It is well known that oil quality is determined by concentrations of specific fatty acids and among the fatty acids it is the ratio of oleic acid to linoleic

acid (O/L) matters a lot from the point of both oxidative stability [1] and nutritional value [2]. Generally the oils having high oleic acid and reduced linoleic acid are the most stable and desirable from nutritional point of view.

Research spanning four decades has shown that saturated fatty acids are hypercholesterolemic, while monounsaturated and polyunsaturated fatty acids are hypocholesterolemic [3]. Saturated and monounsaturated oils are very stable. In contrast, polyunsaturated fatty acids are readily oxidized resulting in unpleasant odour, flavour and discolouration [4]. The rates of oxidation of C18 fatty acids are approximately 1:10:100:200 for stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) respectively [5]. Genotypes with increased O/L ratio reduce the need for oil hydrogenation during industrial processing and thus decreasing the production of harmful trans-fatty acids during such processing [6].

Groundnut oil generally contains 45-50% monounsaturated fatty acids, 30-35% polyunsaturated fatty acids and 17-18% saturated fatty acids [7]. In most commercial groundnuts, the O/L ratio varies from 1:1 to 2.5:1, with spanish types typically at the low end of the scale [8]. Mutations, both spontaneously and induced, have been successful in changing the fatty acid composition of several oilseed crops viz., sunflower [9], soybean [10] and rapeseed [11]. However such efforts are minimal in groundnut except the natural mutants isolated in Florida breeding programme [12] and a marginal improvement in O/L ratio of 5 using gamma rays [13].

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The occurrence of natural mutants as well as the possibility of induced mutations and significant achievements made thereof in different oilseed crops suggest that it is possible to increase oleic acid through induced mutagenesis in groundnut. Present research work was undertaken to improve oleic acid through induced mutagenesis in groundnut (*Arachis hypogaea* L.).

Materials and methods

Induced mutagenesis and genotypes

Two spanish bunch cultivars with relatively better O/L ratio viz., GPBD-4 (1.76) [14] and TPG-41 (2.91) [15] were selected for the study and treated with ethyl methane sulphonate (EMS) and gamma rays at two doses (200 Gy and 300 Gy). Samples of 500 dry, healthy and uniform size seeds were used for mutagenic treatment. For EMS treatment, seeds were initially soaked in distilled water for 4 h and treated with EMS (0.5%) for 8 h at pH 7.0. The treated seeds of both the cultivars were grown during summer 2003-04 along with controls to raise the M₁ generation in experimental field at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad.

The M₂ generation was raised by growing M₁ plant to progeny rows grown during rainy season 2004. Around 900 morphological variants were isolated in the M₂ and advanced to M₃ (rainy season 2005). Of these variants, 600 true breeding mutants with various morphological traits were isolated at M₃ and carried to M₄ (rainy season 2006). Fatty acid analysis was carried out among 600 mutants in M₄ using gas chromatography and were advanced to M₅ (rainy season 2007). Fatty acid analysis of the 600 mutants was again carried out during M₅ using near infra-red reflectance spectroscopy to confirm the fatty acid composition of some of the highly distinct mutants. 20 high oleate mutants showing consistent fatty acid composition across M₄ and M₅ generations were advanced to M₆ (rainy season 2008) for seed multiplication. In the M₇, these high oleate mutants were evaluated along with controls for fatty acid profile, pod yield and other economic traits sown during rabi/summer 2008-09 in a Randomized Block Design with two replications and plot size of 5m X 1.5 m. In each generation the untreated controls were grown after every 25 mutants.

Fatty acid analysis

Gas Chromatography (GC): Sound matured single

seeds from 600 mutants grown during 2006 (M₄) were ground to a fine paste using mortar and pestle. 2 ml of petroleum ether (bp 35-60°C) was added to 0.2 g (approx.) ground paste from each mutant. It was then vortexed and kept overnight for oil extraction. Fatty acids were esterified by saponification-transesterification method [16]. 2 ml of 0.5 N sodium hydroxide in methanol was added, vortexed and heated in a boiling water bath for 5 min to saponify the oil. After cooling, 2 ml of 12% boron trifluoride in methanol was added, vortexed, heated in a boiling water bath for 5 min and cooled. Then, 2 ml of petroleum ether and 2 ml of deionized water were added to tubes. After vortexing, when petroleum ether and water layer separated clearly, 1 ml was taken from top petroleum ether and added to 2 ml sample vial for injection to gas chromatography (Model GC 2010, Shimadzu, Kyoto, Japan). GC was equipped with AOC- 20s auto sampler, FID detector and fitted with a capillary column (Rtx[®]-Wax, Restek, PA, USA). The column oven was programmed for an initial temperature setting of 170°C for 3 min then increased at the rate of 10°C per min to a maximum of 230°C and held for one min. Injector and detector temperatures were set at 250°C. Fatty acids were identified by comparing the retention time to an standard fatty acid methyl ester mixtures (Sigma, Aldrich, USA). Concentration of each fatty acid were recorded by normalization of peak areas and reported as per cent of particular fatty acid.

NIRS analysis

Fatty acid composition of the M₅ mutant populations was determined by Near Infrared Reflectance Spectroscopy (NIRS) analysis. NIR diffused reflectance spectra were collected by a monochromator NIR spectrometer model 6500 (Foss NIR systems, France) with the range from 400 to 2500 nm. For NIRS calibration, 600 mutant entries (M₄) with wide fatty acid composition analyzed by GC were used. Spectra from intact single seeds of the mutant entries were collected with a specially designed adapter using standard monochromator instrument. Calibration equations were developed for the calibration set of 400 mutant entries and were further validated with cross-validation and external-validation set consisting each of 100 mutant entries. Best calibration equations were developed using full spectral information using standard normal variate, detrend scatter correction, 2, 6,4,1 math treatment and modified partial least squares (mPLS) regression model. The r² between NIRS and GC was 0.79 (palmitic acid), 0.91 (oleic acid) and 0.89 (linoleic acid) in cross

validation and 0.77 (palmitic acid), 0.88 (oleic acid) and 0.85 (linoleic acid) in external validation, demonstrating the high reliability of NIRS to predict these fatty acid concentrations in intact single seeds. High oleate mutants were again confirmed for their composition at the M₆/M₇ using GC.

Oil and protein analysis

NIRS was calibrated for oil and protein content estimation using 500 groundnut samples with oil and protein values obtained through Soxhlet and Kjeldal methods, respectively.

Iodine value = (% oleic x 0.8601) + (% linoleic x 1.7321) + (% eicosenoic x 0.7854) [17].

Results and discussion

One of the most important objectives in oilseed breeding has been the genetic modification of seed oil quality by changing the proportion of fatty acids suitable for either nutritional or industrial purposes. Nutritional concerns, functionality in food manufacturing and the need for high stability and extended shelf life had a tremendous impact on developing and commercializing modified oilseeds, so far [18]. Induced mutation is one of the most widely used technique for creating additional variability in seed oil quality.

Induced variability in the present work was found to be skewed towards high levels of oleic acid and low level of linoleic acid in GPBD-4 mutant populations (Fig. 1). Whereas in TPG-41, the variability was found to be skewed towards low levels of oleic acid and high level of linoleic acid (Fig. 1). The differential response of the two genotypes may be attributed to a) genetic background of the genotype, b) interaction between genotype and mutagen and c) the parent TPG-41 already contains higher oleic acid (58-61%) so the gene in respect to higher oleic acid might has been already mutated and there was no scope for further improvement. But the another parent GPBD-4 had low oleic acid content of 48-50 per cent and in this genotype there was lot of scope for the genes to get mutated leading to increased oleic acid. Such a differential response of the genotypes and mutagenic treatments in inducing variability for fatty acids was also reported in Ethiopian mustard [19].

In the present study, mutagenic treatments resulted in high induced variability for palmitic acid, oleic acid and linoleic acid compared to other fatty acids (Table 1). In M₅ generation, GPBD-4 varied greater for oleic acid, linoleic acid and O/L ratio than

TPG-41. There are reports of increasing variability in fatty acid composition by means of mutagenic treatments [20, 10]. In Ethiopian mustard greater variability was found for oleic acid and erucic acid content compared to parents [19]. The range for fatty acid content found in mutant populations of two genotypes exceeded the ranges reported in various groundnut materials by most of the earlier workers. The average O/L ratio among 75 *Arachis* species accessions ranged from 0.35 to 0.43 [21] and among 732 plant introductions, oleic acid and linoleic acid ranged from 31.5 to 60.2% and 19.90 to 45.40% respectively [22]. In general, groundnut oil contains 6-20% palmitic acid, 1-6% stearic acid, 36-71% oleic acid and 20-48% linoleic acid [23].

High oleate mutants of GPBD-4 and TPG-41

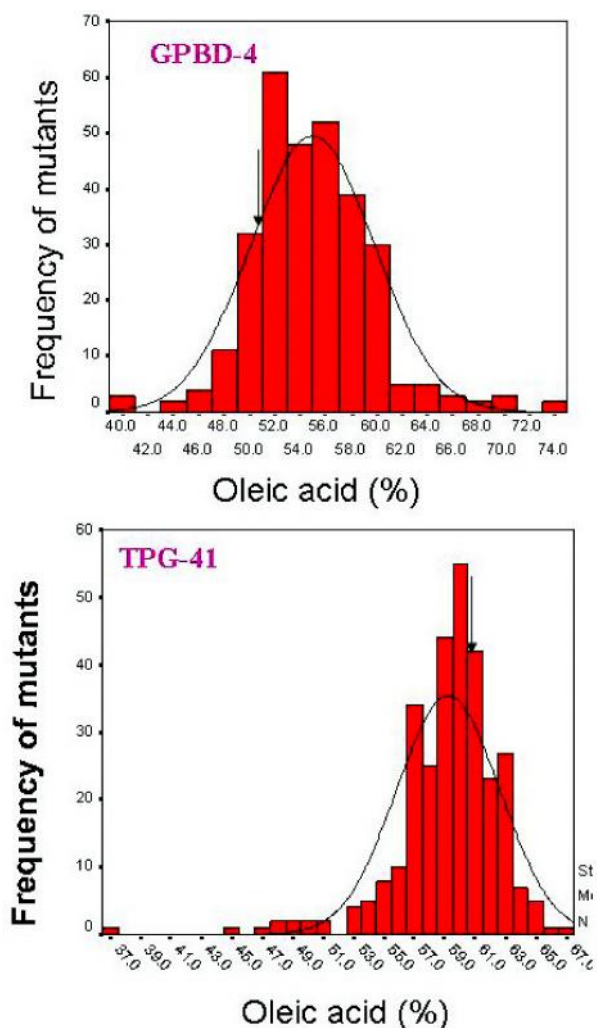


Fig. 1. Frequency distribution of oleic acid (%) in the mutant populations derived from GPBD-4 and TPG-41 in M₅ generation

Table 1. Induced variability for fatty acid profile in mutant populations of groundnut at M₅ generation

Fatty acids	Genotypes	Treatments	Range
Palmitic (C16:0)	GPBD-4	Mutagenic treatment	6.83-12.71
		Control	9.13-11.03
	TPG-41	Mutagenic treatment	7.66-12.45
		Control	8.82-10.45
Stearic (C18:0)	GPBD-4	Mutagenic treatment	2.14-4.17
		Control	2.08-2.65
	TPG-41	Mutagenic treatment	1.45-2.91
		Control	1.32-2.71
Oleic (C18:1)	GPBD-4	Mutagenic treatment	39.01-75.16
		Control	47.62-52.67
	TPG-41	Mutagenic treatment	37.40-68.97
		Control	57.41-62.60
Linoleic (C18:2)	GPBD-4	Mutagenic treatment	9.01-37.82
		Control	26.62-32.57
	TPG-41	Mutagenic treatment	13.60-40.30
		Control	18.01-22.68
Arachidic (C20:0)	GPBD-4	Mutagenic treatment	1.23-2.44
		Control	1.05-1.96
	TPG-41	Mutagenic treatment	1.13-2.04
		Control	1.18-1.70
Behenic (C22:0)	GPBD-4	Mutagenic treatment	2.85-4.77
		Control	3.51-4.02
	TPG-41	Mutagenic treatment	2.32-4.28
		Control	2.76-3.47
Lignoceric (C24:0)	GPBD-4	Mutagenic treatment	1.02-2.06
		Control	1.18-2.05
	TPG-41	Mutagenic treatment	1.14-1.77
		Control	1.32-1.61
O/L ratio	GPBD-4	Mutagenic treatment	1.03-8.34
		Control	1.66-1.82
	TPG-41	Mutagenic treatment	0.95-5.38
		Control	2.75-3.18

isolated during M₄ and M₅ generations were further tested for fatty acid composition at M₆/M₇ using GC. Mutant GE-113 recorded the highest oleic acid, the lowest linoleic acid and higher O/L ratio accounting for 46, 68 and 361% improvement, respectively over the parent GPBD-4 (Table 2) (Fig. 2). Mutant GE-97 was the next best for O/L ratio (7.65) with mean oleic acid content of 73.72% and linoleic acid of 9.63%. Among TPG-41 mutants, mutant T3-109 recorded higher oleic acid, lower linoleic acid with higher O/L ratio resulting in 12.10, 33.65 and 68.72 per cent improvement over its parent respectively (Table 3). Earlier studies on induced mutagenesis in groundnut reported an increase in O/L ratio to 1.65 [24], 3.3 using

X-rays [8], 3.5 using ethyl methane sulfonate [25] and 5 using gamma rays [13]. The O/L ratio recorded in present work almost reached the higher values earlier recorded in two natural mutant lines with 80 per cent oleic acid and 2 per cent linoleic acid [12]. The diets rich in monounsaturated fatty acid (MUFA) lowered total cholesterol by 10 per cent and LDL cholesterol by 14 per cent [3]. High MUFA diet resulted in 79 per cent and 27 per cent reduction in recurrent myocardial effects [26] and type-2 diabetes [27]. The research results clearly indicate that significant genetic improvement for oil quality in groundnut through induced mutagenesis that was not achieved hitherto by recombination either by using wild species or

Table 2. High oleate mutants with desirable fatty acid profile in groundnut cultivar GPBD-4

S.No. Mutants	C16:0	C18:0	C18:1	C18:2	C20:0	C20:1	C22:0	C24:0	C18:1/ C18:2	Iodine value	Protein (%)	Oil content (%)	100-seed weight (g)	Pod yield (q/ha)
1. G [#] E [§] -113	7.18**	2.60	74.48**	9.17**	1.14	1.52	2.92**	1.03**	8.12**	80.96**	30.34	47.56	36.88	25.33
2. GE-97	7.32**	2.30	73.72**	9.63**	1.24	1.68**	3.02**	1.20**	7.65**	81.40**	36.78**	48.09	37.39	21.67**
3. G3-77	7.53**	2.50	70.20**	12.16**	1.17	1.50	3.04**	1.26**	5.77**	82.61**	30.37	47.06	42.58**	25.85
4. G3-15	7.61**	2.80	69.72**	12.24**	1.30	1.31	3.01**	1.50	5.70**	82.18**	32.14	47.20	39.01	26.39
5. G3-109	7.85**	2.18	68.31**	13.81**	1.04**	1.40	2.85**	1.25**	4.95**	83.77**	32.16	46.19	39.16	23.06**
6. GE-34	7.70**	2.39	67.82**	13.88**	1.73	1.30	3.80	1.42	4.88**	87.80**	31.47	47.21	36.88	26.02
7. G3-233	7.91**	3.05**	65.24**	15.34**	1.68	1.22	3.68	1.50	4.25**	83.67**	36.13**	48.13	36.76	24.44
8. GE-75	7.88	2.58	65.09**	16.16**	1.92**	1.25	3.51	1.64	4.03**	89.25**	32.04	47.81	35.93	22.34**
9. G2-212	8.01**	2.51	64.71**	16.08**	1.56	1.40	3.88	1.73	4.02**	84.58**	29.85	48.86	38.21	24.30
10. G3-46	8.16**	2.74	64.32**	16.21**	1.80	1.34	3.52	1.75	3.96**	84.46**	33.84	48.81	38.63	25.28
11. GPBD-4	9.61	2.30	50.87	29.00	1.45	1.31	3.80	1.70	1.76	94.93	31.86	48.25	38.60	27.06
CD (1%)	0.83	0.53	3.21	2.80	0.36	0.21	0.41	0.28	0.48	2.67	1.98	1.40	3.15	3.86

**Significant at 1% level of probability; # -GPBD-4; §- Mutagen treatment (E-EMS, 2-200Gy, 3-300Gy)

Table 3. High oleate mutants with desirable fatty acid profile in groundnut cultivar TPG-41

S.No. Mutants	C16:0	C18:0	C18:1	C18:2	C20:0	C20:1	C22:0	C24:0	C18:1/ C18:2	Iodine value	Protein (%)	Oil content (%)	100-seed weight (g)	Pod yield (q/ha)
1. T [#] 3 [§] -109	7.93**	1.92	67.55**	13.80**	1.28	1.42	2.94**	1.80	4.90**	82.95**	23.66	44.80	62.50	21.25
2. T3-6	8.06**	2.32**	65.43**	15.76**	1.40	1.50	3.00**	1.78	4.15**	84.57**	22.60	45.67	50.63**	18.61**
3. T2-92	8.11**	2.07	65.34**	15.75**	1.25	1.66**	3.10	1.51	4.13**	84.61**	24.78	47.12**	62.54	20.78
4. TE-178	8.31**	2.20	65.28**	16.20**	1.38	1.16	2.92**	1.44	4.03**	85.11**	23.21	47.17**	76.85**	24.40
5. T3-2	9.18	2.52**	64.08**	16.55**	1.26	1.48	3.38	1.48	3.87**	84.70**	23.37	45.37	58.52	21.10
6. TE-160	8.58**	1.98	64.55**	17.00**	1.31	1.20	3.03	1.56	3.80**	85.84**	25.76	46.50	64.70	22.70
7. TE-151	9.10	2.36**	64.26**	16.93**	1.30	1.33	2.91**	1.41	3.80**	85.47**	24.51	46.08	67.22**	23.07
8. T3-17	8.74	2.17	64.20**	16.96**	1.31	1.58	2.98**	1.81	3.78**	85.65**	24.36	46.10	63.21	23.15
9. TE-87	9.10	2.30**	64.00**	17.20**	1.24	1.36	3.40	1.50	3.72**	85.60**	23.80	45.50	65.00	21.50
10. T3-90	8.95	2.50**	63.47**	16.97**	1.20	1.65**	2.86**	2.12	3.74**	85.01**	25.00	45.60	53.40**	21.35
11. TPG-41	9.47	1.94	60.26	20.80	1.48	1.23	3.33	1.47	2.91	88.68	24.10	45.03	60.51	23.80
CD (1%)	0.76	0.32	2.02	1.76	0.41	0.35	0.31	0.33	0.38	2.01	1.95	1.98	5.02	3.53

**Significant at 1% level of probability; # -TPG-41; §- Mutagen treatment (E-EMS, 2-200Gy, 3-300Gy)

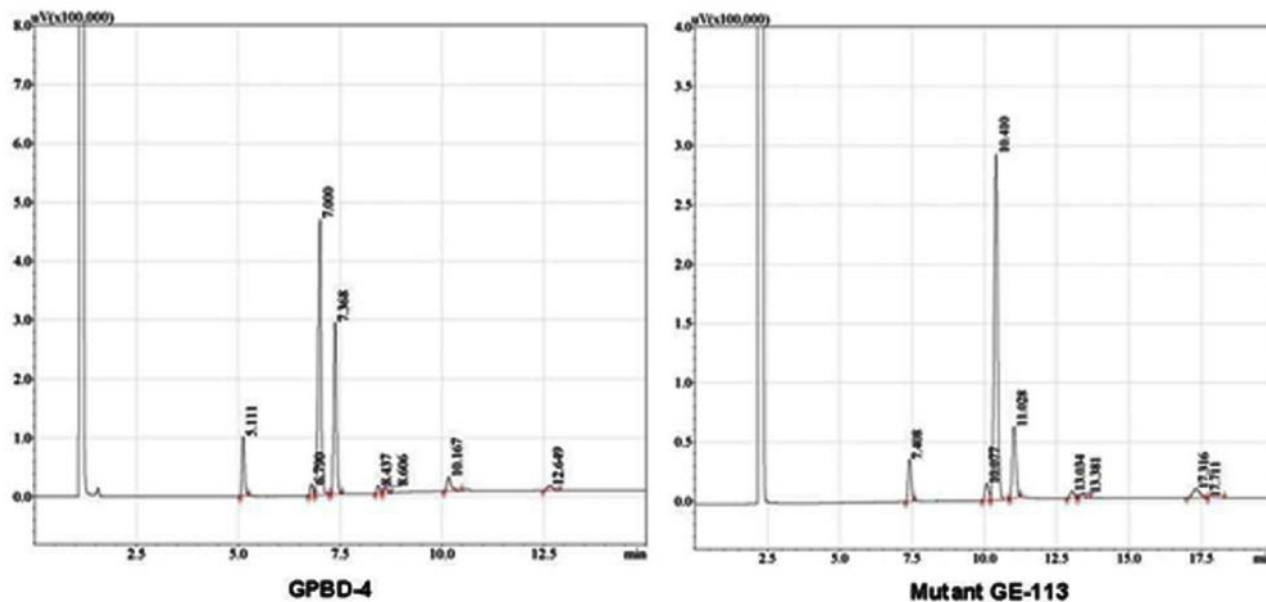


Fig. 2. Chromatogram of parent GPBD-4 and high oleate mutant (GE-113)

cultivated ones. Hence, the wide range of induced variability observed in the present work will strengthen future oil quality improvement programmes. Higher oleic acid in turn higher O/L ratio could be due to differential activity of the enzyme $\Delta 12$ desaturase, which catalyzes the reaction of oleic acid to linoleic acid.

Many associated changes were noted in high oleate mutants. They were characterized by greatly reduced palmitic acid, long chain saturated fatty acids and iodine value. Mutant GE-113 and T3-109 had palmitic acid content of 7.18 and 7.93 per cent respectively with 25.28 and 16.26 per cent reduction over GPBD-4 and TPG-41 respectively. High oleate mutants often had higher proportions of stearic acid with G3-233 (3.05%) significantly higher compared to parent GPBD-4 (2.30%). Some of the high oleate mutants were with significantly lower proportion of behenic and lignoceric acids in GPBD-4 (GE-113, GE-97, G3-77 and G3-109) and only behenic acid in TPG-41 (T3-6, T3-17, T3-109, TE-151, TE-178). Further there was significant reduction in iodine value among all high oleate mutants (Tables 2 and 3). The inverse association between oleic acid and palmitic acid in groundnut has been reported [28]. High intake of saturated fatty acids (palmitic acid) in the diet are associated with increased levels of blood cholesterol, arteriosclerosis and high coronary heart disease risk [29, 30]. Long-chain saturated fatty acids have been implicated in the elevated atherogenic effect [31].

Such of the deficiencies can be mitigated by consumption of high oleate mutants as they have reduced percentage saturation and an increase in the ratio of unsaturated to saturated fatty acids.

Successful development of cultivars with change in fatty acid profile depends on its association with agronomic and seed traits of economic importance. The results indicated that most of the high oleate mutants from both the genotypes were on par with parent for important agronomic and economic features viz., protein content, oil content, 100 seed weight and pod yield per hectare (Tables 2 and 3). Two of the mutants viz., GE-97 (36.78%) and G3-233 (36.13%) found to be significantly superior for protein content compared to parent GPBD-4 (31.86%). Mutants with protein content of 34% have been reported [32]. All the mutants were on par with the control for oil content and 100 seed weight except mutant G3-77 (42.58 g) which recorded significantly increased 100 seed weight compared to parent GPBD-4 (38.60 g). Seven of the ten high oleate mutants were on par with the parent GPBD-4 for pod yield. Highest oleate mutant GE-113 had comparable pod yield as that of its parent (Table 2). In TPG-41, mutant T2-92 and TE-178 recorded significant increased oil content compared to parent. Mutant TE-178 and TE-151 recorded significantly increased 100-seed weight whereas T3-6 and T3-90 recorded decreased 100-seed weight compared to TPG-41. Except T3-6, all other mutants were statistically on par with parent TPG-41 for mean pod

yield per hectare (Table 3).

These results confirm the earlier findings that seed yield, seed oil and protein contents were unaffected by selection for changes in oleic acid content in soybean [33]. Non significant correlation between per cent oil and any of the fatty acids in groundnut was reported [34] hence selection for improved fatty acid composition should not affect oil content of seed. 'SunOleic 95 R' a high oleic acid genotype had pods and seeds very similar to those of Sunrunner [35]. Results of the present findings and reports from earlier works indicate that selection for increased oleic acid is not associated with undesirable agronomic features. Thus, it would be possible to extend the shelf-life of oil and enhance nutritional quality by genetically altering the fatty acid composition.

The lack of variability in both wild species and cultivated species, inherent low oleic acid content of spanish types and limited success of transfer from other species/subspecies due to association of undesirable linkages and lengthy time to develop inter-specific derivatives are the major-factors which limited the oil quality improvement in spanish types. Given the large scale utility of groundnut as oilseed crop and short duration erect spanish bunch cultivars grown on large scale in major rainfed ecosystem of our country necessitates oil quality improvement in spanish types. In this direction, the induced genetic variability in two spanish bunch genotypes along with other desirable agronomic features of the spanish types such as high oil content, acceptable pod yield and yield components and early maturity will aid in wider acceptance and commercialization of the genotypes. The wide range of variation induced with respect to major fatty acids in groundnut in the present investigation is a significant initiative step towards addition of an important source of variability for the development of oils with a new fatty acid composition in groundnut in general and spanish bunch genotypes in particular.

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