Induction of genetic variability for fatty acid composition in a large-seeded groundnut variety through induced mutagenesis

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Fatty acid composition of groundnut (*Arachis hypogaea*) oil is an important trait with reference to human nutrition and oil stability during storage. In general, groundnut oil contains 6–20% palmitic acid, 1–6% stearic acid, 36–71% oleic acid, 20–48% linoleic acid, 1.0–3.5% arachidic acid, 2.7–5.1% behenic acid, 0.6–5.9% lignoceric acid and 0.0–1.5% linolenic acid (Savage and Keenan 1994). Oleic, linoleic and linolenic acids have been reported to lower plasma cholesterol levels and low density lipoproteins (Kris-Etherton et al. 1999). The higher the proportion of polyunsaturated fatty acids, the greater is the oxidation leading to unpleasant odors and tastes, thus limiting the storage quality of the oil (Sanders et al. 1993). Oils with high oleic acid to linoleic acid (O/L) ratio are less prone to oxidation and off-flavors and extend shelf life by delaying the development of rancidity (O'Keefe et al. 1993). Induction of genetic variability for fatty acid composition is desired and is a prerequisite in groundnut for its genetic improvement. Very few studies reported genotypic variability for fatty acids in groundnut germplasm. In this study, we report genetic variability for different fatty acids among gamma ray induced groundnut mutants from a large-seeded variety.

Materials and methods

TPG 41 is a Spanish bunch (*Arachis hypogaea* ssp *fastigiata* var *vulgaris*) large-seeded variety with 120 days maturity period and has been released for irrigated areas in summer season in India (Kale et al. 2004). Seeds (500 each) of TPG 41 were irradiated with 200 and 300 Gy of gamma rays during rainy season (June–September) 2004. The M_2 generation was grown in summer 2005 by advancing M_1 plants as plant-to-row progenies. Based on the breeding behavior in M_3, M_4 and M_5 generations, 69 true breeding mutants for various traits were isolated (Badigannavar et al. 2007). These mutants along with the parent were evaluated for fatty acid profile and per se performance. Experiments were laid out with two replications in randomized complete block design during summer and rainy season 2006. Plant spacing was 30 cm × 10 cm in summer and 45 cm × 10 cm in rainy season.

Sound matured seeds (10 each) in two replicates from 69 mutants and parent were ground to a fine paste using a mortar and pestle. Three ml of petroleum ether (boiling point 60–80°C) was added to 0.2 g ground paste, vortexed and kept overnight. Fatty acids were esterified by saponification-transesterification method (Metcalfe et al. 1966). Two ml of 0.5 N sodium hydroxide in methanol was added, vortexed and heated for 5 min in a boiling water bath (90°C). After cooling, 2 ml 10% boron trifluoride in methanol was added, vortexed and heated in a boiling water bath (90°C) and then 3 ml of petroleum ether and 2 ml of deionized water were added. When petroleum ether and water layer separated clearly, 1 ml was taken from top petroleum ether layer and added to 1.8 ml sample vial for injection to gas chromatography (GC) (Model GC 2010, Shimadzu, Kyoto, Japan). The GC apparatus is equipped with automated sampler and injector, having capillary column of 30 m length (RtxÒ-Wax, Restek, Pennsylvania, USA). The initial column temperature was 150°C and held for 1 min followed by an increase to 210°C at a rate of 20°C per min. Both injector and detector temperatures were set to 250°C. Gas flow rates of 25, 20 and 300 ml per min for nitrogen, hydrogen and air, respectively were maintained. Fatty acids were identified by comparing the retention time of standard fatty acid methyl ester mixture (Supelco, Bellefonte, Pennsylvania, USA) under same temperature condition and gas flow rate. Fatty acid percentages were arcsine transformed and the transformed data were used for analysis of variance using IRRISTAT 2.0 software (IRRI 2003).

Results and discussion

Breeding for modified fatty acid profile as per the requirement of industry is creating considerable interest. In the present study, palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), arachidic (20:0), eicosenoic (20:1), behenic (22:0) and lignoceric (24:0) acids were detected in the 69 induced groundnut mutants of TPG 41. These mutants significantly surpassed parental mean for all the fatty acids except arachidic acid in both rainy and summer seasons (Table 1). Estimation of fatty acids in 69 mutants of TPG 41 indicated greater variability for stearic acid, oleic acid and linoleic acid and lesser variability for palmitic acid, arachidic acid, eicosenoic acid, behenic acid and lignoceric acid. Further, this variability was more pronounced in summer than rainy season (Table 1). Palmitic acid increased in 10 mutants (TGM 3, 13, 18, 22, 40, 44, 57, 64, 77, 83) by 1.3–2.5% compared to parent (8.8%) in both the seasons. Similarly, seven of the mutants (TGM 44, 45, 46, 50, 51, 55, 59) had increased stearic acid by 1.1–2.7%. Oleic acid being an important fatty acid was enhanced in seven mutants (TGM 12, 19, 48, 54, 62, 71, 88) at the cost of linoleic acid by 3–6% resulting in an increase in O/L ratio to 4–5 compared to parent (Table 2). TGM 71 recorded the highest oleic acid and the lowest linoleic acid contents with mean O/L ratio of 5. Earlier studies on induced mutagenesis in groundnut reported an increase in O/L ratio to 1.65 (Badigannavar 2007) and 2.2 (Doo et al. 2008) using gamma rays, 3.3 using X-rays (Sharma et al. 1981) and to 3.5 using ethyl methane sulfonate (EMS) (Dwivedi et al. 1998). The Virginia and runner types (*Arachis hypogaea* var *hypogaea*) generally contain higher oleic acid and lower linoleic acid than Spanish types (Savage and Keenan 1994). Mutants in the present study being Spanish types with large seeds showed higher oleic acid content. In general, oleic acid content among the mutants was more from the crop grown in rainy season than summer, while the reverse was the case for linoleic acid. Such seasonal influence on oleic and linoleic acid contents was observed by Chiou et al. (1995) wherein spring crop had more oleic acid and lesser linoleic acid than the fall crop. The other fatty acids showed minor variation.

Induced mutants of TPG 41 were characterized for pod yield, seed yield, shelling outturn and seed size.

Table 1. Fatty acid content and yield traits among TPG 41 mutants during summer and rainy season 2006.

Description	Season	Mutants	TPG 41	CD (*P*=0.05)
Palmitic acid (%)	Summer	8.0–12.0	9.1	0.90
	Rainy	7.0–11.4	8.5	0.74
Stearic acid (%)	Summer	0.7–5.4	1.1	0.34
	Rainy	0.6–3.2	1.1	0.29
Oleic acid (O) (%)	Summer	44.3–67.8	57.0	3.75
	Rainy	59.0–74.4	66.7	4.18
Linoleic acid (L) (%)	Summer	15.2–32.1	21.9	4.21
	Rainy	10.7–22.9	16.8	3.74
Arachidic acid (%)	Summer	1.1–1.6	1.3	0.05
	Rainy	1.1–1.7	1.5	0.04
Eicosenoic acid (%)	Summer	0.8–1.1	0.8	0.02
	Rainy	0.0–2.6	0.9	0.04
Behenic acid (%)	Summer	0.6–2.7	2.1	0.10
	Rainy	1.7–2.6	2.0	0.08
Lignoceric acid (%)	Summer	0.9–1.5	1.1	0.06
	Rainy	0.9–1.5	1.0	0.05
O/L ratio	Summer	1.38–4.46	2.61	0.66
	Rainy	2.57–6.90	4.00	1.22
100-seed weight (g)	Summer	60.0–96.0	78.0	6.40
	Rainy	48.9–100.0	74.0	7.90
Pod yield (g plant^{-1})	Summer	8.8–39.5	32.3	3.70
	Rainy	27.0–64.7	49.7	6.80
Seed yield (g plant^{-1})	Summer	5.6–27.2	22.7	2.70
	Rainy	16.7–45.4	36.6	5.90
Shelling outturn (%)	Summer	58.0–74.0	70.1	3.20
	Rainy	57.2–75.6	73.7	3.90

Table 2. Fatty acid content and yield traits in selected mutants of TPG 41 during summer (S) and rainy (R) seasons 2006.

Mutants	Palmitic acid (%)			Oleic acid (O) (%)			Linoleic acid (L) (%)			O/L ratio			Pod yield (g plant^{-1})			Seed yield (g plant^{-1})			100-seed weight (g)			Shelling outturn (%)		
	S	R	Mean	S	R	Mean	S	R	Mean	S	R	Mean	S	R	Mean	S	R	Mean	S	R	Mean	S	R	Mean
TGM 12	9.1	8.4	8.7	63.7	70.4	67.1	18.8	14.4	16.6	3.4	4.9	4.0	22.8	45.8	34.3	15.3	31.6	23.5	80.0	83.0	81.5	67.0	69.2	68.1
TGM 13	11.3	11.4	11.3	56.7	59.6	58.2	22.8	20.0	21.4	2.5	3.0	2.7	31.2	58.0	44.6	22.4	42.2	32.3	92.0	80.9	86.5	71.7	72.8	72.3
TGM 18	10.0	10.3	10.1	56.4	60.6	58.5	23.1	21.3	22.2	2.4	2.8	2.6	23.4	46.8	35.1	16.6	32.9	24.7	88.0	81.2	84.6	70.9	70.3	70.6
TGM 19	9.7	7.0	8.4	62.0	70.3	66.1	19.7	12.8	16.3	3.1	5.5	4.1	21.7	52.6	37.2	13.2	30.1	21.6	84.0	57.2	70.6	60.8	57.2	59.0
TGM 35	11.0	7.8	9.4	62.5	66.7	64.6	19.6	15.2	17.4	3.2	4.4	3.7	33.0	37.7	35.3	21.6	27.0	24.3	94.0	100.0	97.0	65.5	71.7	68.6
TGM 36	9.7	8.6	9.2	61.0	67.4	64.2	21.6	15.8	18.7	2.8	4.3	3.4	26.4	48.2	37.3	19.5	33.1	26.3	90.0	73.6	81.8	74.1	68.7	71.4
TGM 42	10.7	8.9	9.8	56.5	68.5	62.5	23.5	15.3	19.4	2.4	4.5	3.2	27.6	60.3	44.0	19.6	42.4	31.0	86.0	82.7	84.4	70.9	70.3	70.6
TGM 48	9.9	7.4	8.7	61.7	70.8	66.3	20.5	11.4	16.0	3.0	6.2	4.1	22.2	54.5	38.4	14.9	35.9	25.4	86.0	76.0	81.0	67.0	65.8	66.4
TGM 49	9.4	7.7	8.5	62.7	66.4	64.5	19.9	15.6	17.8	3.1	4.3	3.6	33.8	50.2	42.0	24.0	34.6	29.3	84.0	70.0	77.0	71.1	68.8	69.9
TGM 54	9.4	7.4	8.4	60.6	70.4	65.5	20.9	12.2	16.5	2.9	5.8	4.0	34.1	54.5	44.3	24.2	38.9	31.5	80.0	65.8	72.9	71.0	71.3	71.2
TGM 55	8.5	8.1	8.3	65.7	65.8	65.8	16.8	16.9	16.9	3.9	3.9	3.9	39.5	46.7	43.1	27.2	32.7	30.0	78.0	64.6	71.3	68.8	70.0	69.4
TGM 62	9.0	8.3	8.6	62.1	69.2	65.7	19.0	13.4	16.2	3.3	5.2	4.1	26.2	64.7	45.4	18.8	45.4	32.1	82.0	61.9	72.0	71.6	70.2	70.9
TGM 67	10.6	8.0	9.3	54.8	71.6	63.2	25.6	12.3	18.9	2.1	5.8	3.3	26.6	32.0	29.3	18.3	20.1	19.2	96.0	72.3	84.2	68.7	62.7	65.7
TGM 71	8.3	7.7	8.0	63.0	74.4	68.7	16.5	10.8	13.6	3.8	6.9	5.0	24.0	38.0	31.0	17.5	26.1	21.8	74.0	71.0	72.5	73.0	68.8	70.9
TGM 79	10.4	9.3	9.8	55.7	67.0	61.3	23.3	16.4	19.8	2.4	4.1	3.1	31.5	56.4	44.0	21.1	43.5	32.3	80.0	72.2	76.1	67.0	77.0	72.0
TGM 86	11.3	7.4	9.3	55.8	69.5	62.7	24.8	13.6	19.2	2.3	5.1	3.3	30.9	62.0	46.5	22.0	38.2	30.1	76.0	66.1	71.0	71.1	61.7	66.4
TGM 88	8.6	8.5	8.5	67.8	67.0	67.4	15.2	15.2	15.2	4.5	4.4	4.4	27.7	56.7	42.2	18.4	36.8	27.6	86.0	73.6	79.8	66.6	65.0	65.8
TPG 41	9.1	8.5	8.8	57.0	66.7	61.9	21.9	16.8	19.3	2.6	4.0	3.2	32.3	49.7	41.0	22.7	36.6	29.6	78.0	74.0	76.0	70.1	73.7	71.9
CD (P=0.05)	0.9	0.7	0.7	3.8	4.2	3.8	4.2	3.7	3.8	0.7	1.2	0.9	3.7	6.8	5.7	2.7	5.9	4.9	6.4	7.9	7.4	3.2	3.9	3.7
CV (%)	2.6	4.1	3.6	4.2	3.8	4.1	6.1	4.8	5.7	4.5	5.2	4.8	6.5	7.9	7.5	6.2	7.2	7.0	5.5	4.2	4.9	2.3	1.6	2.0

Among the 69 mutants, TGM 55 in summer and TGM 42 and TGM 62 in rainy season surpassed significantly parent TPG 41 for pod and seed yields (Table 2). Further, seed size in TGM 13, 18, 35, 36, 42, 48, 67, 88 during summer and in TGM 12, 35, 42 during rainy season increased significantly compared to the parent. Earlier, high yield potential in X-rays, gamma rays and EMS induced groundnut mutants were demonstrated due to mutation for large seeds (Patil 1974, Mouli et al. 1987, Branch 2002, Gowda et al. 2002). Thus, it is evident from the earlier and present studies that it is possible to induce genetic variability through induced mutagenesis for various fatty acids and yield traits. This variability would be effectively utilized to evolve useful groundnut genotypes through recombination breeding.

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