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ORIGINAL PAPER

Improvement of Oil Quality in Soybean [*Glycine max* (L.) Merrill] by Mutation Breeding

Archana Patil · S. P. Taware · M. D. Oak · S. A. Tamhankar · V. S. Rao

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Abstract Low oxidative stability, off-flavor and rancidity are the major drawbacks of soybean oil. Modification of the fatty acid composition of soybean [*Glycine max* (L.) Merrill] oil can improve its quality and value for processors and acceptability among consumers. Mutation breeding of soybean was therefore initiated with the objective of identifying stable soybean mutants with altered fatty acid composition for improved oxidative stability and nutritional quality. Seeds of soybean cultivar 'MACS 450' were treated with γ-radiation and/or ethyl methane sulfonate (EMS). The harvest of M_1 plants was evaluated for fatty acid composition by gas chromatography. Highly significant variation in all the fatty acids except palmitic acid was observed. Treatment of EMS in higher concentrations as well as combined treatment of both the mutagens, i.e., γ-radiation and EMS were effective in increasing the variability for the fatty acid content in soybean oil. The variability was skewed towards high levels of oleic (35–42%) and low levels of linolenic acid (3.77–5.00%). M_3 and M_4 generations of desirable variants were analyzed for the stability of the mutated trait. Only high oleic variants were stable in M_3 and M_4 generations. Based on fatty acid values, oxidative stability index (OSI), nutritional quality index (NQI) and ratio of essential fatty acids (ω_6/ω_3) were calculated for the control and M_2, M_3 and M_4 generations. The ω_6/ω_3 ratio in all the high oleic mutants was within the World Health Organization (WHO) recommended value (5–10%). A significant positive correlation between OSI and oleic acid content ($P < 0.001$) indicated improved oxidative stability of the oil while retaining nutritional quality. These high oleic lines could be utilized further in breeding programs for improvement of soybean oil quality.

Keywords Soybean · Oil quality · Mutation · Fatty acid · γ-Radiation · Ethyl methane sulfonate

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the major oil crops of the world. Soybean seeds contain 20% oil. Its acceptability among consumers is somewhat diminished due to its low oxidative stability, off-flavor and rancidity [1]. Soybean oil contains an average of 110 g/kg palmitic acid, 30 g/kg stearic acid, 240 g/kg oleic acid, 550 g/kg linoleic acid and 70 g/kg linolenic acid [2]. The excessive content of polyunsaturated fatty acids limits the utility of soybean oil as a cooking oil, unless it is hydrogenated [3]. During the process of hydrogenation along with the conversion of unsaturated fatty acids into saturated ones, many positional and *trans*-isomers are also produced. *Trans* fats produced during hydrogenation increase the chances of developing heart disease and are currently a hot issue in human health [4]. Modification of soybean oil composition to reduce polyunsaturated fatty acids can be a viable alternative to improving the oil stability, flavor and eliminate the need for hydrogenation [5]. Generally, oil with a high content of monounsaturated fatty acids (e.g., oleic acid) is less susceptible to oxidative changes during refining, storage and frying. This oil can be heated to higher temperature without producing smoke, so that the food can

A. Patil · S. P. Taware · M. D. Oak · S. A. Tamhankar · V. S. Rao (✉)
Genetics Group, Division of Plant Sciences, Agharkar Research Institute, Agarkar Road, Pune 411004, Maharashtra, India
e-mail: arimacs@pn2.vsnl.net.in

be cooked faster and without absorbing much oil. Therefore, there is increasing interest among food industries and consumers to produce oil crops with high monounsaturated fatty acids (oleic acid) and a low content of polyunsaturated fatty acids (linoleic and linolenic acids).

Development of new lines with desirable alteration to the fatty acid composition of the seed oils can be achieved through conventional breeding, coupled in some cases with mutagenesis without adversely affecting agronomic characteristics of the plant. Such alterations in the fatty acid composition through the use of physical as well as chemical mutagens have been reported for many oil seed crops including soybean [6–11]. However, these soybean lines with altered fatty acid composition are either inaccessible or difficult to adapt to Indian conditions.

The present study was therefore initiated with the objective of developing soybean lines with beneficially altered fatty acid composition from an indigenous soybean variety by mutagen treatment. The stability of the desired mutants was also tested further in the M_3 and M_4 generations.

Materials and Methods

Material

About 3 kg seeds of soybean cultivar MACS 450 were used for mutagenic treatments.

Mutagen Treatments

Seeds of soybean cultivar MACS 450 were treated with ethyl methane sulfonate (EMS) and γ-radiation in varying concentrations. Treatments (150 g, approximately 1,000 seeds per treatment) consisted of 4 different doses of γ-radiation (100, 150, 200 and 250 Gy), 3 different concentrations of EMS (0.05, 0.10 and 0.15%) and combinations of both (Table 1) making of a total 19 mutation treatments. Untreated seed stock of the same cultivar was used as a control. Seeds were irradiated with γ-radiation at Bhabha Atomic Research Center (BARC) Mumbai, India. EMS solutions were prepared in 0.1 M phosphate buffer (pH = 7.0). Seeds were presoaked in air-bubbled water at 22 °C for 16 h to allow uptake of EMS. Presoaked seeds were then treated with EMS for 8 h at room temperature in cloth bags. Treated seeds were rinsed in running water for 2 h and planted wet in the field. The area was irrigated immediately after sowing to prevent the desiccation of the seeds. The seeds were sown in a randomized complete block design in four replications with two rows per treatment. Each row consisted of 100 seeds with a distance of 5 cm within the rows and 45 cm between the rows. About five seeds from each M_1 plant were harvested and bulked for each treatment.

Table 1 Mutagen treatments given to seeds of Soybean var. MACS 450

Physical mutagen (γ-radiation)	
1	100 Gy
2	150 Gy
3	200 Gy
4	250 Gy
Combined (γ-radiation + EMS)	
5	100 Gy + 0.05%
6	100 Gy + 0.10%
7	100 Gy + 0.15%
8	150 Gy + 0.05%
9	150 Gy + 0.10%
10	150 Gy + 0.15%
11	200 Gy + 0.05%
12	200 Gy + 0.10%
13	200 Gy + 0.15%
14	250 Gy + 0.05%
15	250 Gy + 0.10%
16	250 Gy + 0.15%
Chemical mutagen (EMS)	
17	0.05%
18	0.10%
19	0.15%

Fatty Acid Analysis

Approximately 200 M_2 seeds (M_1 harvest) of each treatment were analyzed for fatty acid composition. For fatty acid extraction from M_2 seeds, a part of the cotyledon tissue from each seed was cut with a razor and placed in a separate glass tube for extraction while the embryos were kept intact. The fatty acid extraction was carried out according to the method of Primomo et al. [12].

Fatty acid analysis was carried out on a gas chromatograph with an auto sampler and auto injector (6890 N series, Agilent Technologies Inc., Wilmington, DE, USA) using an HP-Innowax capillary column (J&W Scientific, Agilent Technologies Inc., Wilmington, DE, USA). The temperatures of injector, oven and detector were adjusted to 225, 150 and 275 °C, respectively. The initial oven temperature of 150 °C was ramped by 15 °C/min up to 250 °C. The air, hydrogen and nitrogen (carrier gas) flow rates were set to 400, 30 and 2 mL/min, respectively. Methyl esters of palmitic, stearic, oleic, linoleic and linolenic acids (Sigma Chemical Co., St Louis, MO, USA) were used as standards

to calibrate the method. The signals from the detector were integrated as normalized percentages from the calibration curve by using the HP CHEMSTATION software (Agilent Technologies Inc., Wilmington, DE, USA).

Mutants showing desirable variations in fatty acid composition were advanced to a next generation. The M_3 seeds of these plants were analyzed and stable variants were sown to harvest the M_4 generation. In the M_3 and M_4 generations, ten seeds from each plant were bulked and analyzed in replicates for testing the stability of the mutated trait.

Statistical Analysis

Standard deviation and coefficient of variance were calculated in the M_2 generation for each mutagenic treatment and control. One-way analysis of variance was computed to ascertain fatty acid differences in the M_2 generation of all treatments and the control using Agrobase/4^{TM} (Agronomix Software Inc., MB, Canada). Significant differences between and within treatments means were determined using least significant difference (LSD) values. The nutritional quality index (NQI), oxidative stability index (OSI) and ratio of essential fatty acids, i.e., linoleic acid/linolenic acid (ω_6/ω_3) were calculated for high oleic mutants [13, 14]. In stable high oleic variants, simple correlation coefficients were also calculated for fatty acid content and different quality parameters using Agrobase/4.

Results and Discussion

Variability in Fatty Acid Composition

The utility of any vegetable oil is largely determined by its fatty acid composition. The high content of polyunsaturated fatty acids in soybean oil affects its acceptability among consumers. There are reports of increasing variability in fatty acid composition by means of interspecific hybridization [15] and mutagenic agents [8, 11]. Though wild *Glycine* species showed extensive variations of fatty acid composition, they are not good sources for improving soybean oil quality due to high linolenic acid content [16]. The development of soybean lines with altered fatty acid composition was therefore undertaken in a popular Indian cultivar MACS 450. To our knowledge, this is a first report of its kind in India.

As a result of mutagenic treatments, a wide range of variability for all the fatty acid was observed in M_2 seeds. Analysis of variance of M_2 seeds of all 19 treatments and the control indicated significant variation between and within treatments and the control (Table 2). Stearic acid, oleic acid and linolenic acid contents showed more variability compared to palmitic and linoleic acid (Table 3). The seeds treated with the combination of higher concentration of both EMS and γ-radiation displayed a highly significant coefficient of variation (CV) than the control for all the fatty acids except palmitic acid (Table 3). The CV values for all the fatty acids in mutated material were higher than the corresponding CV values in Indian soybean cultivars [17, 18]. Variation in quantitative traits such as % germination, % leaf abnormalities, % survival, days to maturity, plant height, number of pods per plant, seed weight per plant, seed yield at M_1 generation have also been reported earlier [19].

Table 2 Analysis of variance of fatty acids in M_2 generation of 19 mutation treatments and control

Source	Mean squares					
	df	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
Treatments	19	0.21**	0.10**	14.74**	11.63**	0.40**
Block	03	0.10	0.02	10.57**	5.95**	0.13
Error	57	0.04	0.02	2.30	1.34	0.10

** Significant at probabilities of 0.01

Among the fatty acids, most variation was observed for stearic acid. Seven treatments showed a higher CV for stearic acid as compared to the control (Table 3). Out of these seven treatments five showed highly significant variation with a mean value higher than the control (Table 3). EMS treatment of 0.1% showed a maximum CV of 34% with a wide range of variation (1.8–6.77%). Hence, an EMS treatment of 0.1% seems to be effective for developing high stearic acid mutants. Such mutants will be of importance in confectionaries, margarines and related products.

Thirteen treatments showed larger variation for oleic acid as compared to the control. However, highly significant variation was observed in nine treatments with mean values higher than control. EMS treatments of 0.05 and 0.1% showed greater CV than the other treatments (Table 3). Variation for linoleic acid was found in ten treatments (Table 3). Nine treatments showed highly significant lower mean values for linoleic acid than control. However, it was lesser in comparison to other fatty acids but was greater than palmitic acid. For palmitic acid, a higher CV than the control was observed in only two treatments of 100 Gy γ-radiation and 0.15% EMS. Treatment with 100 Gy γ-radiation showed highly significant variation with higher mean value of palmitic acid than the control. The doses of γ-radiation and EMS concentrations used for mutagenesis in the present study appear to be less effective for inducing wide variations in both linoleic and palmitic acid. Higher concentrations of mutagens could be

Table 3 Fatty acid variation in M_2 generation

Fatty acid	Treatments	Minimum (%)	Average (%)	Maximum (%)	SD
Palmitic acid	Control	8.2	9.2	10.9	0.5
	100 Gy	8.6	9.7**	13.0	0.7
	250 Gy + 0.10% EMS	7.2	8.9#	9.8	0.5
	0.10% EMS	7.7	9.0#	10.0	0.5
	0.15% EMS	7.6	8.9#	11.5	0.6
Stearic acid	Control	1.7	2.2	2.6	0.2
	200 Gy	1.8	2.4	3.8	0.5
	250 Gy	1.7	2.5**	4.5	0.6
	200 Gy + 0.15% EMS	2.0	2.5**	4.0	0.5
	250 Gy + 0.05% EMS	1.8	2.5**	4.2	0.6
	250 Gy + 0.10% EMS	1.7	2.5**	4.5	0.6
	250 Gy + 0.15% EMS	1.9	2.6**	4.1	0.6
	0.10% EMS	1.8	2.3	6.8	0.8
Oleic acid	Control	18.7	26.3	34.4	3.4
	100 Gy	14.6	26.9	40.9	4.9
	200 Gy	20.0	30.0**	43.2	4.8
	250 Gy	23.4	30.7**	40.9	4.7
	100 Gy + 0.10% EMS	19.6	29.5**	41.6	4.8
	100 Gy + 0.15% EMS	18.4	27.2	42.8	5.0
	200 Gy + 0.05% EMS	23.9	31.2**	40.9	3.9
	200 Gy + 0.10% EMS	18.4	30.3**	40.9	5.5
	200 Gy + 0.15% EMS	22.0	30.2**	40.6	4.5
	250 Gy + 0.05% EMS	18.3	31.5**	44.1	5.4
	250 Gy + 0.10% EMS	17.6	29.5**	41.0	4.9
	250 Gy + 0.15% EMS	22.5	30.9**	43.1	5.4
	0.05% EMS	18.2	26.7	47.9	5.4
	0.10% EMS	18.1	26.2	36.6	5.0
Linoleic acid	Control	48.8	55.8	62.3	2.7
	200 Gy	41.1	52.2##	61.6	4.2
	250 Gy	43.8	51.4##	57.4	3.6
	100 Gy + 0.10% EMS	42.5	53.3##	61.1	3.9
	200 Gy + 0.05% EMS	43.6	51.6##	57.2	3.1
	200 Gy + 0.10% EMS	44.4	52.3##	61.5	4.2
	200 Gy + 0.15% EMS	43.2	52.0##	59.8	4.0
	250 Gy + 0.05% EMS	41.5	51.2##	61.8	4.2
	250 Gy + 0.10% EMS	44.6	53.0##	61.0	3.7
	250 Gy + 0.15% EMS	40.9	51.7##	58.7	4.3
	0.05% EMS	37.9	55.6	62.5	4.4
Linolenic acid	Control	5.5	6.5	8.4	0.6
	100 Gy	4.9	6.3	10.4	0.9
	100 Gy + 0.15% EMS	4.6	6.7	9.3	1.1
	200 Gy + 0.05% EMS	4.6	5.8##	7.9	0.7
	200 Gy + 0.10% EMS	4.2	6.1	8.9	1.1
	200 Gy + 0.15% EMS	4.5	5.8##	7.2	0.6
	250 Gy + 0.05% EMS	3.9	5.7##	7.9	0.9
	250 Gy + 0.10% EMS	4.3	6.1	10.2	1.2
	250 Gy + 0.15% EMS	3.9	5.7##	7.9	0.9

** Significantly higher than control at probabilities of 0.01

#, ## Significantly lower than control at probabilities of 0.05 and 0.01, respectively

Table 4 Fatty acid composition of high oleic mutants (HOM 1–11) at M_2, M_3, M_4 generations and control

Mutants	Palmitic (%)	Stearic (%)	Oleic (%)	Linoleic (%)	Linolenic (%)	OSI	NQI	ω_6/ω_3
HOM 1								
M_2	8.6	2.2	36.5	47.1	5.6	0.7	4.9	8.4
M_3	12.1 ± 0.04	3.0 ± 0.03	45.2 ± 0.4	35.4 ± 0.3	4.3 ± 0.2	1.1	2.6	8.2
M_4	11.5 ± 0.1	2.6 ± 0.1	35.0 ± 0.2	45.9 ± 0.2	5.0 ± 0.1	0.7	3.6	9.2
HOM 2								
M_2	9.6	1.8	33.4	49.4	5.7	0.6	4.8	8.6
M_3	8.8 ± 0.2	1.9 ± 0.1	38.6 ± 0.2	45.7 ± 0.2	5.0 ± 0.3	0.8	4.7	9.1
M_4	11.7 ± 0.1	2.7 ± 0.1	36.4 ± 0.6	44.3 ± 0.7	4.8 ± 0.1	0.7	3.4	9.2
HOM 3								
M_2	9.4	2.2	34.5	49.1	4.8	0.6	4.7	10.2
M_3	7.8 ± 0.1	1.4 ± 0.03	51.5 ± 0.5	34.0 ± 0.3	5.3 ± 0.1	1.3	4.3	6.4
M_4	11.3 ± 0.1	2.8 ± 0.1	36.0 ± 0.2	45.2 ± 0.3	4.5 ± 0.1	0.7	3.5	9.9
HOM 4								
M_2	9.4	2.2	34.5	49.1	4.8	0.6	4.7	10.2
M_3	7.5 ± 0.04	1.5 ± 0.03	49.4 ± 0.3	36.0 ± 0.2	5.6 ± 0.1	1.2	4.6	6.5
M_4	11.2 ± 0.05	3.5 ± 0.1	41.1 ± 0.4	40.4 ± 0.5	3.8 ± 0.1	0.9	3.0	10.7
HOM 5								
M_2	9.0	2.0	34.9	48.5	5.6	0.6	4.9	8.6
M_3	8.4 ± 0.04	1.9 ± 0.04	36.3 ± 0.2	46.8 ± 0.1	6.5 ± 0.1	0.7	5.1	7.2
M_4	12.5 ± 0.1	3.0 ± 0.1	34.9 ± 0.4	45.0 ± 0.3	4.5 ± 0.1	0.7	3.2	9.9
HOM 6								
M_2	9.0	1.9	34.9	48.5	5.6	0.6	5.0	8.6
M_3	7.9 ± 0.03	1.4 ± 0.04	47.4 ± 0.3	37.9 ± 0.3	5.3 ± 0.1	1.1	4.5	8.6
M_4	12.1 ± 0.1	3.0 ± 0.1	35.8 ± 0.4	44.1 ± 0.5	5.0 ± 0.1	0.7	3.3	8.7
HOM 7								
M_2	8.3	1.9	42.8	42.0	4.9	0.9	4.6	8.5
M_3	7.5 ± 0.1	1.8 ± 0.04	47.8 ± 0.3	36.7 ± 0.3	6.1 ± 0.1	1.2	4.6	6.0
M_4	11.7 ± 0.1	3.0 ± 0.1	36.7 ± 0.5	43.5 ± 0.5	5.1 ± 0.1	0.8	3.3	8.6
HOM 8								
M_2	8.3	1.9	42.8	42.0	4.9	0.9	4.6	8.5
M_3	8.0 ± 0.05	2.0 ± 0.05	38.2 ± 0.1	44.9 ± 0.2	6.9 ± 0.2	0.7	5.2	6.5
M_4	11.4 ± 0.1	2.8 ± 0.1	37.1 ± 0.2	43.8 ± 0.4	4.9 ± 0.1	0.8	3.4	8.9
HOM 9								
M_2	8.7	2.0	36.4	48.0	4.9	0.7	5.0	9.7
M_3	9.1 ± 0.1	1.9 ± 0.1	36.9 ± 0.4	46.8 ± 0.3	5.3 ± 0.1	0.7	4.7	8.7
M_4	11.2 ± 0.4	3.0 ± 0.4	37.9 ± 0.4	43.3 ± 0.4	4.6 ± 0.1	0.8	3.4	9.5
HOM 10								
M_2	8.8	1.9	35.2	48.5	5.4	0.6	5.0	9.0
M_3	8.8 ± 0.05	1.6 ± 0.04	36.3 ± 0.4	46.5 ± 0.4	6.7 ± 0.1	0.7	5.1	6.9
M_4	11.8 ± 0.05	3.4 ± 0.6	35.1 ± 0.5	45.2 ± 0.6	4.4 ± 0.1	0.7	3.3	10.3
HOM 11								
M_2	8.7	2.6	40.8	42.8	5.1	0.8	4.3	8.3
M_3	10.1 ± 0.1	2.8 ± 0.04	37.2 ± 0.3	44.3 ± 0.2	5.58 ± 0.1	0.7	3.9	7.9
M_4	11.2 ± 0.1	3.3 ± 0.3	37.5 ± 0.7	43.3 ± 0.6	4.7 ± 0.04	0.8	3.3	9.3
MACS 450 (control)								
2003	8.6 ± 0.1	2.3 ± 0.04	26.8 ± 0.3	55.1 ± 0.3	7.3 ± 0.1	0.4	5.7	7.5
2004	8.7 ± 0.04	2.5 ± 0.05	28.4 ± 0.2	53.9 ± 0.2	6.5 ± 0.04	0.5	5.4	8.3
2005	9.0 ± 0.1	2.3 ± 0.1	26.9 ± 0.2	54.7 ± 0.2	7.2 ± 0.1	0.4	5.5	7.6

M_3 and M_4 generations are the mean of three samples

useful to induce more variability in linoleic and palmitic acid.

For linolenic acid, eight treatments showed variation as compared to the control. Four treatments with a combination of both mutagens showed highly significant variation with a mean value lower than the control. It was observed that the seeds treated with the combination of γ-radiation and EMS (250 Gy + 0.05%) showed greater CV giving a wide range of variation (3.93–7.86%) (Table 2). Less than 3% linolenic acid content in the oil is desirable due to its more oxidative stability. The maximum reduction in linolenic acid content was observed with the 100 Gy + 0.05% EMS treatment. The observed linolenic acid content (3.93%) in this treatment is 50% less than the control (8%). Similar values of 3–4% linolenic acid have also been reported previously in two soybean mutants A5 [6] and C1680 [20] produced by chemical mutagenesis.

Stability of Mutants in M_3 and M_4 Generation

Fatty acid variants from the M_2 generation (total 393) with desirable variations such as high stearic/high oleic/low linolenic acid were transferred to the field. The soil was a vertisol type with pH 7.7. Out of these, only 94 could be harvested. Elevated stearic acid and low linolenic acid variants did not show stability for the trait in the M_3 generation, indicating the unstable nature of the trait across environments. This agrees well with the previous report by Hawkins et al. [21] where the normal level of stearic acid was found to be relatively stable as compared to elevated levels across different environments. High oleic acid variants were comparatively stable. Out of 55 high oleate M_2 plants harvested, 11 plants showed stability in the M_3 and M_4 generations (Table 4, Fig. 1). The oleic acid content in the M_4 generation of these 11 mutant plants was 34–42% as compared to 27% in the control cultivar MACS 450 (Fig. 1). The maximum values among these were obtained by combination treatments 100 Gy + 0.05% EMS and 100 Gy + 0.15% EMS. Two other high oleic mutants obtained by X-ray irradiation, viz., M23 (46.1%) and M11 (35.9%) have been reported previously by Rahman et al. [8] and Takagi and Rahman [22], respectively. The oleic acid content in M_4 plants was closer to the respective values in M_2 rather than M_3 (Table 4, Fig. 1). The oleic acid content of the M_3 plants was higher as compared to the M_2 and M_4 plants. This may be because of the environmental effect on the high oleic acid trait. The M_2, M_3 and M_4 generations were taken in 2003, 2004 and 2005, respectively, and the rainfall in 2004 was relatively higher than 2003 and 2005 (data not shown). Previous studies conducted in

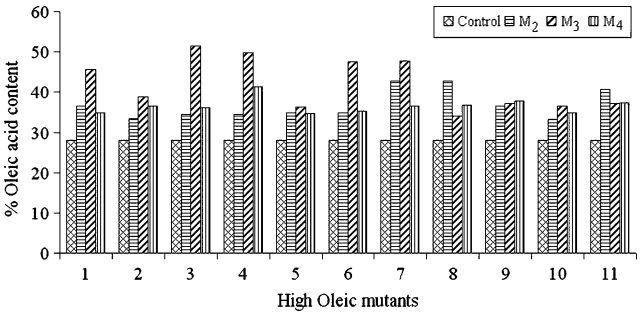

Fig. 1 Oleic acid content in high oleic mutants determined at the M_2, M_3 and M_4 generations and the control

controlled environments have also shown that temperature [23] and precipitation [24] have a major impact on fatty acid levels in soybean seeds. Therefore, effects of environmental factors on these high oleic mutants need to be studied.

The rate of oxidation of linolenic (18:3), linoleic (18:2) and oleic acid (18:1) are in the ratio of 21.6:10.3:1, which indicates that the oil containing high amounts of oleic acid has more oxidative stability. The OSI of high oleic mutants was higher than the control (Table 4). Linoleic acid and linolenic acid are the essential fatty acids. The World Health Organization (WHO) recommends an essential fatty acids ratio of 5–10 in the diet. Hence to check the nutritional quality of high oleic mutants the ratio of essential fatty acids was calculated. The ratio of essential fatty acids (ω_6/ω_3 ratio) in all the high oleic mutants were also within the WHO recommended value of 5–10 (Table 4). The OSI and ω_6/ω_3 ratio values indicate that oil from these mutants will have a good shelf life without affecting nutritional quality.

Correlation Among Fatty Acids and Quality Parameters

Correlation coefficients between the five fatty acids, OSI, NQI and ω_6/ω_3 ratio are listed in Table 5. A highly significant negative correlation between oleic acid and linoleic acid was observed ($P < 0.001$). There was a consistent decrease in the linoleic acid content with a corresponding increase in the oleic acid content. An association between these two fatty acids has also been reported previously in soybean [25], sunflower [26] and safflower [27]. Conversion of oleic acid to linoleic acid takes place by introducing a second double bond in the oleic acid by both plastidial and microsomal ω_6 desaturases. The high oleic and low linoleic acid content of these mutants might be due to the mutation in the seed specific, *fad 2–1* gene, which codes for the microsomal ω_6 desaturase enzyme [28].

A significant positive correlation between OSI and oleic acid content and negative association between OSI and

Table 5 Correlation between fatty acid content and quality parameters in stable high oleic mutants

Fatty acid	Palmitic	Stearic	Oleic	Linoleic	Linolenic	OSI	NQI
Stearic	–0.06						
Oleic	–0.64	0.39					
Linoleic	0.48	–0.49	–0.97***				
Linolenic	0.27	–0.72*	–0.73*	0.74*			
OSI	–0.55	–0.48	0.99***	–0.99***	0.80**		
NQI	–0.20	–0.79**	–0.53	0.68*	0.77**	–0.64	
ω_6/ω_3	–0.10	0.71*	–0.49	–0.49	–0.95***	–0.57	–0.69*

*, **, *** Significant at probabilities of 0.05, 0.01, 0.001, respectively

linoleic acid is well anticipated ($P < 0.001$). There was a negative but non-significant association between OSI and NQI, which indicates that improvement in these two quality parameters could be brought about by selecting desirable recombinants. Rahman et al. [25] developed a recombinant line DHL with high oleic acid and low linolenic acid content by combining the *ol* locus for the high oleic trait from HOLL and *fanx*^{a} locus from LOLL. A strong significant but negative correlation between linolenic acid and ω_6/ω_3 ratio ($P < 0.001$) was also as expected.

Conclusion

Treatments of EMS in higher concentrations as well as combined treatment of both the mutagens, i.e., γ-radiation and EMS were effective in increasing the variability for the fatty acid content in soybean oil. The variability was skewed towards high levels of oleic acid and low levels of linoleic acid, thus improving the oxidative stability of the soybean oil without affecting the nutritional quality. Research on the stability of the high oleic trait across the environments and its inheritance is ongoing. After establishing the stability of the trait, the stable high-oleic mutants could be utilized in breeding programs for the improvement of the soybean oil quality.

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References

1. Frankel EN (1980) Soybean oil flavor stability. In: Erikson DR, Pryde EH, Brekke OL, Mounts TL, Falk RA (eds) Handbook of soy oil processing and utilization. ASA, St Louis, pp 229–244
2. Schnebly SR, Fehr WR (1983) Effects of year and planting dates on fatty acid composition of soybean genotypes. Crop Sci 33:716–719
3. Rakow BD, McGregor DI (1973) Opportunities and problems in modification of levels of rapeseed C18 unsaturated fatty acid. J Am Oil Chem Soc 50:400–403
4. Willet WC, Ascherio A (1994) *Trans* fatty acids: are the effects only marginal? Am J Public Health 84:722–724
5. Lui HR, White PJ (1992) Oxidative stability of soybean oils with altered fatty-acid compositions. J Am Oil Chem Soc 69:528–532
6. Hammond EG, Fehr WR (1983) Registration of A5 germplasm line of soybean. Crop Sci 23:192
7. Auld AD, Heikkinen MK, Erickson DA, Sernyk JL, Romero JE (1992) Rapeseed mutant with reduced levels of polyunsaturated fatty acids and increased levels of oleic acid. Crop Sci 32:657–662
8. Rahman SM, Takagi Y, Kubota K, Miyamoto K, Kawakita T (1994) High oleic acid mutant in soybean induced by X-ray irradiation. Biosci Biotech Biochem 58:1070–1072
9. Ntiamoah C, Rowlin GG, Taylor DC (1995) Inheritance of elevated palmitic acid in flax and its relationship to low linolenic acid. Crop Sci 35:148–152
10. Fernandez-Martinez JM, Mancha M, Osorio J, Graces R (1997) Sunflower mutant containing high levels palmitic acid in high oleic background. Euphytica 97:113–116
11. Fehr WR, Hammond EG (1998) Reduced linolenic acid production in soybeans. US Patent 5,850,030
12. Primomo VS, Falk ED, Ablett GR, Tanner JW, Rajcan I (2002) Inheritance and interaction of low palmitic and low linolenic soybean. Crop Sci 42:31–36
13. Carpenter DL, Lehman J, Manon DS, Slover HT (1976) Lipid composition of selected vegetable oils. J Am Oil Chem Soc 53:713–718
14. Ghafoorunissa KK (2000) Dietary prescriptions. In: Diet and heart disease. National Institute of Nutrition Publication, Hyderabad, pp 23–41
15. Rebetzke GJ, Pantalone VR, Burton JW, Carter TE Jr, Wilson RF (1997) Genotyping variation for fatty acid content in selected *Glycine max* × *Glycine soja* population. Crop Sci 37:1636–1640
16. Hymowitz T, Palmer RG, Hadley HH (1972) Seed weight, protein, oil and fatty acid relationships within the genus *Glycine*. Trop Agric (Trinidad) 49:245–250
17. Patil A, Taware SP, Raut VM (2004a) Quality of Indian soybean in relation to fatty acid composition. Indian J Genet Plant Breed 64:245–246
18. Kumar V, Rani A, Joshi OP (2004) Fatty acid profile of released cultivars of Indian soybean: identification of comparatively low linolenic and high oleic acid cultivars. Indian J Agric Sci 74:388–391
19. Patil A, Taware SP, Raut VM (2004b) Induced variation in quantitative traits due to physical (γ rays), chemical (EMS) and combined mutagen treatments in soybean [*Glycine max* (L.)

Merrill], Soyb Genet Newsl 32. http://www.soygenetics.org/pdf/inducedvariationinquantativetraitsduetophysica,chemi[1].PDF
20. Wilcox JR, Cavins JF (1986) Registration of C1640 soybean germplasm. Crop Sci 26:209–210
21. Hawkins SE, Fehr WR, Hammond EG, Cianzio SR (1983) Use of tropical environments in breeding for oil composition of soybean genotypes adapted to temperate climates. Crop Sci 23:897–899
22. Takagi Y, Rahman SM (1995) Variation of different fatty acids in mutants in comparison with natural soybean. Bull Fac Agric Saga Univ 79:23–27
23. Wolf RB, Cavins JF, Kleiman R, Black LT (1982) Effect of temperature on soybean seed constituents: oil, protein, moisture, fatty acids, amino acids and sugars. J Am Oil Chem Soc 59:230–232
24. Dornbos DL, Mullen RE (1992) Soybean seed protein and oil contents and fatty acid composition adjustments by drought and temperature. J Am Oil Chem Soc 69:228–231
25. Rahman SM, Takehito K, Toyoaki A, Takagi Y (2001) Combining ability in loci for high oleic and low linolenic acids in soybean. Crop Sci 41:26–29
26. Downey RK, Dorrell DG (1971) Genetic control of fatty acid in oil seed crops. Proc Flax Inst U S 41:1–3
27. Knowles PE, Hill AB (1964) Inheritance of fatty acid content in the seed oil of a safflower introduction from Iran. Crop Sci 4:406–409
28. Heppard EP, Kinney AJ, Stecca KL, Miao GH (1996) Developmental and growth temperature regulation of two different microsomal $\omega 6$ desaturase genes in soybean. Plant Physiol 110:311–319