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Correlation Studies and Coefficient of Variation among Fatty Acids and Oil Quality Parameters in Soybean Mutants

Digambar Dadaji Ahire

Post Graduate Department of Botany, New Arts, Commerce and Science College, Ahmednagar- 414 001, Maharashtra, India

Corresponding author: * **digambar12@rediffmail.com**

ABSTRACT

The interrelationships between fatty acids and oil quality parameters were determined by correlation studies to estimate the best selection criteria of mutants for improving oil quality. Correlation analysis results showed that a highly significant ($P < 0.01$) negative correlation of oleic (MUFA) with linoleic (r = -0.847) and linolenic acid (r = -0.692) (PUFAs) contents is important for selection of mutants with high oleic and low linolenic acid content to improve the oxidative stability index (OSI) of soybean oil. A strong significant but negative correlation was found between linolenic acid and the ω6/ω3 ratio (r = -0.764) and a strong significant positive correlation between OSI and oleic acid (r = 0.938) content indicating improved oxidative stability of the oil while retaining nutritional quality. Mutagenic treatments produced significant genetic variation in fatty acid composition and oil quantity without altering the population means. Among all quality parameters, OSI (50.0%) and stearic acid (62.8%) exhibited maximum variation compared to other traits among the fatty acids.

Keywords: correlation coefficient, mutants, oil quality, soybean
Abbreviations: EMS, ethylmethane sulphonate; **MUFA**, monounsaturated fatty acid; **NQI**, nutritional quality index; **OSI**, oxidative stability index; **PUFA**, polyunsaturated fatty acid

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is an annual leguminous species cultivated mainly for its seed oil. The seeds are used in variety of industries, providing products for human consumption, and livestock feed. Seeds of soybean are the major source of proteins (40%) and also consist of carbohydrate (35%), ash (5%) and oil (20%). Oil contains saturated as well as unsaturated fatty acids and shares about 40% of the world's edible vegetable oil (Hildebrand *et al.* 1986). Common soybean cultivar contains about 12% palmetic (16:0), 4% stearic (18:0), 23% oleic (18:1), 53% linoleic (18:2), and 8% linolenic acid (18:3) (Wilcox 1985; Stoltzfus *et al*. 2000; Fehr 2007; Lee *et al*. 2009). It is known that unsaturated fatty acids are healthier than saturated fatty acids (Mazur *et al.* 1999) and polyunsaturated fatty acids (PUFAs), although unstable due to the number of double bonds among their chains, are important for maintenance of cell membranes and making prostaglandins in human body (Simopoulos 1999).

Oleic acid is more stable than other unsaturated fatty acids, it is more resistant to oxidative changes during storage, refining and frying (Laga *et al.* 2004). Scientists have therefore modified the seed trait of soybeans to increase the concentration of oleic acid. Linoleic acid is used in making soaps, emulsifiers and quick-drying oils (Fehr 2007). It has become increasingly popular in cosmetic industries because of its beneficial properties on skin (Letawe *et al.* 1998; Darmstadt *et al.* 2002). The high linoleic acid (ω-6) content of soybean oil is potentially nutritionally negative, because diets high in linoleic acid content may reduce the nutritionally positive effects of the health-beneficial ω-3 fatty acids such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA n-3), and docosahexaenoic acid (DHA) in tissue (Friesen and Innis 2010; Blasbalg *et al*. 2011). High stearic acid (19% to 30%) content oils can be used in margarine oil production (Knowlton 2001). In soybean, elevated stearic acid phenotype is governed by recessive loci manifested via mutagenesis (Rahman *et al*. 2003; Spencer *et al*. 2003; Zhang *et al*. 2008). Two avenues have been successful for increasing stearic acid levels of seed oils: (1) heterologous expression of a stearoyl-ACP thioesterase (Hawkins and Kridl 1998; Facciotti *et al*. 1999) and (2) mutations (Bubeck *et al*. 1989; Rahman *et al*. 1995; Pantalone *et al*. 2002; Zhang *et al*. 2008).

More palmitic acid results in an increase in saturated fatty acids (SFAs), thus increasing the solidity of the oil, which is essential for margarine and shortening production (Takagi *et al*. 1995; Kok *et al*. 1999). Palmitic acid content has been elevated to above 40% of total fatty acids in lines with combinations of high-palmitic-acid mutant alleles (Stoltzfus *et al*. 2000). A reduction of palmitic acid increases the oil nutritional value, as it helps to increase low-density lipoprotein cholesterol in the blood and susceptibility to coronary heart disease (Minihane and Harland 2007). Also, consumption of less palmitic acid shows low occurrences of breast, colon and prostate cancers (Stojsin *et al*. 1998). High linolenic acid content (7-9%) is associated with poor flavor (fishy-painty-grassy-melony) and stability of soy oil (Dutton *et al*. 1951). The high concentration of linoleic acid and linolenic acid is disadvantageous for food production due to the fact that the oil is oxidized easily and the foods go rancid quickly (Warner and Gupta 2005). Research has been concentrated on soybean oil with high oleic acid, low linolenic acid (< 3%), high stearic acid and high palmitic (for industrial uses) and low palmitic acids (for nutritional value). This can be done by changing the degree of unsaturation and by increasing or decreasing the fatty acid chain length (Topfer *et al.* 1995). The most desired phenotype for soybean oil is < 7% saturates (palmitic + stearic), > 55% oleic, and < 3% linolenic because of its multiple uses in many edible and industrial applications (Wilson 2004; Lee *et al*. 2009).

Soybean is highly self pollinated (98%) crop (Ahrent and Caviness 1994). In soybean hybridization is a tedious process, due to small, fragile flowers that, makes it difficult

Received: 28 July, 2011. Accepted: 7 March, 2012.

Original Research Paper

to carry out the process of emasculation injuring the parts of the flower and prone to heavy flower shedding (> 75%) even under favorable conditions (Johnson and Bornad 1976). These coupled with complete self-fertility impose limitations on the success of hybridization programme (Singh *et al*. 2007). When genetic variability is diminished using traditional breeding methods, induced mutation is a good way to increase genetic diversity in soybean. One of the chief advantages of traditional mutagenesis is that it can give rise to many different mutant alleles with different degree of trait modification. Natural genetic variability for fatty acid composition in commercial soybean oil is limited (Rattray 1991), therefore, additional variability needs to be created by mutagenesis. Polyploidy and mutations have played a significant role in the evolution of oilseed crops has been reported by Bhatia *et al*. (1999). Mutagenesis has been an excellent tool for creating variability for fatty acid contents (Takagi *et al*. 1989; Singh and Hymowitz 1999; Velasco *et al*. 1999; Lee *et al*. 2008; Hudson 2012). Oilseed germplasm with modified oil characters have been generated through mutation breeding programs (Beaith *et al*. 2005). Genetic variation for fatty acid composition is vital for genetic improvement of the oil quality in oilseed crops (Hamdan *et al*. 2008). Soybean breeders have made great strides to move an elevated oleic acid phenotype into elite genotypes by exploiting natural variation in oleic acid levels among various sources of soybean germplasm (Takagi and Rahman 1996; Rahman *et al*. 2001; Alt *et al*. 2005a, 2005b). Conventional approaches to raise the oleic acid content in soybean oil has led to the development of "mid-oleic" phenotype, in which seed storage lipids range in oleic acid from 30 to 70%. The conventional approach to develop the mid-oleic phenotype has some drawbacks. First, the genetics of the phenotypes require the stacking of multiple loci (Alt *et al*. 2005a, 2005b), which may complicate the breeding process. Secondly, the "mid-oleic" phenotype is affected by environment, typically requiring growth in warmer climates for stability of the elevated oleic acid trait to be maintained. This is due to the temperature effect on the desaturase activity and expression (Heppard *et al*. 1996; Tang *et al*. 2005). The third drawback associated with the conventional breeding approach for a "mid-oleic" soybean is the germplasm that expresses this phenotype is associated with yield drag (Primomo *et al*. 2002). Traditional plant breeding and genetic manipulations of conventional oilseed crops have resulted in high-oleic oil varieties (Hu *et al*. 2006). The current mutation breeding technology using mild mutagens induces nucleotide base substitutions (point mutations) without causing major physical damage to chromosome structure (Beaith *et al*. 2005).

Recently, genetic engineering and candidate gene-based molecular breeding approaches were used to generate soybeans with oleic acid content over 80% of the total oil (Buhr *et al*. 2002; Dierking and Bilyeu 2009; Hoshino *et al*. 2010; Pham *et al*. 2010; Bolon *et al*. 2011). Anai *et al*. (2012) identified and characterized the mutations corresponding to two of three high-palmitic-acid mutant lines obtained by X-ray irradiation of a soybean population. The objectives of this study were to determine the interrelationships between fatty acids and quality parameters and their coefficient of variation and estimate the best selection criteria of mutants for oil quality improvement.

MATERIALS AND METHODS

Plant material and induction of mutation

Dry seeds of soybean variety 'MACS 450' were subjected to combination treatments of gamma rays and ethylmethane sulphonate (EMS) (50 Gy + 0.2% EMS, 50 Gy + 0.4% EMS, 100 Gy + 0.2% EMS and 100 Gy + 0.4% EMS). For each treatment, 800 seeds were exposed to gamma rays in gamma cell 200 with ^{60}Co (Cobalt-60) source at Bhabha Atomic Research Center (BARC), Mumbai, India and followed by the treatment with EMS prepared in phosphate buffer (pH 7.0) for 4 h. The treated seeds along with the control were sown in experimental field following randomized block design (RBD) with three replications at Botanic Garden, Department of Botany, University of Pune to raise the M_1 generation. The M_1 plants were harvested individually and the seeds obtained were used to raise the M_2 generation as plant-to-row progenies. The M_2 population was carefully screened for morphological mutations (plant habit, growth habit, foliage, flower type, pod type, seed type) maturity (early and late), high yielding (Ahire *et al.* 2005).

Selected mutants from M_2 generation were sown plant to progeny along with control for their multiplication and confirmation of stability in the M_3 generation. Further the true breeding lines and the selections from segregating lines were sown in triplicates in M_4 and M_5 generation with national check cultivar 'JS-335' and control to check agronomic performance, stability and for multiplication of mutants. Bulks of ten seeds were taken from each individual for checking overall plant performance for fatty acids content.

Fatty acid analysis by gas chromatography

In present study, the mutants [morphological (plant habit, growth habit, foliage, flower type, pod type, seed type) maturity (early and late), high yielding and showing yield near to the control] were selected and analyzed by gas chromatography for identification of low linolenic acid and other desirable fatty acid content. The digestion of fresh tissue, transmethylation of lipids, and the extraction of fatty acid methyl esters (FAMEs) were carried out as described by Garces and Mancha (1993) with the modification in use of pinch of sodium sulphate for dehydration in separated upper phase of heptane-containing FAMEs.

1. Preparation of methylating mixture

Methylating mixture contains methanol: benzene: 2, 2-dimethoxy propane (DMP): sulphuric acid (37: 20: 5: 2, v/v/v/v) and stored in amber colored bottle at 4°C. All the chemicals and reagents used in the present study were of analytical/HPLC/GC grade (97-99% pure) (Merck, Mumbai, India).

Sulphuric acid is efficient to prepare methyl esters from free fatty acids and glycerolipids. The limiting factor is the solubility of products during transmethylation reaction. Free fatty acids, polar lipids and resulting methyl esters are readily soluble in hot methanol, but non-polar lipids such as triacylglycerols (TAGs) are slightly soluble (Christie 1989). DMP improves the *trans*-methylation of glycerolipidis by converting the resulting glycerol to isopropylideneglycerol (Mason and Waller 1964). DMP also reacts with excess water and has been used in combine digestion and fatty acid methyl esters (FAMEs) preparation from fresh leaves (Browse *et al.* 1986).

2. Preparation of fatty acid methyl esters (FAMEs)

Soybean seed powder was placed in amber color glass vial and 1.6 ml methylating mixture was added to this 0.9 ml of heptane was added and the vials were caped and placed in water bath for 30 min at 80°C with intermittent shaking at 5-min intervals. Simultaneous digestion and lipid transmethylation takes place in a single phase during heating. This extracts the lipids from tissue and converts them to corresponding FAMEs. Then the vials were cooled to room temperature and form two phases of which upper phase contains the FAMEs. The upper phase was dehydrated using a pinch of sodium sulphate. After dehydration FAMEs was dried by N_2 gas and dissolved in 100 µl methanol and were used for GC analysis.

3. Analysis by gas chromatography

FAMEs were analyzed in a gas chromatograph (Shimadzu 17A Ver. 3 Co. Japan) using Capillary column (P/N 122-7032 DB-Wax. J & W Scientific, Agilent Technologies, Inc., Wilmington, DE, USA; length 30 mm × I.D. 0.25 mm; film 0.25 µm). The temperatures of injector, oven and detector (flame ionization detector, FID) were adjusted to 245, 150 and 251°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

Table 1 Correlation studies between fatty acids and quality parameters.

	Palmitic	Stearic	Oleic	Linoleic	Linolenic	ω6/ω3	OSI
Stearic	0.330**						
Oleic	-0.101*	-0.080					
Linoleic	-0.256**	-0.276**	-0.847**				
Linolenic	-0.336**	-0.196**	-0.692**	0.727**			
ω6/ω3	0.419**	0.084	0.239**	-0.241**	-0.764**		
OSI	0.041	0.051	0.938**	-0.913	-0.689**	0.232**	
NQI	-0.684**	-0.548**	-0.459**	0.787**	0.684**	-0.380**	-0.575**

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.
ω6/ω3: omega6/omega3, OSI: oxidative stability index, NQI: nutritional quality index

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 for calibration. The signals from FID were integrated as normalized percentages from the calibration curve by using Winchrom software (Indtech Instrument Pvt. Ltd., Mumbai).

Identification of fatty acids from soybean samples was done on the basis of retention time of standard fatty acids. An area under each peak and % component was calculated by automatic integrator to give relative comparison from following formula (Colin *et al*. 1974):

Area = (height) × (width at ½ height)

% Component 1 = [(area under peak 1)/(total area)] × 100%

Qualitative and quantitative analysis of oil

Oil quantification of all true breeding phenotypic and biochemical mutants was done by Nuclear Magnetic Resonance (NMR) analyzer (Oxford 4000, Analytical Instrument Ltd.).

The balance between fatty acids were calculated for superior oil quality and compared with recommended limits proposed by FAO (WHO and FAO Joint Consultation 1995).

The nutritional quality index (NQI), oil/oxidative stability index (OSI) and ratio of essential fatty acids, i.e., linoleic acid/ linolenic acid (ω6/ω3) were calculated for high oleic mutants (Carpenter *et al.* 1976; Ghafoorunissa 2000). These values were obtained by using following parameters:

1) Ratio ω6/ω3 = linoleic/ linolenic;
2) Oil Stability Index (OSI) = monounsaturated fatty acid/polyunsaturated fatty acid or oleic/ linoleic + linolenic;
3) Nutritional Quality Index (NQI) = polyunsaturated fatty acid/ saturated fatty acid or (linoleic+ linolenic)/ (palmitic+ stearic);

The observations were recorded on fatty acid composition from total 429 mutant progenies (163 progenies of 50 Gy + 0.2% EMS, 93 progenies of 50 Gy + 0.4% EMS, 44 progenies of 100 Gy + 0.2% EMS and 129 progenies of 100 Gy + 0.4% EMS) along with parent (40 progenies).

Experimental design and statistical analyses

The field experiments were carried out using randomized block design and subjected to statistical analysis and to study the induced variability. The minimum and maximum values, mean value, standard deviation and coefficient of variation (CV) were calculated in the M_3-M_5 generation for each mutagenic treatment and control by using software SPSS 9.0 (SPSS Inc., Chicago, Illinois, USA). For fatty acid analysis each samples were analyzed in three replicates. To assess the nature and magnitude of association between fatty acid profile and quality parameters, simple correlation coefficient were calculated by using bivariate correlation method (Pearson's correlation coefficient) at the probability level of 0.05 and 0.01 to test their significance.

RESULTS AND DISCUSSION

Correlation studies among fatty acids and quality parameters

The correlation studies among fatty acids and quality parameters of the mutants revealed significantly ($P < 0.01$) strong positive correlation of palmitic acid with stearic acid (r =0.330) and the ratio of ω3/ ω6 (r = 0.419). While it had highly significant but negative correlation with oleic acid (r = -0.101, $P < 0.05$), linolenic (r = -0.336), linoleic (r = -0.256) and NQI (r = -0.684) at $P < 0.01$ (**Table 1**). Furthermore correlation between palmitic acid and OSI was non-significant.

A negative, significant correlation ($P < 0.01$) was observed between stearic acid, linoleic (r = -0.276), and linolenic acids (r = -0.196) and NQI (r = -0.548). Correlation between stearic acids and oleic acid; ω3/ ω6, and OSI were non-significant. There were strong positive significant correlations ($P < 0.01$) of oleic acid with OSI (r = 0.938) and ω3/ω6 (0.239) while as anticipated strong and inverse significant ($P < 0.01$) relations were observed between oleic acid and linoleic (r = -0.847) and linolenic acids (r = -0.692) and NQI (r = -0.459) (**Table 1**).

Linoleic acid showed significant ($P < 0.01$) positive correlation with linolenic acid (r = 0.727) and NQI (r = 0.787) as expected and negative significant ($P < 0.01$) correlation with ratio of ω3/ ω6 (r = -0.241). However, it had no correlation with OSI. On the other hand linolenic acid showed positive significant ($P < 0.01$) correlation with NQI (r = 0.684), while it had negative correlation with ratio of ω6/ ω3(r = -0.764) and OSI (r = -0.689).

A significant ($P < 0.01$) but negative correlation was found between ratio of ω6/ω3 and NQI (r = -0.380), whereas it had significant ($P < 0.01$) positive correlation with OSI (r = 0.232). As expected there was strong significant but negative correlation between OSI and NQI (r = -0.575) (**Table 1**).

In the present study, palmitic acid showed positive correlation with stearic acid while negative correlation with oleic, linoleic and linolenic acids. Therefore, due to positive correlation between palmitic and stearic acid, there is great scope for selection of mutants with low saturates (palmitic + stearic) (< 7%) for oil quality improvement or oil with high saturates for industrial uses. In soybean, Stoltzfus *et al*. (2000) found significant positive correlations of palmitic with stearic and linoleic acid contents while it negatively correlated with oleic and linolenic acids. Significant negative correlations between oleate and palmitate were also reported by Rebetzke *et al*. (2001). Also, Primomo *et al*. (2002) reported significant negative correlations between oleate and palmitate only in one of six F_2 soybean populations segregating for different palmitate and linolenate alleles. Cardinal and Burton (2007) detected negative correlations between oleate and palmitate, as well as between oleate and stearate, only in one of three F4–derived populations segregating for the *fapnc*, *fap1*, and *fan* alleles. As reported by Bachlava *et al.* (2008), in soybean palmitate showed significant positive correlations with stearate, while it had significant negative correlation with oleate. Palmitic acid correlated with oleic acid significantly and negatively (r = -0.51) in safflower also reported by Golkar *et al*. (2011).

In the present study SFAs (palmitic and stearic) showed a significant negative correlation with PUFAs (linoleic and linolenic) content. This negative correlation is important to increase PUFAs content and decrease SFAs content or to increase the ratio of PUFAs/SFAs to improve NQI. A highly significant negative correlation of oleic (MUFA) with linoleic and linolenic acid (PUFAs) contents, is also important

Table 2 Mean, minimum and maximum values, standard deviation and coefficient of variation of quality parameters and oil percentage of 429 mutants and parent line.

Statistics	Quality parameters						Oil (%)	
	ω6/ω3 ratio		OSI		NQI			
	M	P	M	P	M	P	M	P
Minimum	3.2	7.0	0.2	0.3	0.5	3.0	8.7	19.3
Maximum	18.3	9.9	2.4	0.6	7.5	4.4	21.0	19.8
Mean	8.4	8.5	0.6	0.4	3.2	3.5	18.8	19.5
SD	1.8	0.9	0.3	0.1	0.9	0.4	1.8	0.2
CV (%)	22.0	10.6	50.0	25.0	26.7	11.4	9.7	1.0

M: mutant/s, P: parent, SD: standard deviation, CV: coefficient of variation. OSI: Oxidative Stability Index, NQI: Nutritional Quality Index

Table 3 Mean, minimum and maximum values, standard deviation and coefficient of variation of fatty acids of 429 mutants and their parent line.

Statistics	Fatty acids profile									
	Saturated						Unsaturated			
	Palmitic acid		Stearic acid		Oleic acid		Linoleic acid		Linolenic acid	
	M	P	M	P	M	P	M	P	M	P
Minimum (%)	6.6	10.1	1.0	2.0	12.9	19.5	18.9	47.0	1.5	5.4
Maximum (%)	28.2	15.1	19.2	5.0	61.9	32.3	61.7	56.3	11.4	7.9
Mean (%)	14.1	13.1	3.5	3.7	27.8	24.0	48.3	52.8	6.0	6.3
SD	2.8	1.1	2.2	0.7	8.7	4.0	8.1	2.7	1.6	0.8
CV (%)	20.2	8.4	62.8	18.9	31.4	16.0	16.8	5.1	27.3	12.7

M: mutant/s, P: parent, SD: standard deviation, CV: coefficient of variation.

to increase MUFA and lower PUFAs content or for selection of mutants with high oleic and low linolenic acid content to improve OSI of soybean oil. These results are in agreement with the earlier results in soybean. Alt *el al.* (2005) showed oleate content of 88 $F_{2:3}$ lines were significantly negatively correlated with palmitate (r = -0.47), stearate (r = -0.38), linoleate (r = -0.97) and linolenate (r = -0.45). A significant negative correlation of oleic acid (MUFA) with linoleic acid (r = -0. 988) and linolenic acid (r = -0.827) contents (PUFAs) was also reported by Wang (2006). A highly significant negative correlation of oleic acid (MUFA) with linoleic acid (r = -0.97) and linolenic acid (r = -0.73) (PUFAs) was observed by Patil *et al.* (2007) at $P < 0.001$ and $P < 0.05$, respectively. Bachlava *et al.* (2008) showed a significant but negative correlation of oleic acid (MUFA) with linoleic acid (r = -0.926) and linolenic acid (r = -0.308) contents (PUFAs) in soybean. The negative association between oleic acid and linoleic acid was also reported in safflower by Mahasi *et al.* (2009) and Golkar *et al*. (2011). High oleic to low linoleic genotypes reduce the need for oil hydrogenation during industrial processing and thus decreasing the production of harmful *trans*-fatty acids during such processing (Corbett 2002).

In the present study, negative association was found between oleic acid and other fatty acids such as palmitic, stearic, linoleic and linolenic. Soybean lines with elevated oleate content can be used to develop lines with elevated oleate combined with low saturated fatty acids, low linolenate, or both low saturates and low linolenate as has been reported by Scherder *et al.* (2008), who used a soybean line M-23 with elevated oleate content to develop lines with elevated oleate combined with low saturated fatty acids, 1% linolenate, or both low saturates and 1% linolenate. The significantly low levels in the saturated fatty acids (palmitic+ strearic) and the reduced levels in linoleic and linolenic acids of the high oleic acid genotypes compared to the parents and the typical cultivar offer the opportunity to develop soybean oils with higher oxidative stability (Pham *et al*. 2011).

The positive correlation between linoleic and linolenic acid was observed in the present investigation. Progress in selection for increased level of linoleic acid and reduced linolenic acid has been rather slow mainly due to a positive correlation between them as reported by Wang (2006). A strong significant but negative correlation was found between linolenic acid and ω6/ω3 ratio and a strong significant positive correlation between OSI and oleic acid content indicating improved oxidative stability of the oil while retaining nutritional quality. A non-significant association between OSI and NQI indicates that improvement in these two quality parameters could be brought about by selecting desirable recombinants. Similar results were obtained earlier by Patil *et al.* (2007) in soybean.

Coefficient of variation

Differences in means between the control samples and mutants were negligible for fatty acid profile, quality parameters and oil content, indicating that means were not affected by the mutagenic treatments. Mutagenic treatments produced significant genetic variation for fatty acid composition and oil quantity without altering the population means, it might be due to mutagens induces nucleotide base substitutions (point mutations) without causing major physical damage to chromosome structure. Among oil quality parameters; OSI exhibited maximum (50.0%) CV (**Table 2**). Stearic acid exhibited maximum CV (62.8%) followed by oleic acid (31.4%) and linolenic acid (27.3%) compare to other fatty acids in mutants (**Table 3**). Similarly Patil *et al*. (2007) reported that greater magnitude of induced variation in soybean was observed for stearic acid, oleic acid and linoleic acid content. Stearic and oleic acid showed greater variation than palmitic, linoleic and linolenic acids that have also been earlier reported by Ishikawa *et al*. (2001) in 60 soybean cultivars. In soybean for stearic acid, at least three SAD genes have been identified, designated *SAD-A*, -*B*, and -*C*. The first two are expressed constitutively (Byfield *et al*. 2006), while *SAD-C* is more highly expressed in seeds (Zhang *et al*. 2008). Brossman and Wilcox (1984) reported that increase in genetic variation was greatest for oleic acid and smallest for plant height. High CV in the fatty acid profile quality parameters and oil content traits in all the mutagenic treatments indicate better chances for selection to be successful. Therefore, these traits have high selection value and can be exploited for the improvement of soybean. There are reports of increasing variability in fatty acid composition by means of interspecific hybridization (Rebetzke *et al*. 1998) and mutagenic treatments (Rahman *et al*. 1994).

ACKNOWLEDGEMENTS

The author gratefully acknowledge the help extended by Department of Botany, University of Pune; Agharkar Research Institute, Pune and the financial assistance received from Board of Research in Nuclear Sciences (Ref. No. 2002/35/9/BRNS/175), Department of Atomic Energy, Government of India for undertaking the present work. The author thanks Dr. Jaime A. Teixeira da Silva for significant improvements to grammar.

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