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Fatty acid profile of released cultivars of Indian soybean (*Glycine max*) with special reference to identification of comparatively low linolenic and high oleic acid cultivars*

VINEET KUMAR[1], ANITA RANI[2] and OM PRAKASH JOSHI[3]

National Research Centre for Soybean, Indore, Madhya Pradesh 452 017

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Soybean [*Glycine max* (L.) Merr.] oil is one of the major sources of vegetable oil in India. In general, oil from common soybean cultivars consists of 11% palmitic acid (16:0), 4% stearic acid (18:0), 23 % oleic acid (18:1), 53% linoleic acid (18:2) and 7% linolenic acid (18:3) (Fehr *et al.* 1992). Linolenic acid, though, a precursor to omega fatty acids, is also considered as the main reason for poor shelf-life of soybean oil because the rate of oxidation of linolenic acid, linoleic acid and oleic acid are in the ratio of 21.6:10.3:1 (Fatemi and Hammond 1980). On the other hand, oleic acid being less susceptible to oxidation imparts oxidative stability to soybean oil. Partial hydrogenation of soybean oil employed by industries to improve oxidative stability results in the production of undesirable *trans* fatty acids (Willet and Ascherio 1994, Lichtenstein *et al.* 2003). Hence globally, soybean cultivars with low linolenic and high oleic acid content are being searched and developed to obviate the need of partial hydrogenation (Ross *et al.* 2000, Rahman *et al.* 2001, Fehr and Curtiss 2004, Kirsten *et al.* 2004).

Soybean varieties in India have largely been released mainly on yield basis and resistance or tolerance to various a biotic and biotic stresses rather than quality parameters. At present, the information on fatty acid composition of Indian soybean genotypes is very scarce (Sharma *et al.* 1997). In the present investigation, mature harvested seeds of 82 genotypes, comprising all the released cultivars (75) and few advanced lines (7) of Indian soybean, were evaluated for fatty acid composition to identify lines possessing comparatively low level of linolenic and high level of oleic acid.

Eightytwo genotypes, comprising all the released cultivars (75) and few advanced breeding lines (7) of soybean were sown in the field of the National Research Centre for Soybean, Indore (22°N), on 25 June 2002 and harvested on their respective maturity. The seeds from single plant of all the varieties and breeding lines were crushed into flour. The seeds from single plant of all the varieties and breeding lines were crushed into flour. Oil was extracted from freshly ground seed flour using petroleum ether (BP 40–60°C) and transesterified in methanol containing 1 N sodium methoxide as catalyst (Luddy *et al.* 1968). Fatty acid methyl esters (FAMEs) prepared were separated and analysed in gas liquid chromatograph (GLC), Shimadzu GC 17A, using capillary column with length and diameter of 30 m and 0.32 mm respectively. Oven temperature of the gas liquid chromatograph was programmed at 140°C for 3.6 min, then increased to 170° C @ 13.5°C/min and maintained for 3.8 min and finally increased to 182°C @ 5°C/min for obtaining best resolution of methyl esters. Temperatures of flame ionization detector and injector were maintained at 240°C. Nitrogen, the carrier gas used, was maintained at a flow rate of 15 ml/min with column pressure at 90 kpa. Peaks for individual fatty acid methyl esters were identified by comparing the retention times with those of standard methyl esters (procured from a Chemical Company). Data given in Table 1 for different saturated and unsaturated fatty acids are means of determination in 3 independent samples.

Palmitic acid, the undesirable saturated fatty acid which increases the blood cholesterol level, was 10.6–17.4%, with a mean value of 12.53 in the genetic material studied but 80% of the varieties or advanced lines studied showed a narrow range of 11.0-13.0%. The highest value for palmitic acid was observed in 'MACS 330', while the lowest in 'Shilajeet'. Stearic acid, a saturated fatty acid but having a neutral effect on cholesterol, ranged from 2.8 to 5.9% with a mean value of 4.18. Oleic acid a monounsaturated fatty acid with good oxidative stability, varied between 18.4 and 48.5% with a mean value of 26.11%. Only 4 varieties or advanced lines, viz 'LSb 1', 'MACS 330', 'MACS 58' and 'Shilajeet', possessed oleic acid more than 35 % (Table 1). 'LSb 1', an early-maturing variety released recently from Southern India, showed the highest oleic acid percentage (48.5), followed by 'MACS 330' while the lowest value for this fatty acid was observed in 'PK 1024'. The polyunsaturated fatty acids, viz linoleic and linolenic acid, ranged between 30.0 and 55.5 % and 4.6 and 9.0% with mean values of 49.01 % and 6.59 % respectively. Linolenic acid, the main reason for poor oxidative stability, was observed minimum in 'MACS

* Short note

[1] Scientist Senior Scale (Plant Biochemistry), [2] Scientist Senior Scale (Plant Breeding), [3] Principal Scientist

Table 1 Levels of individual fatty acid and monounsaturated: polyunsaturated fatty acid ratio of the released cultivars and few advanced breeding lines of Indian soybean

Variety	Fatty acids (%)[a]					Monosaturated: polyunsaturated fatty acids ratio
	Palmitic	Stearic	Oleic	Linoleic	Linolenic	
'ADT 1'	12.5	4.1	30.2	45.6	5.9	0.59
'Alankar'	11.8	4.7	24.5	49.0	7.3	0.44
'Ankur'	12.5	3.5	21.5	53.5	7.8	0.35
'Bhatt Black'	10.8	3.7	31.4	48.0	5.6	0.59
'Bhatt Yellow'	10.9	3.3	28.8	49.2	5.6	0.53
'Bhawali Bold'	16.5	2.8	32.9	42.5	5.3	0.69
'Bragg'	11.7	4.3	21.5	55.0	7.0	0.35
'BS 1'	12.0	4.1	27.5	48.5	5.6	0.51
'Co 1'	12.0	4.7	25.3	48.6	7.0	0.46
'CO Soya 2'	11.7	3.8	30.9	45.1	6.3	0.60
'Hardee'	11.7	4.3	22.3	52.5	6.1	0.38
'Himso 1563'	10.8	4.3	30.2	45.6	5.9	0.59
'His 1'	12.1	3.8	22.6	52.6	7.7	0.37
'GS 1'	13.5	4.9	24.5	48.9	7.2	0.44
'GS 2'	11.5	4.5	22.0	51.0	9.0	0.37
'Improved Pelican'	14.3	4.2	25.0	47.6	7.5	0.45
'IS 9'	12.2	3.9	26.7	50.0	6.4	0.47
'JS 2'	13.5	5.2	23.6	49.0	5.9	0.43
'JS 71-05'	12.0	3.9	24.5	51.6	6.5	0.42
'JS 75-46'	13.6	5.5	22.8	49.5	8.0	0.40
'JS 72-44'	13.0	3.6	23.5	50.5	8.2	0.40
'JS 72-280'	12.4	5.1	30.9	45.6	5.2	0.61
'JS 76-205'	11.5	3.3	24.5	52.0	6.2	0.42
'JS 79-81'	12.2	5.1	27.2	48.6	5.2	0.51
'JS 80-21'	15.5	4.8	30.3	43.3	4.6	0.63
'JS 90 41'	12.5	3.7	31.8	44.8	4.9	0.64
'JS 93-05'	12.0	3.8	27.2	49.6	6.1	0.49
'JS 93-06'	12.8	4.9	31.0	45.5	5.0	0.61
'JS 335	14.0	3.3	22.7	50.0	7.6	0.39
'Kalitur'	12.5	4.6	21.5	53.0	7.0	0.36
'KB 79'	12.4	3.8	28.2	47.5	7.3	0.51
'KHSb 2'	12.0	4.2	31.1	46.2	5.4	0.60
'Lee'	12.6	3.7	25.5	49.0	7.4	0.45
'LSb 1'	11.0	4.4	48.5	30.0	4.8	1.39
'MACS 13'	13.1	4.7	28.0	46.1	6.1	0.54
'MACS 124'	12.5	3.8	28.5	47.0	6.0	0.54
'MACS 57'	11.7	4.2	28.9	44.8	5.9	0.57
'MACS 58'	12.3	5.3	37.7	39.2	4.6	0.86
'MACS 45'	11.5	3.6	23.5	52.0	7.0	0.40
'MACS 330'	17.4	2.9	38.3	34.5	6.9	0.93
'MAUS 1'	13.4	4.3	23.9	51.3	6.2	0.42
'MAUS 2'	12.5	4.1	22.8	51.0	6.9	0.39
'MAUS 32'	12.0	3.8	24.7	51.7	7.0	0.42
'MAUS 61'	17.4	4.0	25.0	46.9	6.2	0.47
'MAUS 61-2'	12.5	4.6	25.2	49.5	6.5	0.45
'MAUS 71'	12.0	4.0	33.4	43.5	6.3	0.67
'MAUS 47'	13.0	3.4	30.0	44.5	6.6	0.59
'Monetta'	11.7	4.0	31.0	45.5	6.4	0.60
'NRC 2'	11.5	4.3	24.5	50.5	7.4	0.42
'NRC 7'	10.9	3.3	29.0	48.4	6.4	0.53
'NRC 12'	15.0	3.9	22.0	50.5	6.8	0.38
'NRC 37'	14.0	4.3	21.1	50.8	8.9	0.35
'Palam Soya'	12.4	5.9	27.0	48.1	5.8	0.50
'PK 262'	12.5	4.9	23.5	51.5	6.5	0.41
'PK 308'	12.2	5.1	27.6	47.5	6.3	0.51
'PK 327'	10.7	4.0	23.0	53.5	7.6	0.38
'PK 416'	14.5	4.4	18.5	55.0	7.2	0.30
'PK 471'	12.1	3.8	24.4	52.2	7.3	0.41
'PK 472'	13.5	3.2	24.5	51.8	6.6	0.42
'PK 564'	11.4	3.9	21.1	54.0	7.9	0.34
'PK 1024'	14.8	4.8	18.4	53.3	7.4	0.30
'PK 1029'	12.0	5.0	23.7	51.8	6.5	0.41
'PK 1042'	11.0	4.3	19.6	55.5	8.7	0.31
'PK 1092'	16.1	4.2	21.5	51.5	6.7	0.37
'Punjab 1'	16.0	4.1	23.5	49.5	6.7	0.42
'Pusa 16'	11.8	3.7	19.4	53.6	8.6	0.31
'Pusa 20'	11.3	4.8	24.0	52.5	5.8	0.41
'Pusa 22'	11.5	3.7	19.9	52.3	8.5	0.33
'Pusa 24'	11.5	4.6	33.9	42.5	5.5	0.71
'Pusa 37'	11.1	3.8	25.2	51.5	6.7	0.43
'Pusa 40'	11.5	4.3	24.0	53.1	6.1	0.41
'RAUS 5'	12.5	3.5	29.0	46.5	6.8	0.54
'Samrat'	12.8	4.4	22.5	52.0	6.0	0.39
'Shilajeet'	10.6	3.5	35.0	44.5	5.4	0.70
'Shiwalik'	11.8	4.0	21.5	53.9	6.8	0.35
'SL 96N'	11.1	4.1	24.6	52.1	6.7	0.42
'Sl 295'	13.0	4.5	29.0	43.5	7.5	0.48
'Sl 459'	12.0	5.4	25.2	49.6	6.2	0.45
'T 49'	12.7	4.6	25.0	49.5	6.5	0.45
'VLS 1'	11.0	4.5	20.0	56.4	7.1	0.31
'VLS 2'	12.0	3.7	32.0	45.0	4.9	0.64
'VLS 21'	13.0	4.3	23.6	50.5	7.4	0.41
'VLS 47'	12.5	4.0	25.2	51.3	6.0	0.44
CD (P=0.05)	1.1	0.4	1.6	2.1	0.6	0.05

[a] Data are means of determination in 3 independent samples

58', while maximum value was observed in 'GS 2'. Linoleic acid was maximum in 'PK 1042' while minimum in 'LSb 1'. 'JS 335', not only the most popular variety of Madhya Pradesh, the soya state of India, but also adaptable in other soybean-growing agroclimatic zones, showed normal levels of oleic acid and linolenic acid content. All the newly released soybean varieties 'MAUS 47', 'MAUS 61', 'MAUS 61-2', and 'MAUS 71' possessed levels of linolenic and oleic acid content as found in regular soybeans.

Xia (1995) reported the ranges of palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid among 70 soybean cultivars cultivated in China as 8.2–13.5, 2.5–5.1, 16.9–27.3, 46.9–58.3, 6.6–13.2, with mean values of 11.0, 3.63, 21.47, 53.99 and 9.93 respectively. In comparison to Chinese cultivars, our cultivars or lines showed comparatively low values for palmitic acid, linoleic acid and linolenic acid, and higher value for oleic acid.

Table 2 Correlation among different fatty acids and monounsaturated : polyunsaturated (M:P) fatty acid ratio

Fatty acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	M:P ratio
Palmitic acid	–0.054	–0.036	–0.202	0.016	–0.018
Stearic acid		–0.084	0.018	–0.151	–0.054
Oleic acid			–0.938***	–0.656***	0.962***
Linoleic acid				0.534***	–0.933***
Linolenic acid					–0.626***

***P=0.001; M:P, monosaturated : polyunsaturated fatty acids

Monounsaturated : polyunsaturated fatty acids (M:P) ratio is considered as an indicator of oxidative stability of a vegetable oil. In general, soybean oil possess monosaturated : polyunsaturated fatty acids ratio of 0.5 compared to 2.3 and 1.0 for canola and peanut oil respectively. In the present investigation, this ratio ranged between 0.31 and 1.39 (Table 1). Highest monosaturated : polyunsaturated fatty acids ratio was observed in 'LSb 1', while the lowest was observed in 'Pusa 16'. Fiftyfive varieties or lines showed monosaturated : polyunsaturated fatty acids ratio less than 0.5, while 23 varieties showed between 0.5 and 0.7 and only 4 varieties ('LSb1', 'MACS 330', 'MACS 58' and 'PUSA 24') showed values more than 0.7.

A very strong negative correlation of oleic acid with linoleic and linolenic acid ($P < 0.001$) was observed (Table 2). This result is in conformity with the report of Liu *et al.* (1995). No association was observed between saturated fatty acids, ie palmitic acid and stearic acid and, with unsaturated fatty acids. Linoleic and linolenic acid content showed strong negative correlation with monosaturated : polyunsaturated fatty acids ratio, while oleic acid showed significant positive correlation with monosaturated : polyunsaturated fatty acids ratio.

None of the varieties possessed linolenic acid below 4 % and oleic acid around 60% or more as desired for improved shelf-life of soybean oil. Globally, low linolenic and high oleic acid content have been achieved through mutational and traditional breeding (Fehr *et al.* 1992, Rahman *et al.* 1994). 'OT 96-15', a low linolenic line (3.42 %) reported by Health Canada is a backcross derived line using the variety 'Maple Glen' and a source of the low linolenic trait (PI 361088B), while transgenic soybean lines, viz 'G 94-1', 'G 94-19', 'G 168', with oleic acid content as high as 80 % have been developed through silencing *Fad* 2-1 gene (fatty acid desaturase) by Du Pont, USA. Oleic acid in soybean being a quantitatively inherited character (Burton *et al.* 1983), 4 varieties or lines, 'LSb 1', 'MACS 330', 'MACS 58' and 'Shilajeet', which showed comparatively higher oleic acid in our studies can be employed for pyramiding genes for high oleic acid as reported earlier (Wilson *et al.* 1981, Burton *et al.* 1983). However, an extensive screening for fatty acid composition of the soybean germplasm and land races available in the country needs to be undertaken for exploring the lines for still higher oleic acid and low linolenic acid content.

SUMMARY

In an experiment conducted during 2002 at Indore, Madhya Pradesh, 82 soybean [*Glycine max* (L.) Merr.] genotypes, comprising all the released cultivars and few advanced lines of Indian soybean, were grown to identify comparatively low linolenic and high oleic acid containing cultivars. Different unsaturated fatty acids, viz oleic, linoleic and linolenic acid were 18.4–48.5, 30.0–55.5 and 4.6–9.0% respectively. Negative association of oleic acid with linoleic ($P < 0.001$) and linolenic acid ($P < 0.001$) was observed along with positive association between linoleic acid and linolenic acid ($P < 0.001$). Of all the genotypes evaluated, only 4 cultivars or lines ('LSb 1', 'MACS 330', 'MACS 58' and 'Shilajeet') showed cmparatively higher oleic acid content, while 'MACS 58', 'LSb 1' and 'Shilajeet' also showed comparatively low linolenic acid. Monounsaturated : polyunsaturated fatty acids ratio, an indicator of oxidative stability of vegetable oils, ranged between 0.31 and 1.39 with most of the cultivars falling below 0.5. The varieties identified for comparatively higher oleic acid may be exploited for gene pyramiding for oleic acid.

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