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Evaluation of early maturing soybean (*Glycine max*) germplasm accessions for fatty acid composition

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Soybean [*Glycine max* (L.) Merr] accounts for 26% of the total oilseed production in India. Soybean oil haS poor shelf-life. Oil industries are demanding soybean varieties of oil content with better natural shelf-life as partial hydrogenation done to increase oxidative stability is not only cost ineffective but also leads to formation of trans fatty acids about which serious health concerns have been raised (Lichtenstein *et al.* 2003). The shelf-life of soybean oil depends upon the oxidative stability of soybean oil which in turn depend on the fatty acid composition as the rate of oxidation of linolenic (C18:3), linoleic (C18:2) and oleic acid (C18:1) are in the ratio of 21.6:10.3:1 (Fatemi and Hammond 1980), i e linolenic acid is the most vulnerable while oleic acid is the least susceptible to oxidation among unsaturated fatty acids. Globally soybean cultivars with low linolenic and high oleic acid content are being searched and developed to obviate the need for partial hydrogenation (Fehr *et al.* 2004, Kristen *et al.* 2004).

Early maturing soybean varieties are preferred by the Indian farmers to avoid late season drought and to fit into the multi–cropping system. In view of this demand, Indian council of Agricultural Research has undertaken breeding programme for early maturity in soybean as a priority activity under ISOPOM (Integrated Scheme for Pulse, Oilseed and Maize). Furthermore, negative correlations observed between oleic acid content and crop maturity have been reported in oilseeds (Fernandez *et al.* 1989, Rani *et al.* 2005). Hence, under ongoing screening of soybean germplasm for fatty acid composition at National Research Centre for Soybean, it was considered worthwhile to evaluate early maturing germplasm accessions on priority for fatty acid composition.

Of the 2500 germplasm lines grown in a single row in 3 m length and 0.45 m breadth in the cropping season 2004 in the fields of National Research Centre for Soybean, germplasm lines maturing in approximately 90 days were selected. All the recommended agronomic practices were followed. Days to maturity of each germplasm line were recorded. Seeds from germplasm lines were hand harvested on their respective maturity. Mature seeds were dried in a seed drier at 40°C till they became moisture free. Dried seeds were ground with metallic pestle and mortar and sieved to get a flour of 100mesh size.

Oil was extracted from sieved soy flour using petroleum ether (boiling point 40–60°C). Methyl esters were prepared from the oil by interesterification in methanol using 1N sodium methoxide as the catalyst following Luddy *et al.* (1968). Prepared fatty acid methyl esters (FAMEs) were separated and analyzed in gas liquid chromatograph, Shimadzu GC 17A using capillary column, SGE BPX70. Oven temperature of the gas liquid chromatograph was programmed at 140°C for 3.6 min and subsequently increased to 170°C @13.5°C per minute and maintained for 3.8 min and finally to 182°C at the rate of 5°C per minute for obtaining best resolution of methyl esters. The temperatures of flame ionization detector and injector were maintained at 240°C. The peaks for fatty acid methyl esters were identified by comparing the retention times with those of standard methyl esters (procured from Sigma-Aldrich). The analysis was done in triplicate samples and the mean values were reported.

Based upon their maturity period in Indian conditions soybean genotypes have been divided into early, mid and late maturing,. Genotypes maturing in less than 90 days are considered as early maturing genotypes. Table1 indicates the days to maturity of the early maturing germplasm lines selected for the study (Table1). Days to maturity varied from 70 days for 8 genotypes, namely EC 481219, 481306, 481309, 251514, 251770, 291448, 39488, 216379 to 90 days for 3 genotypes, viz. EC 333865, 333886, 333922.

Table 1 indicates the fatty acid composition of all the germplasm lines analysed. Palmitic acid (C16:0) ranged from 9.81 for EC 333880 to 19.18 for EC 481306 with a mean value of 12.53 in the germplasm lines studied. Per cent stearic acid (C18:0) content ranged from 2.66 for EC 39488 to 5.4 in EC 333886 with a mean value of 3.87. The oleic acid (C18:1) ranged from 13.59 for EC 481306 to 47.78 for EC39490 with a mean value of 26.60. Six germplasm lines *viz.* EC175330, 39490, AGS2, IC222, P955, WT142 showed oleic acid more than 35%. Among

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le 1 Days to maturity, fatty acid composition, monounsaturated (M): polyunsaturated fatty acids and linoleic (n-6):linolenic acid (n-3) ratio of early maturing soybean germplasm lines grown in cropping season 2004

mplasm	DM	% fatty acid*					M:P	n-6:n-3
		C16:0	C18:0	C18:1	C18:2	C18:3		
481219	70	14.45	4.55	17.38	55.94	7.27	027	7.69
'481306	70	19.18	3.82	13.59	55.16	8.25	0.21	6.69
'481309	70	14.31	3.42	19.67	53.77	8.82	0.31	6.09
'251514	70	14.87	3.04	20.73	54.03	7.33	0.34	7.37
'251770	70	12.08	4.78	20.35	54.86	7.35	0.33	7.46
'291448	70	17.16	3.81	26.9	46.31	5.79	0.52	7.99
'39488	70	18.48	2.66	27.10	44.49	6.25	0.53	7.11
'216379	70	12.28	3.78	25.35	50.97	6.38	0.44	7.98
'216384	75	12.26	3.35	40.50	37.94	5.37	0.94	7.06
'216374	75	11.06	3.43	31.68	46.71	6.77	0.59	6.89
'216376	72	11.87	3.06	28.38	49.97	6.20	0.51	8.05
'39486	75	11.24	3.57	28.14	50.63	5.97	0.49	8.48
'175330	75	12.51	3.85	35.66	42.13	5.27	0.75	7.99
'216385	77	12.67	3.30	31.10	47.28	5.07	0.59	9.32
'C175324	77	10.75	3.46	25.6	53.86	5.80	0.43	9.29
'C210380	71	12.50	4.14	22.0	52.33	8.63	0.36	6.06
'C39490	77	11.81	3.87	47.78	31.49	4.40	1.33	7.16
'C175322	77	10.07	2.76	26.33	53.50	7.34	0.43	7.29
i76	77	10.92	3.77	24.14	54.92	5.73	0.39	9.58
'C175320	77	10.29	4.59	28.77	49.52	6.16	0.52	8.04
i257	77	10.95	3.26	30.31	49.42	5.48	0.55	9.02
'able variety	77	11.10	3.69	24.72	54.15	5.73	0.41	9.45
AGS2	77	9.99	3.10	45.13	36.28	5.20	1.08	6.97
AGS386	75	11.81	3.23	25.29	53.44	5.67	0.43	9.42
AGC191	75	11.23	4.09	23.84	53.53	6.49	0.397	8.25
G214	73	12.38	3.76	26.37	51.14	5.96	0.46	8.58
EC325097	79	11.47	3.31	24.15	54.34	6.49	0.36	8.37
EC333917	81	11.07	4.21	19.8	58.1	6.16	0.31	9.43
EC333880	82	9.81	3.52	33.15	48.19	4.78	0.63	10.08
EC333867	82	10.33	4.31	23.40	54.45	6.85	0.38	7.95
EC33903	83	11.65	4.32	23.05	54.06	6.32	0.38	8.55
E264	84	12.05	3.60	28.80	48.61	6.96	0.52	6.98
EC468441	85	11.89	4.91	30.78	46.57	5.05	0.59	9.22
EC357990	86	10.7	4.4	31.0	46.9	6.14	0.58	7.63
EC329157	86	11.37	3.74	22.26	56.51	6.13	0.36	9.21
TGX8523D	86	11.59	3.85	27.38	48.94	7.77	0.48	6.29
IC222	86	10.71	4.08	38.46	42.10	4.40	0.83	9.56
P955	86	12.76	3.52	35.99	41.72	6.01	0.75	6.94
EC333891	86	14.52	4.01	15.18	57.46	8.83	0.23	6.50
GPC154	86	11.95	3.80	33.91	43.20	6.71	0.68	6.43
HAL	86	12.17	4.04	22.45	51.49	9.37	0.37	5.49
EC313976	86	11.46	3.82	26.61	48.52	8.90	0.46	5.45
WT142	86	10.14	3.66	35.66	44.37	6.77	0.69	6.55
EC338846	86	10.45	3.15	18.41	60.27	7.43	0.27	8.11
EC350665	86	12.31	3.38	25.24	51.42	7.24	0.43	7.10
TGX860-11E	86	14.77	4.68	19.88	54.10	6.03	0.33	8.97
EC333871	86	11.52	4.39	29.94	48.43	5.23	0.56	9.26
EC30968	86	11.45	3.68	26.74	50.34	7.21	0.46	6.98
EC329158	86	12.43	5.25	20.43	55.45	5.81	0.33	9.54
EC333919	86	11.79	4.51	29.52	48.80	4.83	0.55	10.10
PK474	86	13.37	3.61	27.35	47.61	8.07	0.49	5.89
PK416	86	12.83	4.20	18.43	55.35	8.86	0.29	6.24
EC333909	86	11.14	4.23	26.18	51.45	6.23	0.45	8.25
EC333925	86	12.48	4.3	31.02	46.35	5.85	0.59	7.92

3 or w6) ratio ranged from 5.45 for EC313976 to 10.10 for 333919. One-year data indicates that soybean germplasm :s EC39490 and AGS2 that exhibited oleic acid more than % may be employed for developing varieties with improved dative stability.

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Table 1 contd....

Germplasm	DM	% fatty acid*					M:P	n-6:n-3
		C16:0	C18:0	C18:1	C18:2	C18:3		
EC333904	87	12.22	4.58	20.75	54.96	6.89	0.36	7.97
EC333901	87	10.97	4.14	19.86	57.78	6.83	0.31	8.45
EC333865	90	11.50	3.90	23.06	54.51	7.03	0.37	7.75
EC333886	90	10.37	5.40	25.71	51.90	6.19	0.44	8.38
EC333922	90	12.95	4.21	18.27	57.32	7.24	0.28	7.91
Mean		12.53	3.87	26.60	50.42	6.53	0.487	7.88

*Values given are mean of triplicate samples

DM. Drymatter, C 16 : 0 fall from g C 18 : 1, C 18 :2, C18 : 3, M:P,

Table2 Correlations studied between various oil quality parameters

Parameter	C18:0	C18:1	C18:2	C18:3	M:P	n-6:n-3
C16:0	ns	-0.349**	ns	ns	ns	ns
C18:0		ns	ns	ns	ns	ns
C18:1			-0.940***	-0.624***	0.975***	ns
C18:2				0.473***	-0.954***	794***
C18:3					-.0597***	775***
M:P						ns

***P=0.001,**P=0.01; M:P, monounsaturated:polyunsaturated fatty acids; n-6:n-3, linoleic acid:linolenic acid

polyunsaturated fatty acids, linoleic acid (C18:2) ranged from 31.49 in EC 39490 to 60.27 for EC 338846 with a mean value of 50.42 while linolenic acid (C18:3) ranged from 4.40 for IC222 and EC 39490 to 9.37 for HAL with a mean value of 6.53. In soybean varieties, a range of 18.0–48.5, 30.0–55.5 and 4.6–9.0% for oleic, linoleic and linolenic acid respectively has been observed (Kumar *et al.* 2004).

Monounsaturated: polyunsaturated fatty acids (M:P) ratio is considered as an indicator of oxidative stability of a vegetable oil. In general, soybean oil possess M:P ratio of 0.5 or less than 0. 5 compared to 7.0, 2.3, 1.0, 0.5, 0.2 for olive, canola, peanut, corn and sunflower oil respectively. Efforts are being made to breed soybean varieties with higher M:P ratio all over world. In the present investigation M:P ratio ranged from 0.21 for EC481306 to 1.33 for EC39490 (Table 1). It ranged from 0.31 to 1.39 in released varieties of soybean (Kumar *et al.* 2004). Thirty seven germplasm lines exhibited monounsaturated: polyunsaturated ratio less than 0.5, while 16 germplasm lines showed between 0.5–0.7, 4 germplasm lines showed M:P ratio value above 0.7 and two germplasm lines (EC39490,AGS2) exhibited M:P ratio more than 1.0. Another health related oil quality parameter recommended by World Health Organisation is the ratio between n-6/n-3 or w6 /w3), i e linoleic/linolenic acid. It should be between 5-10. Table 1 exhibits n-6:n-3 ratio, which indicates the ratio between linoleic (n-6 or w6) to linolenic acid (n-3 or w3), ranged from 5.45 for EC313976 to 10.10 for EC333919 i e all soybean germplasm lines fell into the recommended range of n-6/n-3 ratio of FAO that is 5-10. Table 2 indicates various correlations studied among fatty acids and oil quality parameters. Palmitic acid observed a significant negative correlation with oleic acid ($P<0.01$). Oleic acid observed highly significant negative correlations with linoleic and linolenic acid ($P<0.001$) and positive with M:P ratio ($P<0.001$). Linoleic acid observed a significant positive correlation with linolenic acid and negative correlation with M:P ratio ($P<0.001$). Linoleic (n-6 or w6)/ linolenic acid (n-3 or w3) observed highly significant negative correlation with linolenic acid ($P<0.001$) and positive correlation with linoleic acid ($P<0.001$). Significant negative correlations observed between oleic acid, linoleic acid and linolenic acid are in consonance with earlier reports (Liu *et al.* 1995, Kumar et al. 2004).

Oleic acid in soybean being a quantitatively inherited character (Burton *et al.* 1983), 2 genotypes EC39490 and AGS2 that exhibited comparatively higher level of oleic acid content in the present study can be employed for pyramiding genes for high oleic acid content. However, an extensive screening for fatty acid composition of soybean germplasm available in the country for exploring lines for still higher oleic acid and low linolenic acid content is underway.

SUMMARY

Among unsaturated fatty acids present in soybean oil, linolenic acid is the most vulnerable while oleic acid is the least susceptible to oxidation. Hence, globally, soybeans with low linolenic acid and high oleic acid are being searched. Fifty nine early maturing soybean germplasm lines were selected from germplasm field of National Research Centre for Soybean and the seeds harvested on their respective maturity were analysed for fatty acid composition and the oil quality parameters, *viz* monounsaturated (M) :polyunsaturated fatty acids (P), a ratio indicating the oxidative stability of vegetable oils, and linoleic acid (n-6 or w6) : linolenic acid (n-3 or w3), a ratio important from health point of view, were computed. Genotypic variation was observed for all the five major fatty acids of soybean, namely palmitic, stearic, oleic, linoleic and linolenic acid content. Six germplasm lines, viz EC175330, EC39490, AGS2, IC222, P955 and WT142 exhibited oleic acid more than 35%. M/P ratio ranged from 0.21 for EC481306 to 1.33 for EC39490 while linoleic acid (n-6 or w6) / linolenic acid