Oil and Protein Quality of Black Seeded Soybean Germplasm

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Sixteen black seeded soybean lines locally known as *Kaley Bhatt*, collected from different parts of Uttarakhand area of India were grown in similar environmental and field conditions along with five creamish-white colored control lines. Nutritional quality of seeds in terms of oil-protein quantity, fatty acid profile of seed oil and amino acid profile of proteins were established and compared. *Kaley Bhatt* accessions produced small sized black seeds with average hundred seed weight of 7.62 g. In general, black seeds were more in protein and less in oil content in comparison to creamish white colored seeds. Fatty acid profiles of the collections were uniform irrespective of seed color and size. Significant and negative association exists between oleic-linoleic and oleic-linolenic acid. Some black seeded accessions were identified for their higher concentrations of linolenic acid (omega-3 fatty acid). Essential amino acid composition of *Kaley Bhatt* accessions varied with in range of 47.75 to 40.73 mol% of total amino acid. Maximum methionine concentration of 2.08 mol % was observed in IC548683.

Key Words: Soybean, Germplasm, Oil, Fatty acid profile, Protein

Introduction

Soybean serves as a major vegetable source of edible protein and oil (Smith *et al.*, 1987). Increased demand for these constituents has stimulated research efforts to develop soybean lines which can produce seed with higher concentration of oil and protein

Agronomically acceptable cultivars producing quality seed in terms of having combine oil and protein concentration of 62% can command significant market value over other related crops. However soybean oil is found to contain relatively high level of lipoxygenase enzyme, which react with linolenic acid (omega-3 fatty acid) present in seed oil and contribute to reduced oxidative stability and poor flavor quality (Mounts *et al.*, 1988). Presence of lesser levels of sulphur containing amino acid in seed proteins makes soybean less suited for human nutrition. Soybean oil also contains appreciable quantity of saturated fatty acids and in some cases; this value is about 16% (Mounts *et al.*, 1994). In modern time, the health issue involving saturated fats and blood serum cholesterol levels has received considerable public attention. Besides that, higher proportion of genetically modified *Brassica*, safflower, sunflower and peanut seed has been released for commercial production that gives high oil with desired quality (Fernandez-Martinez *et al.*, 1989; Mundel and Braun, 1999). Keeping all these developments in view it seems apparent that genetic modification research on soybean must have to be continued as per demand of market, just to keep pace with the technological advances in other related crops.

In this direction, some good progress has been made in developing some genetic altered soybean lines (Burton *et al.*, 1989; Wilson *et al.*, 1976, 1981, 1988; Erickson *et al.*, 1988; Carver *et al.*, 1986), with one or other improved traits by exploiting germplasm resources. It is expected with the active involvement of germplasm collection and evaluation activities towards crop improvement programme will result future production of commercial soybean that will bear little resemblance to the soybean oil protein quality, available today. In continuation of these activities, the present work has been undertaken to study some black seeded soybean lines widely available in different pockets of Uttrakhand (India) for their nutritional quality.

In Uttrakhand region of India, the soybean is known as *Bhatt*. Based on colour, black seeded soybean is known as *Kaley Bhatt*. Similarly, yellowish white and brown seeded soybeans are known as *Safed Bhatt* and *Rata Bhatt*, respectively. Among *Kaley Bhatt* type some are very small in size, climber, tall and giant. Locally it is being grown in margins of agricultural field, mixed cropping with minor millets, *i.e.*, foxtail, finger, barnyard and pseudo-cereals, *i.e.*, amaranth. Others are rounded and medium type.

In the present experiment, sixteen black seeded soybean lines (*Kaley Bhatt*) and five yellowish white color, erect, small dwarf lines (*Safed Bhatt*) were taken for comparison of nutritional quality of the seeds.

Materials and Methods

Plant Materials

Sixteen black seeded soybean accessions collected from different parts of Uttarakhand (India) were grown along with five control lines. All control lines were rounded types and yellowish white seeded.

Accessions were grown in Randomized Block Design with three replications in June, 2007 and harvested after five months. Dried powdered seeds were taken for analysis.

The Soybean seeds were dried to 4-5% moisture level in oven set at 108°C for 16 to 18 h. The oil content of the seed samples were determined by non-destructive method using a Newport NMR analyzer (Model-4000) from Oxford Analytical Instruments Ltd. U.K, equipped with 40ml coil assembly. The instrument was kept in a room of constant temperature (23-25°C). Pure seed oil required to calibrate the instrument was extracted by solvent extraction method. The instrument was run by adjusting audio frequency (AF) gain of 400 and radio frequency (RF) current of 225 µA with a gate width of 1.5 gauss. The NMR responses (signal/ mass) of the seed samples were compared to NMR response of pure oil (100%) for obtaining oil percentage of the soybean accessions.

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Samples of soybean seeds were freshly grounded (Remi homogenizer) and weighed so that 40 mg oil are obtained when extracted with 10 ml solvent mixture consisting of chloroform:hexane:methanol (8:5:2 v/v/v). The extracts obtained were dried at 60°C in nitrogen gas for 30 min. Methyl esters of oil samples were prepared according to the method of Neff *et al.* (1994) with slight modifications as applied for Cruciferous species by Mandal *et al.* (2002). 1 µl of the hexane extract was injected into a highly polar HP Innowax capillary column of 30 m length (inner diameter: 0.32 m, film thickness: 0.5 µm, split: 1:80). A Hewlett Packard gas chromatograph, model 6890 equipped with flame ionization detector (FID) was used. The injector and detector temperatures were 260°C and 275°C, respectively. Oven temperature was programmed from 150°C holding at 1 min to 210°C at the rate of 15°C/min, followed by 210°C to 250°C at the rate of 5°C/min for 12 min Peaks of fatty acid methyl esters were identified by comparing their retention time with that of the known standards, run under similar separation conditions, peak integration was performed applying HP3398A software.

Protein Content

Nitrogen content of the soybean seeds was determined by conventional Kjeldahl method with Kjeltec analyzer (Model-2300) from Foss Tecator, Sweden. Factor 6.25 was used to convert nitrogen to protein.

Amino Acid Profile of Total Seed Protein by High Performance Liquid Chromatography

HPLC based pre-column derivatization technique was used in the present study. Fluorescent active reagent, 6-aminoquinolyl-N-hydroxy succinimidyl carbamate of Millipore Corporation (Cohen and Michaud, 1993) was used for derivatization with protein hydrolysate amino acids.

Hydrolyzing Samples

5 mg powder samples were taken in a clean 6x50 mm sample tube. Tubes were placed in a reaction vial containing 200 µl of constant boiling HCl (6 N) and a crystal of phenol, placed to the bottom of the reaction vial. Samples were hydrolyzed in an oven at 112°C to 116°C for 20 to 24 h. After hydrolysis is over, excess HCl was wiped off from tubes and dried under vacuum.

Derivatizing the Samples

Vacuum dried samples were dissolved in 750 µl, 20 mM HCl solution. To 20 µl of this protein hydrolysate amino acid solution, 20 µl of AccQ·Fluor^{TM} reagent (waters Part no.WAT052880) and 60 µl of AccQ·Fluor^{TM} Borate buffer (waters Part no. WAT052880) was added, vortex thoroughly and mixture was heated for 10 min at 55°C.

HPLC System for Separation of Amino Acid Derivatives

The HPLC system consisted of waters two pumps (Model no. 515), auto sampler and fluorescence detector (Waters 2475). Waters Empower software was used to control the operation and peak integration.

Derivatized sample was run in Waters AccQ·Tag^{TM} column of length 3.9 x150 mm (Part no.–WAT052885). Mobile phases A containing 10% solution of AccQ·Tag^{TM} concentrate (Part no. WAT052890) and phase B consisting of 60% HPLC grade Acetonitrile were used as eluent in gradient conditions : initially A=100%, 2 min=98%,

15 min=93%, 19min=90%, 32 to 37min=67% followed by wash with 100% eluent B for 13 min. and re-equilibration for 10 min at 100% by eluent A.

Detection

Detection of individual amino acid derivative was made by fluorescence detector with excitation at 250 nm and emission at 395 nm with band width 18 nm. The polarity of the detector was positive with gain 10 and sampling rate 1.

Establishment of Factors to Convert Area Percentage of the Individual Amino Acid in Respective Concentration

From the area percentage of the individual amino acid of the standard mixtures (PIERCE Product no. NC10180/ vial No. 88122) run under similar condition, the factors were calculated to convert area percentage of the individual amino acid to respective concentration.

Results and Discussion

Sixteen black seeded soybean genotypes collected from different regions of Uttarakhand state of India were studied along with five normal colored control lines from same state, for major nutritive traits like oil, protein, fatty acid profile of seed oil and amino acid composition of protein, to make comparison between two different types of soybean samples.

Black seeded collections were comparatively very small in size. Hundred seed weight varied within the range of 10.29 to 5.77 g, with the mean value of 7.62 g, against 20.81 g mean seed weight recorded for control collections. The black seeded collections with average oil value of 18.65% was about 2.5% less than that of control line's oil value of 21.23%. But small seeded samples are more in protein content. Average protein value of 40.80% was about 2% more than the average protein value for the five control lines. IC548665, IC548683 and IC548669 were the three black seeded genotypes that contain more than 42% protein in their seeds. Among these, IC548665 and IC548683 showed combine oil plus protein concentration of about 62%. Out of five control lines, four were found having combine oil- protein percentage of about 60.

Fatty Acid Profile of Seed Oil

In general, average fatty acid profiles of the samples were uniform irrespective of seed color and seed size. Palmitic, stearic, oleic, linoleic, and linolenic acid components of the black seeded soybean oils varied within the range of 10.78 to 8.17%, 5.23 to 2.24%, 25.95 to 16.23%, 60.52 to 51.89% and 14.70 to 7.66%, respectively. Values are very similar as mentioned earlier by different workers (Wilson, 1987).

Fatty acid profiles of the accessions along with other quality traits are shown in Table 1. IC548719 with total saturated fatty acid concentration of 11.87% was identified as low total saturated fatty acid containing line. IC419815 and IC548665 identified for their high oleic acid concentration of 25.95 and 22.86%, respectively

Poly unsaturated fatty acid (PUFA) content of the present accessions including control lines, varied within the range of 71 to 61%. Both the experimental and control lines gave average PUFA content of about 66%. Soybean oil is one of the few edible oils where appreciable amount of linolenic acid (omega-3 fatty acid) is present in PUFA components.

Higher level of lipoxygenase enzyme in soybean seed reacts with linolenic acid (omega-3 fatty acid) component of the oil and generates poor flavor if oil is not stored properly. Both linoleic and linolenic fatty acids are essential for human health (Pryde, 1980; Tinoco *et al.,* 1979). The linolenic acid acts as precursor for its conversation to long chain (LC) omega-3 PUFAs mainly eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA). Indu and Ghafoorunissa (1992) indicated that increasing dietary linolenic increases EPA concentrations in plasma phosphor lipids. The beneficial effects of PUFA are mainly due to EPA and DHA, the LC omega-3 PUFAs components. Dietary amounts of linoleic acid as well as the ratio of linoleic to linolenic acid in PUFA, appear to be important for the metabolism of linolenic to LC omega-3 PUFAs. Indu and Ghafoorunissa (1992) also showed that while keeping the amount of dietary linoleic acid constant, 3.7 g linolenic acid appears to have biological effects similar to those of 0.3 g LC omega-3 PUFA with conversion of 11 g linolenic acid to 1 g LC omega-3 PUFA. Thus ratio of 4 (15 g linoleic: 3.7 g linolenic) is appropriate for conversion. This ratio is also consistent with the Lyon Hear study (12). Simopoulos *et al.*, 2003 and Cleland, 1997 reported linoleic to linolenic acid ratio of 3:1 to 5:1 for exerting beneficial effects of PUFA. In the present study, omega-3 fatty acid concentration of *Kaley Bhatt* oils varied from 7.66 to 14.70% and 10.33% was the mean value against 9.56% mean value established for control lines. IC548669 and IC548612 were the two *Kaley Bhatt* collections selected for possessing high linolenic acid

Table 1. Value rich trait of soybean samples

S.No.	Accession No.	100 seed wt. (g)	Protein (%)	Total oil (%)	Palmitic	Stearic	Oleic	Linoleic	Linolenic	linoleic/ linolenic
					Percent of total oil					
1	IC548612	5.77	39.88	19.29	9.14	3.93	17.43	56.22	13.02	4.32
2	IC548623	6.62	39.45	18.34	9.01	3.23	19.09	57.43	11.23	5.11
3	IC548632	5.98	38.69	18.50	9.21	4.36	19.22	55.53	11.67	4.76
4	IC548636	5.83	38.92	18.73	8.74	4.96	20.23	56.67	9.39	6.04
5	IC548640	10.29	40.72	19.26	10.44	4.77	20.23	54.98	9.56	5.75
6	IC548643	**9.12**	**39.50**	20.13	**10.32**	**4.41**	**17.03**	**58.13**	**10.11**	**5.75**
7	IC548665	**9.50**	43.15	**18.66**	**10.73**	**5.17**	**21.70**	**51.89**	**10.57**	**4.91**
8	IC548669	**7.00**	42.47	**16.30**	**9.41**	**3.30**	**16.23**	**57.25**	**14.70**	**3.89**
9	IC548672	9.80	40.99	19.50	10.27	5.23	18.67	56.22	9.56	5.88
10	IC548676	9.26	41.31	19.38	9.59	3.89	19.08	57.75	9.69	5.96
11	IC548683	**10.00**	42.99	**19.58**	**9.63**	**2.24**	**19.75**	**59.37**	**8.99**	**6.60**
12	IC548719	**6.22**	**40.33**	**18.87**	**8.17**	**4.25**	**16.78**	60.52	**10.25**	**5.90**
13	IC548721	7.10	40.59	18.32						
14	IC548724	6.98	41.35	18.79	10.78	2.41	24.07	53.91	8.82	6.11
15	IC419815	**5.92**	**40.72**	**17.60**	**9.35**	**3.42**	25.95	**53.61**	**7.66**	**7.00**
16	IC524256	6.50	41.66	17.10	9.82	3.77	18.65	58.04	9.71	5.98
	Min.	5.77	38.69	16.30	8.17	2.24	16.23	51.89	7.66	3.89
	Max.	10.29	43.15	20.13	10.78	5.23	25.95	60.52	14.70	7.00
	Mean	7.62	40.80	18.65	9.64	3.96	19.61	56.50	10.33	5.60
	Std dev	1.70	1.34	0.98	0.75	0.92	2.65	2.27	1.75	0.84
	% Cv	22.35	3.29	5.258	7.82	23.25	13.53	4.02	16.99	15.01
17	Soybean-02	19.93	38.65	21.46	11.06	3.22	20.62	55.64	9.45	5.89
18	Soybean-21	21.53	36.01	21.95	10.54	4.00	17.45	57.22	10.56	5.42
19	Soybean-47	20.50	40.39	20.27	10.60	3.58	20.28	55.94	9.59	5.83
20	Soybean-54	21.20	38.60	21.61	10.10	4.21	21.35	55.65	8.69	6.40
21	Soybean-77	20.87	39.45	20.87	10.36	2.99	17.33	59.79	9.52	6.28
	Min.	19.93	36.01	20.27	10.10	2.99	17.33	55.64	8.69	5.42
	Max.	21.53	40.39	21.95	11.06	4.21	21.35	59.79	10.56	6.40
	Mean	20.81	38.62	21.23	10.53	3.60	19.41	56.85	9.56	5.96
	Std dev	0.621	1.631	0.665	0.35	0.51	1.88	1.77	0.67	0.39
	% Cv	2.99	4.22	3.13	3.36	14.23	9.69	3.11	6.96	6.57

value of 14.70% and 12.80%, respectively. Corresponding linoleic to linolenic fatty acid ratio were 3.89 and 4.32, respectively. The linoleic to linolenic fatty acid values for other *Kaley Bhatt* collections varied within the range of 3.89 to 7.00 against mean value of 5.96 recorded for five control lines.

Most of the common edible oils produced in modern days are rich in linoleic acid. These soybean oils with more than 10% linolenic acid stand a very good scope for use as an alternative for fish oil and other linolenic acid rich oil.

Amino Acid Composition of Seed Protein

In the present study, 6 aminoquinolyl-N-hydroxysuccinimidyl carbamate flour reagent of Waters (USA, Cat no. WAT 025890) was used for amino acid derivative formation. Afterwards the corresponding amino acid derivative gets separated in column and finally identified and quantified through fluorescence detector in HPLC system.

The Essential amino acid (EAA) composition of *Kaley Bhatt* collections varied with in range of 44.21 to 31.67 mol%, with mean value of 38.07 mol%. This mean value was about 7.36 mol% less when compared with the mean value of five control lines. Black seeded lines were found to contain more percentage of lysine and less percentage of tyrosine amino acid. Methionine content also was in higher side in control lines. More variation in the concentration of methionine was observed in *Kaley Bhatt* collections. IC548683 gave highest methionine concentration of 2.08 mol % against average 1.52 mol % recorded for *Kaley Bhatt* collections. Range and mean of the Essential Amino Acid composition, both for control and black seeded lines are shown in Table 2.

Variation in EAA compositions in black seeded lines in comparison to control lines was mainly due to the difference of lysine and tyrosine concentrations. Average tyrosine concentration of 2.61% was found in black seeded lines in comparison to 4.0% tyrosine found present

Table 2. Range and mean value of essential amino acid concentrations of soybean

EAA	*Kaley Bhatt* collections					Control lines				
	Min	Max.	Mean	Std dev	% Cv	Min	Max.	Mean	Std dev	% Cv
Arginine + Threonine	9.69	12.45	10.89	0.71	6.56	11.89	12.59	12.15	0.26	2.17
Half cystine	0.53	1.76	1.07	0.38	35.40	0.95	1.57	1.2	0.28	23.08
Tyrosine	1.03	3.76	2.61	0.89	34.24	3.51	4.48	4.046	0.49	12.16
Valine	4.97	5.78	5.31	0.25	4.77	5.15	5.4	5.254	0.11	2.15
Methionine	1.17	2.08	1.52	0.27	17.48	1.65	1.74	1.676	0.04	2.37
Lysine	4.53	6.48	5.39	0.57	10.64	4.04	5.26	4.54	0.66	14.48
*Iso*leucine	3.72	4.75	4.30	0.28	6.50	4	4.41	4.188	0.19	4.62
Leucine	6.57	8.24	7.48	0.50	6.62	6.78	7.41	7.08	0.30	4.22
Phenylalanine	3.62	6.68	5.10	0.89	17.47	4.46	6.13	5.304	0.79	14.84
Total EAA	40.73	47.75	43.68	1.75	4.00	43.88	46.75	45.438	1.46	3.21

control lines. On the other, lysine concentrations of black seeded lines varied within the range of 6.48% to 4.53%, 5.39% was the mean value. Average lysine value of 4.54% was observed in control lines. Maximum lysine concentration of 5.26% was observed in one of the creamish white coloured Control line. In this method, response of cystine amino acid was not satisfactory; as a result, good and clear cut cystine peaks were not detected, causing unreliable integration values. This might have contributed high CV% values for the samples. Except cystine, other essential amino acids got well separated and quantified with respect to standard amino acids, run under similar instrument programme. Comparative values rather than literature cited data were given more emphasis for drawing any conclusion about experimental results and for selecting superior line for possessing higher level of any essential amino acid. *Kaley Bhatt* collections were found to contain low tyrosine and high lysine residue in seed protein in comparison to present five normal colored control lines.

Essential amino acid components in seed protein of few selected accessions, which were identified for their high and low protein, high methionine percentage in seed protein and high and low total essential amino acid composition are shown in Table 3 along with their protein values.

The present work was undertaken with the sole objective to quantify different nutritional parameters of *Kaley Bhatt* seeds and to identify any oil-protein related traits which might be considered specific for these black seeded accessions, if compared with any normal colored soybean line. Another objective has been to select value rich germplasm for use as resource material in development of a good soybean seed with improved nutritional quality.

Relationship between Different Quality Traits

Significant and direct associations exist between size and oil content of the seed. Associations between oil and protein content of the seed was also very strong but inverse. Among the fatty acid components, significant and negative association exists between oleic–linoleic and between oleic-linolenic acid as observed by earlier workers (Wolf, 1982).

Table 3. Lines showing high and low EAA, high protein and high methionine content

EAA	IC548665 Mol %	IC548724 Mol %	IC548683 Mol %	IC548632 Mol %	IC548669 Mol %
Argenine +Threonine	11.16	10.67	11.21	10.03	10.86
Half cystine	1.59	0.86	1.76	0.73	0.96
Tyrosine	2.56	2.98	3.76	1.31	3.03
Valine	5.31	5	5.28	5.6	5.42
Methionine	1.39	1.66	**2.08**	1.24	1.51
Lysine	5.81	5.87	4.53	4.87	5.34
*Iso*leucine	4.29	4.02	4.53	4.52	4.42
Leucine	7.42	7.02	7.92	8	7.31
Phenylalanine	4.68	4.26	6.68	6.01	4.9
EAA	**44.21**	**31.67**	**36.54**	**42.31**	**43.75**
Protein %	**43.15**	41.35	**42.99**	**38.69**	**42.47**

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