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Original Article

Influence of growing environment on the biochemical composition and physical characteristics of soybean seed

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Abstract

The biochemical composition and physical appearance of soybean seeds determine the quality of various soy foods. A multilocational field trial with seven Indian cultivars at four growing locations was conducted to study the influence of the growing environment on the biochemical and physical characteristics of soybean seed. Genotypic, locational and genotypic \times locational interaction were found to be significant for protein, oil and unsaturated fatty acids, namely oleic acid, linoleic acid and linolenic acid $(P<0.001)$. Phytic acid and heat-stable antinutritional factor in soybean seed showed significant $(P<0.01)$ locational and genotypic \times locational effects. Among climatic factors, latitude showed a significant positive correlation with oil (P<0.05) and a negative correlation with protein $(P<0.01)$, while rainfall showed a negative correlation with protein content $(P<0.001)$. Average daily mean temperatures during bean development showed a positive correlation with protein $(P<0.05)$ and a negative one with oil and linolenic acid ($P < 0.05$). Among physical characteristics, seed size showed a positive correlation with phytic acid and oleic acid, and a negative correlation with linolenic acid. Over four growing locations, the seed coat colour showed no variation, while light hilum colour cultivars responded differentially. The effect of various climatic factors on physical and biochemical composition of soybean seed as observed in our studies suggests the need for development of location-specific cultivars for food uses of soybean. O 2005 Elsevier Inc. All rights reserved.

Keywords: Soybean; Seed composition; Physical characteristics; Growing environment; Food uses

1. Introduction

Soybean [Glycine max (L.) Merr.] has been known to far-eastern countries as one of the most economic and nutritious food for centuries. Given the various nutraceutical applications of soybean in reducing the risk of major killer diseases such as breast cancer, cardiovascular disease, osteoporosis, diabetes, and its role in alleviating menopausal symptoms [\(Messina, 1997\)](#page-7-0), people in western countries are also seeking to incorporate soy-foods into their regular diet.

The biochemical composition and physical appearance of soybean seeds affect the quality of various soy

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preparations such as soy milk (water extract from soybean), tofu (a gelatinous soy food), soy sprouts, miso and natto (fermented soy products). Generally, higher protein content, low oil content, lighter seed coat and a clear hilum are desirable characteristics for food uses ([Liu et al., 1995a, b](#page-7-0)). Additional specifications vary with the specific type of the soy food. Yield and texture of tofu are affected by seed protein content affects ([Lim](#page-7-0) [et al., 1990](#page-7-0); [Schaefer and Love, 1992\)](#page-8-0). Moreover, large seeded soybeans with clear hilum are preferred in both soy-milk and tofu preparations. In tofu-making, phytic acid, heat-stable anti nutritional factor in soybean seed, which chelates heavy metal ions Ca^{2+} , Mg^{2+} , Zn^{2+} , $Fe³⁺$, also has an important implication. Soybean seeds with a higher phytic acid content necessitate higher requirement of coagulants, namely CaSO₄ ([Schaefer and](#page-8-0) [Love, 1992\)](#page-8-0), thereby affecting the quality of tofu

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([Skurray et al., 1980\)](#page-8-0). Thus, soybean genotypes with a very low phytic acid content are good for tofu preparations. In other soy preparations such as soybean sprouts and natto, small seeded varieties with high protein content are preferred, while large seeded genotypes are preferred for miso and for use as vegetables (Edamame). Although soy foods prepared from seeds have yet to become common in the regular diet of people in many countries, soybean oil extracted from soybean seeds is already one of the major vegetable oils consumed the world over. Soybean oil is a good source of essential fatty acids, namely linoleic and linolenic acid. However, a very high presence (7%) in soybean seed of linolenic acid, a polyunsaturated fatty acid, which is much above the body's requirement, makes soybean oil easily susceptible to oxidation ([Frankel, 1980\)](#page-7-0). The partial hydrogenation of soybean oil being employed by industries to impart fairly good oxidative stability leads to formation of trans fatty acids which have been reported to cause hypercholesterolemic effects ([Zock and Katan, 1992](#page-8-0)). On the contrary, oleic acid in soybean seed, being less prone to oxidation, is a desirable attribute. Thus, soybean genotypes with low linolenic and high oleic acid are in high demand from industries to produce soybean oil with natural oxidative stability.

There are some studies indicating the influence of the growing environment on protein, oil, fatty acid composition, lipoxygenases, trypsin inhibitor ([Chapman et al.,](#page-7-0) [1976](#page-7-0); [Piper and Boote, 1999;](#page-8-0) [Nian et al., 1996](#page-8-0); [Thomas](#page-8-0) [et al., 2003;](#page-8-0) [Kumar et al., 2003](#page-7-0); [Pipolo et al., 2004\)](#page-8-0), while information on the effect of the growing environment on phytic acid and physical characteristics (such as seed size, hilum colour and seed coat colour) is scanty. Although Madhya Pradesh (a central region) is the epicentre of soybean cultivation in India, soybean has recently made inroads into new geographical locations with various latitudes. The intent of this study was to study the effect of growing environment, prevalent over widely differing latitudes, on both the biochemical and physical characteristics of soybean seed.

2. Material and methods

Seven Indian cultivars, namely Hara soya, JS335, KHSb2, Kalitur, NRC37, Pb1 and Shilajeet from the same seed lot were grown in the fields of H.P. Krishi Vishva Vidyalaya, Palampur $(32°N)$; at G.B. Pantnagar University of Agriculture and Technology, Pantnagar $(29°N)$; at the National Research Centre for Soybean, Indore $(22.2^{\circ}N)$; and at the University of Agricultural Sciences, Bangalore (12.6 \textdegree N). They were planted in a split-plot arrangement of a randomized complete block design with three replications. Each plot consisted of two rows 5 m long and 45 cm apart; the plant-to-plant

distance maintained was 5 cm. Seeds were harvested by hand on maturity and were oven dried at 60° C for 5 days until they became moisture-free. These oven-dried seeds were evaluated for protein, oil and fatty acid composition, phytic acid, seed size and hilum and seedcoat colour (Table 1).

2.1. Crude protein analysis

The crude nitrogen of the soy flour ground from dried seeds was determined by the standard micro-Kjeldahl method and it was converted to protein content by using the conversion factor 5.71. Values given in [Table 2](#page-3-0) are the mean of observations in three independent samples.

2.2. Oil extraction and estimation

Soy flour was extracted with n -hexane in an Automatic Soxtherm apparatus for 4 h as per the software programme of the instrument. Oil percentages were determined by weight differences. Values given in [Table](#page-3-0) [2](#page-3-0) are mean of observations in three independent samples.

2.3. Fatty acid analysis

The dried seeds were crushed into flour. Oil was extracted from freshly ground seed flour using petroleum ether (boiling point $40-60$ °C) and transesterified in methanol with 1 N sodium methoxide as catalyst following [\(Ludy et al., 1968\)](#page-7-0). Fatty acid methyl esters (FAMEs) were prepared by separating and analysing in gas liquid chromatograph (GLC), model Shimadzu GC 17A, using a capillary column measuring 30 m \times 0.32 mm. The oven temperature of the GLC was programmed at 140° C for 3.6 min, then increased to 170 °C at the rate of 13.5 °C per minute and maintained for 3.8 min and finally increased to $182 \degree C$ at the rate of 5° C per minute for best resolution of methyl esters. The temperatures of the flame ionization detector (FID) and injector were maintained at 240° C. Nitrogen, the carrier gas used, was maintained at a flow rate of 15 mL/min

Table 1

Different weather parameters during bean development in the cropping season at four growing locations

Location		Rainfall $(mm)^a$		Temperature $(^{\circ}C)$	Soil group	
	a	h	Max	Min	Mean	
Palampur $(32°N)$ 417.0 Pantnagar $(29°N)$ 259.6 Indore $(22°N)$ Bangalore $(12°N)$	329.0 61.0	956.0 918.9 447.7 227.8	25.8 30.2 31.8 27 O	12.2 18.5 21.6 16.2	19.0 24.4 26.7 21.6	Alfisol Mollisoll Vertisol Alfisol

^a(a) During bean development; (b) during whole cropping season.

 $n = 3$.

 $n = 3$.

with column pressure at 90 kPa . The peaks for individual FAMEs were identified by comparing the retention times with those of standard methyl esters (procured from Sigma Chemical Company). Data given in Table 3 for different fatty acids are means of observations in three independent samples.

2.4. Phytic acid analysis

Freshly harvested seeds from cultivars from all four locations were oven-dried at 60° C, ground to pass through a 0.5 mm sieve and subjected to phytic acid analysis following the standard method ([Wheeler and](#page-8-0)

[Ferrel, 1971\)](#page-8-0). Phytate content was calculated from the iron concentration in ferric chloride by assuming a constant Fe:P molecular ratio of 1:1.5 in the precipitate of the extracts. The values given in [Table 4](#page-4-0) are means of observations in three independent samples.

2.5. Physical attributes

Seed size was expressed as weight of 100 oven-dried seeds and given in the tables as the mean value of the observations in three independent samples. Seed coat colour and hilum colour were observed visually using a hand-held lens.

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2.6. Statistical analysis

Correlation studies were conducted by MSTAT-C programme developed by Russell D. Feed.

3. Results and discussion

[Table 1](#page-2-0) shows the different physiographic, climatic factors and soil types at four growing locations.

Among all the genotypes studied at different locations, the percentage of protein content ranged from 32.2% to 42.1% [\(Table 2\)](#page-3-0). Genotypic, locational and genotypic \times locational interaction were found to be significant for protein content $(P<0.001)$. The maximum value for protein was observed in Pb1 at Bangalore, while the minimum value was observed in KHSb2 at Pantnagar. Averaged over seven genotypes, the maximum mean protein content was observed at Indore (Table 5). Averaged over four locations, the maximum mean value for protein content was observed in Pb1 [\(Table 6\)](#page-5-0).

The percentage of oil content among genotypes studied at different locations ranged from 15.4–22.0% ([Table 2\)](#page-3-0). The maximum value for oil was observed in Hara soya at Palampur, while the lowest was observed in Shilajeet at Bangalore ([Table 2\)](#page-3-0). Averaged over seven genotypes, the maximum mean value was observed at Palampur (Table 5). Averaged over four location, Hara soya showed maximum mean value for oil content ([Table 6](#page-5-0)) while minimum values were observed for Pb1 and Shilajeet. Genotypic, locational and genotypic \times locational interaction were found to be significant for oil content $(P<0.001)$.

All the genotypes showed significant variation for individual unsaturated fatty acids over four different locations [\(Table 3\)](#page-3-0). Genotypic, locational and genotypic \times locational interaction for oleic, linoleic and linolenic and palmitic acid were observed to be significant $(P<0.001)$. For stearic acid, the effect of genotype was significant ($P < 0.05$); however, the effect of location and genotypic \times locational interaction was not significant. Averaged over seven genotypes, the minimum mean value for linolenic acid was observed at Pantnagar, while the highest value was observed at Palampur (Table 5). All the cultivars except KHSb2 ([Table 3](#page-3-0)) showed the lowest linolenic acid value at Pantnagar. Maximum mean values for oleic acid were observed at Pantnagar, while the minimum values were observed in Palampur. Averaged over four locations, Shilajeet

 $n = 3$

Table 5

Averaged over seven genotypes, mean percent protein, oil, fatty acid composition, phytic acid and size of soybean seed at four growing locations

Table 6

Averaged over four different growing locations, the mean percent protein, oil fatty acid composition, phytic acid content and seed size of different genotypes of soybean

Genotype	Biochemical parameters		Phytic acid	Seed size $(g/100)$					
	Protein $(\%)$	$\text{Oil } (\%)$		Fatty acid $(\%)$		(mg/g)	dried seeds)		
			16:0	18:0	18:1	18:2	18:3		
Hara soya	36.1	20.7	11.2	4.7	25.0	51.9	6.2	34.1	11.4
JS335	38.7	19.4	12.0	4.0	25.6	49.4	7.2	34.7	11.1
Kalitur	37.7	19.4	11.7	4.4	23.4	52.2	7.0	34.1	9.3
KHS _{b2}	37.4	19.7	12.1	4.3	26.5	49.8	6.4	33.6	10.8
NRC37	36.9	19.7	12.5	4.5	21.5	50.5	8.6	35.4	9.5
Pb1	39.4	17.6	11.5	4.9	26.3	49.2	7.3	34.6	9.3
Shilajeet	37.3	17.6	10.7	4.4	35.3	47.5	5.6	35.2	10.8

 $n = 3$.

Table 7 Physical characteristics of various genotypes of soybean at different locations

Location	Factor	Hara soya	JS335	Kalitur	KHSb2	NRC37	Pb1	Shilajeet
Palampur	Seed size ^a	11.5	11.4	8.8	10.2	8.6	8.8	10.5
	SC^b Hilum	Green Black	Yellow Black	Black Black	Yellow Light brown	Yellow Brown	Yellow Light brown	Yellow wrinkled Light brown
Pantnagar	Seed size ^a SC^b	13.4 Green	11.4 Yellow	11.8 Black	9.8 Yellow	9.0 Yellow	8.2 Yellow	13.7 Yellow
	Hilum	Black	Black	Black	Light brown	Brown	Normal brown	Light brown
Indore	Seed size ^a SC^b Hilum	9.9 Green, compressed Black	9.8 Yellow Black	7.6 Black Black	12.6 Yellow Light brown	8.8 Brown Brown	9.5 Yellow Yellow, light brown	10.5 Yellow Light brown
Bangalore	Seed size ^a SC^b Hilum	10.9 Green Black	11.7 Yellow Black	9.0 Black Black	10.4 Yellow Brown	11.5 Yellow Dark brown	10.6 Yellow Normal brown	8.9 Light brown

^aWeight of 100 dried seeds and mean of triplicate samples.

b Sc—seed coat color.

showed the maximum mean value for oleic acid and the minimum mean values for linoleic acid and linolenic acid (Table 6).

Phytic acid variation in different varieties at different locations ranged from 27.8 to 45.0 mg per gram of soy flour [\(Table 4](#page-4-0)). The maximum value for phytic acid was observed in Shilajeetat, Pantnagar while minimum was observed in Pb1 at Palampur. Averaged over seven genotypes, the maximum mean values for phytic acid were observed at Pantnagar and the minimum at Palampur. Locational and genotypic \times locational effects were found to be significant $(P<0.01)$.

Among physical characteristics, genotype and genotypic \times locational interaction for seed size were observed as significant ($P < 0.001$), while the effect of location on seed size was not significant. Among all the varieties studied under different locations, the maximum value for seed size was observed in Shilajeet at Pantnagar, while minimum value was observed in Kalitur at Indore (Table 7). Averaged over four locations, the maximum mean seed size was observed in Hara soya, while the minimum was observed in Pb1 and Kalitur (Table 6). Seed coat colour of all the genotypes remained the same at all the four growing locations (Table 7). However, varieties responded differentially for hilum colour. Pb1 was light brown at Palampur and Indore; however, it appeared its normal brown colour at Pantnagar and Bangalore. The hilum colour of NRC37 appeared dark brown at Bangalore, while it was its normal brown at the other locations.

Genotypes responded differentially for variation in seed size and biochemical characteristics over the four locations. JS335 was observed to be the most stable for oil, protein and seed size. Hara soya was found to be the most sensitive for oil content, while KHSb2 was the most sensitive for protein content. Pb1 was observed to be the most sensitive for linolenic acid content and Shilajeet was most sensitive for oleic acid, linoleic acid,

phytic acid and seed size. Hara soya was found to be the most stable for fatty acid composition, while Kalitur was the most stable for phytic acid content.

The correlation coefficient between various seeds traits, namely seed size and biochemical components, are shown in Table 8. Significant negative correlation between oil and protein was observed $(P<0.01)$. Seed size showed a positive correlation with desirable oleic acid $(P<0.01)$, while a significant negative correlation with unsaturated fatty acids linoleic $(P<0.01)$ and linolenic acid $(P<0.05)$ is in agreement with earlier reports ([Maestri et al., 1998a, b](#page-7-0); [Liu et al., 1995b\)](#page-7-0). A significant positive correlation $(P<0.05)$ of seed size with antinutritional factor phytic acid was also observed. However, a non-significant correlation of phytic acid with protein content is in contrast to earlier reported significant positive correlations between these two biochemical components in some legumes ([Chitra et](#page-7-0) [al., 1995\)](#page-7-0). Results also indicated negative correlation of oleic acid with linolenic acid $(P<0.001)$ and linoleic acid $(P<0.001)$, but a positive significant correlation between linolenic acid and linoleic acid $(P<0.001)$.

Table 9 shows the correlation analysed between seed size, biochemical parameters and various climatic factors. No significant correlation was observed between seed size and latitude and any of the climatic factors. Significant negative correlation of latitude with protein $(P<0.01)$ and positive correlation with oil $(P<0.05)$ is in agreement with earlier reports ([Breene et al.,](#page-7-0) [1988](#page-7-0); [Maestri et al., 1998a, b](#page-7-0)). Our results did not indicate any significant correlation between latitude and any fatty acid in contrast to the earlier report ([Maestri](#page-7-0) [et al., 1998b\)](#page-7-0) which suggested a positive correlation between latitude and linoleic acid and negative with linolenic acid. This may be because the earlier reported study was carried out over a very narrow range of latitude. Rainfall during bean development showed a positive correlation with oil $(P<0.05)$, and a negative one with protein $(P<0.05)$. Total rainfall during the cropping season in our studies showed a stronger negative correlation with protein $(P<0.001)$ (Table 9). However, a non-significant positive correlation of total rainfall during the season with the oil content observed in our studies is in contrast

Table 8

Correlations studied among various biochemical parameters and seed size^a ($n = 84$)

	Protein	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Seed size	Phytic acid
Oil Protein Palmitic Stearic Oleic Linoleic Linolenic Seed size	$-0.507**$	-0.061^{ns} 0.100 ^{ns}	0.108 ^{ns} -0.144 ^{ns} -0.0166 ^{ns}	-0.251^{ns} 0.067^{ns} -0.363^{ns} -0.163^{ns}	0.255^{ns} $-0.109ns$ 0.219^{ns} 0.096^{ns} $-0.949***$	0.249^{ns} -0.072 ^{ns} 0.120 0.054^{ns} $-0.740***$ $0.632***$	0.082 ^{ns} -0.020^{ns} -0.310^{ns} -0.121^{ns} $0.512**$ $-0.457**$ $-0.407*$	-0.030^{ns} -0.091^{ns} -0.089 ^{ns} 0.01 ^{ns} 0.275^{ns} -0.227^{ns} -0.312 ^{ns} $0.376*$

 $ns = non significant.$

Level of significance.

 $T₁$ \sim

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

 $ns = non significant.$

^aLevel of significance.

^b(a) bean development, (b) cropping season.

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

to the earlier report (Maestri et al., 1998b) which suggested a negative correlation of total rainfall during the growing season with oil content. Furthermore, total rainfall during seed fill has been reported to be positively associated with linoleic acid and negatively with oleic acid (Kane et al., 1997); however, our results did not indicate this.

It has been suggested that temperature is more important than the photoperiod, which is related to latitude, in influencing the linolenic acid content (Howell and Collins, 1957). A number of reports ([Wolf et al., 1982;](#page-8-0) [Rennie and Tanner, 1989\)](#page-8-0) also suggested that soybeans developing under warmer temperatures possess elevated oleic acid and low polyunsaturated fatty acids. In our studies, maximum and minimum temperatures during bean development showing negative correlation with linolenic acid are in agreement with the earlier report (Howell and Collins, 1957). Both maximum and minimum temperatures also showed positive correlations with palmitic acid $(P<0.05)$. In our studies, the average minimum temperature seemed to affect protein and oil as well, as it showed a positive correlation with protein $(P<0.01)$ and a negative correlation with oil $(P<0.05)$. The mean temperature during bean development showed a significant positive correlation with protein and a negative correlation with oil and linolenic acid $(P<0.05)$.

The differences in locational mean values for phytic acid, the principal source of phosphorus (80%) in soybean seed, at different latitudes may be explained on the basis of soil characteristics and temperature. Low mean values for phytic acid at Palampur may be attributed to the alfisollic character of the soils ([Table 1](#page-2-0)) of this location, which possess unfavourable characteristics (acidic pH, high iron and aluminium oxides) for phosphorus mineralization [\(Tripathi et al.,](#page-8-0) [1982](#page-8-0)) accompanied by prevailing low temperatures during bean development ([Table 1\)](#page-2-0), another unfavourable factor for phosphorus mineralization (Chaudhary and Sandhu, 1982). The higher value observed at Pantnagar can be explained by the mollisollic characteristics of the soils of this location, which possess favourable characteristics (neutral pH, high organic matter and phosphorus) coupled with high temperatures during bean development [\(Table 1](#page-2-0)); these are conducive factors for phosphorus mineralization (Biswas and Mukherji, 1992; Chaudhary and Sandhu, 1982). Thus, phytic acid accumulation in seed is affected by interaction of soil type and temperature.

Considering the effect of various climatic factors observed on biochemical composition and physical characteristics of soybean seed in our studies, it may be concluded that environment-specific genotypes need to be developed to enhance food uses of soybean and to obtain soybean oil with more oxidative stability.

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