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Screening soybean (grain and vegetable) genotypes for nutrients and anti-nutritional factors

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Abstract. Fifty six genotypes of grain-type soybean and 17 genotypes of vegetable-type soybean collections were analyzed for protein and oil content, trypsin inhibitor, and lipoxygenase activities. The protein and oil content ranged from 36.9 to 47.9% and from 13.3 to 23.0% for different accessions in grain- and vegetable-type soybeans, respectively. Trypsin inhibitor and lipoxygenase activities ranged from 22.0 to 47.0 trypsin inhibitor units/mg meal and from 482 to 6265 lipoxygenase units/min/mg meal for grain- and vegetable-type soybeans, respectively. Significant correlations ($r = -0.62$ and -0.52, $P < 0.05$) were found between protein and oil, and between protein and trypsin inhibitor. A significant positive correlation ($r = 0.42$, $P < 0.05$) was also calculated for oil and lipoxygenase activity. Several genotypes of soybean and vegetable soybean (plant introductions 423905, 417330, 417223, 171451, 200506, 200523, 417124, 227687, 203402, 445842, 203399, 423852, 416771, FC 31927, Avoyelles, and Sooty) showed good nutritional potential and may be useful in a breeding program to improve the nutritional quality of soybean. Screening for essential amino acids, fatty acids, and trace minerals for selected genotypes is underway.

Introduction

Legume seeds are an important source of dietary protein, oil, and carbohydrates. It is the relative proportions of these constituents that largely determines the nutritional quality of the seeds [5, 38]; that is a high content of both protein and oil is desirable. However, it has been documented that protein and oil are negatively correlated [10, 20, 21, 24]. Although the soybean (*Glycine max* L.) has a high protein content and is used as an alternative to animal protein, the nutritive value of soybean protein is poor due to deficiency of the sulfur-containing amino acids, particularly methionine [5, 7, 25] and the presence of several anti-nutritional factors. Protease inhibitor is one of the anti-nutritional factors found in soybean [11, 36]. It has been reported that animals fed heat-treated

soybeans had better growth and a higher protein efficiency ratio (PER) [4, 13, 14].

Lipoxygenase is considered an anti-nutritional factor because of its adverse effects on the unsaturated fatty acids and also on flavor. This enzyme mediates the oxidation of linoleic and linolenic acids, and consequently causes off-flavor in soybean food products and beverages [2, 29, 31, 37]. However, heat treatment and other food processing techniques inactivate this enzyme and improve the flavor of soy food products [2, 5, 37].

Vegetable-type cultivars of soybean are already popular as a food in the Orient. Also they are reported to be superior to grain-type cultivars in flavor, texture, and cooking, [23, 30] and low in trypsin inhibitor activity [6, 8, 9, 27, 28]. Little is known about the nutritional quality of vegetable-type soybean and no studies have been reported on genetic variations in protein, oil, trypsin inhibitor and lipoxygenase.

The objective of this study was to screen soybean (grain- and vegetable-type) for nutritional and anti-nutritional factors.

Methods and materials

Fifty-six genotypes of grain-type soybean from maturity groups (MG) VI, VII, and VIII, and 17 genotypes of vegetable-type soybean were selected from germplasm collection based on their tolerance to ozone and resistance to the Mexican bean beetles. The selected genotypes of soybean and vegetable soybean were analyzed for protein and oil content. Twenty g sample of seeds was weighed and ground in a centrifugal grinding mill (Cyclotec, model 1093 sample meal, Techator) and passed through a 0.5 mm pore size screen; three samples for each accession were analyzed. Protein content was analyzed by the Kjeldahl method [1]. Oil content was determined by petroleum ether and Goldfisch extractor (Labconco Corp.) according to the method of Quaife and Harris [35]. The defatted meal samples were prepared as described by Mohamed et al. [28] and used to determine trypsin inhibitor and lipoxygenase activity as described below.

Trypsin inhibitor activity was determined according to the method of Kakade et al. [22] using 2-N-Benzoyl-DL arginine p-nitroanilide as substrate. One trypsin unit corresponds to an increase of 0.01 absorbance at 410 nm/10 ml of the reaction mixture under the conditions defined by Kakade et al. [22]. Trypsin inhibitor activity is defined as the number of trypsin units inhibited.

Lipoxygenase activity was assayed by a modification of the spectrophotometric method of Surrey [40] using linoleic acid as substrate.

Defatted soybean sample (0.25 g) was extracted for 30 min with 25 ml of deionized water and centrifuged for 15 min at 18,000 × g. The supernatant was used to prepare the working sample solution; 2 ml was brought to 100 ml with borate buffer (0.10 M, pH 7.0). The linoleic acid stock solution was prepared with equal volumes (50 μl) of linoleic acid and 90% ethanol and brought up to a final volume of 50 ml with deionized water. Five ml of the stock lineoleic acid solution was brought to a final volume of 30 ml with borate buffer; this was used as the working substrate solution. The reaction mixture, consisting of 2.4 ml borate buffer and 0.5 ml of the working substrate solution was warmed to 25 °C in a cuvette using a model DU-8 spectrophotometer (Beckman Instruments, Inc., Columbia, MD). To start, the reaction 0.1 ml of the working sample solution was added to the mixture. The increase in absorbance at 234 nm over a period of 5 min was recorded. One unit of lipoxygenase is defined as that amount which causes an increase of 0.001 absorbance unit per minute at 234 mm under stated conditions.

The data were statistically analyzed and the means were separated using the least significant differences (LSD) test at the 5% level of significance. Simple linear correlations were also calculated using the MSTAT statistical package.

Results and discussion

As shown in Table 1, wide variations were found in the protein and oil content of soybean genotypes from MG VI. The mean % protein was 42.3, and ranged from 39.2% for PI 416.937 to 45.8% for PI 417.330. The mean % oil was 19.9 and ranged from 17.2% for PI 416.937 to 23.7% for PI 210.422. These genotypes were also analyzed for trypsin inhibitor (TI) and lipoxygenase (LP) activities. The activity of TI ranged from 25.0 TI units/mg meal for PI 423.905 to 42.4 TI units/mg meal for PI 201.422; mean TI was 31.21 TI units/mg meal. Lipoxygenase activity ranged from 663 LP units/min/mg meal for PI 417.310 to 3459 units/min/mg meal for PI 71.525, with a mean of 2227 units/min/mg meal.

The data in Table 2 show wide variations in % protein and % oil for selected accessions of soybean from MG VII. PI 229321 had the lowest % protein (37.2), while the highest value was reported for PI 416.900 (45.0%). The mean % protein was 41.5%. Oil content ranged from 15.7% for PI 416.900 to 23.0% for FC 31.732, with a mean of 19.9%. No significant difference was found in protein and oil between MG VI and MG VII. Variation in trypsin inhibitor and lipoxygenase activities are also reported in Table 2. The mean trypsin inhibitor and lipoxygenase actitivities were

Table 1. % protein (16% nitrogen), % oil, trypsin inhibitor and lipoxygenase activity in selected soybean germplasm from maturity group VI

Accessions	% Protein	% Oil	TI[a]	LP[b]
PI 36906	40.7	9.6	29.5	2212
PI 71525	41.8	10.5	31.2	3459
PI 88461	43.1	19.9	30.8	3377
PI 201422	40.5	23.7	42.4	1673
PI 379621	44.1	18.1	30.7	2133
PI 416895	41.0	20.1	31.4	2538
PI 416925	40.2	19.6	31.2	3364
PI 416937	39.2	17.2	29.9	2837
PI 417213	42.7	22.1	33.1	1831
PI 417223	43.6	17.9	30.9	1881
PI 417310	41.4	17.9	32.2	663
PI 417330	45.8	20.2	27.5	1239
PI 417422	43.1	19.3	28.9	2128
PI 417742	44.7	20.9	30.0	2101
PI 423905	44.0	20.9	25.0	2420
PI 423907	41.0	21.5	27.2	2325
SS 7171	41.7	20.3	38.8	1677
Mean	42.32	19.95	31.21	2227
CV %[c]	1.95	2.83	1.64	4.3
LSD ($P < 0.05$)	0.95	0.60	1.80	50

[a] Trypsin inhibitor units/mg protein
[b] Lipoxygenase units/min/mg protein
[c] Variation Coefficient

29.9 TI units/mg meal and 2269 LP/units/min/mg meal, respectively. These means ranged from 24.3 TI units/mg meal for PI 393.547 to 41.4 TI units/mg meal for PI 31.927, and 968 LP units/min/mg meal for PI 31.927 to 3502 units/min/mg meal for cultivar 'Bragg'.

The data in Table 3 for MG VIII show that PI 227687 had the highest level of protein (49%); however, this line was also high in trypsin inhibitor (36.3 TI units/mg meal) and lipoxgenase activity (4084 LP units/min/mg meal) and exceptionally low in oil (13.3%). Cultivar 'Avoyelles' had a high level of oil (23.1%) and was exceptionally low in lipoxygenase activity (482 LP units/min/mg meal). It was moderate in trypsin inhibitor (28.6 TI units/mg meal) and low in protein (41.9%). No significant difference was found among maturity groups for either protein and oil. However, MG VIII was significantly higher in lipoxygenase and had wider range (482 to 6265 LP units/min/mg meal) than either MG VI or VIII. The data also indicate that the selection of these accessions from MG VI, VII, and VIII covered most differences in protein, oil, trypsin inhibitor, and lipoxygenase activity.

Seventeen genotypes of vegetable-type soybean were analyzed for protein,

Table 2. % protein (16% nitrogen), % oil, trypsin inhibitor and lipoxygenase activity in selected soybean germplasm from maturity group VII

Accessions	% Protein	% Oil	TI[a]	LP[b]
PI 7219	41.4	22.5	35.6	1861
PI 31927	41.3	21.0	41.1	968
PI 71564	44.7	20.0	24.4	2125
PI 71608	42.1	19.3	39.7	3266
PI 171451	43.4	19.2	24.4	2003
PI 181565	41.9	19.1	37.5	2844
PI 200474	41.4	21.2	34.2	2815
PI 200506	43.9	21.3	28.1	1556
PI 200523	44.1	18.9	27.8	1559
PI 229321	37.2	22.8	24.4	2300
PI 229358	37.7	20.5	28.1	2751
PI 322690	40.6	19.4	32.5	1469
PI 393547	43.9	18.3	24.3	3481
PI 393550	42.6	19.8	26.2	2162
PI 416900	45.0	15.7	33.4	2802
PI 417063	40.2	19.0	29.8	1093
FC 31732	39.4	23.0	20.4	2007
Bragg	37.3	20.0	35.9	3502
Braxton	39.6	18.0	38.3	2550
Mean	41.56	19.94	29.92	2269
CV %[c]	2.10	2.67	4.12	3.9
LSD ($P < 0.05$)	0.86	0.51	1.20	106

[a] Trypsin inhibitor units/mg protein
[b] Lipoxygenase units/min/mg protein
[c] Variation Coefficient

oil, trypsin inhibitor, and lipoxygenase activity; these data are presented in Table 4. Means % protein and % oil were 42.9 and 19.2, respectively. The protein content ranged from 36.9% for PI 222.397 to 47.9% for Cultivar 'Sooty', and oil ranged from 15.2 for PI 423.852 to 23.4% for Cultivar 'Sango'.

The mean trypsin inhibitor activity was 31.2 TI units/mg meal and ranged from 24.4 TI units/mg meal for PI 423.759 to 47.1 TI units/mg meal for PI 222.397. Lipoxygenase activity ranged from 963 LP units/min/mg meal for PI 203.399 to 4750 LP units/min/mg meal for PI 222.397, with a mean of 2267 LP units/min/mg meal. PI 203.399 was characterized by low lipoxygenase activity (963 LP units/min/mg meal), and moderate protein content (40.3%) and trypsin inhibitor (27.4 TI units/mg meal). The oil content of this PI was also low (17.3%). Cultivar 'Sooty' was characterized by high protein (47.9%) and relatively low lipoxygenase activity. The oil content and trypsin inhibitor were average. No significant difference was

Table 3. % protein (16% nitrogen), % oil, trypsin inhibitor and lipoxygenase activities in selected soybean germplasm from maturity group VIII

Accessions	% Protein	% Oil	TI[a]	LP[b]
PI 159924	39.9	23.0	27.7	2823
PI 181697	40.7	18.8	25.5	2193
PI 194773	41.7	18.5	27.2	5419
PI 203399	40.4	20.9	24.2	3330
PI 203402	38.8	20.2	31.7	991
PI 209837	42.6	20.2	24.4	1545
PI 227687	49.0	13.3	36.3	4084
PI 324068	41.9	20.3	30.5	1062
PI 381657	44.8	15.4	30.4	2389
PI 416806	42.9	17.6	30.9	1754
PI 417061	43.5	15.3	30.5	2695
PI 417124	40.9	19.7	22.0	1459
PI 417134	39.9	18.3	28.3	2912
PI 417136	41.2	17.4	27.5	3147
PI 437668	43.3	18.3	30.3	2432
PI 445842	43.3	18.7	24.7	1283
SS 374161	44.4	19.2	31.8	1851
Avoyelles	41.9	23.1	28.6	482
Cobb	39.3	23.2	26.3	2864
Hutton	44.0	22.2	26.0	6265
Mean	42.56	17.43	28.28	2549
CV %[c]	1.69	3.40	4.91	5.1
LSD ($P < 0.05$)	1.4	0.75	2.01	89

[a] Trypsin inhibitor units/mg meal
[b] Lipoxygenase units/min/mg meal
[c] Variation Coefficient

found between vegetable-type and grain-type soybean in protein, oil, trypsin inhibitor, and lipoxygenase activity.

The protein values reported in this study are higher than those reported for other grain and vegetable-type soybean genotypes [6, 9, 10, 24]. This result may be due to environmental factors and/or varietal differences [9, 42]. The mean oil content of tested genotypes was similar to that reported by Hafez [10] and Deodhar et al. [6]. The variation among the tested genotypes was greater than that reported in earlier studies [26, 27, 29]. A significant negative correlation coefficient ($r = -0.62$) was observed between protein and oil content. This correlation coefficient was lower than that reported by Hafez [10] and similar to that reported by Weiss et al. [42], Krivoruchco et al. [24], and Mohamed et al. [28, 29]. Two anti-nutritional factors, trypsin inhibitor and lipoxygenase, were investigated in this study. The selection of these two factors was based on their adverse effects on

Table 4. % protein (16% nitrogen), % oil, trypsin inhibitor and lipoxygenase activities in selected soybean germplasm

Accessions	% Protein	% Oil	TI[a]	LP[b]
PI 171437	41.8	20.3	29.7	3165
PI 194773	44.8	19.4	38.0	3860
PI 203399	40.3	17.3	27.4	963
PI 222397	36.9	22.3	47.1	4750
PI 416771	46.8	17.6	24.7	1271
PI 416982	44.8	19.8	31.8	1603
PI 417052	41.2	21.0	30.8	1559
PI 417288	42.2	18.5	27.7	1506
PI 423759	38.9	22.4	24.4	1419
PI 423852	40.2	15.2	25.1	1689
Emperor	46.5	17.9	32.7	2292
Kim	40.7	20.4	34.7	3760
Kingston	46.8	18.4	31.3	2424
Sango	38.4	23.4	34.9	2525
Sooty	47.9	19.3	29.4	1143
Ware	43.5	18.1	32.4	3787
Wilson-5	47.8	15.7	28.3	3012
Mean	42.99	19.26	31.23	2267
CV%[c]	2.60	3.41	2.40	5.3
LSD ($P < 0.05$)	1.34	1.02	2.24	150

[a] Trypsin inhibitor units/mg meal
[b] Lipoxygenase units/min/mg meal
[c] Variation Coefficient

protein digestibility and oil quality. Variations in trypsin inhibitors and their inheritance in vegetable and grain-type soybean have been investigated [8, 11, 19, 32, 33, 39]. In earlier studies we indicated that food processing such as microwave heating and gamma irradiation [13, 14] reduces trypsin inhibitor activity. However, the presence of trypsin inhibitor activity was not completely eliminated, and our investigation indicated the presence of another type of proteinase inhibitor, which we called non-protein trypsin inhibitors [11]. The non-protein trypsin inhibitors are small peptide and are heat stable [12]. More research is needed to study the inheritance of these inhibitors in soybean germplasm. Several studies have been reported on the inheritance of lipoxygenase in grain-type soybeans, and soybean lines lacking lipoxygenase-1(PIs 133226, 408251, 157440, 229324, T102, and cultivar Altona) have been identified [15–17, 34]. Lipoxygenase activity is affected by such factors as oil content, temperature, pH, and moisture content [3, 14, 18, 41]. Earlier reports indicate that lipoxygenase activity can be drastically reduced in whole seed by several food processing techniques, such as conventional heating, microwave heating, and gamma irradiation [10, 14, 20].

In soybean with high oil content, the low lipoxygenase activity is highly desired because of the effect of this enzyme on the stability of oil and off-flavor associated with soymeal. In vegetable-type soybeans, which are generally eaten as green immature seed, the presence of lipoxygenase does not affect the nutritional quality and/or the flavor.

In conclusion, the wide variations in protein, oil, trypsin inhibitor, and lipoxygenase activity indicate that it is possible to breed for low anti-nutritional factors, and high protein and oil content, in both vegetable and grain-type soybeans. Several genotypes of grain and vegetable- type soybean (PIs 423905, 417330, 417223, 171451, 200506, 200523, 417124, 227687, 203402, 445842, 203399, 423852, 416771, FC 31927, Avoyelles, and Sooty) show good nutritional potential and may be useful in breeding programs. Through hybridization and selection, the gene(s) responsible for desirable nutritional quality observed in those genotypes could be incorporated into other genotypes which possess the desirable agronomic traits. The screening for essential amino acids, fatty acids, and trace minerals for the germplasm of those selected genotypes is underway.

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