

Associations between isoflavones and protein content in soybean (*Glycine max*) seed

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Abstract

Six soybean cultivars were grown at three locations in Tennessee, USA to determine if there was a correlation between protein content and isoflavone concentration. The cultivars represented two maturity groups (MG 4 and 5) and were selected for this study based on high, intermediate or low seed protein content relative to commercial cultivars with similar adaptation. Four of the six cultivars possessed the glyphosate tolerance trait. Isoflavones were measured using an HPLC method. Protein content did not correlate with any of the major isoflavones detected, daidzin, genistin, glycitin, malonyl daidzin, malonyl genistin, malonyl glycitin, or closely related derivatives of malonyl daidzin or malonyl genistin. Both protein content and total isoflavone concentration varied by cultivar and location ($P < 0.05$).

Media summary

Six soybean cultivars differed by protein content and isoflavone concentration, but there was no correlation of protein content with isoflavone concentration.

Key words

protein, soybean, isoflavone

Introduction

Soybeans contain isoflavones which have been associated with antioxidative and anticancer activity (Messina et al. 1994; Naim et al. 1976). Because protein content is a quality measure in soybeans, we investigated whether there is a correlation between protein content and isoflavone concentration.

Methods

Selection of Cultivars

A subset of six cultivars was selected from a larger set of cultivars that had been tested for yield, seed protein and seed oil at the Ames Plantation, Grand Junction, Tennessee, USA (35.07 lat., 89.15 long.), Knoxville Experiment Station, Knoxville, Tennessee, USA (35.96 lat., 83.91 long.), and Milan Experiment Station, Milan, Tennessee, USA (35.92 lat., 88.76 long.) during 2003. The growing season at all three locations was characterized by very favorable soil moisture conditions throughout all stages of growth. The yield tests were conducted in a randomized complete block design with three replications. The six cultivars were selected on the basis of their average protein content measured across seven environments in Tennessee during 2003. Cultivars USG 5601T and NK Brand S 57-P1 were chosen because of high seed protein relative to other cultivars tested. Holladay and Pioneer 95B43 were chosen because of lower protein content and Morsoy RT 4809 and USG 7403nRR were chosen because of intermediate levels of protein. The latter two cultivars are classified as maturity group 4 cultivars; whereas the other four are classified as maturity group 5. USG 5601T and Holladay are conventional, non-glyphosate herbicide tolerant cultivars and the other four cultivars are glyphosate tolerant.

Analytical Procedures

Seed protein and isoflavone content were determined with three replications from each location. Protein was measured on whole beans using a Foss Infratec 1229 Grain Analyzer. Approximately 60 g seed

samples of each variety were ground in a water-cooled grinder (Foss Knifetec 1095 Sample Mill) at 20 degrees C for 20 sec. Extraction of isoflavones was performed by a modification of the method of Griffith and Collison (2001). Approximately 200 mg of ground seed were extracted on a rotary shaker for 2 h in 2.0 ml of acetonitrile, 1.2 ml of high purity water, and 0.1 ml of an internal standard solution containing 1 mg/ml of apigenin. Then 0.7 ml of water was added and the mixture was centrifuged for 10 min at 2000 g. The supernatant was filtered through a 0.45 µm filter and stored at -80 °C until analysis by a high performance liquid chromatograph (HPLC). A Hewlett-Packard (Palo Alto, CA, USA) HPLC was used with an Agilent (Palo Alto, CA, USA) Zorbax XDB C-18 reverse-phase column (150 H 3.0 mm ID, 3.5-µm) and UV detector at a wavelength of 260 nm. The column temperature was set at 40 °C. Injection volume was 5 µl. Solvent A was 0.1% (v/v) acetic acid in water. Solvent B was 0.1% (v/v) acetic acid in acetonitrile. Gradient elution was from 9% B to 15% B over 10 min, from 15% B to 17% B over an additional 10 min, held at 17% B for 23 min, then purged for 2 min with 90% B and equilibrated for 10 min at 9% B prior to the next sample analysis. Isoflavones were identified by comparison with authentic standards of daidzin, genistin, glycitin, daidzein, genistein, glycitein, malonyl daidzin, and malonyl genistin procured from LC Laboratories (Woburn, MA, USA). Data were analyzed using the SAS statistical software (SAS, 1999).

Results

Protein content

Protein content differed among all cultivars except USG 5601T and Morsoy RT 4809 (Table 1). The highest level, measured in NK Brand S 57-P1, was 13% higher than the lowest level, measured in Holladay. Only the seed yields of USG 5601T and USG 7403nRR differed significantly across the three locations.

Table 1. Seed Protein, isoflavone, and yield levels in six soybean varieties grown at three locations in Tennessee, USA during 2003.

Cultivar	Protein ^z (%)	Total Isoflavones (mg/g)	Yield ^y (kg/ha)
NK Brand S 57-P1	42.9 A ^x	7.2 A	4226 AB
USG 5601T	41.6 B	7.9 A	4494 A
Morsoy RT 4809	41.6 B	3.2 C	4464 AB
USG 7403nRR	40.7 C	4.5 B	4054 B
Pioneer 95B43	38.7 D	5.2 B	4098 AB
Holladay	38.0 E	7.9 A	4300 AB

^zDry weight basis; averaged across the three locations.

^yAdjusted to 13% moisture.

^xMean separation within columns by Duncan's multiple range test, P #0.05.

Isoflavone concentration

Total isoflavone concentration was highest in USG 5601T and Holladay. In USG 5601 T, total isoflavone concentration was 168% higher than in Morsoy RT 4809, the cultivar with the lowest isoflavone concentration. The predominant isoflavones were malonyl daidzin and malonyl genistin, each comprising greater than 38% of the total isoflavone concentration (Table 2). The next highest concentration of an individual isoflavone, malonyl glycitin, contributed about 5% to the total isoflavone concentration. The isoflavones referred to as new malonyl daidzin and new malonyl genistin were identified by comparison with previously published chromatograms (Griffith and Collison 2001). According to their study, these compounds are structurally similar to their malonyl parents and may only differ by the position of hydroxyls on the glucose. These isoflavones appear increasingly with time in extraction solutions of isoflavones but may be only minor components in undisrupted soybeans.

Statistical Analyses

Protein content did not correlate with concentration of total or individual isoflavones (Table 2). Protein and isoflavone content differed by cultivar, location, and cultivar \times location ($P < 0.05$) (Table 3). Several studies have previously documented location and cultivar effects on isoflavone concentration in soybeans (Hoeck et al. 2000; Lee et al. 2002). Our study determined that for the six soybean cultivars tested, protein content did not correlate with isoflavone concentration.

Conclusion

Because protein content did not correlate with isoflavone concentration, the use of protein content as an indicator for isoflavone concentration does not appear to be warranted. A correlation of protein with isoflavones may exist among specific cultivars, and future research may determine if this is the case.

Table 2. Mean isoflavone concentrations and correlation coefficients for isoflavones with protein content.^z

Isoflavone	Mean quantity (mg/g)	Correlation coefficient (r)
Total	6.09	0.08
Daidzin	0.17	-0.01
Genistin	0.24	-0.07
Glycitin	0.12	-0.14
Malonyl daidzin	2.41	0.10
Malonyl genistin	2.32	0.04
Malonyl glycitin	0.30	0.16
New malonyl daidzin	0.24	0.14

New malonyl genistin

0.18

0.06

²Individual isoflavones were at least 1% of total quantity.

Table 3. Analyses of variance for protein content and total isoflavone concentration in soybeans produced at three locations in Tennessee, USA.

Source	DF	SS	MS	F-value	P-value
<i>Model for protein content</i>					
R-square = 0.97					
Cultivar	5	156.1	31.2	111.3	<0.0001
Location	2	43.0	21.5	76.6	<0.0001
Cultivar ? Location	10	26.1	2.6	9.3	<0.0001
Error	29	8.1	0.28		
<i>Model for total isoflavone concentration</i>					
R-square = 0.89					
Cultivar	5	178.3	35.7	35.9	<0.0001
Location	2	24.2	12.1	12.2	0.0001
Cultivar ? Location	10	28.2	2.8	2.8	0.01
Error	30	29.8	0.99		

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