Isoflavone Content Among Maturity Group 0 to II Soybeans

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ABSTRACT: This study reports the isoflavone contents of 210 soybean cultivars grown in South Dakota and explores possible relations between isoflavone contents and agronomic characteristics. Total isoflavone contents (normalized) ranged from 1161 to 2743 µg/g. A number of agronomic characteristics were documented for each variety including maturity group, hilum color, disease resistance, seed weight, yield, maturity (in days), and plant height. Varieties in maturity group I had significantly higher total isoflavones when compared to maturity group 0. Hilum color was related to differences in genistin, daidzein, and genistein content. No differences in isoflavone content were observed based on disease resistance profiles. Genistein content was found to be negatively correlated with yield, days of maturity, and plant height. Weak but significant correlations also existed between these agronomic characteristics and other isoflavones.

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KEY WORDS: Agronomic characteristics, daidzein, genistein, *Glycine max* L. Merrill, glycitein, HPLC, isoflavone.

Isoflavones exert a number of biological influences. Barnes *et al.* (1) documented that rats consuming a soy-based diet developed fewer mammary tumors following administration of the carcinogens *N*-methylnitrosourea and 7,12-dimethylbenz[a]-anthracene than rats fed isonitrogenous and isocaloric diets without soy. The anticarcinogenic effect may have been due to isoflavones. Isoflavones exhibit other influences such as a reduction in risk factors associated with heart disease. Soy protein products, especially those with higher concentrations of isoflavones, were shown to be hypocholesterolemic in human clinical trials (2).

Isoflavone content varies substantially among soybeans according to variety and growth conditions (3,4). Wang and Murphy (3) reported that the effect of crop year had a greater impact on the isoflavone content than did location, possibly due to climate conditions. Tsukamoto *et al.* (4) documented significant isoflavone decreases in the seeds of soybean varieties grown at a high temperature. Other investigators have documented differences in isoflavone content relating to seed physiology. Among the three major parts of the seed, the hypocotyl was found to have the highest concentration of

isoflavones, at about 10 to 20% (w/w) of total seed isoflavones (3–5). In soybean seedlings, leaf flavonoid concentrations are increased by ultraviolet (UV)-B radiation and decreased soil phosphorus (6–8). The relation between isoflavone content and agronomic properties is valuable to soybean breeders for the selection of varieties that are high in individual and total isoflavones. To date, little work has been done on the relation between isoflavone content and agronomic soybean characteristics. The objective of this study was to determine useful associations that may exist between isoflavone concentrations and agronomic characteristics of soybean varieties adapted for growth in the northern United States.

MATERIALS AND METHODS

The soybean sample set $(n = 210)$ was collected from 1994 Crop Performance Tests. The soybean varieties were grown at the South Dakota State University (SDSU) Agronomy Farm in Brookings, South Dakota. The sample set consisted of 41 varieties in maturity group 0, 96 varieties in maturity group I, and 73 varieties in maturity group II. Hilum color and seed mass were determined for each variety. Hilum color among varieties was yellow, green, black, or brown. Yield, days of maturity, plant height, and disease resistance to three strains of *Phytophthora* (root rot) were obtained from the 1994 Crop Performance Tests (9).

Extraction procedure. Soybeans were ground with a Retsch Ultra-Centrifugal Mill Model ZM-1 (Haan, Germany) with a 0.5-mm sieve. One gram of soy meal was added to 6 mL of 80% methanol, and the mixture was stirred for 30 min. The soy meal/methanol mixture was filtered into a 50-mL volumetric flask through Whatman #42 filter paper followed by two washes with 5 mL of 80% methanol, and 80% methanol was used to dilute the filtrate to volume. Extraction conditions (stirring time, number of washes) were optimized by using the soybean variety, Mustang M-1000. The recovery rates for genistein and daidzein were consistently greater than 90%. The coefficients of variance (CV) for intraday and interday determinations were all below 2%. Although inadequate for extraction of acetyl forms of isoflavones (Murphy, P.A., personal communication), the 80% methanol extraction procedure worked well with raw soybeans, as they contained a very low amount of these acetyl derivatives.

Determination of isoflavone content. High-performance

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liquid chromatography (HPLC) was performed with a YMC-Pack ODS-AQ 303 column (5 µm, 25 × 4.6 mm i.d.) (Wilmington, NC). A modified gradient program (10) was used, where solvent A $(0.1\%$ glacial acetic acid in water) was decreased from 85 to 69% over 45 min, while solvent B (0.1% glacial acetic acid in acetonitrile) was increased from 15 to 31%. A flow rate of 1.7 mL/min was used. The detecting UV wavelength was 254 nm.

Genistein, daidzein, and genistin peaks on sample chromatograms (Fig. 1) were confirmed using standards (Sigma Chemical Company, St. Louis, MO). The remaining isoflavones, daidzin, glycitin, malonyl daidzin, malonyl glycitin, malonyl genistin, acetyl daidzin and acetyl genistin, were identified by liquid chromatography–mass spectroscopy (LC–MS), since standards for these seven isoflavones were not commercially available. The LC–MS procedure was performed on the Mustang M-1000. Concentrations of the isoflavones were calculated from standard curves and expressed as micrograms per gram of dry weight. Equations for calculating genistein, daidzein, and genistin content were developed using known concentrations of the standards and their respective peak areas. Concentrations of malonyl genistin, acetyl genistin, and malonyl glycitin were calculated based on the standard curve of genistin with the adjustment of molecular weight ratio. Malonyl daidzin, acetyl daidzin, and daidzin concentrations were calculated from a standard curve with daidzein. Although there were no analytical standards for these isoflavones, the UV absorption per mole of isoflavones was identical (11). All isoflavone concentrations were reported as normalized and on a dry mass basis. Moisture content of the soybean varieties was determined following AOAC Method 925.10 (12), except 1 g of soy meal was used rather than 2 g.

Statistical analysis. Statistical analyses were performed using the SAS software package (13). Differences in individual and total isoflavone content among soybean varieties were determined using PROC ANOVA, with the least significant difference test. PROC GLM was used to examine differences in individual and total isoflavone content among maturity

FIG. 1. A typical high performance liquid chromatogram of isoflavones in soybeans. Peaks are identified as follows: $1 =$ daidzin, $2 =$ glycitin, 3 = genistin, 4 = malonyl daidzin, 5 = malonyl glycitin, 6 = malonyl genistin, $7 =$ daidzein, $8 =$ acetyl genistin, $9 =$ genistein.

groups, hilum color groups, and disease resistance designations. PROC CORR tested potential relations of individual and total isoflavone contents with seed weight, days of maturity, yield, and plant height. Pearson's *r* was reported from PROC CORR as an indicator of the strength and the direction of these relationships. Relations between these variables and individual and total isoflavone content were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

HPLC analysis detected 10 isoflavones including: daidzin, glycitin, genistin, malonyl daidzin, malonyl glycitin, malonyl genistin, daidzein, acetyl genistin, acetyl daidzin, and genistein. Glycitein and acetyl glycitin were not quantified due to low concentrations in these soybean samples.

The isoflavone content of individual soybean varieties is listed in Table 1. The values for total genistein, total daidzein, and total glycitein represent the summation of all of the free and conjugated forms. Differences in isoflavone content were observed between soybean varieties. The five soybean varieties that yielded the highest total isoflavone content were Asgrow A-1395 (2743 µg/g), Prairie Brand PB-137 (2539 µg/g), Stine 2500 (2528 µg/g), Prairie Brand PB-193 (2526 µg/g), and Golden Harvest H-1196 (2520 µg/g). The overall range in total isoflavone content was 1116 to 2743 µg/g. Similar observations were made among other soybean varieties $(3-5.14)$.

Significant differences in individual and total isoflavone content were observed among maturity groups. Maturity group I had significantly higher total isoflavones when compared to maturity group 0 (Table 2). However, the differences between groups I and II and groups II and 0 were not significant. Genistein content in group II varieties was significantly lower than in groups 0 and I. However, daidzein content in group I varieties was higher than in the other two groups. Genistin content was highest in maturity group 0. Maturity group 0 yielded significantly lower malonyl daidzin content than maturity groups I and II. Malonyl genistin content was significantly higher in maturity group I than in maturity group 0. Daidzin, glycitin, malonyl glycitin, and acetyl genistin content did not differ significantly among the three maturity groups.

Maturity group was not considered as a variable in previous studies. It is evident, from the results presented in this study, that the isoflavone composition of samples varies among maturity groups. The underlying mechanism for these observations has not been determined.

Isoflavone content also varied according to hilum color (Table 3). Genistin content was significantly higher in varieties with a green hilum when compared to varieties having black and brown hilums. Soybean varieties with black hilums contained lower levels of daidzein than varieties with yellow hilums. Genistein content was lowest in varieties having black hilums. Total isoflavone content did not differ significantly among hilum color groups. Although differences in

TABLE 1 (continued)

a The values for total genistein, total daidzein, and total glycitein represent the summation of all free and conjugated forms.

content were observed for individual isoflavones on the basis of hilum color, no discernible trend was found that could provide a suitable explanation for these findings.

Past research has demonstrated that isoflavones can function as fungicides (15). To test this premise, the relation between disease resistance to three strains of *Phytophthora* (root rot) and isoflavone content was investigated for the soybean sample set. No differences in isoflavone content were detected for susceptible, unknown, and resistant varieties.

A weak, but significant, negative correlation was observed between seed mass and the malonyl glycitin content of the samples $(r = -0.18)$. Correlations between seed weight and the other isoflavones were insignificant. This indicated that seed weight was a poor indicator for isoflavone content of the sample.

Yield (bushels/acre) for each variety was obtained from the 1994 Crop Performance Tests. Weak, but significant, cor-

relations were observed between yield and daidzin $(r = 0.19)$, glycitin $(r = 0.20)$, malonyl daidzin $(r = 0.36)$, malonyl glycitin ($r = 0.21$), malonyl genistin ($r = 0.16$), daidzein ($r =$ − 0.20), acetyl daidzin (*r* = 0.21), and total isoflavone content $(r = 0.20)$. A stronger correlation was observed between yield and genistein $(r = -0.44)$. This indicated that high-yield varieties tended to have lower genistein concentration. Yield was not found to be correlated significantly with genistin and acetyl genistin.

The number of days required for seed maturity was recorded for each variety in the soybean sample set as part of the 1994 Crop Performance Tests. This differs from maturity groups, which are categorical designations for the number of days required for seed maturity. Days of maturity yielded insignificant correlations with daidzin, glycitin, malonyl glycitin, malonyl genistin, acetyl genistin, and total isoflavone content. Weak, but significant, linear correlations were ob-

a Values with the same letter, in the same column, are not significantly different (*P* < 0.05). LSD, least significant difference (*P* < 0.05). *^b*Isoflavone contents are expressed in µg/g.

*a,b*For footnotes see Table 2.

served for genistin $(r = -0.27)$, malonyl daidzin $(r = 0.17)$, and daidzein $(r = -0.33)$. For genistein, days of maturity yielded the strongest linear correlation $(r = -0.59)$, indicating that maturity of seeds has a negative relationship with genistein content in soybean seeds. This follows the results from maturity group comparisons in that longer maturity resulted in a reduction of genistein content.

Plant height was measured from the soil surface to the top node of the main stem for the 1994 Crop Performance Tests. Weak, but significant, correlations were observed between plant height and genistin $(r = -0.29)$, malonyl daidzin $(r =$ 0.15), or daidzein $(r = -0.23)$. Insignificant linear correlations were found between plant height and daidzin, glycitin, malonyl glycitin, malonyl genistin, acetyl genistin, and total isoflavones. The strongest linear correlation was observed for genistein $(r = -0.46)$. This indicated that taller plants tend to have lower genistein content. Similar to yield, plant height was found to be a poor indicator of total isoflavone content. This has also been confirmed by Tsukamoto *et al.* (4). However, compared to other isoflavones, plant height, yield, and maturity were better indicators of genistein content. Environmental factors such as crop year, location, rainfall, and temperature apparently influence the relations observed between agronomic seed characteristics and isoflavone content.

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