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Correlations of Oil and Protein with Isoflavone Concentration in Soybean [*Glycine max* (L.) Merr.]

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Twelve isoflavones were detected by high-performance liquid chromatography in seeds of 17 soybean [*Glycine max* (L.) Merrill] cultivars grown at three locations. 6′′-*O*-Malonyldaidzin and 6′′-*O*-malonylgenistin together constituted 71−81% of total isoflavones, which ranged in concentration from 2038 to 9514 μg/g and averaged 5644 μg/g across locations and cultivars. The total as well as several individual isoflavones had a moderate negative correlation with oil across locations and cultivars. Six cultivars had a moderate or strong negative correlation of total isoflavones with oil. Five cultivars had a moderate or strong positive correlation of total isoflavones with protein. These results suggest that judicious selection of germplasm for soybean breeding may facilitate development of soybean lines with desirable isoflavone concentrations.

KEYWORDS: ***Glycine max* (L.) Merrill; isoflavone; oil; protein; soy; soybean; phytoestrogen**

INTRODUCTION

The production of soybeans [*Glycine max* (L.) Merrill] in 2002/2003 was estimated at 197 million metric tons worldwide, of which 74.8 million metric tons were produced in the United States (*1*). Traditionally, soybean value has been determined by protein and oil content (*2*). Isoflavone concentration, however, may emerge as another determinant of soybean value. Isoflavones are phenylpropanoid-derived compounds that constitute about 0.3 to more than 0.8% of soybean seed on a dry weight basis (*3*). Because isoflavones exert estrogen-like effects, they are frequently referred to as phytoestrogens. Many research studies have indicated that isoflavones and consumption of isoflavone-containing foods are associated with a wide variety of health benefits, including prevention of breast and prostate cancers, cardiovascular disease, and reduced symptoms of diabetes and postmenopausal bone loss (*4*−*8*). Other studies have raised concerns about toxic health effects of isoflavones and isoflavone-containing foods. One concern is that the isoflavone genistein is apoptotic, potentially a useful benefit with regard to cancerous cells, but which may be problematic for normal cells (*9*). Moreover, at a concentration of 25 mol/L, genistein (but not daidzein) exhibited clastogenic activity in human cultured lymphocytes cells (*10*). Also, a longitudinal study undertaken in Hawaii reported that high levels of midlife tofu consumption correlated with cognitive impairment later in life (*11*). Reviews that address the risks and benefits of isoflavones generally conclude that more research is required to elucidate the specific effects of different isoflavone dosages on human metabolic functions (*12*−*14*).

Isoflavones exist in soybeans and soy foods as aglycones (daidzein, genistein, and glycitein), glucosides (daidzin, genistin, and glycitin), acetylglucosides (6′′-*O*-acetyldaidzin, 6′′-*O*-acetylgenistin, and 6′′-*O*-acetylglycitin), and malonylglucosides (6′′-*O*-malonyldaidzin, 6′′-*O*-malonylgenistin, and 6′′-*O*-malonylglycitin) (*15*). Isoflavone concentrations in soybean seed are affected by a variety of factors. Although the malonylglucosides are the predominant isoflavones in soybeans, concentrations of the glucoside, acetylglucoside, and aglycone forms tend to increase during extraction, processing, and cooking (*15*, *16*). Genotype, plot location, year, and their interactions affect isoflavone levels (*17*, *18*). Location differences may be explained by differences in soil fertility or water availability. Concentrations of daidzein, genistein, and total isoflavones in soybean increased with potassium fertilization of low potassium soils (*19*), and irrigation enhanced isoflavone content of early- and late-planted soybeans as much as 2.5-fold (*20*). Year differences may be attributed to climatic variability. Tsukamoto et al. showed that isoflavone content is higher in soybeans when temperatures are cooler during seed development (*21*). Light may have a role in regulating isoflavone metabolism. Light induced large accumulations of 6′′-*O*-malonylgenistin in subepidermal cells of soybean cotyledons, an effect that was enhanced by the application of the putative defense signaling compounds methyl jasmonate and glutathione (*22*). Applications of a native cell wall glucan from *Phytophthora sojae* (Kauf. and Gerde.) increased levels of daidzein, a precursor of the pterocarpan phytoalexin glyceollin, supporting the hypothetical role of isoflavones in defense responses (*22*).

Because soybean value is highly related with oil and protein content and because there is considerable evidence that isofla-

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Table 1. Soybean Cultivars Grown to Evaluate Oil, Protein, and Isoflavone Content

no.[a]	cultivar	brand	type[b]	relative maturity[c]
1	52-C2	Armor	C	5.2
2	5601T	UniSouth	C	5.6
3	DP 4748 S	Deltapine	C	4.7
4	HBK 4944 CX	Hornbeck	C	4.9
5	Holladay	U.S. Department of Agriculture	C	5.5
6	Manokin	University of Maryland	C	5.0
7	7403nRR	UniSouth	R	4.0
8	7440nRR	UniSouth	R	4.4
9	AG 4502 (RR)	Asgrow	R	4.5
10	AG 5501 (RR)	Asgrow	R	5.5
11	HBK R 4920 (RR)	Hornbeck	R	4.9
12	RT 4809 (RR)	Morsoy	R	4.8
13	TVX 49R2Y4 (RR)	Terral	R	4.9
14	95B43 (RR)	Pioneer	R	5.4
15	DP 5414 RR	Deltapine	R	5.4
16	HBK R 5823 (RR)	Hornbeck	R	5.8
17	S 57-P1 (RR)	N. K. Brand	R	5.7

[a] Number designations are used to identify cultivars throughout this article. [b] C = conventional (non-Roundup Ready); R = Roundup Ready. [c] Relative maturity is the maturity designation of the cultivar relative to other cultivars and is determined by optimal adaptation in geographical zones (*23*).

vones exert effects on human health, our primary objective was to determine whether oil or protein content in soybeans correlates with individual or total isoflavone concentration. Any such correlations could be useful for breeders selecting for oil, protein, and isoflavone content.

MATERIALS AND METHODS

Field Experiment. The 17 soybean cultivars tested in this experiment included conventional and Roundup Ready soybeans from maturity groups 4 and 5. Maturity group classifications of soybean varieties are assigned by plant breeders during the yield testing phase of development. They are determined by comparing the maturity of the experimental lines relative to already established check varieties and/or a regression equation derived by Pioneer Hibred International (Johnston, IA). Maturity group numerals correlate to geographical zones and increase in magnitude moving from northern to southern zones (*23*). Cultivars are identified by number designations throughout this text (**Table 1**). The 17 cultivars are a subset of a larger set of cultivars from maturity groups 4 and 5 that were tested at each of the locations. The subset was chosen on the basis of high or low seed protein content and to represent a range in maturity within maturity groups 4 and 5.

Soybeans were planted in spring 2003 in a randomized complete block design with three replications at each of three locations in Tennessee: Ames Research and Education Center, Grand Junction (latitude 35.07° N, longitude 89.15° W), Knoxville Research and Education Center, Knoxville (latitude 35.96° N, longitude 83.91° W), and Milan Research and Education Center, Milan, Tennessee (latitude 35.92° N, longitude 88.76° W) (**Table 2**). The experimental sites were fertilized with 67.2−89.6 kg/ha of P_20_5 and K_20. Weed control for the Roundup Ready cultivars consisted of glyphosate herbicide applied as a burndown at the rate of 2.372 L/ha approximately 2 weeks prior to planting and again as a postemergence treatment at approximately 4 and 8 weeks after planting. Weed control for the conventional cultivars consisted of glyphosate burndown Planting, and harvest dates are listed in **Table 2**.

Near-Infrared Reflectance Spectroscopy (NIR) of Seed Protein and Oil. Samples of approximately 60 g of seeds of each cultivar were ground in a water-cooled grinder (Knifetec 1095 Sample Mill, Foss Tecator, Hoganas, Sweden) at 20 °C for 20 s. Seed protein and oil were measured by NIR using an Infratec 1229 Grain Analyzer (Foss Tecator, Hoganas, Sweden). Individual data values are an integration of five scans. Measurements were taken on each replication of each cultivar from each location. NIR is a well-established method for measurement of oil and protein in oilseeds. The correlation coefficient when comparing oil measured in soybean by NIR vs nuclear magnetic resonance (NMR) was 0.97, and when protein was measured by NIR and the Kjeldahl method, the correlation coefficient was 0.94 (*24*).

Isoflavone Analysis. Extraction of isoflavones was performed by a modification of the method of Griffith and Collison (*25*). Approximately 200 mg of ground soybean seed was 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 the flavone apigenin (Sigma, St. Louis, MO) in dimethyl sulfoxide. 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 nylon filter (Fisher Scientific, Pittsburgh, PA) and stored at −80 °C until analysis by high-performance liquid chromatography (HPLC). A Hewlett-Packard (Palo Alto, CA) model 1050 HPLC was used with an Agilent (Palo Alto, CA) Zorbax XDB C-18 reverse phase column (150 mm × 3.0 mm ID, 3.5 μm) and UV detector at a wavelength of 260 nm. The column temperature was set at 40 °C. The injection volume was 5 μL. Solvent A was 0.1% (v/v) acetic acid in water, and solvent B was 0.1% (v/v) acetic acid in acetonitrile. The gradient elution ranged from 9 to 15% B over 10 min, from 15 to 17% B over an additional 10 min, was 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 daidzein, genistein, glycitein, daidzin, genistin, glycitin, 6″-*O*-acetyldaidzin, 6″-*O*-acetylgenistin, 6″-*O*-acetylglycitin, 6″-*O*-malonyldaidzin, 6″-*O*-malonylgenistin, and 6″-*O*-malonylglycitin procured from LC Laboratories (Woburn, MA). Two additional chromatographic peaks

Table 2. Location Information for Tennessee Research and Education Centers Where Soybean Variety Tests Were Conducted in 2003[a]

experiment station	location	cultivars	planting date	harvest date	soil type
Ames Plantation	Grand Junction	1–6	May 12	October 16	Lexington silt loam
		7–10	May 15	September 23	(fine-silty, mixed,
		11–13	May 15	October 3	active, thermic
		14, 15	May 12	October 13	Ultic Hapludalfs)
		16, 17	May 12	October 29	
Knoxville	Knoxville	1–6	April 29	October 15	Sequatchie fine sandy
		7–10	April 29	September 19	loam (fine-loamy,
		11–13	April 29	September 26	siliceous, semiactive,
		14, 15	April 29	October 13	thermic Humic
		16, 17	April 29	October 23	Hapludults)
Milan	Milan	1–6	May 23	October 22	Loring silt loam
		7–10	May 23	September 29	(fine-silty, mixed,
		11–13	May 23	October 3	active, thermic,
		14, 15	May 23	October 20	Typic Epiaqualfs)
		16, 17	May 23	October 23	

[a] Cultivars are a subset of a larger set of cultivars of similar maturity tested at each location. The subset was chosen based on seed protein levels.

Table 3. Mean Yield, Oil, Protein, and Isoflavone Concentration of Soybeans Grown at Ames Plantation Research and Education Center[a]

				isoflavone concentration (μg/g)														
cultivar	yield (kg/ha)	oil (%)	protein (%)	daidzein	genistein	glycitein	daidzin	genistin	glycitin	6″-*O*-malonyl-daidzin	unknown 1[b]	6″-*O*-malonyl-genistin	unknown 2[c]	6″-*O*-malonyl-glycitin	6″-*O*-acetyl-daidzin	6″-*O*-acetyl-genistin	6″-*O*-acetyl-glycitin	total isoflavones
1	3434	18.4	41.7	17	16	0	184	268	94	2699	226	2548	201	330	46	0	0	6629
2	3964	18.4	42.1	14	13	1	182	224	100	2746	263	2253	184	266	41	0	0	6286
3	3840	19.1	41.9	17	17	0	134	192	81	1995	198	2003	147	238	33	0	0	5055
4	3589	19.3	41.2	15	16	6	149	214	92	2351	229	2417	204	334	58	0	0	6085
5	3299	19.6	39.7	25	23	2	227	299	99	3200	296	2944	243	301	58	0	0	7716
6	3235	19.3	41.1	20	20	0	190	216	96	2508	272	2339	135	287	98	0	0	6181
7	3562	20.4	41.8	14	20	2	135	222	141	1894	182	2094	184	441	59	4	0	5392
8	4198	21.3	39.0	20	24	0	151	185	137	1547	145	1533	99	457	70	4	0	4372
9	3906	19.6	42.4	14	16	7	132	162	117	1169	126	1266	107	401	35	6	0	3561
10	4619	18.7	41.4	24	18	0	141	210	60	2146	164	1863	143	252	33	2	0	5056
11	3955	20.0	41.1	11	6	5	115	175	107	1602	148	1696	137	326	33	3	0	4364
12	3881	19.4	42.2	7	7	2	90	143	85	1276	134	1408	116	232	25	4	0	3531
13	2994	18.9	43.0	8	5	0	93	114	78	1416	148	1323	109	249	22	0	0	3566
14	4621	18.6	40.3	12	14	0	153	218	100	2308	226	2130	153	302	34	0	0	5649
15	4691	17.2	44.0	22	15	0	179	223	65	2533	188	2024	173	324	42	0	0	5786
16	4361	19.0	40.3	10	12	2	167	180	82	2668	261	2360	82	335	143	6	11	6319
17	5136	18.3	42.1	11	16	9	139	244	97	2011	225	2262	189	401	67	0	0	5671
$LSD_{0.05}$	826	0.4	1.0	10	8	NS[d]	NS	71	NS	1041	108	720	72	126	NS	NS	NS	1996

[a] Yield and isoflavone concentrations are reported on a dry weight basis. [b] Tentatively identified as a different form of 6″-*O*-malonyl-daidzin. [c] Tentatively identified as a different form of 6″-*O*-malonyl-genistin. [d] NS = analysis of variance model for cultivar was not significant at $P < 0.05$.

Table 4. Mean Yield, Oil, Protein, and Isoflavone Concentration of Soybeans Grown at Knoxville Research and Education Center[a]

				isoflavone concentration (μg/g)														
cultivar	yield (kg/ha)	oil (%)	protein (%)	daidzein	genistein	glycitein	daidzein	genistin	glycitin	6″-*O*-malonyl-daidzin	unknown 1[b]	6″-*O*-malonyl-genistin	unknown 2[c]	6″-*O*-malonyl-glycitin	6″-*O*-acetyl-daidzin	6″-*O*-acetyl-genistin	6″-*O*-acetyl-glycitin	total isoflavones
1	5010	19.6	39.5	10	27	7	168	261	97	1900	167	2483	120	345	103	3	0	5690
2	5707	19.0	40.8	18	21	0	274	306	145	3372	309	2674	205	465	55	0	0	7845
3	4924	20.4	39.7	17	39	5	59	106	93	645	113	1184	131	338	57	0	0	2786
4	5165	20.5	39.3	27	61	0	166	310	109	1541	164	2128	138	350	117	4	0	5115
5	5158	20.8	36.7	23	32	3	226	286	103	2763	264	2551	196	325	60	0	0	6831
6	4863	20.3	39.0	15	20	11	209	202	67	2384	202	2023	157	229	24	0	0	5543
7	4235	22.4	38.6	11	28	6	92	233	155	793	95	1358	109	478	41	0	0	3398
8	5043	22.2	37.6	3	2	0	111	167	181	1359	116	1281	104	620	26	0	0	3969
9	4592	20.2	41.4	7	12	4	88	143	107	778	100	984	92	362	42	5	0	2723
10	4954	19.9	40.0	28	28	0	184	245	128	2617	249	2256	182	383	45	0	0	6347
11	4746	22.0	38.3	7	14	3	72	135	95	829	112	1300	75	342	62	0	0	3046
12	4771	21.0	39.4	7	15	3	54	63	101	537	56	716	131	325	27	3	0	2038
13	3452	19.7	42.4	10	12	1	96	116	83	1007	95	799	50	214	15	0	0	2498
14	3694	20.3	37.3	12	23	2	78	205	117	1197	147	1864	146	385	38	2	0	4218
15	3513	18.5	42.3	20	14	2	201	192	76	2267	267	1986	172	271	47	6	0	5521
16	5339	20.3	40.6	12	7	0	164	210	109	2713	250	2749	218	419	64	0	0	6915
17	3805	19.2	42.9	9	17	0	206	238	118	3000	249	2978	225	515	45	0	0	7601
$LSD_{0.05}$	558	0.5	0.6	9	11	NS[d]	55	71	44	609	77	393	77	137	NS	NS	NS	1208

[a] Yield and isoflavone concentrations are reported on a dry weight basis. [b] Tentatively identified as a different form of 6″-*O*-malonyl-daidzin. [c] Tentatively identified as a different form of 6″-*O*-malonyl-genistin. [d] NS = analysis of variance model for cultivar was not significant at $P < 0.05$.

were tentatively identified as different forms of 6″-*O*-malonyldaidzin and 6″-*O*-malonylgenistin based on comparison to previously published chromatograms and our confirmation that these peaks increase in standard solutions with time (*25*).

Statistics. Data were analyzed using SAS statistical software (Version 9.1, 2002, SAS Institute, Cary, NC). Analyses of variance using the general linear model procedure were performed on each variable (oil, protein, and individual and total isoflavones). Least significant differences were determined for each variable at each location and then with the location trials combined. Correlation analysis was conducted to examine trends of oil and protein concentration with isoflavone concentration for total isoflavones and for individual isoflavones with concentrations ≥1% of total isoflavones. The strength of the correlation was defined as weak for $|r| \leq |0.3|$, moderate for $|0.3| < r < |0.7|$, and strong for $r \geq |0.7|$.

RESULTS

Oil, Protein, and Isoflavone Concentrations. Soybean oil, protein, and isoflavone concentrations at each location are given in **Tables 3−5**. Oil concentrations were consistently highest in cultivars 7 and 8, comprising 22.4 and 22.2%, respectively, of soybean seed in the Knoxville trial. In contrast, cultivar 15 was lowest in oil at all locations, comprising just 17.0% of soybean seed at the Milan Experiment Station. Protein concentrations were not as consistent across locations. The highest protein concentration at the Ames Plantation trial was 44.0% (cultivar 15) and the lowest was 39.0% (cultivar 8). Cultivars 13, 15, and 17 were highest in protein at Knoxville (42.4, 42.3, and 42.9% respectively) and Milan Experiment Stations (43.6, 43.2, and 43.9%, respectively). The lowest protein concentrations were measured in cultivar 5 (36.6%) in Knoxville and cultivars 5 and 14 (37.7 and 38.5%, respectively) in Milan. The oil concentration had a moderate negative correlation ($r = -0.68$, $P < 0.01$, data not shown) with protein concentration across locations.

At the Ames Plantation, the total isoflavone concentrations ranged from 3531 μg/g (cultivar 12) to 7716 μg/g (cultivar 5).

Table 5. Mean Yield, Oil, Protein, and Isoflavone Concentration of Soybeans Grown at Milan Research and Education Center[a]

				isoflavone concentration (μg/g)														
cultivar	yield (kg/ha)	oil (%)	protein (%)	daidzein	genistein	glycitein	daidzin	genistin	glycitin	6′′-*O*-malonyl-daidzin	unknown 1[b]	6′′-*O*-malonyl-genistin	unknown 2[c]	6′′-*O*-malonyl-glycitin	6′′-*O*-acetyl-daidzin	6′′-*O*-acetyl-genistin	6′′-*O*-acetyl-glycitin	total isoflavones
1	3905	18.7	39.7	13	15	0	199	337	119	3181	264	3235	245	362	53	0	0	8024
2	4252	18.0	41.9	12	9	0	273	282	133	4643	399	3063	241	406	50	2	0	9514
3	4033	19.2	42.0	9	6	0	93	187	89	1834	164	2321	173	283	36	0	0	5194
4	3991	19.6	40.1	17	19	0	249	291	122	3440	274	2967	224	379	51	0	0	8034
5	4136	19.6	37.7	17	12	0	252	328	113	4055	332	3443	267	398	59	0	0	9275
6	3825	19.6	39.3	21	16	3	308	325	127	4209	290	3373	273	329	55	6	0	9335
7	4376	20.3	41.7	6	8	2	94	210	115	1386	123	1960	161	452	46	0	0	4562
8	4818	21.0	39.8	8	2	5	191	245	191	2937	221	2632	237	723	45	7	0	7442
9	4481	19.2	42.6	6	0	4	130	174	92	1890	166	1825	145	339	34	0	0	4805
10	4372	19.0	40.5	15	12	0	152	203	91	2903	246	2101	166	283	30	0	0	6203
11	4099	20.3	40.8	0	0	0	99	176	112	1561	142	1913	145	358	32	0	0	4539
12	4738	19.5	42.4	5	7	2	92	160	105	1315	120	1549	135	362	26	4	0	3882
13	2998	18.5	43.6	5	0	0	107	137	87	1838	148	1640	144	230	25	0	0	4359
14	3977	19.0	38.5	7	7	1	127	286	97	2093	196	2502	188	311	33	0	0	5849
15	4220	17.0	43.2	13	10	0	236	204	77	3703	320	2604	201	244	41	0	0	7656
16	4411	18.6	41.4	9	6	0	175	232	84	3428	248	2854	206	281	40	0	0	7562
17	3750	17.2	43.9	8	10	0	195	368	108	3489	249	3109	233	476	45	0	0	8290
$LSD_{0.05}$	556	0.5	1.0	6	8	NS[d]	69	87	54	962	120	778	65	190	16	NS	NS	2171

[a] Yield and isoflavone concentrations are reported on a dry weight basis. [b] Tentatively identified as a different form of 6′′-*O*-malonyl-daidzin. [c] Tentatively identified as a different form of 6′′-*O*-malonyl-genistin. [d] NS = analysis of variance model for cultivar was not significant at $P < 0.05$.

Table 6. Mean Yield, Oil, Protein, and Isoflavone Concentration of Soybeans from All Locations[a]

				isoflavone concentration (μg/g)														
cultivar	yield (kg/ha)	oil (%)	protein (%)	daidzein	genistein	glycitein	daidzin	genistin	glycitin	6′′-*O*-malonyl-daidzin	unknown 1[b]	6′′-*O*-malonyl-genistin	unknown 2[c]	6′′-*O*-malonyl-glycitin	6′′-*O*-acetyl-daidzin	6′′-*O*-acetyl-genistin	6′′-*O*-acetyl-glycitin	total isoflavones
1	4116	18.9	40.3	14	19	2	184	289	103	2593	219	2755	189	346	67	1	0	6781
2	4508	18.5	41.6	15	14	0	243	271	126	3587	324	2663	210	379	48	1	0	7882
3	4266	19.6	41.2	14	21	2	95	162	88	1491	158	1836	150	286	42	0	0	4345
4	4248	19.8	40.2	20	32	2	188	271	108	2444	222	2504	189	355	75	1	0	6411
5	4310	20.0	38.0	21	22	1	235	304	105	3339	297	2979	235	342	59	0	0	7941
6	3975	19.7	39.8	18	19	5	235	248	97	3034	255	2579	188	282	59	2	0	7020
7	4057	21.1	40.7	10	19	3	107	222	137	1358	133	1804	151	457	48	1	0	4451
8	4687	21.5	38.8	10	9	2	151	199	170	1948	160	1815	146	600	47	4	0	5261
9	4326	19.7	42.1	9	9	5	117	160	105	1279	131	1358	115	368	37	4	0	3696
10	4508	19.2	40.6	23	19	0	159	219	93	2555	220	2073	164	306	36	1	0	5869
11	4267	20.8	40.1	6	7	3	95	162	104	1331	134	1637	119	342	42	1	0	3983
12	4463	19.8	41.6	6	10	2	79	122	97	1043	103	1225	127	307	26	4	0	3150
13	3148	19.0	43.0	8	5	1	99	122	83	1420	130	1254	101	231	21	0	0	3474
14	4097	19.3	38.7	10	15	1	119	236	105	1866	190	2165	162	332	35	1	0	5239
15	4141	17.6	43.2	18	13	1	205	206	73	2834	258	2205	182	280	43	2	0	6321
16	4704	19.3	40.8	10	8	1	169	207	92	2936	253	2654	168	345	82	2	4	6932
17	4230	18.2	43.0	10	15	3	180	283	108	2833	241	2783	216	464	52	0	0	7188
$LSD_{0.05}$	592	0.7	1.0	5.6	9.2	NS[d]	43	50	28	703.9	62	482	50	89	31	NS	NS	1352

[a] Yield and isoflavone concentrations are reported on a dry weight basis. [b] Tentatively identified as a different form of 6′′-*O*-malonyl-daidzin. [c] Tentatively identified as a different form of 6′′-*O*-malonyl-genistin. [d] NS = analysis of variance model for cultivar was not significant at $P < 0.05$.

In Knoxville, concentrations ranged from 2038 μg/g (cultivar 12) to 7845 μg/g (cultivar 2). At the Milan Experiment Station, the lowest total isoflavone concentration measured was 3882 μg/g (cultivar 12) and the highest was 9514 μg/g (cultivar 2). Across locations, the highest total isoflavone concentrations occurred in cultivars 2, 5, 6, 16, and 17 and the lowest in cultivars 9 and 11−13 ($P < 0.05$; **Table 6**).

Daidzein, genistein, glycitein, daidzin, genistin, glycitin, 6′′-*O*-acetyldaidzin, 6′′-*O*-malonyldaidzin, 6′′-*O*-malonylgenistin, and 6′′-*O*-malonylglycitin were found in all cultivars at all locations. 6′′-*O*-Acetylgenistin and 6′′-*O*-Acetylglycitin were detected in only a few cultivars and never contributed more than 0.2% to total isoflavone concentration (6′′-*O*-acetylgenistin, cultivar 9, Knoxville). The predominant individual isoflavones detected in all cultivars were 6′′-*O*-malonyldaidzin and 6′′-*O*-malonylgenistin. At the Ames Plantation, 6′′-*O*-malonyldaidzin comprised 33−44% (cultivars 9 and 15, respectively) of total isoflavones and 6′′-*O*-malonylgenistin comprised 35−40% (cultivars 15 and 17, respectively) of total isoflavones. At the Knoxville Experiment Station, 6′′-*O*-malonyldaidzin ranged from 23% of total isoflavones in cultivar 3 to 43% of the total in cultivar 6, whereas 6′′-*O*-malonylgenistin varied from 32 to 44% (cultivars 13 and 14, respectively) of total isoflavones. This trend continued in soybeans from Milan where 6′′-*O*-malonyldaidzin was 30−49% (cultivars 7 and 2, respectively) of total isoflavones and 6′′-*O*-malonylgenistin was 32−44% (cultivars 2 and 3, respectively) of total isoflavones.

We observed minor peaks in analyses of 6′′-*O*-malonyldaidzin and 6′′-*O*-malonyl-genistin standard solutions that increased with time. On the basis of retention time, these peaks also appeared in soybean seed samples and were tentatively identified as different forms of 6′′-*O*-malonyl-daidzin and 6′′-*O*-malonyl-genistin.

The aglycones daidzein, genistein, and glycitein were only a minor fraction of total isoflavones. Across locations, daidzein and genistein were each ≤0.5% of total isoflavone concentration

Table 7. Interactions between Cultivars and Locations for Oil, Protein, Total, and Individual Isoflavone Concentrations of 17 Soybean Cultivars across Three Locations[a]

variable	cultivar	location	cultivar × location
oil	**	**	**
protein	**	**	**
yield	**	**	**
total isoflavones	**	**	*
daidzein	**	**	*
genistein	**	**	**
glycitein	NS	NS	NS
daidzin	**	**	*
genistin	**	**	*
glycitin	**	*	NS
6′′-*O*-malonyldaidzin	**	**	**
6′′-*O*-malonylgenistin	**	**	NS
6′′-*O*-malonylglycitin	**	*	NS
6′′-*O*-acetyldaidzin	**	NS	NS
6′′-*O*-acetylgenistin	NS	NS	NS
6′′-*O*-acetylglycitin	NS	NS	NS

[a] NS, not significant. *,** significant at $P < 0.05$ or 0.01.

and glycitein was ≤0.08% of total isoflavones except in cultivar 9 where it was 0.14%.

Location, Cultivar, and Interactive Effects. Seed oil, protein, yield, total isoflavone, daidzein, genistein, daidzin, genistin, and 6′′-*O*-malonyldaidzin concentrations were significantly influenced by location, cultivar, and location × cultivar ($P < 0.05$; **Table 7**). Glycitin, 6′′-*O*-malonylglycitin, and 6′′-*O*-malonylgenistin varied with location and cultivar but not location × cultivar whereas 6′′-*O*-acetyldaidzin differed only by cultivar.

Correlations of Oil, Protein, and Yield with Isoflavone Concentration. Across locations and cultivars, oil concentration had moderate negative correlations with total isoflavones, daidzin, 6′′-*O*-malonyldaidzin, and 6′′-*O*-malonylgenistin but moderate positive correlations with glycitin and 6′′-*O*-malonylglycitin ($P < 0.01$; **Table 8**). The correlation of oil with total isoflavone concentration was nonsignificant at the Ames Plantation ($r = -0.26$, $P = 0.06$) and Milan ($r = -0.25$, $P = 0.08$) but significant at Knoxville ($r = -0.48$, $P < 0.01$). These relationships are depicted graphically in **Figure 1** where the overall relationship of total isoflavone and oil concentrations is described by the equation: total isoflavones = 19940 − 732 × oil. There was a moderate negative correlation of daidzein and derivative isoflavones (sum of daidzein, daidzin, 6′′-*O*-malonyl daidzin, 6′′-*O*-acetyl daidzin, glycitein, glycitin, 6′′-*O*-malonyl glycitin, and 6′′-*O*-acetyl glycitin) with oil concentration. Similarly, there was a moderate negative correlation of genistein and derivative isoflavones (sum of genistein, genistin, 6′′-*O*-malonyl genistin, and 6′′-*O*-acetyl genistin) with oil concentration.

Cultivars 3, 9, and 11−13 had strong negative correlations of oil with total isoflavones. Concentrations of daidzin, genistin, and their malonyl conjugates generally had moderate or strong negative correlations with oil in these same cultivars. 6′′-*O*-Malonylglycitin had a strong positive correlation with oil concentration in cultivar 16. No correlations of glycitin with oil concentration were significant for individual cultivars.

Genistin, glycitin, and 6′′-*O*-malonylglycitin had weak negative correlations with protein concentration across cultivars and locations (**Table 9**). Protein concentrations in cultivars 3, 8, 9, 11, and 17 had strong positive correlations with total isoflavones ($P < 0.05$). 6′′-*O*-Malonyldaidzin had a strong positive correlation with cultivars 3, 8, 9, 11, 12, 14, and 17. Correlations of daidzein and its derivative isoflavones with protein and genistein and derivative isoflavones with protein, generally were strong and positive in these same cultivars.

Yield did not correlate with total isoflavones but had weak positive correlations with genistein ($r = 0.22$, $P < 0.01$) and 6′′-*O*-malonylglycitin ($r = 0.22$, $P < 0.01$).

DISCUSSION

The concentrations of oil and protein measured in this study were similar to the mean values for soybean lines evaluted in the Soybean Uniform Tests Northern and Southern Regions from 1948 to 1998 (*26*). The negative correlation of oil with protein concentration in soybean seed has been widely reported (*27*, *28*).

Table 8. Correlation Coefficients[a] for Isoflavone Concentration (μg/g) with Oil Concentration (%)

cultivar	daidzin	genistin	glycitin	malonyl daidzin	malonyl genistin	malonyl glycitin	total isoflavones	daidzein and derivative isoflavones[b]	genistein and derivative isoflavones[c]
1	−0.39	−0.19	0.00	−0.69*	−0.36	−0.06	−0.55	−0.68*	−0.37
2	−0.01	0.14	0.14	−0.46	−0.30	0.27	−0.35	−0.42	−0.26
3	−0.78**	−0.75*	0.22	−0.94**	−0.84**	0.59	−0.92**	−0.95**	−0.84**
4	−0.08	0.76*	0.19	−0.56	−0.44	0.03	−0.48	−0.52	−0.34
5	−0.15	−0.29	0.00	−0.48	−0.61	0.01	−0.50	−0.44	−0.59
6	−0.01	−0.22	−0.65	−0.13	−0.31	−0.40	−0.25	−0.20	−0.27
7	−0.36	0.38	0.61	−0.53	−0.51	0.41	−0.47	−0.50	−0.48
8	−0.66*	−0.58	−0.14	−0.65	−0.66*	−0.21	−0.62	−0.64	−0.66*
9	−0.55	−0.43	0.26	−0.84**	−0.79**	0.35	−0.79**	−0.84**	−0.78**
10	0.40	0.29	0.64	0.12	−0.33	0.63	0.29	0.19	0.35
11	−0.85**	−0.66	−0.37	−0.94**	−0.67*	0.15	−0.82**	−0.91**	−0.73*
12	−0.73*	−0.66	0.20	−0.85**	−0.69	0.24	−0.73*	−0.82**	−0.66
13	−0.13	−0.18	0.03	−0.71*	−0.79**	−0.30	−0.73*	−0.67*	−0.77**
14	−0.74*	−0.27	0.13	−0.84**	−0.46	0.33	−0.67*	−0.81**	−0.45
15	0.05	−0.02	0.14	−0.41	−0.21	0.19	−0.27	−0.33	−0.19
16	−0.14	−0.02	0.58	−0.47	0.07	0.93**	−0.13	−0.41	0.11
17	−0.06	−0.83**	0.05	−0.40	−0.25	0.10	−0.34	−0.38	−0.33
All cultivars	−0.33**	−0.23**	0.37**	−0.49**	−0.38**	0.33**	−0.41**	−0.46**	−0.37**

[a] For individual isoflavone concentrations ≥1% of total isoflavone concentration; *, ** correlation significant at $P < 0.05$ or 0.01, respectively. [b] Correlation based on sum of concentrations of daidzein, daidzin, 6′′-*O*-malonyl daidzin, 6′′-*O*-acetyl daidzin, glycitein, glycitin, 6′′-*O*-malonyl glycitin, and 6′′-*O*-acetyl glycitin. [c] Correlation based on sum of concentrations of genistein, genistin, 6′′-*O*-malonyl genistin, and 6′′-*O*-acetyl genistin.

Table 9. Correlation Coefficients[a] for Isoflavone Concentration (μg/g) with Protein Concentration (%)

cultivar	daidzin	genistin	glycitin	malonyl daidzin	malonyl genistin	malonyl glycitin	total isoflavones	daidzein and derivative isoflavones[b]	genistein and derivative isoflavones[c]
1	0.23	0.02	–0.20	0.15	–0.08	–0.12	0.04	0.14	–0.08
2	–0.15	–0.29	–0.32	0.15	0.05	–0.42	0.07	0.12	0.01
3	0.48	0.66	–0.46	0.77**	0.76*	–0.58	0.75*	0.75*	0.75**
4	–0.38	–0.83**	–0.17	0.09	0.10	0.13	0.04	0.04	–0.03
5	–0.07	–0.02	–0.27	0.06	0.20	–0.21	0.09	0.05	0.18
6	–0.55	–0.17	0.28	–0.42	–0.11	0.26	–0.27	–0.38	–0.11
7	0.24	–0.39	–0.67*	0.44	0.39	–0.47	0.37	0.41	0.35
8	0.76*	0.68*	0.32	0.80**	0.79**	0.40	0.77*	0.79**	0.79**
9	0.47	0.35	–0.19	0.70*	0.66	–0.17	0.67*	0.70*	0.66
10	–0.58	–0.43	–0.61	–0.51	–0.57	–0.61	–0.59	–0.55	–0.56
11	0.82**	0.61	0.39	0.90**	0.62	–0.09	0.78**	0.89**	0.64
12	0.66	0.62	–0.28	0.79*	0.65	–0.20	0.68	0.76*	0.65
13	–0.14	0.12	–0.06	0.33	0.34	–0.25	0.30	0.30	0.34
14	0.72*	0.08	–0.02	0.78**	0.29	–0.18	0.56	0.76	0.27
15	–0.31	–0.13	–0.16	–0.04	–0.19	–0.12	–0.14	–0.02	–0.19
16	–0.06	0.32	–0.08	0.49	0.38	–0.54	0.36	0.39	0.38
17	0.72*	0.85**	0.18	0.89**	0.77*	0.42	0.85**	0.88**	0.82**
All cultivars	–0.06	–0.18*	–0.25*	0.04	–0.07	–0.18*	–0.03	0.02	–0.08

[a] For individual isoflavone concentrations ≥1% of total isoflavone concentration; *, ** correlation significant at $P < 0.05$ or 0.01, respectively. [b] Correlation based on sum of concentrations of daidzein, daidzin, 6′′-*O*-malonyl daidzin, 6′′-*O*-acetyl daidzin, glycitein, glycitin, 6′′-*O*-malonyl glycitin, and 6′′-*O*-acetyl glycitin. [c] Correlation based on sum of concentrations of genistein, genistin, 6′′-*O*-malonyl genistin, and 6′′-*O*-acetyl genistin.

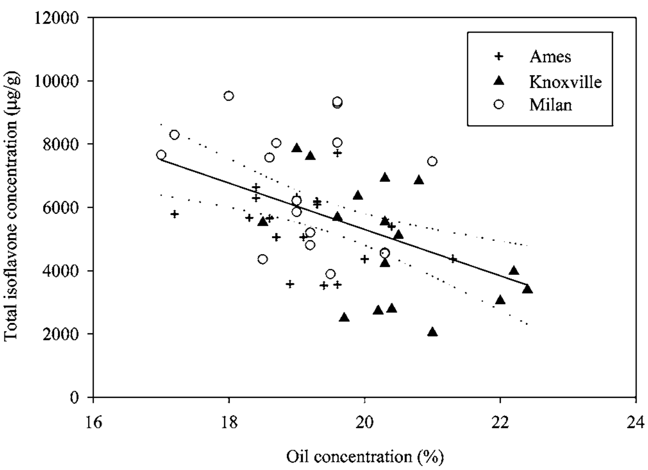

Figure 1. Moderate negative correlation (Pearson $r = -0.41$, statistically significant at $P < 0.01$) was revealed between oil concentration (%) and total isoflavone concentration (μg/g) across locations. This correlation may be approximated by the equation isoflavones = 19940 − 732 × oil. Confidence bands were constructed at the 95% confidence interval.

Our results confirm those of others who have reported high levels of 6′′-*O*-malonyldaidzin and 6′′-*O*-malonylgenistin in soybean seed isoflavones (*29*, *30*). The low concentrations of acetyl isoflavones that we detected are typical in soybean seed although these compounds can constitute over 25% of total isoflavones in processed soy products (*16*). Similarly, although the relatively small amounts of daidzein, genistein, and glycitein that we detected are typical, extraction procedures that utilize hot methanol dramatically increase their levels at the expense of their malonyl conjugates (*16*). The two peaks tentatively identified as different forms of 6′′-*O*-malonyl-daidzin and 6′′-*O*-malonyl-genistin were first described by Griffith and Colson, who reported the emergence of these peaks in authentic standard solutions and suggested that they differ from their parent malonylated isoflavones by the attachment site of the hydroxyl group on the glucose moiety (*25*). Other investigators have since confirmed the presence of isomers of glycoside malonates of daidzein and genistein in soybean (*31*).

The effects of cultivar and location on individual and total isoflavone content have been widely documented (*17*, *18*, *29*, *30*). Location differences may result from variable environments because environmental stresses such as nutrient deficiency, prolonged cold, pathogen infection, and exposure to UV light may influence phenylpropanoid regulatory enzymes (*32*). Our results are in agreement with Hoeck et al. who identified a significant genotype and location effect on 6′′-*O*-malonylgenistin in soybeans grown in Iowa, and the genotype × location interaction was nonsignificant (*18*). In contrast, this interaction was significant for soybeans cultivated in Korea at different locations and times (*30*). These dissimilar results may be attributed to variable environmental factors during the different studies.

Daidzein and genistein are synthesized by the activity of isoflavone synthase from liquiritigenin and naringenin, respectively, which have *p*-coumaroyl-CoA as a common precursor (*33*). The elucidation of glycitein biosynthesis is incomplete, but it appears that daidzein is a precursor of glycitein (*34*). Isoflavone conjugates are formed from the aglycone isoflavone forms. Thus, the isoflavones detected in this study orginate from either daidzein or genistein. The fact that the correlation of oil concentration with daidzein and its derivative isoflavones ($r = -0.46$) and the correlation of oil with genistein and its derivative isoflavones ($r = -0.37$) were similar in sign and magnitude suggests that the daidzein and genistein pathways contributed about equally to the correlation of oil with total isoflavones ($r = -0.41$).

Daidzin, 6′′-*O*-malonyldaidzin (derivatives of daidzein), and 6′′-*O*-malonylgenistin (derivative of genistein) had negative correlations with oil whereas glycitin and 6′′-*O*-malonylglycitin (derivatives of glycitein) were positively correlated with oil across cultivars and locations. These results suggest that the glycitein biosynthetic pathway segregates with oil biosynthetic pathways differently than do the daidzein and genistein pathways. It is noteworthy that Kassem et al. (*35*) identified a quantitative trait locus (QTL) for glycitein on linkage group D1a in soybean seed of a recombinant inbred line (RIL) population derived from the cross of Essex by Forrest that is tightly linked (map distance ≤ 10 cM) to a QTL governing oil synthesis found in a RIL of an Essex by Williams cross (*36*, *37*). Although protein did not correlate with total isoflavones across cultivars and locations, total isoflavones in cultivars 3, 8, 9, 11, and 17 had a moderate or strong positive correlation with protein. A QTL regulating daidzin biosynthesis exists on linkage group K in RILs from Essex by Forrest (*35*) and is tightly linked to a QTL controlling protein synthesis in RILs of Essex by Williams (*36*, *37*). These findings provide possible genetic bases for the correlation data that we report here.

Other investigators have reported a negative correlation of isoflavones with protein content. In one study, total isoflavones had a moderate negative correlation ($r = -0.47$) with protein in F2 plants derived from the cross between soybean cultivars BARC-8 and IAC-100 (*38*). In another study, which used cultivar NC111, total isoflavone concentration increased whereas protein decreased with increasing levels of nitrogen fertilization (*39*). We used a wider range of genetic material, which may explain why total isoflavones in five of 17 cultivars had positive correlations with protein, yet the cultivars together did not reveal a correlation of isoflavones with protein. It is interesting that genistin, glycitin, and 6″-*O*-malonyl glycitin had weak negative correlations with protein concentration across cultivars and locations, but evidently, these compounds were present at insufficient levels to result in a negative correlation of total isoflavones with protein. Additional work is needed to clarify the relationships among isoflavones and protein across a large number of cultivars.

Because biological organisms develop adaptive advantages for species survival, optimal growing conditions for soybean would translate to the higher seed yields that in turn promote survival of successive generations. Soybean flavonoids and isoflavonoids are believed to be important signals that attract *Rhizobia* bacteria, which form nitrogen-fixing root nodules (*40*). Hence, it is plausible that the positive correlations of yield with genistein and 6″-*O*-malonylglycitin reflect the survival advantages afforded by seed yield and seed chemical composition favorable to increased root nodulation.

The present study reports a moderate negative correlation of oil with total and several individual isoflavones. Concentrations of glycitin and 6″-*O*-malonylglycitin were positively correlated with oil, suggesting that their biosynthetic pathways segregate with oil synthetic pathways differently than those of other isoflavones. Our finding that total isoflavones have strong correlations with oil and protein in certain cultivars suggests that these cultivars may be a valuable germplasm resource in breeding efforts to optimize isoflavone concentrations. As the health benefits and risks of soy and isoflavone consumption become clarified, the relationships of total and individual isoflavones with quality factors such as oil and protein content will become increasingly important in soybean improvement programs.

LITERATURE CITED

(1) *Agricultural Statistics*; National Agricultural Statistics Service, United States Department of Agriculture, United States Government Printing Office: Washington, DC, 2004.

(2) Wilson, R. F. Developing agronomic high-protein soybeans. Agro-Food-Industry Hi-Tech September/October 2001.

(3) Lee, S. L.; Ahn, J. K.; Kim, S. H.; Kim, J. T.; Han, S. J.; Jung, M. Y.; Chung, I. M. Variation in isoflavone of soybean cultivars with location and storage duration. *J. Agric. Food Chem.* **2003**, *51*, 3382−3389.

(4) Lamartiniere, C. A. Protection against breast cancer with genistein, a component of soy. *Am. J. Clin. Nutr.* **2000**, *71*, 1705S−1707S.

(5) Santibáñez, J. F.; Navarro, A.; Martinez, J. Genistein inhibits proliferation and in vitro invasive potential of human prostatic cancer cell lines. *Anticancer Res.* **1997**, *17*, 1199−1204.

(6) Anderson, J. W.; Johnstone, B. M.; Cook-Newell, M. E. Meta-analysis of the effects of soy protein intake on serum lipids. *N. Engl. J. Med.* **1995**, *333*, 276−282.

(7) Mezei, O.; Banz, W. J.; Steger, R. W.; Peluso, M. R.; Winters, T. A.; Shay, N. Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese Zucker rats and murine RAW 264.7 cells. *J. Nutr.* **2003**, *133*, 1238−1243.

(8) Picherit, C.; Coxam, V.; Bennetau-Pelissero, C.; Kati-Coulibaly, S.; Davicco, M.; Lebecque, P.; Barlet, J. Daidzein is more efficient than genistein in preventing ovariectomy-induced bone loss in rats. *J. Nutr.* **2000**, *130*, 1675−1681.

(9) Kumi-Diaka, J.; Nguyen, V.; Butler, A. Cytotoxic potential of the phytochemical genistein isoflavone (4′,5′,7-trihydroxyisoflavone) and certain environmental chemical compounds on testicular cells. *Biol. Cell* **1999**, *91*, 515−523.

(10) Kulling, S. E.; Rosenberg, B.; Jacobs, E. The phytoestrogen coumoestrol and genistein induce structural chromosomal aberrations in cultured human peripheral blood lymphocytes. *Arch. Toxicol.* **1999**, *73*, 50−54.

(11) White, L. R.; Petrovitch, H.; Ross, W.; Masaki, K.; Hardman, J.; Nelson, J.; Davis, D.; Markesbery, W. Brain aging and midlife tofu consumption. *J. Am. Coll. Nutr.* **2000**, *19*, 242−255.

(12) Bouker, K. B.; Hilakivi-Clarke, L. Genistein: Does it prevent or promote breast cancer? *Environ. Health Perspect.* **2000**, *108*, 701−708.

(13) Sirtori, C. R. Risks and benefits of soy phytoestrogens in cardiovascular diseases, cancer, climacteric symptoms and osteoporosis. *Drug Saf.* **2001**, *24*, 665−682.

(14) Munro, I. C.; Harwood, M.; Hlywka, J. J.; Stephen, A. M.; Doull, J.; Flamm, W. G.; Aldercreutz, H. Soy isoflavones: A safety review. *Nutr. Rev.* **2003**, *61*, 1−33.

(15) Wang, H.; Murphy, P. A. Isoflavone content in commercial soybean foods. *J. Agric. Food Chem.* **1994**, *42*, 1666−1673.

(16) Coward, L.; Smith, M.; Kirk, M.; Barnes, S. Chemical modification of isoflavones in soyfoods during cooking and processing. *Am. J. Clin. Nutr.* **1998**, *69* (Suppl.), 1486S−1491S.

(17) Eldridge, A. C.; Kwolek, W. F. Soybean isoflavones: Effect of environment and variety on composition. *J. Agric. Food Chem.* **1983**, *31*, 394−396.

(18) Hoeck, J. A.; Fehr, W. R.; Murphy, P. A.; Welke, G. A. Influence of genotype and environment on isoflavone contents of soybean. *Crop Sci.* **2000**, *40*, 48−51.

(19) Vyn, T. J.; Xinhua, Y.; Bruulsema, T. W.; Jackson, C. C.; Rajcan, I.; Brouder, S. M. Potassium fertilization effects on isoflavone concentrations in soybean [*Glycine max* (L.) Merr.]. *J. Agric. Food Chem.* **2002**, *50*, 3501−3506.

(20) Bennett, J. O.; Yu, O.; Heatherly, L. G.; Krishnan, H. B. Accumulation of genistein and daidzein, soybean isoflavones implicated in promoting human health, is significantly elevated by irrigation. *J. Agric. Food Chem.* **2004**, *52*, 7574−7579.

(21) Tsukamoto, C.; Shimada, S.; Igita, K.; Kudou, S.; Kokubun, M.; Okubo, K.; Kitamura, K. Factors affecting isoflavone content in soybean seeds: Changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. *J. Agric. Food Chem.* **1995**, *43*, 1184−1192.

(22) Graham, T. L.; Graham, M. Y. Signaling in soybean phenylpropanoid responses. *Plant Physiol.* **1996**, *110*, 1123−1133.

(23) Poehlman, J. M. Breeding soybeans. In *Breeding Field Crops*, 2nd ed.; AVI Publishing Company, Inc.: Westport, CT, 1979; pp 257−276.

(24) Hymowitz, T.; Dudley, J. W.; Collins, F. I.; Brown, C. M. Estimations of protein and oil concentration in corn, soybean, and oat seed by near-infrared light reflectance. *Crop Sci.* **1974**, *14*, 713−715.

(25) Griffith, A. P.; Collison, M. W. Improved methods for the extraction and analysis of isoflavones from soy-containing foods and nutritional supplements by reversed-phase high-performance liquid chromatography and liquid chromatography−mass spectrometry. *J. Chromatogr. A* **2001**, *93*, 397−413.

(26) Yaklich, R. W.; Vinyard, B.; Camp, M.; Douglass, S. Analysis of seed protein and oil from Soybean Northern and Southern Uniform Tests. *Crop Sci*. **2002**, *42*, 1504−1515.

(27) Brim, C. A.; Burton, J. W. Recurrent selection in soybeans. II. Selection for increased percent protein in seeds. *Crop Sci.* **1979**, *19*, 494−498.

(28) Chung, J.; Babka, H. L.; Graef, G. L.; Staswick, P. E.; Lee, D. J.; Cregan, P. B.; Shoemaker, R. C.; Specht, J. E. The seed protein, oil, and yield QTL on soybean linkage group I. *Crop Sci.* **2003**, *43*, 1053−1067.

(29) Wang, H.; Murphy, P. A. Isoflavone composition of American and Japanese Soybeans in Iowa: Effects of variety, crop year, and location. *J. Agric. Food Chem.* **1994**, *42*, 1674−1677.

(30) Lee, S. J.; Ahn, J. K.; Kim, S. H.; Kim, J. T.; Han, S. J.; Jung, M. Y.; Chung, I. M. Variation in isoflavone of soybean cultivars with location and storage duration. *J. Agric. Food Chem.* **2003**, *51*, 3382−3389.

(31) Wu, Q.; Wang, M.; Sciarappa, W. J.; Simon, J. E. LC/UV/ESI-MS analysis of isoflavones in edamame and tofu soybeans. *J. Agric. Food Chem.* **2004**, *52*, 2763−2769.

(32) Dixon, R. A.; Paiva, N. L. Stress-induced phenylpropanoid metabolism. *Plant Cell* **1995**, *7*, 1085−1097.

(33) Yu, O.; Jung, W.; Shi, J.; Croes, R. A.; Fader, G. M.; McGonigle, B.; Odell, J. T. Production of the isoflavones genistein and daidzein in nonlegume dicot and monocot tissues. *Plant Physiol.* **2000**, *124*, 781−793.

(34) Latunde-Dada, A. O.; Cabello-Hurtado, F.; Czittrich, N.; Didierjean, L.; Schopfer, C.; Hertkorn, N.; Werck-Reichhart, D.; Ebel, J. Flavonoid 6-hydroxylase from soybean (*Glycine max* L.), a novel plant P-450 monooxygenase. *J. Biol. Chem.* **2001**, *19*, 1688−1695.

(35) Kassem, M. A.; Meksem, K.; Iqbal, M. J.; Njiti, V. N.; Banz, W. J.; Winters, T. A.; Wood, A.; Lightfoot, D. A. Definition of soybean genomic regions that control seed phytoestrogen amounts. *J. Biomed. Biotechnol.* **2004**, *1*, 52−60.

(36) Hyten, D. L.; Pantalone, V. R.; Sams, C. E.; Saxton, A. M.; Landau-Ellis, D.; Stefaniak, T. R.; Schmidt, M. E. Seed quality QTL in a prominent soybean population. *Theor. Appl. Genet.* **2004**, *109*, 552−561.

(37) Song, Q. J.; Marek, L. F.; Shoemaker, R. C.; Lark, K. G.; Concibido, V. C.; Delannay, X.; Specht, J. E.; Cregan, P. B. A new integrated genetic linkage map of the soybean. *Theor. Appl. Genet.* **2004**, *109*, 122−128.

(38) Chiari, L.; Piovesan, N. D.; Naoe, L. K.; Inês, C. J.; Viana, J. M. S.; Moreira, M. A.; Barros, E. G. Genetic parameters relating isoflavone and protein content in soybean seeds. *Euphytica* **2004**, *138*, 55−60.

(39) Wilson, R. F. Developing agronomic high-protein soybeans. Agro-Food-Industry Hi-Tech. 2001, September/October, 17−22.

(40) Phillips, D. A. Flavonoids: plant signals to soil microbes. In *Phenolic Metabolism in Plants, Recent Advances in Phytochemistry*; Stafford, H. A., Ibrahim, R. K., Eds.; Plenum Press: New York, 1992; Vol. 26, pp 201−231.

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