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Isoflavone Concentrations and Composition of Soybean Varieties Grown in Upland and Lowland Regions of Vietnam

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ABSTRACT Health beneficial properties of soybean [*Glycine max* (L.) Merr.] isoflavones are well known. The objectives of this study were to determine and compare the isoflavone composition and concentrations of soybean varieties grown in different cultivated regions of Vietnam (i.e., upland and lowland). Total and individual isoflavone composition and concentrations were determined by high-performance liquid chromatography (HPLC). Total isoflavone concentrations varied from 1153 to 6604 μg g^{-1}, and averaging 3354 μg g^{-1} across environments and varieties. In the lowland region, the highest total isoflavones concentration was observed in M103 cultivar (5653 μg g^{-1}) and the lowest in VX9-3 (1153 μg g^{-1}), whereas in the upland region the highest and lowest concentrations were in M103 (6604 μg g^{-1}) and DT93 (1938 μg g^{-1}), respectively. Across varieties, average total isoflavones concentration was higher in the upland than lowland region (3728 vs. 2980 μg g^{-1}). The malonylglucosides and acetylglucosides concentrations in upland soybean varieties were higher than those from the lowland region. Despite the presence of Genotype (*G*) x Environment (*E*) interactions, varieties with consistently high (M103) and low (VX9-3, DT93) isoflavone concentrations across environments were identified. This is the first report of isoflavones in Vietnamese soybean varieties, revealing large variation in isoflavones concentration and profile among different varieties and cultivated regions. Results will be useful in selecting high-isoflavones soybean varieties for growth in tropical regions.

Keywords : Isoflavones, soybeans, environmental factors, lowland, upland, Vietnam

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Soybeans (*Glycine max* L.) have been grown and consumed in tropical countries as well as temperate countries. They have many food, feed and industrial applications. In Vietnam, the cultivated areas and production have increased in recent years. Total soybean production increased from 173.7 thousand tons in 2001 to 275.5 thousand tons in 2007, with the cultivated area increasing by 49.8 thousand ha during this period (National general statistics office, 2008). As a food, soybean is processed in many forms, including tofu, soup, paste, soy milk, bean-sprout as well as various beverages; it also used for industrial purposes and is used as an animal feed. The benefits to human health of soybean based foods have been known for a long time in Vietnam and are also being widely recognized worldwide (Wei *et al.*, 1995; Anthony *et al.*, 1998; Vesper *et al.*, 1999). Soybeans are a major source of protein and oil, but are also containing a variety of secondary metabolites which contribute to human health (Lichtenstein, 1998; Messina *et al.*, 1994; Setchell and Cassidy, 1999; Chung *et al.*, 2000; Peterson and Barns, 1993).

Soybean is known to contain a large number of bioactive phytochemicals including isoflavones, saponins, phytosterols, protease inhibitors, inositol hexaphosphates, sphingolipids, phenolic acids, and trypsin inhibitors (Wei *et al.*, 1995; Vesper *et al.*, 1999; Messina *et al.*, 1994; Chung *et al.*, 2000). Soybean isoflavones are believed to have potential in preventing several important diseases, including cancer and heart diseases (Akitha *et al.*, 2009; Lee *et al.*, 2003a; Kuo *et al.*, 1997; Chiechi *et al.*, 2002; Suthar *et al.*, 2001; Kris-Etherton *et al.*, 2002; Kennedy *et al.*, 1995), while nullifying or reducing some of the side effects of with menopause (Chiechi *et al.*, 2002; Suthar *et al.*, 2001; Kennedy *et al.*, 1998; Koratkar *et al.*,

1997; Lee *et al.*, 1995; Slavin *et al.*, 1997; Holt, 1997).

Beneficial health properties of isoflavones have increased interest for soybean varieties with high isoflavones concentrations. Genetic, agronomic and environmental factors have been reported to affect isoflavones concentration and profile (Liang *et al.*, 2007; Hoeck *et al.*, 2000). Temperature during the seed filling stage was reported as one of the most principle factor in determining seeds isoflavones concentrations (Tsukamoto *et al.*, 1995; Kitamura *et al.*, 1991).

Vietnam is a tropical and sub-tropical country with various environmental conditions. Soybean is grown locally in both upland and lowland areas and it can be planted all year round with two or three crops in some areas. Isoflavones concentration and profile of soybeans grown in contrasting areas have not been reported for this country. Overall, reports on soybean isoflavones of late-maturing varieties and of the effects of the environment in tropical areas remain scarce. In addition, there has been no report contrasting isoflavones content of soybeans grown in lowland and upland conditions. Therefore, the objectives of this study were to compare isoflavones concentration and profile of seven soybean varieties grown in both upland and lowland tropical fields of Vietnam with the purpose of identifying factors which may impact isoflavones content of soybean grown in tropical areas.

MATERIAL AND METHOD

Experimental sites

Experiments were conducted at two contrasting sites located in the midland and mountainous areas of northeastern Vietnam (Table 1). The soil types of the two areas were a condensed mixed sand and clay in the upland area and an ancient alluvia sandy loam in the lowland field. The exact location and environmental specifications of the upland and lowland conditions are described in Table 1.

Table 1. Environmental conditions during seed-filling stages within two regions of Vietnam.

Region	Environmental conditions						
	Latitude North	Altitude (meter)	Slope (degree)	Temperature during ripening[a]	Precipitation during ripening[b]	Relative humidity (RH)[c]	Sunshine duration[d]
Lowland	21°-21° 07'	< 300	< 10	27.8	122.2	74.6	97.8
Upland	22°-22° 20'	300-750	11-25	25.2	163.8	81.2	159.4

[a,b,c,d] Average temperature (degree/month), precipitation (mm/month), RH (%/month), and sunshine duration (h/month), respectively, from April (beginning seed) to June (fully matured seed) 2006.

Table 2. Agronomic characteristics of seven soybean varieties.

Variety	Seed coat color	100 seed weight (gram)	Yield (kg/ha)	Cultivated season	Growing period (day)	Plant height (cm)	National variety registered (year)
AK02	Yellow	10.34	1.000-1.200	Spring, Summer, Winter	75-85	30-40	1987
DN42	Bright-yellow	13-14	1.400-1.600	Spring, Winter	90-95	50-60	1996
DT84	Bright-yellow	15-18	1.300-1.800	Spring, Summer, Winter	85-95	50-60	1995
DT93	Bright-yellow	12.5-14	1.200-1.400	Spring, Summer, Winter	75-85	45-60	1997
M103	Dark-yellow	17.53	1.700−2.000	Spring, Summer, Winter	85-90	30-40	1994
V48	Yellow	12−13.5	1.400−1.500	Spring, Winter	85-90	35-45	1995
VX9-3	Yellow	14-15	1.200-1.500	Spring, Summer, Winter	90-95	35-45	1990

Plant growth and collection

Seven soybean varieties commonly grown in the northern region of Vietnam were used. Varieties DT84, VX9-3, V48, M103, AK02, DT93, and DN42 were obtained from the National Seed Bank in Hanoi, Vietnam. These soybean varieties have shown stable yields for many years in the national registered variety trials and differ in terms of maturity, height, and other agronomic characteristics (Table 2). No rhizobium was artificially inoculated because soybean was planted as preceding crop. Appropriate agrochemicals were used to control weeds, diseases, and insects. Fertilizers were applied prior to seeding at the recommended rates of 8, 8, and 12 kg per 1000 m^2 for N, P_2O_5, and K_2O, respectively. Plants were thinned to the target density of 444,000 plants/ha, each plot consisted of three rows (3.75 m long and 0.6 m between rows) and the experiment consisted of a completely randomized block design with three replicates. Soybean seeds were harvested, air dried and stored in a dehumidified room were shipped to Konkuk University, Seoul, Korea for analysis of isoflavones.

Preparation of seeds for HPLC analyses

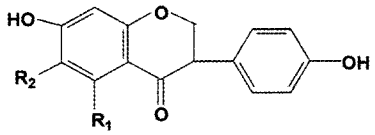
Soybean seed samples used for HPLC analyses were prepared following the method of Lee *et al.* (2003a). Each 2 gram sample of whole soybean seed was ground by a mill grinder, mixed with 2 mL of 0.1N HCl and 10 mL of acetonitrile (ACN) in 125 mL crew-top flask and stirred for 2 h at room temperature, the solution was then filtered through a Whatman No.42 filter paper. The filtrate was dried under vacuum at temperature below -30℃, and then redissolved in 10 mL of 80% HPLC grade methyl alcohol (MeOH) in distilled water. The redissolved sample was filtered through a 0.45 μm filter unit (cameo 13N syringe-filter, nylon) and transferred to a 1 mL vial.

Quantitative analysis of isoflavones using HPLC

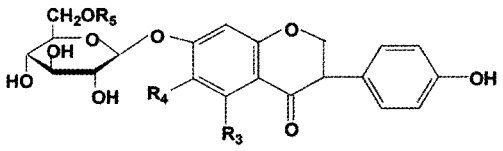
Isoflavone quantification was conducted following the method of Hoeck *et al.* (2000). A linear HPLC gradient was employed: solvent A was 0.1% glacial acetic acid in HPLC-grade distilled water and solvent B was 0.1% acetic acid in ACN. Following the injection of 20 μL of the sample, solvent B was increased from 15% to 35% over 50 min, then held at 35% for 10 min. The solvent flow rate was 1 mL min^{-1}. The HPLC system consisted of a Young-Lin M-930 liquid chromatography pump and an M-720 detector (Young-Lin Instruments Co., Ltd., AhnYang, Korea). The column for quantitative analysis was an YMC-Pack ODS-AM-303 (250 × 4.6 mm I.D) and the UV absorption was measured at 254 nm. ACN, MeOH, acetic acid, and water (HPLC grade) were purchased from J. T. Baker (NJ, USA).

Calibration curves of the 12 isoflavones

Twelve isoflavone standards (Fig. 1) were purchased from LC Laboratories (Woburn, MA, USA). Calibration curves for each standard were built using five concentrations (1, 25, 50, 75 and 100 ppm). A high linearity was obtained for each curve. Daidzein, genistein, glycitein, daidzin, genistin, glycitin, acetyldaidzin, acetylgenistin, acetylglycitin, malonyldaidzin, malonylgenistin and malonylglycitin were identified by their retention times, and their concentrations were calculated by comparing peak areas of samples with those of the standards. Total isoflavones were the sum of the four groups of isoflavones:



aglycons	R_1	R_2
daidzein	H	H
genistein	OH	H
glycitein	H	OCH_3



glucosides	R_3	R_4	R_5
daidzin	H	H	H
genistin	OH	H	H
glycitin	H	OCH_3	H
6"-*O*-malonyldaidzin	H	H	$COCH_2COOH$
6"-*O*-malonylgenistin	OH	H	$COCH_2COOH$
6"-*O*-malonylglycitin	H	OCH_3	$COCH_2COOH$
6"-*O*-acetyldaidzin	H	H	$COCH_3$
6"-*O*-acetylgenistin	OH	H	$COCH_3$
6"-*O*-acetylglycitin	H	OCH_3	$COCH_3$

Fig. 1. Chemical structures of the 12 soybean isoflavones.

aglycones, glucosides, acetylglucosides, and malonylglucosides.

Statistical analyses

The analysis of isoflavones by HPLC was done for each of the three replicates for each variety at each site, with each sample being run in triplicate. Analyses of variance were performed using PROC GLM of the SAS software (2000). Comparisons between sample means were determined using the least significant difference (LSD) test at a probability level of 0.05.

RESULTS AND DISCUSSION

In this study, a total of seven soybean varieties grown in both upland and lowland fields were used to examine their isoflavone concentrations and profile. The isoflavones in soybean seeds were determined from peak areas of the HPLC chromatograms (Fig. 2). Total isoflavone concentrations ranged from 1153 to 5653 μg g^{-1}, with an average of 2887 μg g^{-1} across all varieties and conditions (Table 3). Averaged over the seven varieties, the mean total isoflavones concentration was 2980 and 3728 μg g^{-1}, in lowland and upland conditions, respectively, differences between the two areas being significant.

Among the seven varieties, M103 had the highest total isoflavones concentration in both conditions (i.e., 5653 and 6604 μg g^{-1} in the lowland and upland conditions, respectively); whereas VX9-3 and DT93 had the lowest concentrations (i.e., average of 1365 and 2055 in the lowland and upland conditions, respectively) (Table 3). M103 had 112% higher total isoflavone concentrations across fields, than all other varieties (i.e., 6129 vs. 2891 μg g^{-1}). Differences in total isoflavones concentrations between the two conditions were observed for all varieties. Except AK02, total isoflavones concentration was 41% higher overall for all other varieties when grown upland than lowland. The total isoflavone of AK02 was however 76% higher when grown lowland than upland.

Of the four isoflavone groups, the malonylglucosides and glucosides were found in the highest concentrations in varieties grown upland and lowland, respectively (Table 4). Across varieties in the lowland, aglycons, glucosides, malonylglucosides, and acetylglucosides represented 14, 42, 41, and 3% of the total, respectively; in the upland, these proportions were 11, 41, 45, and 3%.

There were significant differences between the four isoflavone groups among varieties between lowland and the upland

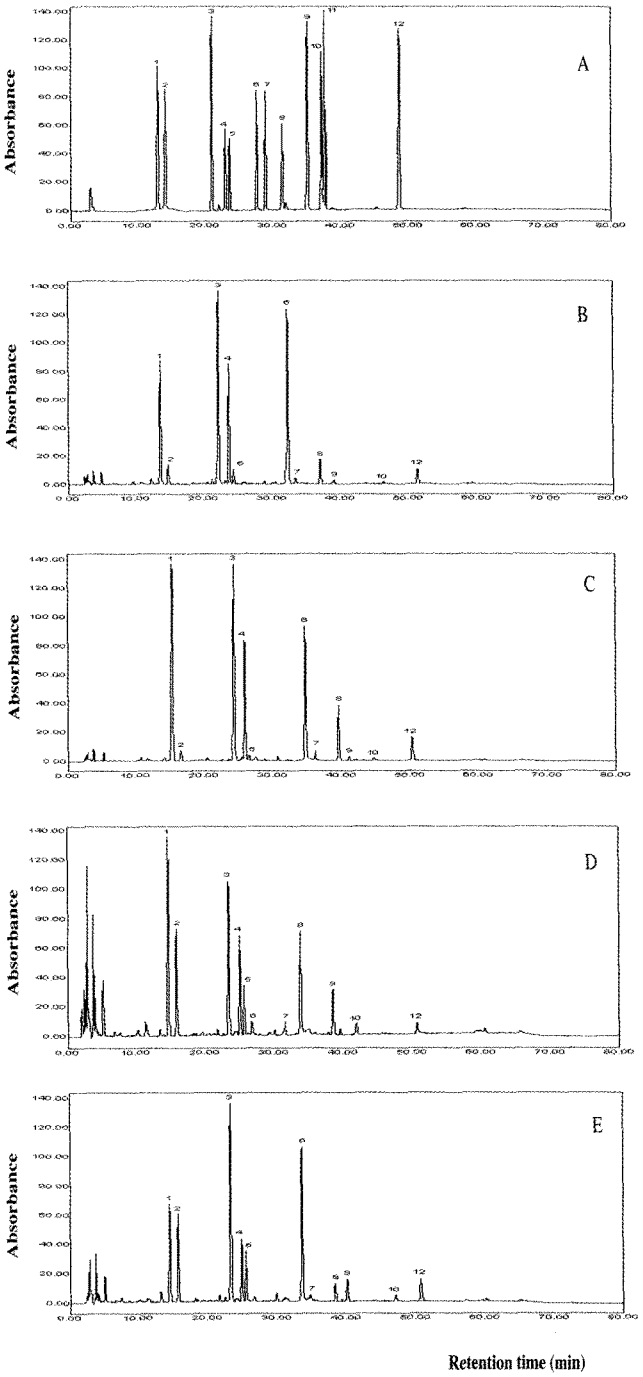


Fig. 2. Representative HPLC chromatograms: A, Standard; B, M103 lowland; C, M103 upland; D, VX9-3 lowland; E, VX9-3 upland; 1, daidzin; 2, glycitin; 3, genistin; 4, malonyldaidzin; 5, malonylglycitin; 6, acetyldaidzin; 7, acetylglycitin; 8, malonylgenistin; 9, daidzein; 10, glycitein; 11, acetylgenistin; 12, genistein.

Table 3. Isoflavones concentration (μg g^{-1}) and profile of seven soybean cultivars grown in lowland and upland regions of Vietnam (n=3).

Variety	Cultivated region	Aglycons			Glucosides			Malonylglucosides			Acetylglucosides		
		Daidzein	Genistein	Glycitein	Daidzin	Genistin	Glycitin	Daidzin	Genistin	Glycitin	Daidzin	Genistin	Glycitin
AK02	L*	474	166	3.2	163	1397	54	111	1434	63	71	27.8	14.2
	U**	176	99	7.1	103	843	192	37	540	139	68	27.2	18.5
DN42	L	102	52	2.5	66	597	125	36	539	135	75	26.2	15.7
	U	112	34	10.8	119	889	109	164	1800	196	72	nd	23.3
DT84	L	214	68	11.4	151	1058	94	125	1390	121	112	nd	9.3
	U	235	35	12.3	261	1076	107	309	1867	176	212	nd	16.0
DT93	L	42	23	3.9	49	431	140	36	573	184	52	28.4	14.4
	U	54	20	9.6	64	548	147	53	737	199	94	nd	14.3
M103	L	387	118	7.8	238	1966	86	227	2429	122	52	nd	21.1
	U	984	210	8.7	502	2380	59	290	2045	65	30	nd	32.0
V48	L	832	189	17.7	266	1163	159	62	508	88	73	nd	18.9
	U	729	nd	7.6	379	1901	48	249	1827	66	56	nd	29.8
VX9-3	L	161	20	4.2	78	322	92	35	285	88	37	nd	11.5
	U	110	64	21.2	66	699	133	43	774	163	76	nd	26.0
Average	L	316	91	7	144	991	107	90	1023	114	67	27	15
	U	343	77	11	213	1191	114	164	1370	143	87	27	23
$LSD_{(0.05)}$	L	65.9	29.2	8.1	24.1	190.5	9.9	13.6	206.6	8.8	29.5	25.1	7.9
	U	22.1	7.2	4.3	21.3	91.3	9.3	15.9	112.6	12.1	52.6	4.4	6.1

*L: Lowland; **U: Upland.

conditions. Varieties AK02, M103, and VX9-3 had the largest difference among isoflavone groups between the two regions (Table 4). For VX9-3, the glucoside, malonylglucoside, and acetylglucoside groups were found in higher concentrations when grown upland than lowland, whereas, the malonylglucoside group of AK02 grown upland was 55% lower than when grown lowland. For M103, difference was only observed for the aglycon group, concentration from the lowland and upland fields being 513 and 1203 μg g^{-1}, respectively.

Across seven soybean varieties, glucosides, the total individual of four isoflavone groups, were found the highest (42% of total) and acetylglucosides were the lowest (3% of the total) concentration, and there were significant differences between each isoflavone group (Table 4). Most differences were observed for the malonylglucosides, the highest concentration was 2777 μg g^{-1} for M103, whereas the lowest was 408 μg g^{-1} for VX9-3. Differences between the concentration of isoflavone groups among varieties were also observed for the glucosides and aglycones. In the aglycon group, the highest isoflavone concentration was 1038 μg g^{-1} for V48, whereas the lowest was 69 μg g^{-1} for DT93. Finally, glucosides were found in the highest concentration in M103 (2290 μg g^{-1}) and lowest in VX9-3 (491 μg g^{-1})

On the other side, the four isoflavone groups within two conditions (i.e. upland and lowland), including malonylglucosides (45%) and glucosides (41%) were in higher concentrations than were the other groups in the two fields. Great differences were observed for the malonylglucosides, the highest concentration being 277.38 μg g^{-1} in M103 within the upland condition, and the lowest was 407.93 μg g^{-1} in VX9-3, in lowland condition. Differences were also observed for glucosides and aglycones. For aglycones, the highest isoflavone concentration was 1202.60 μg g^{-1} in M103, in upland condition whereas the lowest was 69.40 μg g^{-1} in DT93, in lowland condition. Glucosides were found higher concentrations in M103, in upland and lowland areas, the concentrations were 2940.14 μg g^{-1} and 2289.78 μg g^{-1-1}, respectively. Besides, the glucosides concentrations of V48 in the two fields were also found higher than those of other varieties, the concentrations were 2328.48 μg g^{-1} in upland and 1587.73

Table 4. Total concentration (μg g^{-1}) of individual isoflavone groups in varieties grown from lowland and upland regions.

Variety	Cultivated region	Aglycons	Glucosides	Malonyl-glucosides	Acetyl-glucosides
AK02	L*	642.42	1613.00	1607.26	113.43
	U**	281.98	1138.71	715.84	114.08
DN42	L	155.74	788.63	709.01	117.23
	U	156.93	1116.76	2159.92	95.31
DT84	L	293.62	1302.57	1635.63	120.90
	U	281.73	1444.18	2351.00	227.95
DT93	L	69.40	619.32	792.81	94.90
	U	83.22	758.19	989.11	107.74
M103	L	512.76	2289.78	2777.38	73.24
	U	1202.6	2940.14	2399.20	62.15
V48	L	1038.84	1587.73	657.38	91.94
	U	736.52	2328.48	2142.27	85.59
VX9-3	L	205.38	491.34	407.93	48.60
	U	194.52	898.06	979.69	102.41
Average	L	416.88	1241.77	1226.77	94.32
	U	419.64	1517.79	1676.72	113.60
$LSD_{(0.05)}$	L	91.00	222.83	226.71	38.63
	U	28.68	120.19	134.73	54.51

μg g^{-1} in lowland. The lower concentrations of glucosides were found in VX9-3, in lowland and followed by DT93 in lowland, with the concentrations of 491.34 μg g^{-1} and 619.32 μg g^{-1}, respectively.

Overall, the total isoflavones concentrations we observed were higher than that reported in others studies, even despite the fact that plants were grown in a warm tropical climate (Seguin *et al.*, 2004; Lee *et al.*, 2003b, Kim *et al.*, 2005). Total isoflavone concentrations of a given cultivar are usually greater in cooler environments, as high temperatures during seed formation are well documented to reduce isoflavone concentrations (Aussenac *et al.*, 1998; Carrao-Panizzi *et al.*, 1999; Lozovaya *et al.*, 2005; Seguin *et al.*, 2007). Results thus indicate that Vietnamese soybeans cultivars have high potential regarding their total isoflavone content, especially M103, which had unusually high total isoflavones concentrations. Also, isoflavone concentrations in soybean varieties from the upland condition were much higher than those from the lowland condition, lower temperatures in the upland region might have favored isoflavone accumulation in seeds. It should be noted however that, among the seven varieties evaluated, AK02 had lower total isoflavone concentrations when grown upland, where temperature average was higher compared to the upland (24.6 vs. 21.0℃). This result suggests that some varieties might be more responsive to factors other than temperature. There may be some other factors such as latitude, altitude, slop, and average relative humidity (RH) might also have played an important role in the differences in isoflavone concentrations between the two conditions.

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