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Effects of year, site, genotype and their interactions on various soybean isoflavones

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Abstract

The popularity of soybeans is increasing as soybean products are now regarded as healthy foods, partly due to the isoflavones contained in their seeds. This study was initiated to study the isoflavone content in soybean seeds as influenced by genotype, year, site and their interactions. Fifteen cultivars were grown at three sites (Seoul, Suwon and Kyongsan) in Korea in 1998–2000, and harvested seeds were measured to determine the content of nine different isoflavones. This study led to the following conclusions. The main environmental effects (year and year-by-site interactions) and genotype-by-environment interactions (genotype-by-year and genotype-by-year-by-site interactions) were the most important sources of variation for the content of various isoflavones in soybean seeds. Significant differences among genotypes in isoflavone content exist that can only be reliably detected through multi-site and multi-year tests. The genotype 'Geomjeong 1' had consistently higher total isoflavone content. While the genotype 'Jangyeob' also had high total isoflavone content, it was less stable than 'Geomjeong 1'. There were no major genetically determined negative associations among the isoflavones, except for that between glycitin (GLY) and malonylgenistin (MGIN). On the contrary, a strong negative environmental association was observed between genistein (GEIN) (along with glycitein (GLIEN)) and daidzin (DIN) (along with genistin (GIN)). Both genetically and environmentally, total isoflavone content was most closely associated with malonylgenistin (MGIN).

Keywords: Soybean; Isoflavones; Genotype-by-environment interaction

1. Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the major upland summer crops in Korea. Due to its high protein and oil content, it has long been a popular part of the Korean diet. The benefits of soybean food to human health have been known for a long time and are now widely recognized worldwide (Holt, 1997; Kyoko, 1998). Soybean foods are known to be beneficial in treating several common human diseases including cancer (Holt, 1997). Recently, soybean constituents with biological activity, such as anticarcinogens, antioxidatives, antihaemolytics, antifungal substances, and tyrosine protein kinase inhibitors, have received attention (Akiyama et al., 1987; Kudou et al., 1991; Peterson

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and Barnes, 1991; Coward et al., 1993; Chung et al., 2000).

Soybean isoflavones are a large class of phytochemicals, primarily polyphenols, with antioxidative activities (Kyoko, 1998). As important secondary metabolic compounds in soybean seeds, isoflavones have been reported to play essential roles in preventing certain types of cancers and in reducing the risk of cardiovascular diseases (Anderson et al., 1995; Anthony et al., 1996; Schultz, 1998). They can also reduce the activity of hemolysis (Naim et al., 1976), inhibit in vitro growth of human breast cancer and prostate cancer cell lines (Choi et al., 1996), and express estrogenic activities in animals (Murphy et al., 1999; Song et al., 1999). Isoflavones may be transformed with some functional groups, such as the malonyl and acetyl glycosides, and the malonylated isoflavone glycosides are major isoflavone constituents in soybean seed. They are thermally unstable and easily converted into corresponding isoflavone glycosides (Kudou et al., 1991; Wang and Murphy, 1994a,b; Song et al., 1998). Isoflavonoids have similar structures to both endogenous and synthetic estrogens and show estrogenic activities (Barnes et al., 1998). The anticancer function of soybean isoflavones was shown to be associated with genistein, which inhibits protein tyrosine kinase and DNA topoisomerase and binds weakly to estrogen receptors (Messina et al., 1994). The efficiency of daidzein for urinary recovery was shown to be significantly greater than that of genistein (Xu et al., 1994).

Wang and Murphy (1994b) reported total isoflavone contents from 1176 to 3309 μg g^{-1} across years, and from 1176 to 1749 μg g^{-1} across sites within the same year for single soybean cultivars. Choi et al. (1996) reported total isoflavone contents from 458 to 2317 μg g^{-1} among cultivars in a single year. Tsukamoto et al. (1995) showed that the isoflavone content was significantly lower in seeds that developed in high temperatures during seed fill than in seeds exposed to low temperatures. Hoeck et al. (2000) studied the effects of genotype, environment, and genotype × environment interactions on the isoflavones of six soybean cultivars over 2 years and reported significant genotype × environment interactions. However, they concluded that the main genotypic effects of total and individual isoflavones were large enough for effective cultivar improvement.

Although the soybean has been a major crop in Korea, the effects of various internal and external factors on soybean isoflavones have not been studied. This study was undertaken to (1) study the effects of genotype, crop year, site and their interactions on the contents of different isoflavones under Korean conditions; (2) investigate the interrelationships among different isoflavones; (3) identify soybean genotypes that may be used in improving the isoflavone level of Korean soybeans.

2. Material and methods

2.1. Soybean seed samples

Fifteen popular soybean cultivars were grown at three sites: Seoul, Suwon, and Kyongsan in Korea, in 1998–2000, respectively. The cultivars differed in maturity, height, seed weight, growth habit, and other characteristics (Table 1). The soil was a silt clay loam at all sites. As the previous crop was soybean, no artificial inoculation was needed. The planting arrangement was 60 cm × 15 cm per plot and the plants were thinned to a uniform density 14 days after planting. Appropriate pesticides were used to control weeds, disease, and insects, and fertilizers were applied prior to ploughing at the recommended rates of 8, 8, and 12 kg/1000 m^2 for N, P_2O_5, and K_2O, respectively. Each plot contained three rows (3.75 m long and 0.6 m between rows) and the experiment consisted of a completely randomized design with three (1998) or four (1999 and 2000) replicates. Soybean seeds were harvested from each replicate for each genotype at each year and site, and stored at room temperature for isoflavone determination.

2.2. Isoflavone assay

2.2.1. Isoflavone standards

Genuine standards of daidzin, daidzein, genistin and genistein were purchased from a commercial source (Aldrich Chemical, USA). Glycitin, glycitein, malonyldaidzin, malonylgenistin and malonylglycitin were isolated and purified using a modified method of Wang and Murphy (1994a). One hundred grams of dried ground soybean seed was defatted with *n*-hexane (400 ml) for 4 h at room temperature, and the defatted

Table 1
Soybean genotype characteristics used in this study

Genotypes	Flowering date (month, day)	Maturity type	Weight of 100 grains (g)	Seed coat color	Hilum color	Growth type	Stem length (cm)
Taekwang	7, 20	Late	25.6	Yellow	Yellow	Determinate	82
Myeongjunamul	7, 23	Late	11.4	Yellow	Brown	Determinate	65
Danbaek	7, 29	Late	14.0	Yellow	Grey-brown	Determinate	84
Daweon	7, 16	Middle	9.4	Yellow	Black	Determinate	50
Muhan	7, 20	Late	21.0	Yellow	Brown	Indeterminate	129
Jangyeob	8, 40	Late	23.0	Yellow	Yellow	Determinate	50
Hwangkeum	8, 30	Late	25.0	Yellow	Brown	Determinate	50
Hwaeomput	6, 20	Early	31.0	Yellow	Brown	Determinate	37
Pureun	7, 13	Middle	13.5	Green	Green	Indeterminate	101
Hannam	8, 10	Middle	12.0	Yellow	Yellow	Semi-determinate	66
Geomjeong 1	7, 21	Middle	29.0	Yellow	Black	Determinate	82
Jinpum 2	7, 14	Late	22.0	Yellow	Yellow	Determinate	68
Suwon 157	7, 10	Late	20.0	Yellow	Yellow	Determinate	67
Shinpaldal 2	7, 40	Early	20.0	Yellow	Brown	Determinate	64
SS 2	7, 20	Early	20.0	Yellow	Yellow	Determinate	66

soybean powder was air-dried and extracted with 80% (v/v) methyl alcohol (600 ml) in distilled water for 4 h at room temperature, then filtered through a Whatman no. 42 filter paper. The filtrate was then transferred to a 250 ml round-bottomed flask and concentrated using a rotary evaporator at 30 °C. The concentrate was re-dissolved in 150 ml of 80% methyl alcohol and 10 ml of the extract was loaded onto a Sephadex LH-20 (Amersham Pharmacia Biotech AB) column with eluent of 30% (v/v) ethyl alcohol. The elutions were collected using a Waters-made fraction collector and measured at an absorbance of 254 nm. The contents of the tubes of each separated fraction were combined and a high-performance liquid chromatography (HPLC) was used to determine the isoflavone forms. Further purification of the isoflavones was performed with a semi-preparative column (YMC-Pack ODS-AM-323, 250 mm × 10 mm ID), and nine standard compounds were chromatographed individually and as a mixture.

2.2.2. *Isoflavone extraction and HPLC assay*

Two grams of ground soybean seed, with seed coat, were mixed with 2 ml of 0.1 N HCl and 10 ml of acetonitrile (ACN) in a 125 ml screw-top flask, stirred for 2 h at room temperature, and filtered through a Whatman no. 42 filter paper. The filtrate was dried under vacuum at temperatures below 30 °C, and then re-dissolved in 10 ml of 80% HPLC grade methyl alcohol in distilled water. The re-dissolved sample was filtered through a 0.45 μm filter unit (Cameo 13N syringe-filter, nylon) and then transferred to 1 ml vials. An HPLC analysis was conducted according to the method of Wang and Murphy (1994a,b). A linear HPLC gradient was used: solvent A was 0.1% glacial acetic acid in distilled water, and solvent B was 0.1% glacial acetic acid in ACN. Following the injection of 20 μl of the sample, solvent B was increased from 15 to 35% for 50 min, then held at 35% for 10 min. The solvent flow rate was 1 ml/min. The HPLC system consisted of a Young-Lin M930 liquid chromatograph pump and an M720 detector (Young-Lin Instruments), and the column for quantitative analysis used was a YMC-Pack ODS-AM-303 (250 mm × 4.6 mm ID). The HPLC chromatograms of genotype Geomjeong 1 in each of the 3 years are shown in Fig. 1.

2.3. *Statistical analysis*

The SAS procedure MIXED (SAS, 1996) was used to conduct factorial analyses of variance and estimate the variance components of genotype, year, site and their interactions for each of the nine isoflavones and four derived variables. Genotypic values of various isoflavones were calculated from the raw data and displayed in a genotype-by-trait bi-plot (Yan and Kang, 2002; Yan and Rajcan, 2002) to visualize the genetic correlations among the various isoflavones.

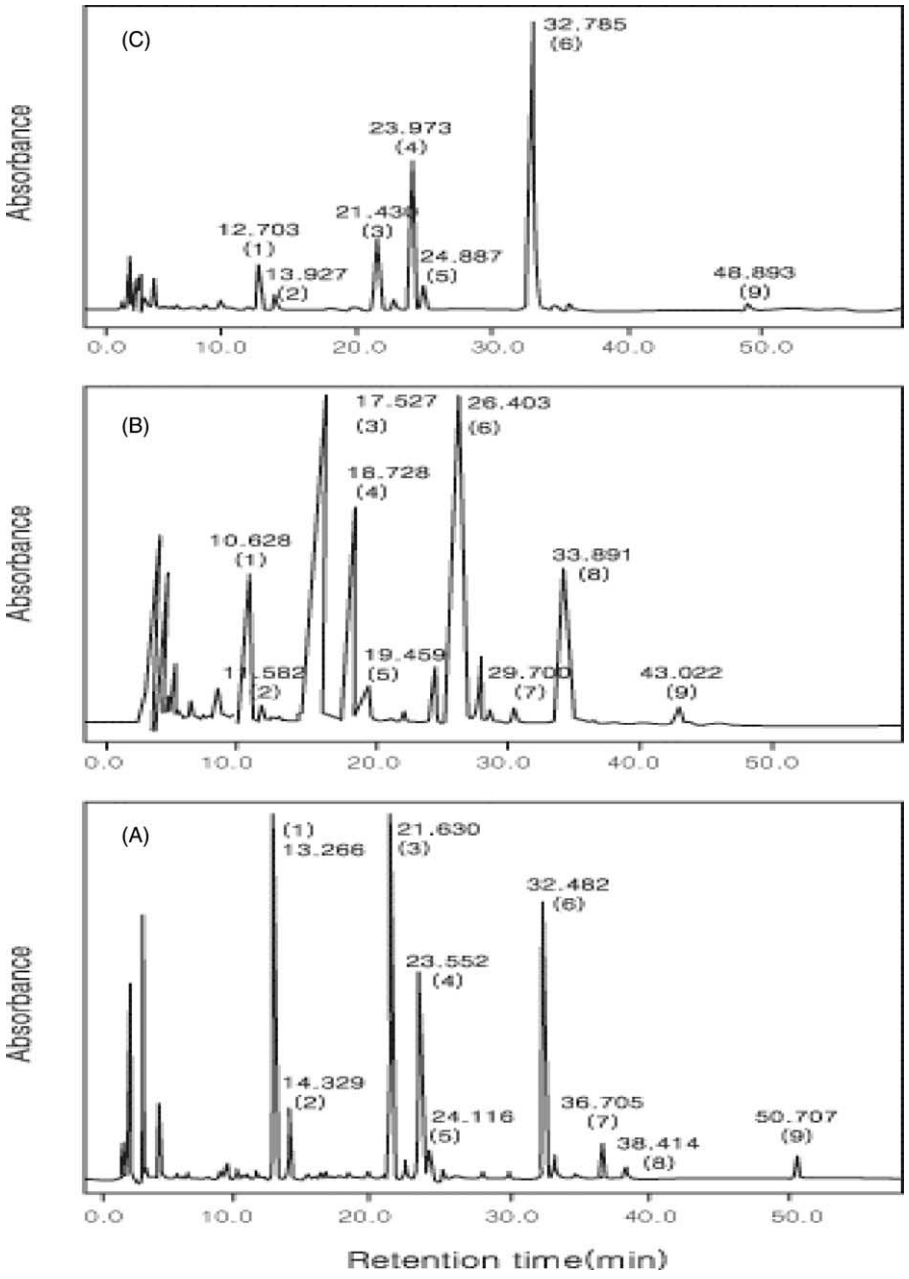

Fig. 1. Comparison of HPLC chromatograms between 1998 (A), 1999 (B), and 2000 (C) of Geomjeong 1 cultivar extracts. 1, daidzin; 2, glycitin; 3, genistin; 4, malonyldaidzin; 5, malonylglycitin; 6, malonylgenistin; 7, daidzein; 8, glycitein; 9, genistein.

Likewise, environmental values of the various isoflavones were calculated and visualized using an environment-by-trait bi-plot to visualize the environmental correlations among the isoflavones. A genotype-by-environment table for total isoflavone content was also derived from the raw data and displayed in a GGE bi-plot to visualize the mean and stability of the genotypes (Yan et al., 2000; Yan, 2001, 2002). The bi-plots

were generated and visualized using GGE bi-plot software (Yan, 2001), a demonstration version of which is available at http://ggebiplot.com/.

3. Results and discussion

3.1. Results of analyses of variance

The main effects of year, site, genotype and all possible interactions between them are all significant at the $P < 0.001$ level for all isoflavones, as indicated by the F-statistics (Table 2). The relative magnitudes of the different sources of variation, however, vary greatly, as indicated by the variance components as percentages of the total variation (Table 3). Compared to the F-values in Table 2, the variance components in Table 3 took into consideration, to some extent, the degrees of freedom associated with each variance source. The latter is, therefore, more informative as to the relative importance of the effects. Based on Table 3, the genotype-by-environment interaction dominated the variation for all isoflavones, except glycitin (GLY), genistin (GIN) and glycitein (GEIN), for which the main environmental effect was the predominant source of variance. Among the main environmental effects, the year effect and/or year-by-site interaction were the main sources of variation. Within the genotype-by-environment interactions, the genotype-by-year and genotype-by-year-by-site interactions were the predominant sources.

Table 2
F-values from the analysis of variance for different isoflavones[a]

Source	d.f.	DIN	GLY	GIN	MDIN	MGLY	MGIN	DEIN	GLIEN	GEIN	TDZ	TGE	TGLIEN	TISO
Genotype (Geno)	14	104	151	57	141	213	55	144	11	4	251	64	121	250
Environment (Env)														
Year (Yr)	2	999	1861	4253	662	2163	305	881	16	167	1361	920	2150	3212
Site	2	66	829	280	130	278	3	321	18	37	275	28	559	326
Yr × site	4	57	705	39	123	609	65	75	26	35	71	37	626	166
Geno × Env														
Geno × Yr	28	102	108	84	151	282	42	166	13	5	192	60	158	195
Geno × site	28	31	103	73	49	92	22	100	3	3	65	39	45	90
Geno × Yr × Site	56	33	92	70	84	123	19	121	4	3	83	32	61	104

[a] All significant at the 0.001 level. The isoflavone codes are: DIN, daidzin; GLY, glycitin; GIN, genistin; MDIN, malonyldaidzin; MGLY, malonylglycitin; MGIN, malonylgenistin; DEIN, daidzein; GLIEN, glycitein; GEIN, genistein. TDZ = DIN + MDIN + DEIN, TGE = GIN + MGIN + GEIN, TGLIEN = GLY + MGLY + GLIEN and TISO = TDZ + TGE + TGLIEN.

Table 3
Variance components as a percentage of the total variance for various isoflavones[a]

Source	DIN	GLY	GIN	MDIN	MGLY	MGIN	DEIN	GLIEN	GEIN	TDZ	TGE	TGLIEN	TISO
Genotype (Geno)	0.8	0.0	0.0	0.0	0.0	3.5	0.0	0.0	0.0	4.9	0.0	0.0	3.3
Environment (Env)	26.4	99.5	58.2	13.1	27.8	21.6	14.8	16.1	65.8	19.6	31.4	96.2	35.1
Year (Yr)	24.1	31.4	55.5	9.9	16.6	16.6	11.5	0.0	36.2	16.6	31.2	39.6	31.4
Site	0.4	0.0	2.6	0.2	0.0	0.0	3.3	0.0	1.2	3.0	0.0	0.0	1.8
Yr × site	2.0	68.1	0.0	3.0	11.2	5.0	0.0	16.1	28.4	0.0	0.2	56.6	1.8
Geno × Env	72.8	0.4	41.8	86.9	72.2	74.9	85.2	83.9	34.2	75.5	68.6	3.8	61.6
Geno × Yr	30.4	0.0	0.1	22.2	21.8	21.7	10.2	40.4	8.0	24.7	14.8	3.8	14.6
Geno × site	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	3.5	0.0	0.0
Geno × Yr × site	42.4	0.4	41.7	64.6	50.3	51.4	75.0	43.5	26.1	50.7	50.3	0.0	47.0

[a] DIN, daidzin; GLY, glycitin; GIN, genistin; MDIN, malonyldaidzin; MGLY, malonylglycitin; MGIN, malonylgenistin; DEIN, daidzein; GLIEN, glycitein; GEIN, genistein. TDZ = DIN + MDIN + DEIN, TGE = GIN + MGIN + GEIN, TGLIEN = GLY + MGLY + GLIEN and TISO = TDZ + TGE + TGLIEN.

Table 4
Genotypic values of various isoflavones across environments[a]

Genotypes	DIN	GLY	GIN	MDIN	MGLY	MGIN	DEIN	GLIEN	GEIN	TDZ	TGE	TGLIEN	TISO
Taekwang	466.7	93.3	653.0	1664.8	214.7	1981.3	95.4	14.3	12.3	2226.9	2646.6	322.2	5195.7
Myeongjunamul	348.2	75.6	556.2	1210.0	172.6	1518.2	34.4	7.1	13.9	1592.6	2088.4	255.4	3936.4
Danbaek	495.8	110.1	493.2	1441.2	246.7	1520.3	34.6	6.7	9.3	1971.6	2022.8	363.5	4357.9
Daweon	321.5	56.4	654.8	1204.3	134.1	1321.1	42.2	18.3	11.9	1568.0	1987.8	208.7	3764.5
Muhan	319.5	98.6	585.3	1527.8	382.1	1676.2	33.0	10.8	14.8	1880.4	2276.3	491.5	4648.2
Jangyeob	608.9	102.7	720.5	1806.3	463.0	1715.2	153.5	10.8	13.9	2568.8	2449.6	576.5	5594.9
Hwangkeum	405.2	78.7	597.3	1736.4	150.7	1734.2	47.2	10.5	13.3	2188.9	2344.8	239.9	4773.5
Hwaeomput	369.0	144.4	773.5	1551.2	235.2	1638.1	80.3	9.8	31.5	2000.6	2443.1	389.4	4833.0
Pureun	479.0	147.7	591.0	1495.8	231.4	1564.1	34.4	16.5	12.7	2009.2	2167.7	395.7	4572.6
Hannam	439.0	106.6	550.9	1560.2	210.0	1534.3	55.0	11.0	15.4	2054.3	2100.6	327.6	4482.4
Geomjeong 1	571.5	176.9	618.2	1798.9	266.8	1843.6	62.9	58.7	16.2	2433.3	2478.1	502.4	5413.8
Jinpum 2	480.8	184.4	614.2	1468.9	206.8	1467.9	37.5	16.5	12.1	1987.2	2094.1	407.7	4489.0
Suwon 157	441.9	149.6	655.0	1701.0	246.1	1646.9	38.6	12.0	15.4	2181.6	2317.4	407.7	4906.7
Shinpaldal 2	310.5	218.3	535.7	1252.3	195.0	1201.6	32.2	15.0	13.9	1595.1	1751.2	428.3	3774.6
SS 2	392.5	146.4	728.8	1462.4	306.3	1330.0	34.9	18.0	16.4	1889.7	2075.3	470.7	4435.7
Mean	430.0	126.0	621.8	1525.4	244.1	1579.5	54.4	15.7	14.9	2009.9	2216.2	385.8	4611.9
Maximum	608.9	218.3	773.5	1806.3	463.0	1981.3	153.5	58.7	31.5	2568.8	2646.6	576.5	5594.9
Minimum	310.5	56.4	493.2	1204.3	134.1	1201.6	32.2	6.7	9.3	1568.0	1751.2	208.7	3764.5

[a] DIN, daidzin; GLY, glycitin; GIN, genistin; MDIN, malonyldaidzin; MGLY, malonylglycitin; MGIN, malonylgenistin; DEIN, daidzein; GLIEN, glycitein; GEIN, genistein. TDZ = DIN + MDIN + DEIN, TGE = GIN + MGIN + GEIN, TGLIEN = GLY + MGLY + GLIEN and TISO = TDZ + TGE + TGLIEN.

The main genotypic effect, main site effect, and genotype-by site-interaction were not important for any of the isoflavones. There were only sizable variances in the main genotypic effect for malonylgenistin (MGIN), daidzin (DIN), malonyldaidzin (MDIN), daidzein (DEIN) and total isoflavones (TISO = TDZ + TGE + TGLIEN) (Table 3). Even for these five variables, the main genotypic effect was no more than 5% of the total variation. Therefore, selection for isoflavones in a single environment, or at multiple sites within a single year, is not likely to be effective.

3.2. Interrelationships between different isoflavones

3.2.1. Genotypic values of various isoflavones

The genotypic values of the various isoflavones, averaged across all environments, are presented in Table 4. Mean, maximum, and minimum values for each isoflavone are also included. Presenting the data in a genotype-by-trait bi-plot makes it much easier to understand (Figs. 2 and 3). Figs. 2 and 3 show different ways to visualize the same bi-plot, with Fig. 2 best visualizing the genetic associations between various isoflavones. The cosine of the angle between the vectors (the lines that connect the bi-plot origin and the isoflavones) of two isoflavones approximates the genetic correlation coefficients between them. A 90° angle means a zero correlation (completely independent), a 0° angle means a correlation of +1, and a 180° angle means a correlation of −1. An acute angle indicates a positive correlation, while an obtuse angle indicates a negative correlation. Fig. 2 shows that most isoflavones are either positively correlated or independent of each other. Therefore, genetically, there were no major negative associations among the isoflavones, with possible exceptions of glycitin (GLY) and malonylgenistin (MGIN), and of glycitin (GLY) and TGE (GIN + MGIN + GEIN). Malonylgenistin (MGIN) and TGE are closely associated, reflecting the fact that malonylgenistin (MGIN) is a major component of TGE. Similarly, malonyldaidzin (MDIN), TDZ (DIN + MDIN + DEIN) and TISO (TDZ + TGE + TGLIEN) are closely associated, reflecting the fact that malonyldaidzin (MDIN) is a major component of TDZ (DIN + MDIN + DEIN), which, in turn, is a major component of TISO (TDZ + TGE + TGLIEN). Therefore, Fig. 2 reveals that malonyldaidzin (MDIN) is the most important component of total isoflavones (TISO = TDZ + TGE + TGLIEN). Isoflavones with short vectors are less variable among genotypes. For

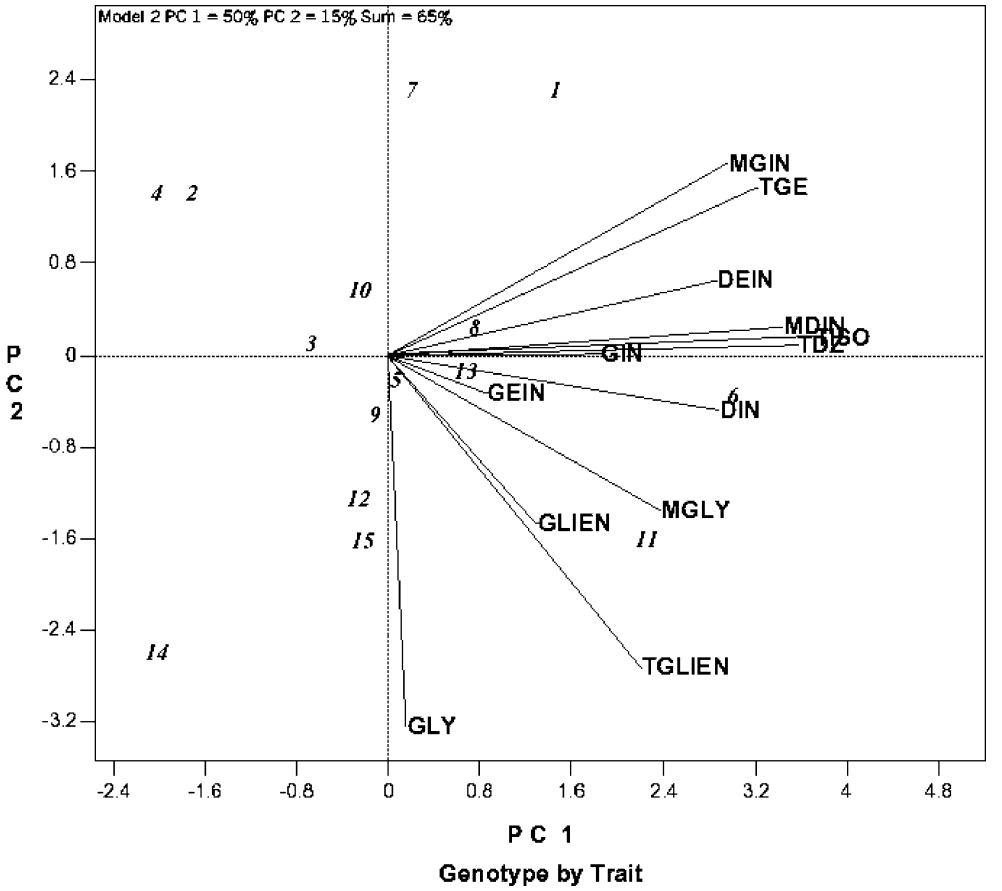

Fig. 2. A genotype-by-isoflavone type bi-plot showing the genetic relations between different isoflavones. The genotype codes are: 1, Taekwang; 2, Myeongjunamul; 3, Danbaek; 4, Daweon; 5, Muhan; 6, Jangyeob; 7, Hwangkeum; 8, Hwaeomput; 9, Pureun; 10, Hannam; 11, Geomjeong 1; 12, Jinpum 2; 13, Suwon 157; 14, Shinpaldal 2; 15, SS 2. The isoflavone codes are: DIN, daidzin; GLY, glycitin; GIN, genistin; MDIN, malonyldaidzin; MGLY, malonylglycitin; MGIN, malonylgenistin; DEIN, daidzein; GLIEN, glycitein; GEIN, genistein. TDZ = DIN + MDIN + DEIN, TGE = GIN + MGIN + GEIN, TGLIEN = GLY + MGLY + GLIEN, and TISO = TDZ + TGE + TGLIEN.

example, genistein (GEIN) has the shortest vector, so the variation among genotypes in genistein (GEIN) content should be very small, which can be verified from Table 2.

Fig. 3 shows which genotype had the highest values for each isoflavone. Genotype 'Jangyeob' (6) had the highest values for 10 of the 13 isoflavones, or groups of isoflavones, including total isoflavones. These include malonylgenistin (MGIN), TGE (GIN + MGIN + GEIN), daidzein (DEIN), malonyldaidzin (MDIN), genistin (GIN), daidzin (DIN), genistein (GEIN), malonylglycitin (MGLY), TDZ (DIN + MDIN + DEIN) and TISO (TDZ + TGE + TGLIEN). Genotype 'Geomjeong 1' (11) had the highest value for glycitein (GLIEN) and TGLIEN (GLY + MGLY + GLIEN), and the genotype 'Shinpaldal 2' (14) had the highest glycitin (GLY) content. Interestingly, 'Shinpaldal 2' is one of the genotypes that had the lowest total isoflavones (the other genotypes include 'Daweon' and 'Myeongjunamul'). The statements are largely consistent with the genotype-by-isoflavone two-way data (Table 4). Minor inconsistencies are expected because the bi-plot only explained 65% of the total variation.

3.2.2. *Environmental values of various isoflavones*

Analogous to Table 4, the environmental values, i.e. the average of different isoflavones across all genotypes in each environment (year–site combination),

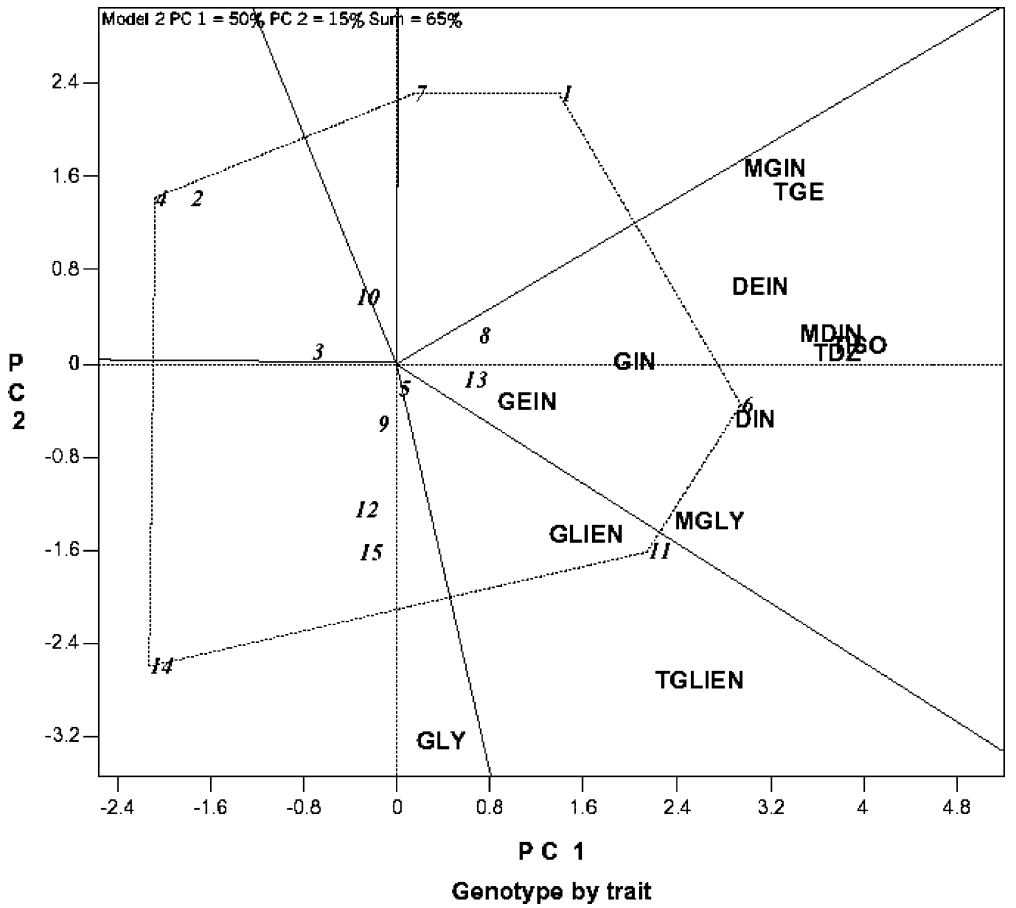

Fig. 3. A genotype-by-isoflavone type bi-plot showing which genotype had the highest value of which isoflavone or isoflavone groups. The genotype codes are: 1, Taekwang; 2, Myeongjunamul; 3, Danbaek; 4, Daweon; 5, Muhan; 6, Jangyeob; 7, Hwangkeum; 8, Hwaeomput; 9, Pureun; 10, Hannam; 11, Geomjeong 1; 12, Jinpum 2; 13, Suwon 157; 14, Shinpaldal 2; 15, SS 2. The isoflavone codes are: DIN, daidzin; GLY, glycitin; GIN, genistin; MDIN, malonyldaidzin; MGLY, malonylglycitin; MGIN, malonylgenistin; DEIN, daidzein; GLIEN, glycitein; GEIN, genistein. TDZ = DIN + MDIN + DEIN, TGE = GIN + MGIN + GEIN, TGLIEN = GLY + MGLY + GLIEN, and TISO = TDZ + TGE + TGLIEN.

are presented in Table 5, which also includes the mean, maximum, and minimum values for each isoflavone. Information contained in this table is better appreciated when presented in an environment-by-trait bi-plot (Fig. 4). Fig. 4 displays 82% of the total variation of the trait-standardized data in Table 4.

From Fig. 4, it can be seen that TDZ (DIN+ MDIN + DEIN), TGE (GIN + MGIN + GEIN), TISO (TDZ + TGE + TGLIEN), malonyldaidzin (MDIN), malonylglycitin (MGLY), malonylgenistin (MGIN), daidzein (DEIN), glycitein (GLIEN), and glycitin (GLY) form a large, closely associated group of isoflavones. Within this group, the total isoflavones, TISO (TDZ + TGE + TGLIEN), is most closely correlated with TGE (GIN + MGIN + GEIN), malonyldaidzin (MDIN) and TDZ (DIN + MDIN + DEIN) and to a lesser extent, with malonylglycitin (MGLY), malonylgenistin (MGIN), daidzein (DEIN), glycitein (GLIEN), and glycitin (GLY). Genistein (GEIN) and glycitein (GLIEN) are highly positively correlated, highly negatively correlated with daidzin (DIN) and genistin (GIN), but independent of isoflavones such as glycitin (GLY). They are also slightly negatively associated with total isoflavones.

All isoflavones, except genistein and glycitein, were higher in 1999 at all three sites, particularly at Kyong-

Table 5
Environmental values of various isoflavones across genotypes[a]

Environments	DIN	GLY	GIN	MDIN	MGLY	MGIN	DEIN	GLIEN	GEIN	TDZ	TGE	TGLIEN	TISO
00Kyongsan	371.4	131.4	468.7	1396.3	178.1	1442.3	75.9	44.6	49.9	1843.6	1960.9	354.1	4158.6
00Seoul	280.6	118.2	339.5	1519.8	228.1	1805.0	30.9	18.3	20.0	1831.3	2164.5	364.5	4360.3
00Suwon	255.9	128.0	333.8	1524.7	248.0	1729.6	28.2	3.4	16.5	1808.9	2079.9	379.4	4268.2
98Kyongsan	532.6	52.8	579.3	1528.4	131.6	1442.4	21.9	13.0	11.0	2082.8	2032.6	197.4	4312.8
98Seoul	425.3	43.4	601.9	1297.6	127.5	1283.8	21.0	12.6	10.6	1743.8	1896.4	183.5	3823.7
98Suwon	432.3	44.5	458.5	1137.4	129.6	1164.3	21.5	14.7	11.1	1591.2	1633.9	188.7	3413.8
99Kyongsan	504.4	330.2	990.0	1901.0	532.8	1712.5	127.9	8.3	4.6	2533.3	2707.2	871.3	6111.8
99Seoul	539.5	123.0	958.7	1617.3	259.7	1635.4	72.8	15.7	3.6	2229.7	2597.7	398.3	5225.6
99Suwon	553.1	103.1	809.7	1653.3	275.7	1788.4	65.0	9.3	3.5	2271.4	2601.7	388.1	5261.2
Mean	432.8	119.4	615.6	1508.4	234.6	1556.0	51.7	15.5	14.5	1992.9	2186.1	369.5	4548.4
Maximum	553.1	330.2	990.0	1901.0	532.8	1805.0	127.9	44.6	49.9	2533.3	2707.2	871.3	6111.8
Minimum	255.9	43.4	333.8	1137.4	127.5	1164.3	21.0	3.4	3.5	1591.2	1633.9	183.5	3413.8

[a] DIN, daidzin; GLY, glycitin; GIN, genistin; MDIN, malonyldaidzin; MGLY, malonylglycitin; MGIN, malonylgenistin; DEIN, daidzein; GLIEN, glycitein; GEIN, genistein. TDZ = DIN + MDIN + DEIN, TGE = GIN + MGIN + GEIN, TGLIEN = GLY + MGLY + GLIEN and TISO = TDZ + TGE + TGLIEN.

san (Fig. 4), which is in sharp contrast with the 1998 season. Genistein and glycitein were the highest, while daidzin and genistin were the lowest in 1999, particularly at Kyongsan. The yearly difference in isoflavone content seems to be associated with the temperature during seed development; the average September temperature during these 3 years was 12.7 °C (1998), 18.0 °C (1999) and 13.9 °C (2000). This result is similar to the study by Tsukamoto et al. (1995), who reported that seed maturing at low temperatures had greater isoflavone concentrations than seed maturing at high temperatures.

3.2.3. *Mean and stability of the genotypes in terms of total isoflavones*

In order to evaluate the genotypes in terms of isoflavone content, GGE bi-plots were generated and examined for each of the 13 variables. However, only the GGE bi-plot for total isoflavone is presented here (Fig. 5). It is the ''average environment coordinate'' view of the bi-plot, which is suitable for simultaneous visualization of both mean performance and stability of the genotypes (Yan, 2001, 2002; Yan and Kang, 2002). Fig. 5 indicates that genotypes 'Geomjeong 1' (11) and 'Jangyeob' (6) had the highest average total isoflavone content, and genotypes 'Hwaeomput' (8), 'Taekwang' (1), and 'Suwon 157' (13) also had above-average total isoflavone content. Genotypes 'Shinpaldal 2', 'Myeongjunamul', and 'Daweon' had the lowest total isoflavone values. Fig. 5 also displays the stability of the genotypes in their total isoflavone content across all environments. For example, although genotypes 'Geomjeong 1' and 'Jangyeob' were similar in mean total isoflavone content, they differed in stability, with 'Geomjeong 1' being more stable than 'Jangyeob'. Compared with 'Geomjeong 1', genotype 'Jangyeob' had higher total isoflavone content in 99Kyongsan, but lower in 99Seoul. The genotypes 'Geomjeong 1' and 'Jangyeob' had the highest total isoflavone content, as can be seen in Fig. 3, the figure that was used to examine which genotype had the highest values for each isoflavone.

Also obvious from Fig. 5 is the difference among the 3 years in discriminating the genotypes. Little difference among genotypes was observed in 2000, as all three sites are located near the bi-plot origin. In contrast, huge genotypic differences in isoflavone content existed in 1999, as evidenced by the long vectors of 99Kyongsan, 99Seoul and 99Suwon. Moreover, there were large genotype-by-site interactions in 1999, as evidenced by the wide angle between 99Kyongsan and 99Seoul (Fig. 5). These interactions are responsible for the large genotype-by-year-by-site interactions identified in the analysis of variance (Table 3). There was little genotype-by-site interaction for total isoflavones in 1998, as all three sites are closely located (Fig. 5). Since genotype-by-year-by-site interaction is

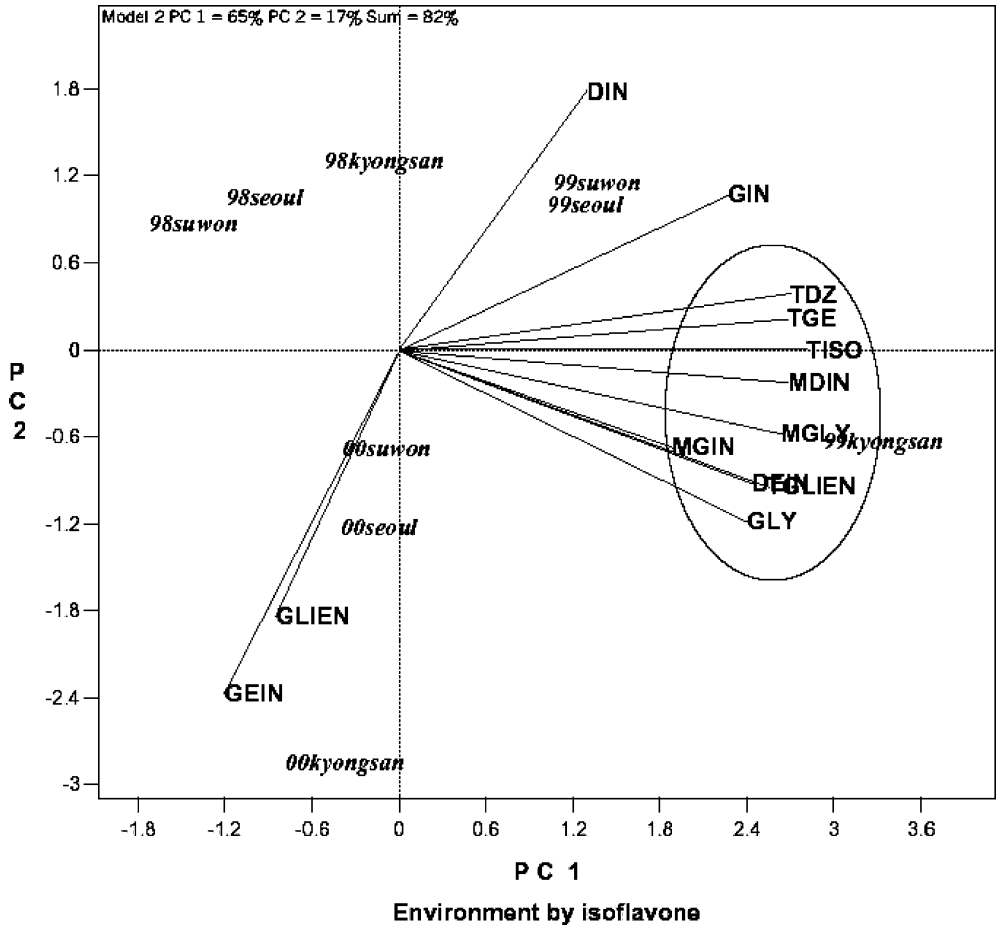

Fig. 4. An environment-by-isoflavone type bi-plot showing the environmental relations between different isoflavones. The isoflavone codes are: DIN, daidzin; GLY, glycitin; GIN, genistin; MDIN, malonyldaidzin; MGLY, malonylglycitin; MGIN, malonylgenistin; DEIN, daidzein; GLIEN, glycitein; GEIN, genistein. TDZ = DIN + MDIN + DEIN, TGE = GIN + MGIN + GEIN, TGLIEN = GLY + MGLY + GLIEN, and TISO = TDZ + TGE + TGLIEN.

the most important source of isoflavone variation, reliable cultivar evaluation for isoflavones can only be achieved through testing at multiple sites and in multiple years. Cultivar evaluation would be much easier if the causes for the genotype-by-year-by-site interaction were known.

It is well known that the isoflavone content in soybean seed is a key factor in its value as a healthy food. This study leads to the following conclusions. First, the main environmental effects (year effect and year-by-site interaction) and genotype-by-environment interactions (genotype-by-year and genotype-by-year-by-site interactions) were the most important sources of variation for the various isoflavones. Significant differences among the genotypes in isoflavone content exist, which can be reliably detected only through multi-site and multi-year tests. Genotype 'Geomjeong 1' had consistently higher total isoflavone content; genotype 'Jangyeob' also had high total isoflavone content, but was less stable than 'Geomjeong 1'. Secondly, no major genetically determined negative association was detected among the various isoflavones, except for that between glycitin and malonylgenistin. On the contrary, a highly negative environmental association was observed between genistein (along with glycitein) and daidzin (along with genistin). Both

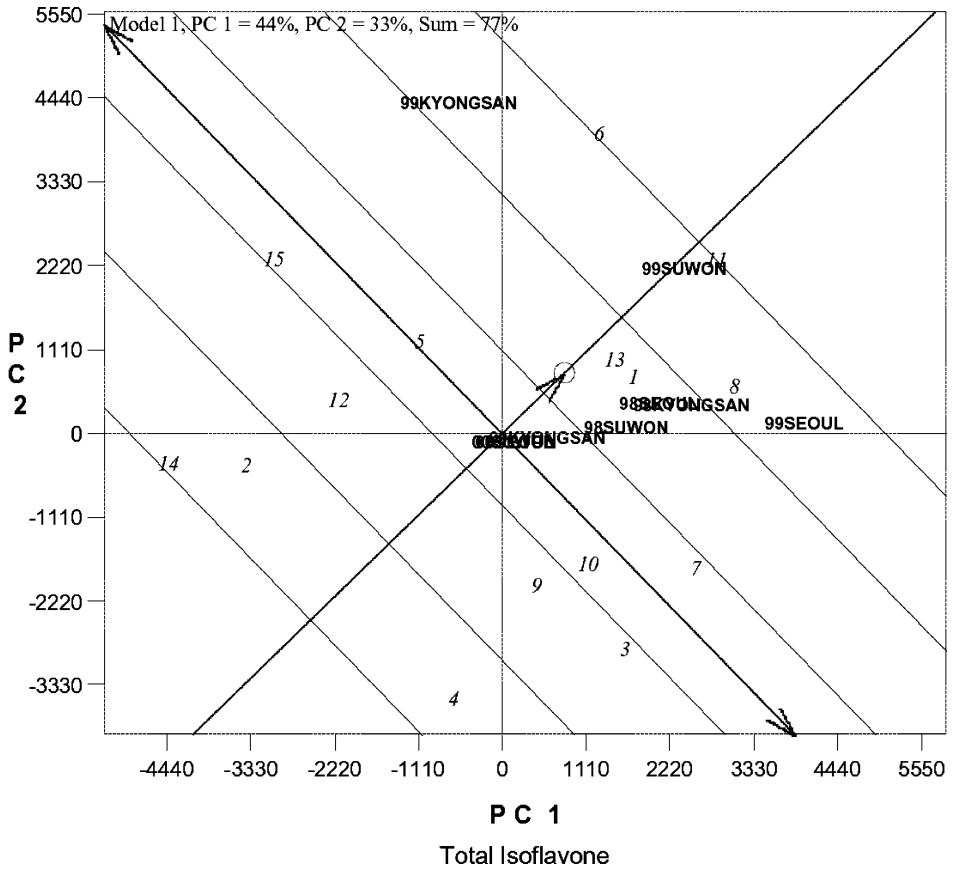

Fig. 5. A GGE bi-plot showing the mean and stability of the genotypes in terms of total isoflavone content and specific genotype-by-environment interactions. Each environment is a year–site combination, e.g. 00Seoul refers to Seoul in 2000. Each environment is a year–site combination. The genotype codes are: 1, Taekwang; 2, Myeongjunamul; 3, Danbaek; 4, Daweon; 5, Muhan; 6, Jangyeob; 7, Hwangkeum; 8, Hwaeomput; 9, Pureun; 10, Hannam; 11, Geomjeong 1; 12, Jinpum 2; 13, Suwon 157; 14, Shinpaldal 2; 15, SS 2.

genetically and environmentally, total isoflavone was most closely associated with malonyldaidzin.

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References

Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shiuya, M., Fudami, Y., 1987. Genistein a specific inhibitor of tyrosine protein kinases. J. Biol. Chem. 262, 5592–5595.

Anderson, J.W., Johnstone, B.M., Cook-Newell, M.E., 1995. Meta-analysis of the effects of soy protein intake on serum lipids. New Engl. J. Med. 333, 276–282.

Anthony, M.S., Clarkson, T.B., Hughes, C.L., Morgan, T.M., Burke, G.L., 1996. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J. Nutr. 126, 43–50.

Barnes, S., Kim, H., Peterson, T.G., Xu, J., 1998. Isoflavones and cancer—the estrogen paradox. J. Kor. Soybean Dig. 15, 81–93.

Choi, J.S., Kwon, T.W., Kim, J.S., 1996. Isoflavone contents in some varieties of soybean. J. Kor. Food Biotechnol. 5, 167–169.

Chung, I.M., Kim, K.H., Ahn, J.K., Chi, H.Y., Lee, J.O., 2000. Screening for antioxidative activity in soybean local cultivars in Korea. Kor. J. Crop Sci. 45, 328–334.

Coward, L., Barnes, N.C., Setchell, K.R., Barnes, S., 1993. Genistein, daidzein, and their β-glycoside conjugates: anti-tumor isoflavones in soybean foods from American and Asian diets. J. Agric. Food Chem. 41, 1961–1967.

Hoeck, J.A., Fehr, W.R., Murphy, P.A., Welke, G.A., 2000. Influence of genotype and environment on isoflavone contents of soybean. Crop Sci. 40, 48–51.

Holt, S., 1997. Soya: the health food of the next millennium. J. Kor. Soybean Dig. 14, 77–90.

Kudou, S., Fleury, Y., Welti, D., Magnolato, D., Uchida, T., Kiamura, K., Okubo, K., 1991. Malonyl isoflavone glycosides in soybean seeds (*Glycine max* Merrill). Agric. Biol. Chem. 55, 2227–2233.

Kyoko, S., 1998. Soybean food industry for the 21st century. J. Kor. Soybean Dig. 15, 94–104.

Messina, M., Persky, V., Setchell, K., Barnes, S., 1994. Soy intake and cancer risk: a review of the in vitro and in vivo data. Nutr. Cancer 21, 113–131.

Murphy, P.A., Song, T.T., Buseman, G., Barua, K., Beecher, G.R., Trainer, D., Holden, J., 1999. Isoflavones in retail and institutional soy foods. J. Agric. Food Chem. 47, 2697–2704.

Naim, M., Benjamin, G., Aron, B., Yehudith, B., 1976. Antioxidative and antihemolytic activities of soybean isoflavones. J. Agric. Food Chem. 24, 1174–1177.

Peterson, G., Barnes, S., 1991. Genistein inhibition of the growth of human breast cancer cell: independence from estrogen receptors and the multi-drug resistance gene. Biochem. Biophys. Res. Commun. 179, 661–667.

SAS Institute, 1996. SAS User's Guide Basics, 5th ed. SAS Institute, Cary, NC.

Schultz, W.B., 1998. Food labelling: health claims: soy protein and coronary heart disease. Fed. Reg. 63, 62977–63015.

Song, T.T., Barua, K., Buseman, G., Murphy, P.A., 1998. Soy isoflavone analysis: quality control and a new internal standard 1–3. Am. J. Clin. Nutr. 68, 1474S–1479S.

Song, T.T., Hendrich, S., Murphy, P.A., 1999. Estrogenic activity of glycitein, a soy isoflavone. J. Agric. Food Chem. 47, 1607–1610.

Tsukamoto, C., Shinji, S., Kazunori, I., Shigemitsu, K., Makie, K.M., Kazuyoshi, O., Keisuke, K., 1995. 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. 43, 1184–1192.

Wang, H.J., Murphy, P.A., 1994a. Isoflavone content in commercial soybean foods. J. Agric. Food Chem. 42, 1666–1673.

Wang, H.J., Murphy, P.A., 1994b. Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year, and location. J. Agric. Food Chem. 42, 1674–1677.

Xu, X., Wang, H.J., Murphy, P.A., 1994. Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. J. Nutr. 124, 825–832.

Yan, W., 2001. GGE biplot—a Windows application for graphical analysis of multi-environment trial data and other types of two-way data. Agron. J. 93, 1111–1118.

Yan, W., 2002. Singular value partitioning for biplot analysis of multi-environment trial data. Agron. J. 94, 990–996.

Yan, W., Kang, M.S., 2002. GGE Biplot Analysis: A Graphical Tool for Breeders, Geneticists, and Agronomists. CRC Press, Boca Raton, FL.

Yan, W., Rajcan, I., 2002. Biplot evaluation of test sites and trait relations of soybean in Ontario. Crop Sci. 42, 11–20.

Yan, W., Hunt, L.A., Sheng, Q., Szlavnics, Z., 2000. Cultivar evaluation and mega-environment investigation based on GGE biplot. Crop Sci. 40, 597–605.