Selection of Useful Germplasm Based on the Variation Analysis of Growth and Seed Quality of Soybean Germplasms Grown at Two Different Latitudes

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ABSTRACT We conducted this study to select useful germplasms for investigating the agricultural performances and content variations of proximate composition, fatty acid, and isoflavone of the soybean germplasms grown at two different latitudes, Suwon (37°16’N) and Yeoncheon (38°12’N), Republic of Korea. The days to flowering of the soybeans grown at Suwon was early by 1 day compared with that at Yeoncheon whereas the days to maturing of the soybeans were not different by latitudes. The Yeoncheon-cultivated soybeans were greater in growth and yield than the Suwon. The crude oil in range of 17.0% to 20.0% had a positive correlation with the yield. There was a strong negative correlation between oleic acid and saturated fatty (palmitic and stearic) acid contents in the seeds produced at Suwon but the Yeoncheon were not significant. The linoleic acid in seeds of the germplasms was contained by 7.3-fold more than the linolenic acid. The malonylg enistin content was the highest among the detected isoflavones and Deapung had the highest total isoflavone content. The content of total isoflavones in the Yeoncheon-producted seeds was high more than that in the Suwon whereas those of Hei nong 44(hou), Tachikogane, and B152 grown at Suwon were significantly lower rather than those at Yeoncheon. However, the difference of latitude seemed to play an important role in the content variations of growth, yield, and fatty acids and isoflavones in the germplasms. Eventually, Tie dou 44 and WIR3722, which were stably outstanding in all the investigated aspects regardless of the growing locations, were selected as useful germplasms.

Keywords Soybean, Germplasm, Variation, Fatty acid, Isoflavone

INTRODUCTION

As economic margin is growing up due to economic growth and increase of the income, interest and demand of health supplement food and diet for healthy longevity are increasing. Accordingly, scale of a domestic market for health supplement food has approached up to a net sales of 1,800 billions won in 2012 (MFDS 2013) and studies for cultivation, production, and improvement of high functional crop also are consistently growing up (Kim 2013).

There are many reports that ingestion of soyfood prevents senescence, cancer, and cardiovascular disease including arteriosclerosis because soybean plentifully contains diverse functional components such as isoflavone and saponin (Hawrylewicz et al. 1995; Klein et al. 1995; Messina 1995; Sirtori et al. 1995). Since legume protein is used as a main component for numerous soy products, analysis of protein in soybean seed is an essential procedure for its quality evaluation. Furthermore, legume protein, which is contained by 40% in soybean seed like carbohydrate, is most of vegetable protein source with the highest quality (Kim 2002). Because soybean seed that contains oil of 20% following protein and carbohydrate produces vegetable oil in scodd following parm, it is a very
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Important crop in economic perspective (USDA 2016). Soybean oil supplies diverse fat soluble vitamins as a nutritional composition and calorie source (Liu 1999). However, soybean oil contains five fatty acids, palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic acid (18:3), which entirely affect to use and physicochemical property of soybean oil depending on its content and composition (Fehr 2007). Fatty acid of soybean is composed of saturated (palmitic acid, stearic acid) and unsaturated (oleic acid, linoleic acid, linolenic acid) fatty acids, in contents of about 16% and 84%, respectively (Lee et al. 2007). Although linolenic acid (ω-3), one of the essential fatty acids, is slightly contained within about 8.0% of total fatty acids in soybean seed compared with linoleic acid (ω-6), it is known that deficiency of ω-3 results in abortion, and decreased visual acuity and learning ability (Gebauer et al. 2006). Isoflavone that is closely related to phytoestrogens-plant-derived compounds with estrogenic activity have grown more popular as a dietary supplement. Legume, particularly soybean, is the most common source of isoflavonoids in human food. Moreover, it was well known that isoflavone-rich diets are associated with antitumor effect (Coward et al. 1993), improving effect in cognitive function (Zhao and Brinton 2007), effect on menopausal symptoms (Jou et al. 2008), and bone-sparing effects (Ishimi 2009). In soybeans, isoflavones are present as aglycones and glycosides: soy isoflavone glycosides are 3 glycosides (daidzin, genistin, glycitin), 3 malonylated glycosides (6"-O-Malonyl daidzin, 6"-O-Malonyl genistin, 6"-O-Malonyl glycitin), and 3 acetylated glycosides (6"-O-Acetyl daidzin, 6"-O-Acetyl genistin, 6"-O-Acetyl glycitin) while its aglycones are called daidzein, genistein, and glycitein, respectively (Kudou et al. 1991; Zhu et al. 2005).

However, soybean is a very outstanding material crop for nutritional health food and soybean consumption is increasing as of 2011 whereas the gross production of domestic soybeans as of 2005 (183,000 ton) was consistently decreasing during the 10 years to 2015 (103,000 ton) (KOSTAT 2016). Accordingly, breeding objectives to develop soybean variety for resolving the imbalance of production and demand of soybeans are diversified with not only high yielding ability but also usability such as high functionality, and tolerance to diseases, insect pests, and stresses (Kim et al. 2006). It was reported that in spite of the same soybean variety, variations of flowering time (Kumar et al. 2008), and oil and fatty acid (Shin et al. 2009; Carrera et al. 2011), crude protein (Kumar et al. 2006), and isoflavone (Tsukamoto et al. 1995; Carrera et al. 2011) contents obviously occur by cultivated environmental conditions. Therefore, one of breeding strategies to achieve the goal to develop soybean varieties with high quality is the selection and application of useful soybean germplasms, which are excellent in yielding ability and principal component contents, and which are remarkably stable in agricultural traits and biochemical compositions affected by environmental factors.

For this reason, we carried out this study to select useful germplasms via investigating agricultural characteristics and analysis of principal component contents of the germplasms that were collected in world wide including China, a major producer of soybean, and then to obtain basic informations from the useful germplasms for breeding of superior soybeans with high quality.

MATERIALS AND METHODS

Crop materials and cultivation methods

The experimental soybean materials for this study are a total of 153 germplasm accessions and 3 Korean domestic varieties as check varieties, which were collected in world wide, China (92), North Korea (23), Japan (14), Russia (11), Canada (3), Czech (3), France (3), Netherlands (1), Germany (1), Hungary (1), Kyrgyzstan (1), and which were distributed from the National Agrobiodiversity Center, Rural Development Administration (RDA), Republic of Korea. After sowing in plant pots on June 4th, 2015, the germinated seedlings were transplanted at 70×15 cm spacing to the soybean plots in two different experimental test fields, Suwon (37°16'N) and Yeoncheon (38°12'N), difference in distance of about 1° latitude, at growth stage V1, June 15th, 2015. The soybean plots were treated with a basic granular fertilizer at N-40, P2O5-70, K2O-60 (kg/ha) before transplanting and were managed by
the soybean standard cultural practices of National Institute of Crop Science after transplanting. Agronomic yield-related and quality characteristics were measured according to the agricultural science technology standards for investigation of research of RDA, Republic of Korea (RDA 2012).

**Analysis of principal composition contents**

The harvested soybean seeds were ground into powder under size of 1.0 mm in an ultraspeed-centrifuge mill (ZM 100, Retsch, Haan, Germany) and then used for analyzing principal composition contents.

Proximate compositions were analyzed according to the recommendation of Association of Official Analytical Chemists (AOAC). The drying method by heating (105°C) under normal atmospheric pressure and the direct ashing incineration method by heating (600°C) were used for measuring contents of moisture and crude ash, respectively (AOAC 1990). Crude oil was extracted by diethyl ether using Soxhlet extractor (Soxtect System HT 1043 extraction unit, Foss Tecator, Höganäs, Sweden) and then the extract was weighed for measuring the content (Kim et al. 2007). Content of crude protein was measured according to semimicro-Kjeldahl method using Auto Sampler System (Kjeltec 2400 AUT, Foss Tecator) (Kim et al. 2007). After calculating the contents of the ahead mentioned components, carbohydrate content was calculated by the following arithmetic operation: 100 − (contents of moisture+crude protein+crude oil+crude ash) (AOAC 1990).

We employed and modified the method of Garcés and Mancha (1993) to extract fatty acids from soybean seed. The ground seeds of 0.3 g were mixed with a reagent containing methanol:heptane:benzene:2,2-dimethoxypropane:H₂SO₄=37:36:20:5:2 (v/v) then heated at 80°C to be able to simultaneously occur digestion and lipid trans-methylation reactions in a single phase. After heating, the hot plate was turned off for cooling down the sample. The cooled upper phase containing the fatty acid methyl esters (FAMEs) was injected into a GC-MS system Agilent 6890 GC & 5973 MS (HP Co., Wilmington, DE, USA) equipped with a HP-Innowax capillary column (30 m×0.25 mm×0.25 μm, cross-linked polyethylene glycol, HP Co.). We used a standard linear regression model prepared with FAME Mix (C14-C22, Supelco, Bellefonte, PA, USA) to quantify amount of each fatty acid. Separation was started from 1 μl injection in pulsed splitless mode (2 minutes initial at 150°C, ramped at 4°C minutes⁻¹ to 280°C) with nitrogen as a carrier gas (constant flow, 1.0 ml min⁻¹).

Isoflavones were analyzed using a modification of the method of Kudou et al. (1991). The milled sample of 1.0 g was added with 20 ml of 50% methanol then shaken with 200 rpm for 24 hours at room temperature. After filtering the shaken sample using 0.20 μm syringe filter, 20 μl of the filtered extract was used directly to analyze contents of isoflavones using HPLC (Agilent 1100 series, Agilent Technologies, Inc., Santa Clara, CA, USA). Isoflavones were separated on a Lichropher 100 RP-18e column (125 mm×4.0 mm×5 μm; Merck Millipore, Kenilworth, NJ, USA) with the eluent A (0.1% acetic acid in HPLC-grade distilled water) and B (0.1% acetic acid in acetonitrile) gradient elution, which concentration of the eluent A reached to 10%, 20%, 25%, 35%, and 50% at 0, 20, 30, 40, and 50 minutes, respectively, with a flow rate of 1.0 ml/min and detected with 1100 DAD diode array detectors (Agilent 1100 series, Agilent Technologies, Inc.). We used 12 standard isoflavones (daidzein, genistein, glycitein, daidzin, genistin, glycitin, acetyldaidzin, acetylgenistin, acetylglycitin, malonyldaidzin, malonylgenistin, and malonylglycitin) produced by LC laboratories (Woburn, MA, USA) and each standard isoflavone was diluted with 2.5, 5, 10, 50, 100 g/ml to calculate regression curves for measuring content of each isoflavone. We confirmed each isoflavone in soybean seed compared with the retention time of each standard isoflavone.

**Statistical analysis**

ANOVA was conducted with SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Multiple comparisons between samples were performed by the least significance difference method based on three independent biological each (n=3).
RESULTS

Weather conditions

The accumulated temperatures of Suwon and Yeoncheon regions during the whole period of growth of the germplasms after transplanting, from June 15th to October 5th, 2015, were 2,727.4°C and 2,457.4°C, respectively, which were higher by 13.2°C and 96.6°C, respectively, than those of the normal years (1981-2010) (Table 1). The respective amounts of precipitation of Suwon and Yeoncheon regions declined to 38.7% and 71.8% relative to that of the normal years (Table 1). The sunshine duration of Yeoncheon region at the normal years was greater by 26.5 hours than that of Suwon and those of Yeoncheon and Suwon regions increased by 292.6 and 170.4 hours in 2015 more than in the normal years, respectively (Table 1).

Growth and yield characteristics

The days to flowering of the Suwon- and Yeoncheon-grown germplasms were longer by about 15 and 16 days, respectively, compared with those of the Suwon- and Yeoncheon-grown domestic varieties, Daepung, Cheongja, and Pungsannamul (Table 2). While the soybeans grown at Suwon bloomed 1 day earlier than that at Yeoncheon, the days to maturing was not different between two regions (Table 2). The germplasms were smaller in stem length relative to the domestics and the Yeoncheon-cultivated germplasms was higher than the Suwon (Table 2). Yield per plant of the Yeoncheon-harvested germplasms was also greater by about 10.0 g than that of the Suwon but the

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Table 1. Weather conditions of Suwon (SW) and Yeoncheon (YC) from July 15th to October 5th, normal years (1981-2010) and 2015, growth duration of the soybeans grown at all two regions.

| Factor                        | Year                  | Region | Month   |       |       | Total  |
|-------------------------------|-----------------------|--------|---------|------|------|--------|
|                               |                       |        | June    | July | August | September | October |       |
| Accumulated temperature (°C)  | Normal years (1981-2010) | SW     | 360.7   | 769.7| 791.7  | 624.5     | 84.2    | 2,630.8 |
|                               |                       | YC     | 344.3   | 728.8| 738.5  | 560.4     | 72.2    | 2,444.2 |
|                               | 2015                  | SW     | 378.0   | 789.1| 812.5  | 662.6     | 85.2    | 2,727.4 |
|                               |                       | YC     | 352.7   | 736.7| 748.3  | 548.4     | 71.3    | 2,457.4 |
| Precipitation (mm)            | Normal years (1981-2010) | SW     | 94.5    | 350.6| 298.8  | 151.3     | 9.8     | 905.0   |
|                               |                       | YC     | 92.7    | 401.5| 341.2  | 141.9     | 8.7     | 986.0   |
|                               | 2015                  | SW     | 21.0    | 225.8| 71.0   | 6.9       | 25.3    | 350.0   |
|                               |                       | YC     | 56.7    | 456.8| 140.6  | 32.7      | 21.5    | 708.3   |
| Duration of sunshine (hours)  | Normal years (1981-2010) | SW     | 89.2    | 136.7| 166.5  | 181.6     | 32.1    | 606.1   |
|                               |                       | YC     | 84.2    | 128.2| 165.0  | 178.7     | 31.0    | 587.1   |
|                               | 2015                  | SW     | 124.8   | 165.8| 205.7  | 241.6     | 38.6    | 776.5   |
|                               |                       | YC     | 138.6   | 197.7| 246.5  | 256.6     | 40.3    | 879.7   |

Table 2. Analysis of agronomic traits of Korean cultivars and 153 soybean germplasm accessions grown at Suwon (SW) and Yeoncheon (YC).

| Variety                        | Days to flowering (days, DAS\(^a\)) | Days to maturing (days, DAS) | Stem length (cm) | Yield per plant (g) |
|-------------------------------|-------------------------------------|------------------------------|------------------|---------------------|
|                               | SW        | YC   | SW      | YC   | SW   | YC   | SW   | YC   |
| Avg. of Korean cultivars      | 52.0      | 54.7 | 112.0   | 113.3| 58.0 | 63.7 | 35.0 | 39.3 |
| Avg. of 153 germplasms        | 37.2      | 38.4 | 80.3    | 82.3 | 43.3 | 55.3 | 22.3 | 32.9 |
| Avg.                         | 37.5      | 38.7 | 81.0    | 82.9 | 43.6 | 55.5 | 22.6 | 32.1 |
| \(^a\)DAS: days after sowing (n=3), Avg.: average, ns: non significant (\(P \geq 0.05\)), LSD: least significant difference (at \(*P < 0.05\) significant).
domestics were greater in yield per plant over 6.0 g compared with the germplasms regardless of the growing locations (Table 2). However, because the average of yield per plant of the soybeans grown at Suwon was 22.6 g (Table 2), we preferentially selected the 23 germplasms among the 153 germplasms in company with 3 domestic varieties, which were produced over the average at all two regions (Table 3). Among the selected soybeans, yields per plant of Jilin 53, He 96-448, Tie dou 44, Ao mame, Tachikogane, Jijori, and WIR3722 were stably greater at all two regions relative to all the selected soybeans and/or the domestic varieties (Table 3). We used the 26 selected soybeans to more accurately select useful germplasms via analysis of principal components.

**Proximate analysis**

The moisture, crude ash, and carbohydrate contents and calorie in seeds of the selected soybeans were distributed differentially by cultivated regions. Number of the Yeoncheon-grown soybeans which had over 7.0 g of
moisture and 5.0 g of crude ash in 100 g seeds was smaller than that of the Suwon whereas no. of the Yeoncheon which contained over 34.0 g of carbohydrate and 440 kcal of calorie in 100 g seeds was bigger rather than that of the Suwon (Fig. 1). The crude protein and oil contents depending on regions showed a negative correlation (Fig. 2). The regional variations of crude protein and oil contents were not significant but the respective differences in contents of crude protein and oil by varieties were different by 7.0 and 6.0 g/100 g. When the contents of crude oil of the soybeans were in range of 17.0 g/100 g to 20.0 g/100 g, it showed a strong positive correlation (R=0.6825) between the yield per plant and the crude oil content (Fig. 3). The 6 germplasms grown at Suwon, including Qian wu ta, and the 8 germplasms grown at Yeoncheon, including Jin 200, were selected as useful germplasms, which were greater than the crude protein content in seeds of other investigated soybeans, and which the contents of crude oil were in range

Fig. 1. Distribution of moisture (A), crude ash (B), and carbohydrate (C) contents, and calorie (D) in seeds of the soybeans grown at Suwon (□) and Yeoncheon (■). Moisture (g/100 g, n=3): average=7.2 (□), 6.8 (■), P=0.0001, LSD=0.18; crude ash (g/100 g, n=3): average=5.2 (□), 4.9 (■), P=0.0000, least significant difference (LSD)=0.08; carbohydrate (g/100 g, n=3): average=34.0 (□), 34.4 (■), P=0.1844, LSD=non significant; calorie (kcal/100 g, n=3): average=438.6 (□), 442.5 (■), P=0.0007, LSD=2.04.

Fig. 2. Correlation between crude protein and crude oil contents in seeds of the soybeans grown at Suwon (□) and Yeoncheon (■). Crude protein (g/100 g, n=3): P=0.0000, LSD=0.08; crude oil (g/100 g, n=3): P=0.0000, least significant difference (LSD)=0.06, correlation coefficients between crude protein and crude oil contents: R (Suwon, □) = -0.7412, and R (Yeoncheon, ■)=-0.589.
Fatty acid analysis

The investigated soybeans contained a palmitic acid content of 11.4% to 14.5%, a stearic acid content of 2.4% to 6.8%, an oleic acid content of 18.5% to 36.4% (Fig. 4A, B). It showed a strong negative correlation between oleic acid and saturated fatty acid (palmitic and stearic acid) contents in seeds of the Suwon-investigated soybeans (Fig. 4A, B) but not in seeds of the Yeoncheon. However, the ω-6 (linoleic acid) was higher by a fold of 7.3 than ω-3, and the ratio of ω-6 to ω-3 (linoleic acid) in seeds of the Yeoncheon-investigated germplasms was lower rather than that of the Suwon (Fig. 4C). CS 02678 among the
germplasms grown at Yeoncheon had the lowest $\omega$-6/$\omega$-3 ratio, a fold of 5.9, and the ratio (6.3) of WIR3722 among the germplasms grown at Suwon was the lowest (Fig. 4C).

 Isoflavone analysis

Distributions of isoflavone aglycone contents in seeds of the investigated soybeans by cultivated regions were not different whereas the aglycones were detected in little or a little level of content (Fig. 5). Malonyldaidzin and malonylgenistin in seeds of the investigated soybeans were contained more by 3 and 6 folds relative to daidzin and genistin, respectively, but malonylglycitin and glycitin were similarly detected (Fig. 6A-C). However, malonylated isoflavone glycoside of genistein, 6"-O-malonylgenistin, was the highest in content (Fig. 6B). While analyzing the variation of content of isoflavones depending on regions, it was confirmed that the distribution of content of each glycoside in seeds of the Yeoncheon-cultivated germplasms was wide more than that of the Suwon but in case of the malonylated isoflavone glycosides were not different at all two regions (Fig. 6A-C). The ratio of glycoside contents in the Yeoncheon-harvested seeds to that in the Suwon-harvested seeds was relatively higher compared with other isoflavones, aglycones and malonylated isoflavone glycosides, and regional variations of contents of daidzin and genistin in the domestic varieties were greater relative to that in the collected germplasms except for glycitin (Table 4). Daepung had the highest total isoflavone content but Qian wu ta was the lowest regardless of region (Fig. 6D). The content of total isoflavones in seed of He feng 95-873 among the collected germplasms was the highest in line with the level of Daepung regardless of region (Fig. 6D). However, the content of total isoflavones in the Yeoncheon-investigated soybeans was generally higher relative to that in the Suwon (Fig. 6D). Hei nong 44 (hou) was the highest variety in variation of total isoflavone contents regardless of region, and the content of this variety grown at Suwon declined by 40% rather than that at Yeoncheon unlike other varieties (Table 4, Fig. 6D).

**DISCUSSION**

Although the genotypic and phenotypic characteristics of crops are primarily related to climate, the response of crops to the different weather variables is quite complex...
Fig. 6. Correlation between malonyldaidzin and daidzin contents (A), malonylgenistin and genistin contents (B), malonylglycitin and glycitin contents (C), and content of total isoflavones (D) in seeds of the soybeans grown at Suwon and Yeoncheon. Malonyldaidzin ($\mu$g/g, n=3): $P=0.0000$, least significant difference (LSD)=14.30; daidzin ($\mu$g/g, n=3): $P=0.0000$, LSD=2.67; malonylgenistin ($\mu$g/g, n=3): $P=0.0000$, LSD=4.89; malonylglycitin ($\mu$g/g, n=3): $P=0.0000$, LSD=7.42; correlation coefficients between malonyldaidzin and daidzin contents: R (Suwon, □)= 0.6697 ($P=0.0000$), and R (Yeoncheon, ●)=0.7983 ($P=0.0000$); correlation coefficients between malonylgenistin and genistin contents: R (Suwon, □)=0.5414 ($P=0.0000$), and R (Yeoncheon, ●)=0.8049 ($P=0.0000$); correlation coefficients between malonylglycitin and glycitin contents: R (Suwon, □)=0.7361 ($P=0.0000$), and R (Yeoncheon, ●)=0.8112 ($P=0.0000$); total isoflavone content ($\mu$g/g, n=3): $P=0.0000$, LSD=28.55.

DW: dry weight.

and difficult to describe. Nevertheless, there were reported that economical production (Ok et al. 2008), seed yield and yield components (Frederick et al. 2001; Kumar et al. 2008), and oil content (Dornbos and Mullen 1992) of soybean are highly related to weather conditions such as accumulated temperature, precipitation, and sunshine duration. During the growth duration of the soybeans at two different latitudes, Suwon and Yeoncheon, the accumulated temperature and sunshine duration in 2015 increased relative to those in the normal years (1981-2010) whereas the precipitation in 2015 severely declined rather than that of normal years. However, our results were similar to previous results that the soybeans grown at low latitude are earlier in flowering and maturing periods and are lower in biomass and yield than that at high latitude (Lima et al. 2000), that drought-stress affect to decrease in seed yield and yield components (Frederick et al. 2001), and that the increase of sunshine duration results in the increase of soybean yield (Kumar et al. 2008). For these results, although we assumed that weather conditions are likely to affect the agronomic characteristics and chemical compositions but could not indicate a critical major environmental factor on regional variation of yield and growth because numerous weather conditions, especially precipitation and sunshine duration, were complicatedly different depending on cultivation years and regions. Therefore, in the future study we need to accurately evaluate the effect on variation of soybean growth, yield, and component contents by each weather condition such as precipitation, accumulated temperature, sunshine duration, and so on.

Even though the crude ash and moisture in seeds of the investigated soybeans were contained in range of those in
Table 4. The isoflavone ratio between the Yeoncheon to the Suwon-grown germplasm seeds.

| Accession name | Daidzein | Genistein | Glycitein | Daidzin | Genistin | Glycitin | Malonyl daidzin | Malonyl genistin | Malonyl glycitin | Total |
|----------------|----------|-----------|-----------|---------|----------|----------|-----------------|-----------------|-----------------|-------|
| Daepung        | 0.8      | 1.2       | 1.1       | 2.1     | 4.6      | 1.3      | 1.1             | 0.9             | 1.2             | 1.3  |
| Cheongja       | -        | 0.8       | 0.9       | 1.6     | 2.9      | 1.1      | 0.9             | 0.8             | 0.8             | 0.9  |
| Pungamamuul    | 0.7      | 1.2       | 1.4       | 2.0     | 3.4      | 1.6      | 1.0             | 1.1             | 1.0             | 1.1  |
| Avg. of Korean cultivars | 0.8 | 1.1 | 1.1 | 1.9 | 3.6 | 1.3 | 1.0 | 0.9 | 1.0 | 1.1 |
| Qianwu ta      | -        | -         | 1.0       | 1.4     | 0.0      | 1.9      | 1.3             | 0.9             | 1.4             | 1.4  |
| Mudanjiang     | 1.0      | 1.5       | 1.2       | 2.1     | 4.4      | 1.6      | 1.3             | 1.0             | 1.3             | 1.5  |
| Hei nong 34    | 0.7      | 1.5       | 1.3       | 1.8     | 2.8      | 1.7      | 1.2             | 1.4             | 1.2             | 1.4  |
| Jin 200        | 0.9      | 0.9       | 0.8       | 1.5     | 2.2      | 2.0      | 0.8             | 1.5             | 1.0             | 1.1  |
| Jin nong 8     | -        | 1.2       | 1.1       | 1.8     | 2.0      | 1.4      | 1.2             | 0.6             | 1.0             | 1.1  |
| He 96-448      | 1.0      | 1.2       | 1.2       | 1.8     | 3.0      | 1.3      | 3.0             | 1.0             | 1.2             | 1.5  |
| Tie dou 44     | 0.6      | 1.3       | 1.1       | 1.0     | 1.3      | 1.1      | 1.4             | 0.9             | 1.0             | 1.1  |
| 06Y2           | 0.8      | 2.5       | 1.5       | 1.7     | 2.4      | 1.3      | 1.3             | 1.0             | 1.2             | 1.3  |
| CS 02678       | -        | 2.2       | 1.2       | 2.1     | 3.4      | 2.4      | 1.4             | 2.0             | 1.4             | 1.6  |
| Ha jiao 01-5314| -        | 1.7       | 0.9       | 1.6     | 4.2      | 1.0      | 1.3             | 0.8             | 1.6             | 1.5  |
| He feng 95-873 | -        | 1.6       | 1.0       | 1.8     | 2.0      | 1.4      | 1.1             | 1.0             | 1.0             | 1.2  |
| Hei nong 44(hou)| -        | 1.3       | 1.0       | 0.7     | 0.1      | 0.5      | 0.7             | 0.7             | 0.5             | 0.6  |
| Gong yin 94141 | -        | 1.7       | 1.4       | 1.6     | 3.6      | 1.5      | 1.4             | 1.6             | 1.1             | 1.3  |
| Jin 180        | 1.2      | 1.2       | 6.7       | 1.6     | 2.5      | 1.7      | 1.0             | 1.1             | 1.0             | 1.2  |
| Sui nong 10    | -        | 2.3       | 1.1       | 2.4     | 3.7      | 2.3      | 1.4             | 1.3             | 1.4             | 1.7  |
| Gidayunude     | 1.1      | 1.9       | 1.0       | 1.5     | 5.0      | 2.2      | 1.0             | 1.2             | 1.2             | 1.3  |
| Ao mame        | -        | 0.7       | 0.9       | 1.5     | 1.7      | 1.0      | 0.9             | 0.9             | 0.7             | 0.9  |
| Tachikogane    | -        | 1.1       | 1.0       | 1.3     | 2.4      | 1.4      | 0.7             | 0.6             | 0.8             | 0.9  |
| Nui 2 hu       | 0.9      | 2.0       | 1.2       | 1.9     | 6.2      | 1.6      | 1.3             | 1.1             | 1.4             | 1.5  |
| Ijori          | -        | -         | 1.2       | 1.8     | 5.7      | 0.9      | 1.3             | 1.2             | 1.3             | 1.4  |
| WIR3722        | 0.9      | 2.0       | 1.5       | 1.0     | 0.9      | 0.9      | 1.2             | 1.1             | 1.2             | 1.1  |
| B152           | 1.1      | -         | 0.7       | 1.4     | 2.4      | 1.4      | 0.7             | 0.9             | 0.6             | 0.7  |
| Avg. of germplasms | 0.9 | 1.4 | 1.1 | 1.6 | 2.8 | 1.5 | 1.2 | 1.1 | 1.1 | 1.2 |
| Avg.           | 0.9      | 1.3       | 1.1       | 1.6     | 2.9      | 1.5      | 1.2             | 1.1             | 1.1             | 1.2  |

Avg.: average.

seeds of the domestic and the imported soybean cultivars of previous study (Ryoo et al. 2004), the contents of the domestics and the germplasms were different by variety categories, the domestics and the germplasms, more than by regions. The carbohydrate content of the investigated soybeans was not significantly different by regions. Especially, our finding is coincident with the previous report that carbohydrate is highly contained by 35% in soybean seed (Medic et al. 2014) whereas the importance of carbohydrate in soybean seed is low in quality because the carbohydrate contains some impurities such as saccharide and cellulose (Kim 2002). However, since soy protein is used as a main ingredient of numerous soy products and a high quality plant protein source (Kim 2002), and soybean oil is used to supply diverse fat soluble vitamins and essential fatty acids for human being (Liu 1999), analysis of the components, soy protein and oil, play an important role in assessment of respective soybean varieties. Previous studies similarly reported in common with our results that it has a significant negative correlation between contents of crude protein and oil (Kumar et al. 2006; Kim et al. 2007), and that yield of soybean shows a positive correlation with crude oil while the oil is in content range of 16.1% to 20.0% (Peng et al. 2001) or 18.0% to 20.0% (Wang 2006). Moreover, result of Hong et al. (2010a) is identical with our result that the contents of crude protein and oil were significantly changed by varieties whereas the contents were not varied by regions. In combination with these results, we confirmed that the contents of proximate compositions in seeds of the
investigated germplasms were highly changed by varieties more than by environmental condition, different latitudes.

Because it was known that palmitic and stearic acids are related to cholesterol synthesis (Lee et al. 2007), and oleic acid is responsible for the hypotensive (blood pressure reducing) effects (Terés et al. 2008), one of soybean breeding goals is to decrease contents of palmitic and stearic acids and to increase content of oleic acid. However, using 517 germplasms, Choung (2006) reported that oleic acid content shows a strong negative correlation with palmitic acid and stearic acid contents, in line with our result caused by the Suwon-investigated germplasms but not by the Yeoncheon. Furthermore, Simopoulos (2002) reported that excessive amounts of ω-6 and a very high ω-6/ω-3 ratio stimulate the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increase of ω-3 levels facilitate suppressive effects. According to Simopoulos (2002), the ω-6/ω-3 ratios of the Suwon- and the Yeoncheon-investigated germplasms were upon a midway point between ratio of 5/1 (a beneficial effect on patients with asthma) and ratio of 10/1 (adverse consequences) but the ratio was widely varied by regions and varieties. Although the content variation of fatty acids in seeds of the domestic black soybean cultivars is strongly affected by genetic factor (Hong et al. 2010a), for our results, it could be assumed that the content of fatty acids in the investigated germplasms was changed by regions and varieties. This result implies that the cultivated environmental condition and genetic difference act as critical factors for content variation of fatty acid and for determining use and physicochemical property of soy fat and oil.

The contents of the extracted isoflavone aglycones in seeds of the investigated soybeans were unlikely to be identical with previous studies (Moon et al. 1996; Hong et al. 2012). Moreover, while the contents of malonylated isoflavone glycosides in the extract significantly decreased at 80°C, an increase in other isoflavone glycosides was reported (Kudou et al. 1991). The regional difference in distribution of each glycoside and each malonylated isoflavone glycoside contents indicated that the contents of isoflavones were affected along with regions and that each malonylated isoflavone glycoside was individually converted into each isoflavone glycoside by environmental factor. The regional variation of each isoflavone glycoside showed that the cultivated regional difference eventually lead to change of composition ratio of isoflavone glycosides in the germplasm seeds. In addition, for the significant difference in content of total isoflavones depending on varieties, it was assumed that the genetic variation also acts as a critical factor for determining the content of total isoflavones in the germplasm seeds.

According to the analysis of the components in our study, the variations of component contents in seeds of the germplasms were determined by genetic and/or environmental factor as critical factor(s). However, we confirmed that useful germplasms among the investigated germplasms can be selected depending on breeding objectives via analysis of agricultural traits and component contents. Ultimately, Tie dou 44 and WIR3722 among the investigated germplasms were outstanding germplasms, which were great and stable in yield and growth and which the content variations by environmental factors, such as latitudes and weather conditions, were lower relative to other soybean germplasms.

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