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ARTICLE

Nutritional composition analysis for beta-carotene-enhanced transgenic soybeans (*Glycine max* L.)

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Abstract Nutritional composition is important for assessing the safety of genetically modified (GM) crops for human consumption. Three beta-carotene-enhanced soybean lines were developed by introducing the *β*-conglycinin promoter::*Phytoene synthase-2A-Carotene desaturase*/t35S gene cassette into the genome of the commercial Kwangan (*Glycine max* L.) soybean variety. Transgenic soybeans were successfully detected on beta-carotene productions ranged from 170.47 to 213.58 μg/g. Comparative assessments of nutrition were conducted with 3 transgenic soybeans, their non-GM counterpart, and several commercial soybean varieties. Results indicated that most levels of proximate, fatty acids, amino acids, and vitamins showed non-significant differences between transgenic soybeans and their counterpart, and fit within the reference ranges established for other commercial soybeans and Organization for Economic Cooperation and Development Guidelines. However, significant differences on levels of crude fat, carbohydrate, *δ*-tocopherol, and oleic acid of transgenic soybeans comparing to those of non-transgenic counterpart Kwangan cannot eliminate the influences of transgene insertion. Alternations on compositions should be definite by further studies, such as transcriptome and metabolome profiling.

Keywords Beta-carotene enhanced · Genetically modified · Nutritional composition · Soybean (*Glycine max* L.) · Substantial equivalence

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Introduction

Carotenoids such as beta-carotene, beta-cryptoxanthin, and alpha-carotene are widely known as dietary precursors of vitamin A [1]. Many studies in metabolite genetic engineering have been conducted by introducing the *phytoene synthase* (*Psy*) or *carotene desaturase* (*CrtI*) gene, or bicistronic expression of both genes in various crops, such as canola, tomatoes, maize, and soybeans [2–7], with the aim of overcoming vitamin A deficiency in infants and pregnant women in Africa and Southeast Asia via food intake [8]. Recently, three biotechnology-derived soybean products including "7-1-1," "9-1-2," and "10-19-1" were previously designed to enhance beta-carotene production in Korea [3, 9]. These genetically modified (GM) soybean lines contain a *bar* gene (isolated from *Streptomyces hygroscopicus*) conferring glufosinate-ammonium tolerance and express phosphinothricin-N-acetyltransferase for herbicide resistance, as well as a bicistronic expression system encoding *Psy* from *Capsicum* and *CrtI* from *Pantoea,* which conducts carotenoid biosynthesis.

For the development of GM crops, it is important to demonstrate that a food derived from GM crops is not only safe, but also has the same nutritional values or characteristics as their conventional comparators [10]. Kim et al. [11] conducted a substantial equivalence study for comparing nutritional values with carotenoid-biofortified transgenic rice, developed by using a bicistronic expression cassette containing *Psy* and *CrtI* [12], and the results revealed that carotenoid-biofortified transgenic rice was substantially equivalent to the non-transgenic counterpart rice variety (Nakdong) by profiling 52 polar metabolites.

However, soybeans (*Glycine max* L.) are different from rice because they have a native carotenoid-biosynthesis pathway, which begins with the condensation of 2

geranylgeranyl diphosphate molecules. This reaction yields phytoene and is catalyzed by phytoene synthase. Subsequently, lycopene is formed by catalysis with 4 enzymes including phytoene desaturase, zeta-carotene isomerase, zeta-carotene desaturase, and prolycopene isomerase, after which enzymatic steps are performed by lycopene beta-cyclase to produce alpha-carotene and beta-carotene [5, 6]. Introduction of *Psy* and *CrtI* into the soybean genome might enhance the levels of useful carotenoid metabolites. However, as a precursor for tocopherols and chlorophyll, or a substrate for both phytoene synthase and gibberellin pathway, the level of geranylgeranyl diphosphate would be alternated, further causing redistributions of secondary metabolites involved in the isoprenoid-biosynthesis pathway and abscisic acid-biosynthesis pathway [6].

In transgenic tomato, canola, *Arabidopsis*, and soybean studies, overproduction of beta-carotene caused changes in plant phenotypes, fatty acid compositions, and protein contents [5, 6, 13–15]. Transgenic tomatoes developed by introducing the plant phytoene synthase gene exhibited dwarfism, which was attributed to reduced gibberellin levels [13]. Transgenic canola [6] with a bacterial phytoene synthase (*crtB*) gene showed decreased levels of tocopherol and chlorophyll, but increased levels of oleic acid (18:1). Lindgren et al. [14] reported that transgenic *Arabidopsis* with phytoene synthase gene insertion showed increased levels of chlorophyll and abscisic acid, which led to delayed seed germination and seed dormancy. Schmidt et al. [5] showed that transgenic soya bean seeds with a bacterial phytoene synthase gene insertion had both elevated oleic acid (18:1) and protein contents and reduced levels of the phytohormone abscisic acid. Three transgenic soybean lines used in this study were previously reported to show accumulation of beta-cryptoxanthin, violaxanthin, and abscisic acid [3, 9]. Therefore, studying the nutritional composition of a beta-carotene-enhanced transgenic soybean is necessary to determine the food safety of transgenic crops and can help understand the effect of foreign gene insertion on native carotenoid biosynthesis. Ensuring the nutritional quality and equivalence of GM soybeans is very important for humans and livestock.

Based on internationally accepted guidelines proposed by the Organisation of Economic Cooperation and Development (OECD), essential macro- and micronutrients, known toxicants and antinutrients, selected secondary metabolites, and measurements of proximates in harvested seeds should be conducted to assess for the emergence of new soybean varieties [16]. Thus, we analyzed key nutrient contents in the seeds of 3 beta-carotene-enhanced transgenic soybean lines, their conventional counterparts, and 3 Korean commercial soybean varieties grown in the same field. Comparative analysis, statistical analysis, and principal component analysis (PCA) were performed to explore nutritional differences between the transgenic lines and their counterpart variety.

Materials and methods

Plant materials and sample preparation

Beta-carotene-enhanced soybeans were developed by performing *Agrobacterium tumefaciens*-mediated transformation using the conventional Kwangan soybean variety (*Glycine max.* L.). After seed reproduction and molecular characterization, 3 transgenic soybean lines including 7-1-1, 9-1-2, and 10-19-1 with single-copy gene insertions and agronomic traits similar to the counterpart variety were selected for further studies [3, 9, 15]. Field studies were conducted in 2015 at the Genetically Modified Organism experimental field of the National Academy of Agricultural Science in the Jeonju region of Korea to produce soybean seed samples from the 3 transgenic soybean lines, the isogenic non-transgenic counterpart Kwangan variety, and 3 non-transgenic commercial soybean varieties (*Glycine max.* L. cultivars "Poongsan," "Poongwon," and "Haepum") for comparative analysis. The experimental design was a randomized complete block with 4 replicated plots of each entry, and the test plots were managed according to local standard cropping practices, such as insect and weed control during soybean growing season. The row length was 8 m, the row spacing was 0.5 m, and the plant spacing was 0.4 m. Plots were manually harvested when the plants reached physiological maturity. Samples of each soybean line or variety were harvested from 4 plots and naturally dried in a greenhouse for 2 weeks. Whole soybean seeds were ground to obtain a fine powder using a planetary mono mill (Pulverisette 6; Fritsch, Idar-Oberstein, Germany). The powder was stored at −80 °C before analysis.

Analysis of proximates

The moisture content was measured by calculating the weight loss after oven-drying the samples at 135 ± 2 °C for 2 h [17]. Crude protein levels were estimated by determining the total nitrogen content using the Kjeldahl method, as previously described [18]. Crude fat contents were determined using the Soxhlet extraction method [19]. Ash contents were analyzed by combustion at 600 °C and gravimetric quantitation of the remaining non-volatile matter [20]. Crude fiber levels were determined by measuring the weight loss following ignition of dried residue after sample digestion with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions [21]. Total carbohydrate levels were calculated using the following equation: %

carbohydrates = 100% − (% protein + % fat + % ash). Soluble and insoluble dietary fiber levels were estimated by enzymatic–gravimetric methods using amylase, protease, and amyloglucosidase according to the association of analytical communities (AOAC) method 991.43 [22].

Determination of amino acid and fatty acid analysis

Amino acids other than cysteine and methionine were analyzed with an automatic amino acid analyzer (L-8500-A, Hitachi, Japan) directly after protein hydrolysis with hydrochloric acid. For the sulfur-containing amino acids cysteine and methionine, oxidative pretreatment was performed with performic acid before hydrolysis [23].

Fatty acid levels including those of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidonic acid (C20:0), and gadoleic acid (C20:1) were determined by lipid extraction and saponification with 0.5 N sodium hydroxide in methanol, followed by methylation in 14% boron trifluoride/methanol [24]. The resulting methyl esters were extracted with pentane and analyzed using a Shimadzu GC-2010 gas chromatography instrument (Shimadzu, Kyoto, Japan).

Mineral element analysis

Mineral elements including calcium, potassium, phosphorus, sodium, magnesium, zinc, iron, manganese, and copper were extracted from samples and determined according to AOAC guidelines [25]. Samples were ashed in an electric furnace at 600 °C and treated with hydrochloric acid. Each element was measured by inductively coupled plasma-optical emission spectrometry (Integra XL inductively coupled plasma-optical emission spectrometer, GBC Co., Melbourne, Australia).

Determination of beta-carotene, tocopherol, and vitamin C contents

Beta-carotene and tocopherol extractions and analyses were conducted in accordance with methods described by Park et al. [26]. Briefly, beta-carotene and tocopherol were extracted by mixing 3 mL of ethanol containing 0.1% ascorbic acid (w/v) with 100 mg and 50 mg freeze-dried soybean powders, respectively, vortexing for 20 s, incubating for 5 min at 85 °C in a water bath, saponification with 80% potassium hydroxide (w/v) in the 85 °C water bath for 10 min, followed by the addition of 1.5 mL cold deionized water. Next, 1.5 mL hexane was added to each extract and centrifuged at 1200×*g* to separate the upper layers, and then, each extract was dried under a nitrogen stream and re-dissolved in 50:50 (v/v) dichloromethane/methanol. Beta-carotene was separated in a C30 YMC column (250 × 4.6 mm, 3 mm; YMC Co., Kyoto, Japan) and quantitated using an Agilent 1100 high-performance liquid chromatography (HPLC) instrument (Massy, France) equipped with a photodiode array detector. The separation and analyses were performed following the descriptions by Park et al. [26].

For tocopherol, the separated upper layer was dried in a centrifugal concentrator (CVE-2000; Eyela, Tokyo, Japan). To derivatize samples, 30 mL of N-methyl-N-trimethylsilyl trifluoroacetamide was mixed with 30 mL of pyridine and incubated at 60 °C for 30 min at a mixing frequency of 1200 rpm using a 5355 compact thermomixer (Eppendorf, Hamburg, Germany). Then, gas chromatography and time-of-flight (TOF) mass spectrometry were performed using a 7890A gas chromatograph (Agilent, Atlanta, GA, USA) coupled to a Pegasus HT TOF mass spectrometer (Leco, St. Joseph, MI, USA) to determine the tocopherol levels. Tocopherols including α-tocopherol, *β*-tocopherol, *γ*-tocopherol, and *δ*-tocopherol were separated on a 30 m × 0.25 mm inside diameter, fused-silica capillary column coated with 0.25-mm CP-SIL 8 CB low bleed (Varian Inc., Palo Alto, CA, USA). The separation and analyses conditions followed the methods described by Park et al. [26].

Vitamin C was extracted using an ultrasonic-assisted extraction method as described by Stan et al. [27], with some modifications. Briefly, each 1 g of finely ground soybean seed powder was extracted twice for 30 min with 8% metaphosphoric acid in an ultrasonic Transsonic T 310 bath at 35 kHz and an installed power of 95 W. The mixtures were centrifuged at 13,000×*g* for 10 min, and the supernatants were filtered. The filtered extracts were separated on a Grace Alltima C18 column (100 × 3 mm, 3 μm) and analyzed using an Agilent 1100 HPLC instrument (Massy, France) equipped with a photodiode array detector. The separation and analyses conditions were performed followed the methods described by Stan et al. [27].

Statistical analysis

The experimental results were analyzed using a *t* test to differentiate significance at the $p < 0.05$ and $p < 0.01$ levels, by using Microsoft Excel (Microsoft, Redmond, WA, USA). Quantification data from 7 proximates, 18 amino acids, 9 minerals, 6 vitamins, and 7 fatty acids were analyzed by PCA (BioPAT-SIMCA version 13; Umetrics, Umeå, Sweden) to evaluate differences in multivariate data among groups. The PCA output consisted of score plots to visualize the contrast between different samples and loading plots to explain the cluster separation.

Results and discussion

Proximates

Regarding the proximate, the levels of crude ash, crude protein, crude fat, crude fiber, carbohydrates, insoluble dietary fiber, soluble dietary fiber, and total dietary fiber were measured in seed samples from the 3 transgenic soybean lines (7-1-1, 9-1-2, and 10-19-1), their counterpart Kwangan, and 3 commercial non-transgenic soybeans (Poongsan, Poongwon, and Haepum) as references shown in Table 1. There was no significant difference in ash, crude fiber, soluble dietary fiber, and total dietary fiber contents were not significantly different between transgenic lines and the non-transgenic counterpart Kwangan variety ($p > 0.05$). The 3 transgenic lines showed trends toward reduced crude fat and increased carbohydrates, comparing to that observed in the Kwangan counterpart. Moreover, both crude fat and carbohydrate levels showed statistically significant differences at $p < 0.05$ or $p < 0.01$ between the transgenic lines and Kwangan, respectively. However, the levels of ash, crude protein, crude fat, crude fiber, carbohydrates, and total dietary fiber from the transgenic soybean lines were within the reference ranges of the OECD consensus documents and literature for commercial soybean varieties [16]. Even though the levels of insoluble and soluble dietary fiber of commercial soybean varieties were not available in the OECD consensus document and in the literature [28–34], transgenic soybean lines showed non-significant differences compared with the parental non-transgenic variety Kwangan, and insoluble dietary fiber contents of the transgenic lines were within the reference ranges with the Poongsan, Poongwon, and Haepum varieties used in this study.

Amino acids

The levels of 18 amino acids were measured as a percentage of the total amino acids, and t test analysis was performed between transgenic soybean lines and the Kwangan counterpart (Table 2). Overall, the profile of 18 amino acids in the transgenic soybean lines showed non-significant differences compared to the non-transgenic Kwangan counterpart. Ten amino acids in the transgenic soybean lines were within the ranges of 4 non-transgenic varieties, except that the levels of aspartic acid, glycine, histidine, leucine, lysine, methionine, phenylalanine, and valine were slightly higher than those in the non-transgenic Kwangan counterpart. However, all amino acid levels were within the reported values from the OECD and previous studies [28–34].

Table 1 Comparison and statistical analysis of the proximate compositions measured in soybean samples

Component (%)	Commercial non-transgenic soybean c.v.			Parental c.v.	Beta-carotene-enhanced transgenic soybeans			OECD (ILSI, 2010)	Literature range[e]
	Poongsan	Poongwon	Haepum	Kwangan[c]	7-1-1	9-1-2	10-19-1		
Ash	5.01 ± 0.07	5.19 ± 0.03	5.03 ± 0.02	4.96 ± 0.02	5.14 ± 0.08	5.06 ± 0.04	5.13 ± 0.05	3.90–7.00	3.80–7.00
Crude protein	35.05 ± 0.21	33.63 ± 0.22	34.08 ± 0.01	39.39 ± 0.25	38.40 ± 0.40*[b]	39.81 ± 0.28	38.55 ± 0.36	33.20–45.50	32.00–48.40
Crude fat	17.27 ± 0.21	17.65 ± 0.14	18.63 ± 0.16	14.29 ± 0.29	12.94 ± 0.28*	12.40 ± 0.38**	13.29 ± 0.37*	8.10–23.60	8.10–24.70
Crude fiber	4.75 ± 0.06	4.69 ± 0.07	4.63 ± 0.05	4.79 ± 0.20	4.92 ± 0.08	5.01 ± 0.05	5.31 ± 0.12	NA[d]	4.10–13.90
Carbohydrates	42.66 ± 0.27	43.52 ± 0.33	42.27 ± 0.18	41.36 ± 0.50	43.53 ± 0.43**	42.72 ± 0.48*	43.02 ± 0.54*	29.60–50.20	29.30–50.20
IDF[a]	19.59 ± 0.22	20.52 ± 0.41	18.30 ± 0.20	17.46 ± 0.53	18.52 ± 0.24	18.76 ± 0.54*	17.95 ± 0.20	NA	NA
SDF	2.44 ± 0.27	2.34 ± 0.24	2.40 ± 0.37	2.37 ± 0.37	2.16 ± 0.23	2.17 ± 0.17	2.23 ± 0.25	NA	NA
TDF	22.03 ± 0.26	22.85 ± 0.24	20.71 ± 0.13	19.93 ± 0.50	20.67 ± 0.44	20.93 ± 0.43	20.18 ± 0.33	15.90–22.90	NA

[a] *IDF* insoluble dietary fiber, *SDF* soluble dietary fiber, *TDF* total dietary fiber

[b] The values indicate the mean ± standard deviation ($n = 4$). p values were obtained using Student's t test; * and ** indicate significant differences between the transgenic lines and the non-transgenic parental Kwangan variety at the 5 and 1% probability levels, respectively

[c] Non-transgenic parental variety of beta-carotene-enhanced transgenic soybeans

[d] *NA* not available in the OECD references

[e] Literature cited from McCann et al. [33], Harrigan et al. [31], Lundry et al. [32], Berman et al. [28–30], and Zhou et al. [34]

Table 2 Comparison and statistical analysis of the amino acid levels measured in soybean samples

Component (% protein)	Commercial non-transgenic soybean c.v.			Parental c.v.	Beta-carotene-enhanced transgenic soybeans			OECD (ILSI, 2010)	Literature range[c]
	Poongsan	Poongwon	Haepum	Kwangan[b]	7-1-1	9-1-2	10-19-1		
Alanine	1.63 ± 0.03	1.53 ± 0.05	1.55 ± 0.02	1.70 ± 0.03	1.71 ± 0.07[a]	1.86 ± 0.02	1.80 ± 0.12	1.51–2.10	1.43–2.10
Arginine	2.88 ± 0.06	2.61 ± 0.09	2.71 ± 0.02	3.65 ± 0.08	3.40 ± 0.09	3.65 ± 0.18	3.41 ± 0.20	2.28–3.40	2.15–3.46
Aspartic acid	4.20 ± 0.06	3.98 ± 0.14	4.09 ± 0.03	4.67 ± 0.08	4.67 ± 0.20	4.78 ± 0.22	4.69 ± 0.24	3.81–5.12	3.81–5.12
Cysteine	0.63 ± 0.02	0.59 ± 0.02	0.53 ± 0.01	0.59 ± 0.01	0.60 ± 0.03	0.61 ± 0.04	0.59 ± 0.01	0.37–0.81	0.31–0.81
Glutamic acid	6.39 ± 0.10	5.96 ± 0.24	6.33 ± 0.04	6.96 ± 0.10	6.86 ± 0.31	6.91 ± 0.27	6.83 ± 0.25	5.84–8.20	5.84–8.72
Glycine	1.59 ± 0.02	1.50 ± 0.05	1.53 ± 0.01	1.65 ± 0.03	1.65 ± 0.07	1.70 ± 0.06	1.66 ± 0.08	1.46–1.99	1.41–1.99
Histidine	0.98 ± 0.02	0.90 ± 0.03	0.95 ± 0.01	1.07 ± 0.02	1.09 ± 0.04	1.12 ± 0.04	1.09 ± 0.05	0.87–1.17	0.86–1.24
Isoleucine	1.38 ± 0.01	1.32 ± 0.05	1.37 ± 0.01	1.42 ± 0.03	1.39 ± 0.06	1.44 ± 0.05	1.42 ± 0.06	1.53–2.07	1.49–2.08
Leucine	2.80 ± 0.02	2.68 ± 0.10	2.79 ± 0.01	2.90 ± 0.04	2.85 ± 0.13	2.96 ± 0.15	2.91 ± 0.13	2.59–3.62	2.39–3.62
Lysine	2.37 ± 0.03	2.24 ± 0.08	2.30 ± 0.01	2.42 ± 0.04	2.43 ± 0.10	2.49 ± 0.09	2.43 ± 0.10	2.28–2.83	2.19–3.15
Methionine	0.40 ± 0.01	0.40 ± 0.01	0.38 ± 0.01	0.42 ± 0.01	0.43 ± 0.02	0.44 ± 0.02	0.43 ± 0.10	0.43–0.68	0.39–0.68
Phenylalanine	1.76 ± 0.01	1.67 ± 0.08	1.73 ± 0.01	1.82 ± 0.03	1.82 ± 0.07	1.87 ± 0.08	1.85 ± 0.07	1.63–2.34	1.62–2.44
Proline	2.04 ± 0.04	1.86 ± 0.07	1.97 ± 0.03	2.10 ± 0.04	2.08 ± 0.11	2.12 ± 0.09	2.09 ± 0.10	1.68–2.28	1.63–2.81
Serine	1.94 ± 0.03	1.85 ± 0.07	1.88 ± 0.01	2.02 ± 0.03	1.98 ± 0.08	1.98 ± 0.14	2.02 ± 0.08	1.10–2.48	1.11–2.48
Threonine	1.51 ± 0.02	1.43 ± 0.05	1.47 ± 0.01	1.54 ± 0.02	1.55 ± 0.06	1.46 ± 0.21	1.54 ± 0.06	1.14–1.86	1.14–1.86
Tyrosine	1.20 ± 0.02	1.15 ± 0.05	1.19 ± 0.01	1.29 ± 0.02	1.23 ± 0.06	1.27 ± 0.10	1.23 ± 0.13	1.01–1.61	0.79–1.61
Tryptophan	0.42 ± 0.01	0.39 ± 0.01	0.42 ± 0.01	0.40 ± 0.02	0.42 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.36–0.50	0.36–0.63
Valine	1.43 ± 0.01	1.35 ± 0.05	1.42 ± 0.01	1.47 ± 0.03	1.47 ± 0.06	1.52 ± 0.04	1.48 ± 0.06	1.59–2.20	1.57–2.20

[a] Values indicate the mean ± standard deviations ($n = 4$). p values were obtained using Student's t test; * and ** indicate significant differences between the transgenic lines and the non-transgenic parental Kwangan variety at the 5 and 1% probability levels, respectively

[b] Non-transgenic parental variety of beta-carotene-enhanced transgenic soybeans

[c] Literature cited from McCann et al. [33], Harrigan et al. [31], Lundry et al. [32], Berman et al. [28–30], and Zhou et al. [34]

Fatty acids

Three saturated fatty acids (C16:0, C18:0, and C20:0) and 4 unsaturated acids (C18:1, C18:2, C18:3, and C20:1) were determined, and t test analysis was performed for transgenic soybean lines and the Kwangan counterpart (Table 3). Slightly higher fatty acid levels were observed in the transgenic soybean lines than in the non-transgenic Kwangan counterpart, but statistical analysis by the t test indicated that no significant differences occurred between them. All 7 fatty acids measured with the transgenic soybean lines were out of the range of values observed in 4 non-transgenic commercial soybean varieties, but were within the range of values reported by the OECD and in previous studies [28–34]. One exception was that a 36% increase of oleic acid (C18:1) production was observed in transgenic soybean line 7-1-1, compared to that observed in the non-transgenic Kwangan counterpart, with a significant difference at the $p < 0.05$ level by t test analysis. However, the same trend was not observed with the other 2 transgenic lines. A transgenic soya bean containing the *phytoene synthase* gene (*crtB*) from the bacteria *Pantoea ananatis* was detected an elevated amount of oleic acid and declined amount of linoleic acid [5]. Transcript profiling analysis suggested that the suppression of Δ12 fatty acid desaturase (FAD2) likely accounts of the elevated amount of oleic acid in the *crtB* transgenic seeds. In the present study, FAD2 expression is repressed or not in transgenic soybean lines and needs a further study by means of transcript profiling.

Minerals

Nine mineral compositions were analyzed, and statistically significant differences in the levels of calcium, potassium, sodium, and iron were observed between the transgenic soybean lines and their non-transgenic Kwangan counterpart (Table 4). All minerals except for sodium and copper showed levels within the ranges provided by the OECD and previous literature, but the copper level did not reach a significant difference between the transgenic lines and their non-transgenic counterpart. Furthermore, the sodium contents of the transgenic lines were outside the range reported by the OECD and in previous studies [28–34], but the values were still within the range of 4 commercial non-transgenic soybean varieties used in this study.

Table 3 Comparison and statistical analysis of fatty acid levels measured in soybean samples

Component (mg/g) (%)	Commercial non-transgenic soybean c.v.			Parental c.v.	Beta-carotene-enhanced transgenic soybeans			OECD (ILSI, 2010)	Literature range (%)[d]
	Poongsan	Poongwon	Haepum	Kwangan[c]	7-1-1	9-1-2	10-19-1		
Palmitic acid (C16:0)	3.98 ± 0.15	3.92 ± 0.39	4.16 ± 0.30	3.79 ± 0.06	4.03 ± 0.17	3.49 ± 0.24	3.49 ± 0.45	0.67–2.78	9.55–15.77
	(16.25 ± 0.31)	(16.17 ± 0.32)	(16.62 ± 0.40)	(16.30 ± 0.12)	(15.03 ± 0.85)[a]	(14.74 ± 0.73)	(15.63 ± 0.61)		
Stearic acid (C18:0)	1.02 ± 0.07	1.05 ± 0.11	1.18 ± 0.08	1.02 ± 0.01	1.25 ± 0.07	1.33 ± 0.03	1.28 ± 0.21	0.28–1.13	2.70–5.88
	(3.78 ± 0.08)	(3.91 ± 0.06)	(4.27 ± 0.12)	(3.99 ± 0.12)	(4.25 ± 0.45)	(5.10 ± 0.27)	(5.18 ± 0.05)		
Oleic acid (C18:1)	4.73 ± 0.36	3.66 ± 0.65	3.96 ± 0.58	7.53 ± 0.05	10.24 ± 1.42*[b]	7.49 ± 0.34	7.45 ± 1.63	1.36–6.56	14.30–40.43
	(17.59 ± 0.71)	(13.70 ± 0.91)	(14.36 ± 0.67)	(29.53 ± 0.50)	(34.69 ± 2.71)	(28.93 ± 2.29)	(30.16 ± 1.71)		
Linoleic acid (C18:2)	14.35 ± 0.59	15.09 ± 1.77	15.13 ± 1.39	10.96 ± 0.32	11.21 ± 0.63	11.13 ± 0.93	10.19 ± 1.35	3.46–13.36	42.30–58.80
	(53.80 ± 0.41)	(57.15 ± 0.50)	(55.45 ± 0.38)	(43.28 ± 0.42)	(38.39 ± 1.32)	(43.14 ± 2.16)	(41.94 ± 1.51)		
Linolenic acid (C18:3)	2.21 ± 0.07	2.31 ± 0.16	2.45 ± 0.28	1.65 ± 0.09	2.10 ± 0.15	1.91 ± 0.22	1.62 ± 0.42	0.30–2.19	3.00–12.52
	(8.36 ± 0.13)	(8.84 ± 0.47)	(9.04 ± 0.17)	(6.55 ± 0.25)	(7.24 ± 0.17)	(7.45 ± 0.64)	(6.63 ± 0.66)		
Arachidonic acid (C20:0)	0.04 ± 0.001	0.05 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.08 ± 0.01	0.11 ± 0.05	0.09 ± 0.02	0.02–0.11	0.16–0.48
	(0.15 ± 0.01)	(0.16 ± 0.03)	(0.19 ± 0.03)	(0.22 ± 0.02)	(0.24 ± 0.01)	(0.39 ± 0.20)	(0.32 ± 0.04)		
Gadoleic acid (C20:1)	0.02 ± 0.001	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.001	0.05 ± 0.01	0.07 ± 0.05	0.04 ± 0.01	NA	0.14–0.35
	(0.07 ± 0.01)	(0.07 ± 0.01)	(0.08 ± 0.03)	(0.13 ± 0.01)	(0.15 ± 0.02)	(0.24 ± 0.18)	(0.15 ± 0.03)		

[a] Means percentage of total fatty acids; literature range was cited by percentage data

[b] Values indicate the mean ± standard deviation ($n = 4$). p values were obtained using Student's t test. * and ** indicate significant differences between the transgenic lines and the non-transgenic parental Kwangan variety at the 5 and 1% probability levels, respectively

[c] Non-transgenic parental variety of beta-carotene-enhanced transgenic soybean

[d] Literature cited from McCann et al. [33], Harrigan et al. [31], Lundry et al. [32], Berman et al. [28–30], and Zhou et al. [34]

Table 4 Comparison and statistical analysis of the mineral levels measured in soybean samples

Component	Commercial non-transgenic soybean c.v.			Parental c.v.	Beta-carotene-enhanced transgenic soybeans			OECD (ILSI, 2010)	Literature range[c]
	Poongsan	Poongwon	Haepum	Kwangan[b]	7-1-1	9-1-2	10-19-1		
Calcium, mg/g	1.91 ± 0.23	2.69 ± 0.06	1.92 ± 0.06	2.50 ± 0.03	2.88 ± 0.06**[a]	2.65 ± 0.09**	2.60 ± 0.08**	1.20–3.10	1.17–5.10
Magnesium, mg/g	2.37 ± 0.34	2.48 ± 0.04	2.27 ± 0.03	2.20 ± 0.03	2.32 ± 0.07	2.22 ± 0.06	2.25 ± 0.00	2.20–3.10	2.05–3.13
Phosphorus, mg/g	8.02 ± 1.17	6.52 ± 0.09	6.60 ± 0.10	7.37 ± 0.16	7.40 ± 0.08	7.22 ± 0.10	7.40 ± 0.13	5.00–9.40	4.71–9.35
Potassium, mg/g	21.19 ± 2.92	19.37 ± 0.20	19.91 ± 0.33	17.48 ± 0.51	17.63 ± 0.35*	17.34 ± 0.03*	18.46 ± 0.42**	18.70–23.20	16.50–25.10
Sodium, mg/g	0.35 ± 0.07	0.33 ± 0.10	0.28 ± 0.02	0.41 ± 0.08	0.34 ± 0.01**	0.35 ± 0.13	0.23 ± 0.11**	NA	0.04–0.30
Copper, μg/g	12.71 ± 1.60	12.91 ± 0.62	11.52 ± 0.81	22.93 ± 0.90	24.02 ± 0.31	21.82 ± 0.42	22.91 ± 0.33	1.10–15.30	6.26–18.60
Iron, μg/g	91.70 ± 12.20	90.60 ± 2.50	80.80 ± 1.80	112.80 ± 1.90	123.30 ± 7.60**	107.10 ± 4.10*	106.60 ± 3.40**	60.00–110.00	37.34–151.00
Manganese, μg/g	21.30 ± 3.01	23.90 ± 0.70	23.40 ± 0.62	55.07 ± 1.12	52.51 ± 1.01	45.21 ± 6.12**	53.24 ± 3.63	0.00–59.00	17.70–71.80
Zinc, μg/g	42.72 ± 6.11	43.71 ± 1.00	35.23 ± 1.02	66.03 ± 2.02	68.22 ± 1.33	64.24 ± 1.32	68.62 ± 1.51	10.90–67.70	31.50–75.80

[a] Values indicate the mean ± standard deviation ($n = 4$), p values were obtained using Student's t test. * and ** indicate significant differences between the transgenic lines and the non-transgenic parental Kwangan variety at the 5 and 1% probability levels, respectively

[b] Non-transgenic parental variety of beta-carotene-enhanced transgenic soybeans

[c] Literature cited from McCann et al. [33], Harrigan et al. [31], Lundry et al. [32], Berman et al. [28–30], and Zhou et al. [34]

Vitamins

Water-soluble vitamin C, fat-soluble vitamin E (including α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol), and beta-carotene (provitamin A) levels were analyzed for all soybean samples. The mean values and t test results are shown in Table 5. Beta-carotene was one of the main metabolic products due to the introduction of *Psy* and *CrtI* into the commercial soybean variety Kwangan, so that beta-carotene production in the 3 transgenic lines 7-1-1, 9-1-2, and 10-19-1 ranged from 170.47 to 213.58 μg/g, suggesting the successful expression of foreign genes. Comparing to 0.46 μg/g of non-transgenic counterpart Kwangan, more than 300-fold high levels of beta-carotene were produced. Previous studies reported that production levels of beta-carotene were very different depending on inserted foreign genes such as *phytoene synthase*, *lycopene synthase*, *lycopene beta-cyclase,* or *carotene desaturase* [5, 6, 13, 14]. However, higher productions of beta-carotene could have greater effects on the native carotenoid-biosynthesis pathway of soybeans, which would upset a genomic balance of intrinsic gene network.

In general, large variations in vitamin contents were observed among the commercial varieties. No significant difference was observed in the levels of vitamin C, α-tocopherol, β-tocopherol, γ-tocopherol, and total tocopherol between the transgenic soybean lines and their non-transgenic counterpart, but a significant difference was observed in the δ-tocopherol level. Except for β-tocopherol (for which reported levels are unavailable), all other values were within the ranges of the 4 commercial soybean varieties and those provided by the OECD or reported in the literature [28–34]. Previously findings suggested that geranylgeranyl diphosphate located upstream of beta-carotene biosynthesis functions as a precursor molecule for tocopherol biosynthesis and the gibberellins pathway. The total tocopherol (vitamin E) contents of the transgenic soybeans were not significantly altered in the present study. However, the transgenic lines did not show significant differences in terms of the plant type, plant height, and agronomic traits, although moderately high abscisic acid levels have been reported (compared to the non-transgenic Kwangan counterpart) by Qin et al. [15]. Therefore, we can conclude that the *Psy* and *CrtI* gene insertions had little effect on the upstream production of geranylgeranyl diphosphate, but may affect the abscisic acid-biosynthesis pathway.

Nutritional difference analysis performed using PCA

To assess the overall experimental variation and to examine differences in nutritional compositions among

Table 5 Comparison and statistical analysis of vitamin levels measured in soybean samples

Component (μg/g)	Commercial non-transgenic soybean c.v.			Parental c.v.	Beta-carotene-enhanced transgenic soybeans			OECD (ILSI, 2010)	Literature range[c]
	Poongsan	Poongwon	Haepum	Kwangan[b]	7-1-1	9-1-2	10-19-1		
Vitamin C	0.40 ± 0.19	0.17 ± 0.06	0.11 ± 0.01	1.02 ± 0.07	0.84 ± 0.08	0.73 ± 0.16	1.22 ± 0.10	NA	0.20–2.10
α-Tocopherol	1.36 ± 0.18	3.06 ± 0.07	1.52 ± 0.29	2.89 ± 0.78	2.03 ± 0.20	3.04 ± 0.72	4.34 ± 0.56*	0.19–6.17	0.19–8.08
β-Tocopherol	0.50 ± 0.07	1.42 ± 0.07	0.87 ± 0.09	2.01 ± 0.30	1.47 ± 0.14	2.04 ± 0.20	2.52 ± 0.20	NA	NA
γ-Tocopherol	18.66 ± 3.21	20.80 ± 0.77	18.40 ± 1.83	20.69 ± 5.33	20.00 ± 1.21	22.47 ± 4.31	19.90 ± 1.88	NA	7.75–21.50
δ-Tocopherol	8.07 ± 0.55	7.43 ± 0.35	7.94 ± 0.57	9.66 ± 1.10	7.59 ± 0.67*[a]	7.05 ± 0.40*	5.87 ± 0.25**	NA	4.97–10.40
Total tocopherol	28.59 ± 3.86	32.72 ± 1.22	28.73 ± 2.64	31.67 ± 1.40	31.10 ± 1.78	34.60 ± 5.45	32.63 ± 2.81	NA	16.00–30.40
β-Carotene	0.36 ± 0.06	0.37 ± 0.01	0.17 ± 0.01	0.46 ± 0.12	184.71 ± 28.86**	213.58 ± 27.04**	170.47 ± 21.34**	0.37–0.44	NA

[a] Values indicate the mean ± standard deviations ($n = 4$). p values were obtained using Student's t test. * and ** indicate significant differences between the transgenic lines and the non-transgenic parental Kwangan variety at the 5 and 1% probability levels, respectively

[b] Non-transgenic parental variety of beta-carotene-enhanced transgenic soybeans

[c] Literature cited from McCann et al. [33], Harrigan et al. [31], Lundry et al. [32], Berman et al. [28–30], and Zhou et al. [34]

the 3 transgenic soybean lines, their non-transgenic counterpart, and 3 reference varieties, PCA was conducted by using the quantification data from 48 components of all soybean samples. PCA revealed that the 2 highest ranking principal components cumulatively accounted for 63% of the total variance. The first 2 principal components, accounting for 51.3% of the total variance, revealed a clear separation between the reference-variety group, the transgenic soybean lines, and the non-transgenic Kwangan counterpart (Fig. 1A). The score and loading plots corresponding to principal component 1 (PC1) indicated that the reference varieties contained high levels of insoluble dietary fiber, total dietary fiber, lipids C18:3, C18:2, and C16:0, and potassium with high and positive PC1 loading. However, the transgenic soybean varieties and the counterpart Kwangan had high levels of proteins, C18:1, most amino acids, zinc, manganese, iron, beta-tocopherol, vitamin C, and fiber with high and negative PC1 loading (Fig. 1A, B). Examination of the score and loading plot of principal component 2 (PC2) showed a relatively non-clear separation between the transgenic soybean lines and their counterpart variety (Fig. 1A) and collectively accounted for 11.7% of the total variance. PC2 showed high and positive levels of carbohydrates, ash, and calcium in the transgenic soybean lines (Fig. 1B). These results indicated that variations of nutritional compositions were more affected by differences in varieties, but not by differences in the transgenic soybean lines and non-transgenic counterpart.

In conclusion, the transgenic soybean lines showed substantial equivalence in most proximates, amino acids, fatty acids, minerals, and vitamins compared to the non-transgenic counterpart Kwangan variety. Furthermore, all nutrients were within the ranges of 4 non-transgenic soybean varieties or the ranges provided by the OECD and reported in the literature. However, several compositions of the transgenic lines, such as crude fat, carbohydrates, calcium, potassium, iron, and δ-tocopherol, were shown in significant differences compared to those of the non-transgenic counterpart variety. Bellaloui et al. [35, 36] studied that the soybean seed protein, oil, fatty acids, sugar, and minerals could be affected by some agricultural practices such as the planting date, irrigation, fertilizer, and soil conditions. In the present study, *Psy* and *CrtI* gene insertion, or cultivation environments and agricultural practices, or both of them should be responsible for minerals, δ-tocopherol, and proximate alternations, which is difficult to give a definite answer. In case of oleic acid levels, a significant increase was only shown on transgenic soybean line 7-1-1, which indicated that transgene inserted locations had some effects on fatty acid levels and their related pathways. It at least makes known that two transgenic soybeans might be a different case, even the same gene

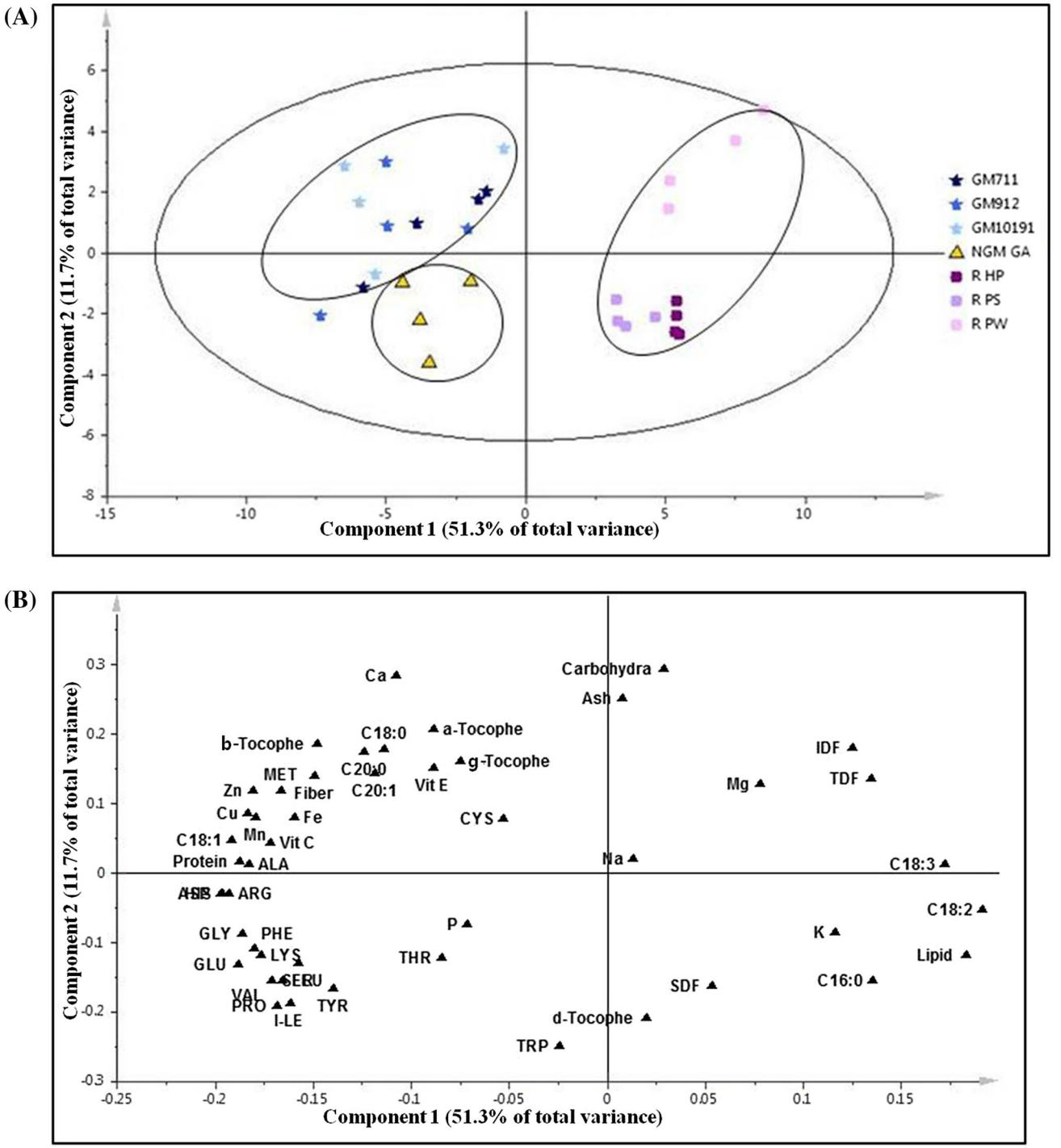

Fig. 1 PCA for 48 compositions consisting of 8 proximates, 18 amino acids, 9 minerals, 7 fatty acids, and 6 vitamins in soybean seed samples. (**A**) PCA score plot; (**B**) loading plot; the cumulative eigenvalues of components 1 and 2 were 0.513 and 0.117, respectively; GM711, transgenic line 7-1-1; GM912, transgenic line 9-1-2; GM10191, transgenic line 10-19-1; NGM KA: non-transgenic counterpart Kwangan; R HP: reference variety Haepum; R PS: reference variety Poongsan; R PW: reference variety Poongwon. ALA: alanine; ARG: arginine; ASP: aspartic acid; CYS: cysteine; GLU: glutamic acid; GLY: glycine; HIS: histidine; ILE: isoleucine; LEU: leucine; LYS: lysine; MET: methionine; PHE: phenylalanine; PRO: proline; SER: serine; THR: threonine; TYR: tyrosine; TRP: tryptophan; VAL: valine; Ca: calcium; Mg: magnesium; S: phosphorus; P: potassium; Na: sodium; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc

cassette insertions. A systemic transcriptome and metabolome profiling should be carried out in the further studies.

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References

1. Bauernfeind JC, Adams CR, Marusich WL (1981) Carotenoids and other vitamin A precursors in animal feed. In: Bauernfeind JC (ed) Carotenoids as colorants and vitamin A precursors. Academic Press, New York, pp 563–743
2. Aluru M, Xu Y, Guo R, Wang Z, Li S, White W, Wang K, Rodermel S (2008) Generation of transgenic maize with enhanced provitamin A content. J Exp Bot 59:3551–3562
3. Kim M, Kim JK, Kim HJ, Pak JH, Lee J, Kim DH, Choi HK, Jung HW, Lee JD, Chuang YS, Ha SH (2012) Genetic modification of the soybean to enhance the beta-carotene content through seed-specific expression. PLoS ONE. doi:10.1371/journal.pone.0048287
4. Römer S, Fraser PD, Kiano JW, Shipton CA, Misawa N, Schuch W, Bramley PM (2000) Elevation of the provitamin A content of transgenic tomato plants. Nat Biotechnol 18:666–669
5. Schmidt MA, Parrott WA, Hildebrand DF, Berg RH, Cooksey A, Pendarvis K, He Y, McCarthy F, Herman EM (2015) Transgenic soya bean seeds accumulating b-carotene exhibit the collateral enhancements of oleate and protein content traits. Plant Biotechnol J 13:590–600
6. Shewmaker CK, Sheehy JA, Daley M, Colburn S, Ke DY (1999) Seed-specific overexpression of phytoene synthase: increase in carotenoids and other metabolic effects. Plant J 20:401–412
7. Zhu C, Naqvi S, Breitenbach J, Sandmann G, Christou P, Capell T (2008) Combinatorial genetic transformation generates a library of metabolic phenotypes for the carotenoid pathway in corn. Proc Natl Acad Sci USA 105:18232–18237
8. Zimmermann R, Qaim M (2004) Potential health benefits of Golden Rice: a Philippine case study. Food Policy 29:147–168
9. Qin Y, Kweon SJ, Chung YS, Ha SH, Shin KS, Lim MH, Kwon TR, Cho HS, Park SK, Woo HJ (2015) Selection of beta-carotene enhanced transgenic soybean containing single-copy transgene and analysis of integration sites. Korean J Breed Sci 47:111–117
10. EFSA, GMO Panel Working Group on Animal Feeding Trials (2008) Safety and nutritional assessment of GM plants and derived food and feed: the role of animal feeding trials. Food Chem Toxicol 46(Suppl 1):S2–70
11. Kim JK, Park SY, Lee SM, Lim SH, Kim HY, Oh SD, Yeo YS, Cho HS, Ha SH (2013) Unintended polar metabolite profiling of carotenoid-biofortified transgenic rice reveals substantial equivalence to its non-transgenic counterpart. Plant Biotechnol Rep 7:121–128
12. Ha SH, Liang YS, Jung H, Ahn MJ, Suh SC, Kweon SJ, Kim DH, Kim YM, Kim JK (2010) Application of two bicistronic systems involving 2A and IRES sequences to the biosynthesis of carotenoids in rice endosperm. Plant Biotech J 8:928–938
13. Fray RG, Wallace A, Fraser PD, Valero D, Hedden P, Bramley PM, Grierson D (1995) Constitutive expression of a fruit phytoene synthase gene in transgenic tomatoes causes dwarfism by redirecting metabolites from the gibberellin pathway. Plant J 8:693–701
14. Lindgren LO, Stålberg KG, Höglund AS (2003) Seed-specific overexpression of an endogenous *Arabidopsis* phytoene synthase gene results in delayed germination and increased levels of carotenoids, chlorophyll, and abscisic acid. Plant Physiol 132:779–785
15. Qin Y, Shin KS, Seo JK, Lim MH, Chung YS, Cho HS, Lee SK, Woo HJ (2016) Assessment of the response of beta-carotene-enhanced transgenic soybeans to *soybean mosaic virus*. Plant Breed Biotech 4:158–169
16. Organization for Economic Cooperation and Development (OECD) Environment, Health and Safety Publications (2012) Revised consensus document on compositional considerations for new varieties of [*Glycine max* (L.) Merr.]: key food and feed nutrients, antinutrients, toxicants and allergens. Series on the safety of novel foods and feeds. No. 25. Environment Directorate. Organisation for economic co-operation and development, Paris, France
17. AOAC (2005a). Moisture content. AOAC official methods 930.15. AOAC, Gaithersburg, MD, USA
18. AOAC (2005b) Nitrogen (total) in fertilizers. AOAC official methods 955.04. AOAC, Gaithersburg, MD, USA
19. AOAC (2005c) Fat (crude) or ether extract in meat. AOAC official methods 960.39. AOAC, Gaithersburg, MD, USA
20. AOAC (2005d) Ash of flour. AOAC official methods 923.03. AOAC, Gaithersburg, MD, USA
21. AOAC (2005e) Total, soluble, and insoluble dietary fiber in foods. AOAC official methods 991.43. AOAC, Gaithersburg, MD, USA
22. Lee S, Prosky L, De vries J (1992) Determination of total, soluble, and insoluble dietary fiber in foods: enzymatic-gravimetric method, MES-TRIS buffer: collaborative study. J AOAC Int 75:395–416
23. AOAC (2005f) Protein efficiency ratio. AOAC official methods 982.30. AOAC, Gaithersburg, MD, USA
24. AOCS (1997) Fatty acid composition by gas chromatography. Official methods and recommended practices of the American Oil Chemists. Method Ce 1-62. AOCS, Champaign, IL, USA
25. AOAC (2000) Determination of lead, calcium, copper, iron, zinc, sodium, magnesium, phosphorus, potassium, manganese in food. AOAC official methods 999.11. AOAC, Gaithersburg, MD, USA
26. Park SY, Park WT, Park YC, Ju JI, Park SU, Kim JK (2012) Metabolomics for the quality assessment of Lycium chinense fruits. Biosci Biotech Biochem 76:2188–2194
27. Stan M, Soran ML, Marutoiu C (2014) Extraction and HPLC determination of the ascorbic acid content of three indigenous spice plants. J Anal Chem 69:998–1002
28. Berman KH, Harrigan GG, Riordan SG, Nemeth MA, Hanson C, Smith M, Sorbet R, Zhu E, Ridley WP (2009) Compositions of seed, forage, and processed fractions from insect-protected soybean MON 87701 are equivalent to those of conventional soybean. J Agric Food Chem 57:11360–11369
29. Berman KH, Harrigan GG, Riordan SG, Nemeth MA, Hanson C, Smith M, Sorbet R, Zhu E, Ridley WP (2010) Compositions of forage and seed from second-generation glyphosate- tolerant soybean MON 89788 and insect-protected soybean MON 87701 from Brazil are equivalent to those of conventional soybean (*Glycine max*). J Agric Food Chem 58:6270–6276
30. Berman KH, Harrigan GG, Nemeth MA, Oliveira WS, Berger GU, Tagliaferro FS (2011) Compositional equivalence of insect-protected glyphosate-tolerant soybean MON 87701 × MON 89788 to conventional soybean extends across different world regions and multiple growing seasons. J Agric Food Chem 59:11643–11651
31. Harrigan GG, Ridley WP, Riordan SG, Nemeth MA, Sorbet R, Trujillo WA, Breeze ML, Schneider RW (2007) Chemical composition of glyphosate-tolerant soybean 40-3-2 grown in Europe remains equivalent with that of conventional soybean (*Glycine max* L.). J Agric Food Chem 55:6160–6168

32. Lundry DR, Ridley WP, Meyer JJ, Riordan SG, Nemeth MA, Trujillo WA, Breeze ML, Sorbet R (2008) Composition of grain, forage, and processed fractions from second-generation glyphosate-tolerant soybean, MON 89788, is equivalent to that of conventional soybean (*Glycine max* L.). J Agric Food Chem 56:4611–4622
33. McCann MC, Liu K, Trujillo WA, Dobert RC (2005) Glyphosate-tolerant soybeans remain compositionally equivalent to conventional soybeans (*Glycine max* L.) during three years of field testing. J Agric Food Chem 53:5331–5335
34. Zhou J, Berman KH, Breeze ML, Nemeth MA, Oliveira WS, Braga DPV, Berger GU, Harrigan GG (2011) Compositional variability in conventional and glyphosate-tolerant soybean (*Glycine max* L.) varieties grown in different regions in Brazil. J Agric Food Chem 59:11652–11656
35. Bellaloui N, Bruns HA, Abbas HK, Mengistu A, Fisher DK, Reddy KN (2015) Agricultural practices altered soybean seed protein, oil, fatty acids, sugars, and minerals in the Midsouth USA. Front Plant Sci 6:1–14
36. Bellaloui N, Mengistu A, Walker ER, Young LD (2014) Soybean seed composition as affected by seeding rates and row spacing. Crop Sci 54:1782–1795