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Comparative analysis of chemical compositions between non-transgenic soybean seeds and those from plants over-expressing *AtJMT*, the gene for jasmonic acid carboxyl methyltransferase

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ABSTRACT

Transgenic overexpression of the *Arabidopsis* gene for jasmonic acid carboxyl methyltransferase (*AtJMT*) is involved in regulating jasmonate-related plant responses. To examine its role in the compositional profile of soybean (*Glycine max*), we compared the seeds from field-grown plants that over-express *AtJMT* with those of the non-transgenic, wild-type (WT) counterpart. Our analysis of chemical compositions included proximates, amino acids, fatty acids, isoflavones, and antinutrients. Overexpression of *AtJMT* in the seeds resulted in decreased amounts of tryptophan, palmitic acid, linolenic acid, and stachyose, but increased levels of gadoleic acid and genistein. In particular, seeds from the transgenic soybeans contained 120.0–130.5% more genistein and 60.5–82.1% less stachyose than the WT. A separate evaluation of ingredient values showed that all were within the reference ranges reported for commercially available soybeans, thereby demonstrating the substantial equivalence of these transgenic and non-transgenic seeds.

1. Introduction

Jasmonic acid (JA) and its derivatives, commonly referred to as jasmonates, are important phytohormones in the signaling pathway that regulates the expression of plant defense genes against pathogen infection, insect attack, and diverse environmental stresses (Reymond & Farmer, 1998; Wasternack & Parthier, 1997). They also participate in the control of plant morphogenesis, including tuberization, flowering, fruit ripening, and seed development (Albrechtová & Ullmann, 1994; Koda, 1997). Methyl jasmonate (MeJA) is a major volatile derivative of JA that mediates interplant communications for defense responses (Farmer & Ryan, 1990). When converted into JA-isoleucine conjugate in neighbouring plants, airborne MeJA stimulates defensive systems (Tamogami, Pakwal, & Agrawal, 2008). Due to its volatility, MeJA is much more effective than JA in activating the jasmonate response (Jang et al., 2014).

In plants, JA is biosynthesized from linolenic acid via the octadecanoid pathway and further metabolized to MeJA by jasmonic acid carboxyl methyltransferase (JMT) (Creelman & Mullet, 1997). Overexpression of the *Arabidopsis thaliana* JMT gene (*AtJMT*) in transgenic *Arabidopsis* increases the level of endogenous MeJA and the accumulation of JA-responsive genes (Seo et al., 2001). In addition, it strongly promotes resistance against a fungal pathogen, *Botrytis cinerea*, in transgenic plants when compared with the non-transformed wild type (WT) (Seo et al., 2001). In *Panax ginseng*, overexpression of *AtJMT* elicits the accumulation of ginsenoside-biosynthetic genes as well as a MeJA-responsive gene, and further enhances root growth in transgenic plants (Kim et al., 2012). When *AtJMT* is introduced into *Oryza sativa* or *Solanum tuberosum*, levels of MeJA and JA-biosynthetic genes are elevated in the transgenic plants and overexpression controls organ development (Kim et al., 2009; Sohn et al., 2011). Overexpression of *AtJMT* in transgenic soybean (*Glycine max*) promotes expression of the gene for chalcone synthase, a key enzyme for defense-related responses

Abbreviations: AOAC, Association of Official Analytical Chemists; GM, genetically modified; JA, jasmonic acid; JMT, jasmonic acid carboxyl methyltransferase; MeJA, methyl jasmonate; MFDS, Ministry of Food and Drug Safety; WT, wild type.

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(Moon et al., 2011). These results suggest that *AtJMT* acts as a key trigger of jasmonate-related plant responses.

Although the role of *AtJMT* in provoking the activation of JA, MeJA, and JA-biosynthetic genes is well-understood, few studies have elucidated the relationship between the expression of this gene and changes in the compositional profiles of transgenic crops. However this topic is of considerable importance in view of safety concerns. Approval of transgenic crops as food sources requires rigorous safety assessments, with an emphasis on the analysis of nutritional composition. Compositional evaluations of transgenic crops have been carried out using the "substantial equivalence" concept developed by the Organization for Economic Co-operation and Development (OECD, 1993), which has proven that a novel transgenic crop is as nutritious and safe for food products as is its conventional counterpart (Gayen, Sarkar, Datta, & Datta, 2013).

Soybean is a critical food crop that provides high amounts of proteins, oils, and biologically active components. Its consumption is increasing worldwide because of a variety of health benefits (Friedman & Brandon, 2001). Compositional analyses have primarily focused on seeds from transgenic soybean plants that were developed to improve their resistance to specific herbicides or insects (Berman et al., 2009; McCann, Liu, Trujillo, & Dobert, 2005). However, few investigations have been made into the chemical composition of transgenic soybeans that regulate plant metabolism by inducing hormonal stimuli. Here, we examined the compositional differences between transgenic soybean that over-expresses *AtJMT* and its non-transgenic counterpart. Plants were grown under conventional field conditions and their levels of proximates, amino acids, fatty acids, isoflavones, and antinutrients were analyzed from seed extracts.

2. Materials and methods

2.1. Transformation and molecular characterization

The full-length *AtJMT* complementary DNA (cDNA) (Seo et al., 2001) was fused with the CaMV 35S promoter gene and inserted into pCAMBIA1305.1 (Cambia, Brisbane, QLD, Australia) at the *Pst*I and *Eco*RI sites. The DNA construct was transformed via *Agrobacterium tumefaciens* transcription factor, NTL4, into cotyledonary nodes from soybean cultivar 'Bert'. The insertion of a single-copy gene into transgenic lines '#3' and '#10' was confirmed by Southern blotting (Fig. 1A) and expression of the transgene was subsequently verified using northern blotting (Fig. 1B). For Southern blot analysis, genomic DNA (2 μg) from leaves of homozygous T_3 lines was digested with *Eco*RI and *Pst*I, separated on 0.8% agarose gels, and transferred onto nylon membranes for hybridization with a ^{32}P-labeled *AtJMT* probe. For northern blots of T_3 transgenic soybeans, 2 μg of total RNA was separated on 1.3% agarose formaldehyde gels and moved to GeneScreen Plus membranes (PerkinElmer, Waltham, MA, USA). Blots were probed with the random primer-extended *AtJMT* cDNA.

2.2. Soybean samples

Seeds of transgenic lines '#3' and '#10' that over-express *AtJMT* and their non-transgenic WT host 'Bert' were sown on plastic trays and placed in a greenhouse set for day/night temperatures of 28 ± 3 °C/22 ± 3 °C and long-day (16 h light/8 h dark) conditions. In June 2012, two-week-old seedlings were transplanted into six replicated plots (18 plants per plot) in an experimental field at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Republic of Korea (36°43′N, 127°26′E; elevation 35 m). Experiments followed a randomized complete block design, and plants were spaced at 0.4 m × 1.0 m. Prior to transplanting, all plots were fertilized with 5.7, 3.0, and 7.0 kg per 10 ac of N, P, and K, respectively. In September, whole seeds were collected from the plants in each plot and air-dried. The 100-seed weight for the WT was 14.8 g, while weights for lines '#3' and '#10' were 15.1 g and 14.7 g, respectively. The harvest index, estimated as the ratio of seed weight to total above-ground dry matter, was 53.9% for the WT, 52.5% for line '#3', and 52.7% for line '#10'. Neither the 100-seed weight nor the harvest index differed significantly among the transgenic and WT samples. For the compositional analyses, dried seed samples were homogenized by grinding to a fine powder and then stored at 4 °C.

2.3. Analysis of chemical compositions

2.3.1. Proximates

The contents of moisture, crude protein, crude fat, crude ash, and crude fibre were determined according to the AOAC official methods. Briefly, moisture content was obtained by calculating the weight loss after powdered seed samples were oven-dried at 105 °C to a constant weight (AOAC, 2000). Crude protein and crude fat were analyzed by the Kjeldahl and the Soxhlet extraction methods, respectively (AOAC, 2005, 2006). Ash content was determined gravimetrically after the samples were ignited in an oven at 600 °C (AOAC, 2005). Crude fibre was estimated based on the loss upon ignition of the dried residue after samples were digested with 1.25% each of sulfuric acid and sodium hydroxide solutions (AOAC, 2006). Carbohydrate contents were calculated by the following equation:% carbohydrates = 100% − (% crude protein + % crude fat + % crude ash).

2.3.2. Amino acids

All amino acids were analyzed according to the procedure stipulated by the Korean Food Code (MFDS, 2011). The samples were hydrolyzed in aqueous hydrochloric acid and placed in an automatic Biochrom 30 Amino Acid Analyzer (Biochrom, Cambridge, UK). For tryptophan, levels were measured by a high-performance liquid chromatography (HPLC) system (1100 Series; Agilent, Santa Clara, CA, USA) after hydrolysis with a sodium hydroxide solution.

2.3.3. Fatty acids

Individual fatty acids were prepared by lipid extraction and saponification, followed by methylation, according to the AOAC method (AOAC, 2005). Samples were then evaluated with a gas chromatograph (7890 GC; Agilent; see Supplementary Fig. S1).

2.3.4. Isoflavones

Isoflavones were extracted with a hydrochloric acid/alcohol solution based on the procedure described by Akitha Devi et al. (2009). The extracts were heat-refluxed, concentrated, and analyzed using an HPLC system (1200 Series; Agilent; see Supplementary Fig. S2). Samples were quantified by comparing their areas with an external standard curve containing daidzein, genistein, and glycitein.

2.3.5. Antinutrients

The contents of raffinose and stachyose were determined according to a procedure specified by the Korean Food Code (MFDS, 2011). After the samples were extracted with distilled water, acetonitrile was added. The mixture was then filtered and analyzed using an HPLC system (1100 Series). Phytic acids were extracted with 3% trichloroacetic acid according to the modified protocol of Wheeler and Ferrel (1971). Their contents were determined through analysis with an ultraviolet–visible (UV–Vis) spectrophotometer (Optizen 2120 UV Plus; Mecasis, Daejeon, Korea),

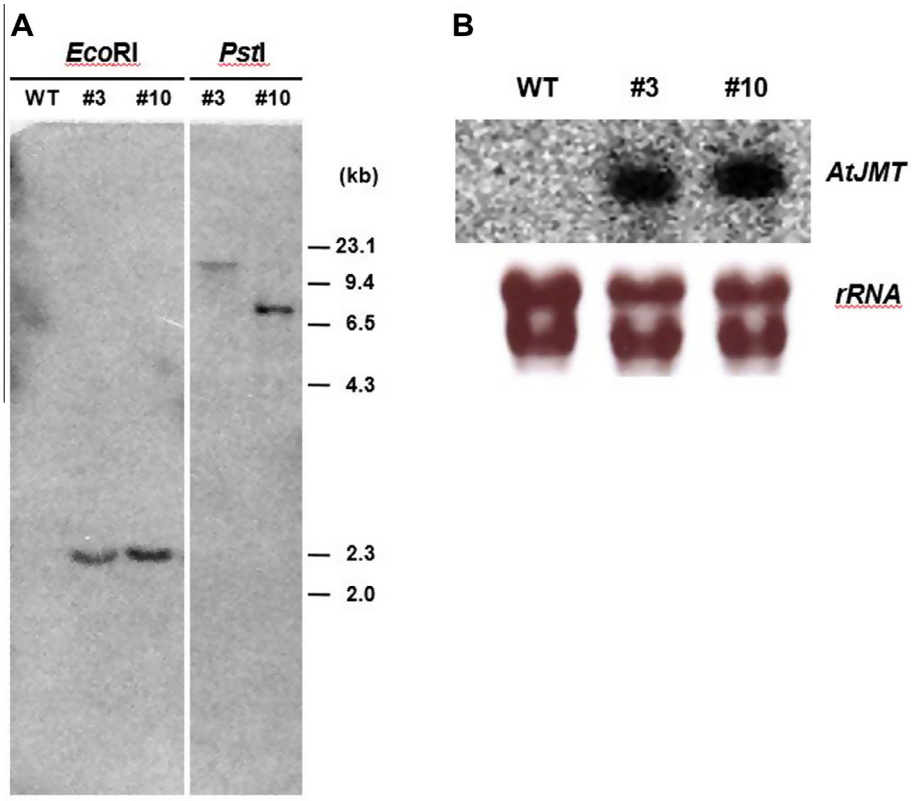

Fig. 1. Southern and northern blot analyses of transgenic soybeans over-expressing *AtJMT*. (A) Genomic DNA isolated from leaves of wild type (WT) and T_3 transgenic lines #3 and #10 was digested with *Eco*RI or *Pst*I and separated by agarose gel electrophoresis. A region of unrelated lines was excised from the blot for clarification, leaving a gap in the gel between areas for each restriction enzyme. (B) Total RNA isolated from WT and T_3 transgenic lines #3 and #10 was separated on formaldehyde agarose gel and blots were verified with random primer-extended cDNA.

where absorbance was measured at 480 nm. Trypsin inhibitor activity (TIA) in the seeds was monitored according to the method of Anderson and Wolf (1995). Samples extracted in 0.01 N sodium hydroxide were mixed with trypsin and benzoyl-DL-arginine-*p*-nitroanilide hydrochloride, and absorbance was read at 410 nm. Values for TIA were expressed as trypsin inhibitor units per milligram of extracted sample.

2.4. Statistical analysis

All statistical analyses were conducted using STATISTICA (Version 8.0; StatSoft Inc., Tulsa, OK, USA). Significant differences were examined by one-way ANOVA, followed by Tukey's HSD tests ($P < 0.05$).

3. Results and discussion

Chemical composition is the main consideration when analyzing the safety of food derived from genetically modified (GM) crops. Such assessments utilize a comparative approach to evaluate substantial equivalences between GM products and their corresponding untransformed controls (OECD, 1993). We compared the levels of key nutrients, antinutrients, and antioxidants between transgenic soybean over-expressing *AtJMT* and its WT counterpart. The compositional profiles for transgenic soybean were also compared with values previously reported (OECD, 2012).

3.1. Proximates

Levels of moisture, crude protein, crude fat, crude ash, and carbohydrates did not differ significantly between the transgenic lines and WT soybeans (Table 1). Although crude fibre contents varied significantly among genotypes, Tukey's HSD tests did not show any statistical differences between the means.

Table 1
Proximate compositions for soybean seeds from *AtJMT*-transgenic lines (#3 and #10) and wild-type (Bert) counterpart.

Component	*P*-value	AtJMT #3	AtJMT #10	Bert	Ref. range[A]
Moisture	ns	7.2 ± 0.2	7.3 ± 0.2	7.2 ± 0.3	4.7–34.4
Crude protein	ns	31.8 ± 1.1	32.5 ± 0.9	32.3 ± 0.7	33.2–46.2
Crude fat	ns	18.6 ± 1.5	18.2 ± 1.7	17.5 ± 1.9	8.1–27.4
Crude ash	ns	5.8 ± 0.1	5.7 ± 0.3	5.7 ± 0.1	3.9–7.0
Crude fiber	*	11.5 ± 1.4[a]	14.6 ± 1.6[a]	11.4 ± 3.4[a]	5.8–7.8
Carbohydrates	ns	36.6 ± 1.2	36.3 ± 1.8	37.3 ± 2.1	26.0–50.2

All data are expressed as % dry weight of sample except for moisture, which is % fresh weight. Data are means ($n = 6$) ± standard deviations. *P*-values were obtained from one-way ANOVA (ns, not significant; *, $P < 0.05$). Within a row, values are not significantly different (Tukey's HSD test, $P < 0.05$).
[A] Source: OECD (2012).

3.2. Amino acids

Except for phenylalanine and tryptophan, levels of amino acids did not differ significantly between genotypes (Table 2). The tryptophan content was 8.2% lower for transgenic line '#3' than for the WT. Tukey's HSD tests revealed significant differences in levels of phenylalanine between the two transgenic lines but not between the WT and either transgenic line. Although tryptophan contents differed significantly between transgenic and non-transgenic soybean ($P < 0.05$), all measured amounts were within reference

Table 2
Amino acid compositions (g per 100 g dry weight) for soybean seeds from *AtJMT*-transgenic lines (#3 and #10) and wild-type (Bert) counterpart.

Component	*P*-value	AtJMT #3	AtJMT #10	Bert	Ref. range[A]
Alanine	ns	1.39 ± 0.04	1.40 ± 0.04	1.41 ± 0.02	1.51–2.10
Arginine	ns	2.36 ± 0.10	2.40 ± 0.10	2.38 ± 0.08	2.19–3.62
Aspartic acid	ns	3.69 ± 0.15	3.75 ± 0.10	3.73 ± 0.09	3.81–5.59
Cysteine	ns	0.20 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.37–0.81
Glutamic acid	ns	5.82 ± 0.22	5.97 ± 0.18	5.92 ± 0.17	5.84–8.61
Glycine	ns	1.35 ± 0.03	1.36 ± 0.04	1.37 ± 0.03	1.46–2.06
Histidine	ns	0.86 ± 0.03	0.87 ± 0.06	0.87 ± 0.01	0.85–1.26
Isoleucine	ns	1.49 ± 0.05	1.51 ± 0.04	1.51 ± 0.03	1.53–2.16
Leucine	ns	2.52 ± 0.08	2.59 ± 0.06	2.58 ± 0.06	2.59–3.62
Lysine	ns	2.14 ± 0.10	2.11 ± 0.05	2.06 ± 0.23	1.56–2.87
Methionine	ns	0.45 ± 0.02	0.45 ± 0.02	0.44 ± 0.01	0.43–0.74
Phenylalanine	*	1.60 ± 0.06[a]	1.68 ± 0.05[b]	1.66 ± 0.03[ab]	1.63–2.45
Proline	ns	1.63 ± 0.09	1.72 ± 0.09	1.65 ± 0.06	1.68–2.60
Serine	ns	1.70 ± 0.05	1.72 ± 0.05	1.72 ± 0.04	1.10–2.48
Threonine	ns	1.20 ± 0.03	1.20 ± 0.03	1.21 ± 0.01	1.14–1.93
Tryptophan	**	0.35 ± 0.01[a]	0.41 ± 0.03[b]	0.38 ± 0.01[b]	0.36–0.63
Tyrosine	ns	1.07 ± 0.03	1.10 ± 0.03	1.11 ± 0.03	1.01–1.68
Valine	ns	1.59 ± 0.05	1.61 ± 0.05	1.60 ± 0.03	1.55–2.22

Data are means ($n = 6$) ± standard deviations. *P*-values were obtained from one-way ANOVA (ns, not significant; *, $P < 0.05$; **, $P < 0.01$). Within a row, values followed by the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).
[a] Source: OECD (2012).

Table 3
Fatty acid compositions (% of total fatty acids) for soybean seeds from *AtJMT*-transgenic lines (#3 and #10) and wild-type (Bert) counterpart.

Component	*P*-value	AtJMT #3	AtJMT #10	Bert	Ref. range[A]
C14:0	ns	0.09 ± 0.00	0.09 ± 0.00	0.11 ± 0.02	
C16:0	***	10.95 ± 0.05[a]	11.15 ± 0.10[b]	11.30 ± 0.09[c]	8.00–15.39
C18:0	ns	3.71 ± 0.07	3.67 ± 0.08	3.72 ± 0.08	0.53–9.45
C18:1	ns	23.67 ± 1.43	24.64 ± 0.73	22.92 ± 1.44	15.18–33.95
C18:2	ns	53.10 ± 1.29	51.92 ± 0.67	53.08 ± 1.16	38.74–59.82
C18:3	**	6.98 ± 0.15[a]	6.97 ± 0.06[a]	7.34 ± 0.25[b]	2.02–11.17
C20:0	ns	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.10–0.96
C20:1	***	0.21 ± 0.00[b]	0.21 ± 0.01[b]	0.20 ± 0.00[a]	
C22:0	*	0.36 ± 0.01[a]	0.36 ± 0.02[a]	0.34 ± 0.01[a]	
C24:0	ns	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	

Data are means ($n = 6$) ± standard deviations. *P*-values were obtained from one-way ANOVA (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Within a row, values followed by the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).
[A] Source: OECD (2012).

ranges, indicating that this difference was not considered biologically relevant. Tryptophan is a common precursor of phytohormone auxins that are involved in many aspects of plant growth and development. Studies on the role of phytohormones in modulating defense mechanisms have revealed that auxins can make plants more susceptible to diseases by antagonizing other hormone signaling pathways (Robert-Seilaniantz, Grant, & Jones, 2011). Therefore, the reduction of tryptophan in overexpressing transgenic soybean causes a subsequent decline in auxin levels, which may then lead to an enhancement of defense responses in plants.

3.3. Fatty acids

Levels for six out of ten individual fatty acids were similar between transgenic soybean and the corresponding control (Table 3). However, when compared with the WT, overexpressing lines '#3' and '#10' had 3.1% and 1.3% less palmitic acid (C16:0) and 4.9% and 5.0% less linolenic acid (18:3), respectively, while their levels of gadoleic acid (20:1) were 5.9% and 5.7% higher, respectively. Whereas all of those differences were significant, Tukey's HSD tests for behenic acid (C22:0) showed no significant difference between WT and transgenic lines. The values for all ten fatty acids measured in transformed plants were within the reported reference ranges.

3.4. Isoflavones

Isoflavone contents in soybean seeds are affected by numerous environmental factors, and can also vary by genotype (Tsukamoto et al., 1995). Based on OECD guidelines, the recommended levels of total isoflavone in soybean range from 678.7 to 9797.9 $\mu g\ g^{-1}$ (OECD, 2012). We analyzed three major isoflavone aglycones – daidzein, genistein, and glycitein – and found that only genistein differed significantly, showing levels being 130.5% and 120.0% higher in lines #3 and #10, respectively, than in the WT (Table 4).

Previous studies with *Lupinus albus* and *Pueraria candollei* have shown that exogenous JA applications can strongly induce an increase in isoflavone levels (Katagiri, Hashidoko, Ibrahim, & Tahara, 2001; Korsangruang, Soonthornchareonnon, Chintapakorn, Saralamp, & Prathanturarug, 2010). Furthermore, MeJA can promote the transcription of an isoflavone-biosynthetic gene and the production of flavonoids in plants (Gundlach, Muller, Kutchan, & Zenk, 1992). Therefore, we would have expected to find considerably enhanced accumulations of genistein in our overexpressing soybean lines, even though all measured values of isoflavones were well within the reference ranges reported by the OECD. Although the elevated levels of isoflavone may not be problematic from a safety standpoint, this response deserves attention because the status of isoflavone in soybean has a critical impact on its nutritive functions. As soybean seeds are rich in isoflavones, these food products may have a crucial role in preventing oxidation-related diseases in humans (Park, Shin, Park, & Kang, 2005). In particular, genistein appears to interfere with estrogen- and androgen-mediated carcinogenesis and to suppress tumor angiogenesis and metastasis (Banerjee, Li, Wang, & Sarkar, 2008). Dietary intake of genistein also diminishes the impact of complex metabolic disorders, e.g., diabetes, obesity, osteoporosis, and cardiovascular diseases. Biosynthesis of isoflavone is controlled by the phenylpropanoid pathway and this metabolic process is correlated with the defense system in plants (Yu & McGonigle, 2005). The generation of isoflavones is stimulated by insect and pathogen attacks, as well as in response to environmental stresses (Lozovaya et al., 2005; Naoumkina et al., 2007). Accordingly, our findings lead

Table 4
Isoflavone compositions (μg g^{-1} dry weight) for soybean seeds from *AtJMT*-transgenic lines (#3 and #10) and wild-type (Bert) counterpart.

Component	*P*-value	AtJMT #3	AtJMT #10	Bert	Ref. range[A]
Daidzein	ns	38.6 ± 13.4	39.2 ± 7.6	51.8 ± 10.4	0.1–153.5
Genistein	*	9.9 ± 2.6[b]	9.5 ± 2.9[b]	4.3 ± 4.1[a]	0.5–31.5
Glycitein	ns	26.5 ± 3.5	27.5 ± 3.0	30.9 ± 5.5	6.7–58.7

Data are means ($n = 6$) ± standard deviations. *P*-values were obtained from one-way ANOVA (ns, not significant; *, $P < 0.05$). Within a row, values followed by the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).
[A] Source: OECD (2012).

Table 5
Contents (g 100 g^{-1} dry weight) of raffinose, stachyose, and phytic acid, as well as trypsin inhibitor activity (TIA) in soybean seeds from *AtJMT*-transgenic lines (#3 and #10) and wild-type (Bert) counterpart.

Component	*P*-value	AtJMT #3	AtJMT #10	Bert	Ref. range[A]
Raffinose	ns	0.3 ± 0.3	0.2 ± 0.3	0.7 ± 0.3	0.1–1.6
Stachyose	**	1.3 ± 0.7[a]	0.6 ± 1.1[a]	3.2 ± 1.0[b]	0.6–5.1
Phytic acid	ns	1.9 ± 0.1	2.0 ± 0.2	2.1 ± 0.1	0.6–2.5
TIA[B]	*	40.6 ± 6.7[b]	31.6 ± 4.3[a]	37.3 ± 3.4[ab]	19.6–184.0

Data are means ($n = 6$) ± standard deviations. *P*-values were obtained from one-way ANOVA (ns, not significant; *, $P < 0.05$; **, $P < 0.01$). Within a row, values followed by the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).
[A] Source: OECD (2012).
[B] Expressed in trypsin inhibitor units per milligram of extracted sample.

us to suggest that this increase in the endogenous level of MeJA in transgenic soybeans that over-express *AtJMT* is sufficient to activate the defense system that then promotes isoflavone biosynthesis.

3.5. Antinutrients

Soybean seeds contain diverse anti-nutritional factors, e.g. a trypsin inhibitor, phytic acid, lectin, raffinose, and stachyose. Therefore, we must quantify the antinutrients present in soybeans because of their deleterious effects on human health. Higher levels of antinutrients reduce nutritional value, as well as the availability and digestibility of soybean protein (Herkelman, Cromwell, Stahly, Pfeiffer, & Knabe, 1992; Liener, 1994). We compared the seed contents of raffinose, stachyose, phytic acid, and trypsin inhibitor between transgenic soybean and the WT and found that they differed significantly for stachyose and the trypsin inhibitor (Table 5). Tukey's HSD tests indicated that stachyose levels were 60.5% and 82.1% lower for transgenic lines '#3' and '#10', respectively, than for the WT. For the trypsin inhibitor, however, levels did not differ significantly between the WT and transgenic lines, but differences were significant between the two transgenic lines when compared to each other. Zalewski et al. (2010) have shown that treating *Lupinus* seeds with a low level of MeJA leads to reduced raffinose contents. Bogatek, Côme, Corbineau, Ranjan, and Lewak (2002) have demonstrated that JA participates in the regulation of oligosaccharide hydrolysis and the catabolism of its products. We noted here that, although overexpression of *AtJMT* resulted in decreased stachyose accumulations, all levels measured in the seeds from both transgenic and non-transgenic soybean plants were within the OECD-specified range. Few studies have been conducted to elucidate any clear relationships between antinutrients and jasmonates. However, our data seem to indicate that MeJA significantly controls stachyose contents in soybean seeds. Stachyose can adversely affect the energy availability in swine and poultry due to gas production and resulting flatulence (OECD, 2012). Therefore, reducing the stachyose content in our transgenic soybeans may help to improve nutrient absorption if flatulence and indigestion are prevented.

4. Conclusions

Based on the principle of substantial equivalence, we compared chemical compositions between transgenic soybean over-expressing *AtJMT* and its non-transgenic counterpart. Levels of proximates, amino acids, fatty acids, isoflavones, and antinutrients were similar between all tested genotypes. However, the amounts of gadoleic acid and genistein were higher while those of tryptophan, palmitic acid, linolenic acid, and stachyose were lower in both transgenic lines. In particular, transgenic soybeans had 120–130.5% more genistein and 60.5–82.1% less stachyose than the WT. Nevertheless, all values for key nutrients, antinutrients, and antioxidants measured in transgenic soybeans were within the reference ranges reported for commercially available soybeans. Therefore, we conclude that transgenic soybeans over-expressing *AtJMT* are compositionally equivalent to those of conventionally produced seed. We also believe that researchers can use such responses by *AtJMT*-overexpressing plants to improve soybean seed quality by enhancing genistein contents while simultaneously reducing their levels of stachyose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.09.046.

References

Akitha Devi, M. K., Gondi, M., Sakthivelu, G., Giridhar, P., Rajasekaran, T., & Ravishankar, G. A. (2009). Functional attributes of soybean seeds and products, with reference to isoflavone content and antioxidant activity. *Food Chemistry, 114*, 771–776.
Albrechtová, J. P. T., & Ullmann, J. (1994). Methyl jasmonate inhibits growth and flowering in *Chenopodium rubrum*. *Biologia Plantarum, 36*, 317–319.
Anderson, R. L., & Wolf, W. J. (1995). Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. *The Journal of Nutrition, 125*, 581–588.
AOAC (2000). *Official Methods of Analysis* (17th ed.). Gaithersburg, MD, USA: Association of Official Analytical Chemists.
AOAC (2005). *Official Methods of Analysis* (18th ed.). Gaithersburg, MD, USA: Association of Official Analytical Chemists.
AOAC (2006). *Official Methods of Analysis* (18th ed.). Gaithersburg, MD, USA: Association of Official Analytical Chemists.
Banerjee, S., Li, Y., Wang, Z., & Sarkar, F. H. (2008). Multi-targeted therapy of cancer by genistein. *Cancer Letters, 269*, 226–242.
Berman, K. H., Harrigan, G. G., Riordan, S. G., Nemeth, M. A., Hanson, C., Smith, M., et al. (2009). Compositions of seed, forage, and processed fractions from insect-protected soybean MON 87701 are equivalent to those of conventional soybean. *Journal of Agricultural and Food Chemistry, 57*, 11360–11369.
Bogatek, R., Côme, D., Corbineau, F., Ranjan, R., & Lewak, S. (2002). Jasmonic acid affects dormancy and sugar catabolism in germinating apple embryos. *Plant Physiology and Biochemistry, 40*, 167–173.
Creelman, R. A., & Mullet, J. E. (1997). Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology, 48*, 355–381.
Farmer, E. E., & Ryan, C. A. (1990). Interplant communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Sciences of the United States of America, 87*, 7713–7716.
Friedman, M., & Brandon, D. L. (2001). Nutritional and health benefits of soy proteins. *Journal of Agricultural and Food Chemistry, 49*, 1069–1086.
Gayen, D., Sarkar, S. N., Datta, S. K., & Datta, K. (2013). Comparative analysis of nutritional compositions of transgenic high iron rice with its non-transgenic counterpart. *Food Chemistry, 138*, 835–840.

Gundlach, H., Muller, M. J., Kutchan, T. M., & Zenk, M. H. (1992). Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Proceedings of the National Academy of Sciences of the United States of America, 89*, 2289–2393.

Herkelman, K. L., Cromwell, G. L., Stahly, T. S., Pfeiffer, T. W., & Knabe, D. A. (1992). Apparent digestibility of amino acids in raw and heated conventional and low trypsin inhibitor soybean for pigs. *Journal of Nutritional Science, 70*, 818–826.

Jang, G., Shim, J. S., Jung, C., Song, J. T., Lee, H. Y., Chung, P. J., et al. (2014). Volatile methyl jasmonate is a transmissible form of jasmonate and its biosynthesis is involved in systemic jasmonate response in wounding. *Plant Biotechnology Report, 8*, 409–419.

Katagiri, Y., Hashidoko, Y., Ibrahim, R. K., & Tahara, S. (2001). Activation of isoflavone biosynthesis in excised cotyledons of *Lupinus* seedlings by jasmonoids and excess light. *Zeitschrift für Naturforschung, 56*, 1038–1046.

Kim, Y. S., Han, J. Y., Lim, S., Kim, H. J., Lee, M. H., & Choi, Y. E. (2012). Overexpressing Arabidopsis jasmonic acid carboxyl methyltransferase (*AtJMT*) results in stimulation of root growth and ginsenoside heterogeneity in *Panax ginseng*. *Plant Omics Journal, 5*, 28–32.

Kim, E. H., Kim, Y. S., Park, S. H., Koo, Y. J., Choi, Y. D., Chung, Y. Y., et al. (2009). Methyl jasmonate reduces grain yield by mediating stress signals to alter spikelet development in rice. *Plant Physiology, 149*, 1751–1760.

Koda, Y. (1997). Possible involvement of jasmonates in various morphogenic events. *Physiologia Plantarum, 100*, 639–646.

Korsangruang, S., Soonthornchareonnon, N., Chintapakorn, Y., Saralamp, P., & Prathanturarug, S. (2010). Effects of abiotic and biotic elicitors on growth and isoflavonoid accumulation in *Pueraria candollei* var. *candollei* and *P. candollei* var. *mirifica* cell suspension cultures. *Plant Cell, Tissue and Organ Culture, 103*, 333–342.

Liener, I. E. (1994). Implications of antinutritional components in soybean. *Critical Reviews in Food Science and Nutrition, 34*, 31–67.

Lozovaya, V. V., Lygin, A. V., Ulanov, A. V., Nelson, R. L., Daydé, J., & Widholm, J. M. (2005). Effect of temperature and soil moisture status during seed development on soybean seed isoflavone concentration and composition. *Crop Science, 45*, 1934–1940.

McCann, M. C., Liu, K., Trujillo, W. A., & Dobert, R. C. (2005). Glyphosate-tolerant soybeans remain compositionally equivalent to conventional soybeans (*Glycine max* L.) during three years of field testing. *Journal of Agricultural and Food Chemistry, 53*, 5331–5335.

MFDS (2011). *Korean Food Code*. Cheongwon, Korea: Ministry of Food and Drug Safety.

Moon, T. H., Cheong, J. J., Kim, C. H., Koo, Y. J., Jung, C., Seo, J. S., et al. (2011). *Development of Transgenic Crops Resistant to Biological and Environmental Stresses*. Seoul, Korea: Ministry of Education, Science and Technology.

Naoumkina, M., Farag, M. A., Sumner, L. W., Tang, Y., Liu, C. J., & Dixon, R. A. (2007). Different mechanisms for phytoalexin induction by pathogen and wound signals in *Medicago truncatula*. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 17909–17915.

OECD (1993). *Safety Evaluation of Foods derived by Modern Biotechnology, Concepts and Principles*. Paris, France: Organization for Economic Co-operation and Development.

OECD (2012). *Revised Consensus Document on Compositional Considerations for New Varieties of Soybean [Glycine max (L.) Merr.]: Key Food and Feed Nutrients, Anti-nutrients, Toxicants and Allergens*. Organization for Economic Co-operation and Development.

Park, E., Shin, J. I., Park, O. J., & Kang, M. H. (2005). Soy isoflavone supplementation alleviates oxidative stress and improves systolic blood pressure in male spontaneously hypertensive rats. *Journal of Nutritional Science and Vitaminology, 51*, 254–259.

Reymond, P., & Farmer, E. E. (1998). Jasmonate and salicylate as global signals for defense gene expression. *Current Opinion in Plant Biology, 1*, 404–411.

Robert-Seilaniantz, A., Grant, M., & Jones, J. D. G. (2011). Hormone crosstalk in plant disease and defense: More than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology, 49*, 317–343.

Seo, H. S., Song, J. T., Cheong, J. J., Lee, Y. H., Lee, Y. W., Hwang, I., et al. (2001). Jasmonic acid carboxyl methyltransferase: A key enzyme for jasmonate-regulated plant responses. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 4788–4793.

Sohn, H. B., Lee, H. Y., Seo, J. S., Jung, C., Jeon, J. H., Kim, J. H., et al. (2011). Overexpression of jasmonic acid carboxyl methyltransferase increases tuber yield and size in transgenic potato. *Plant Biotechnology Report, 5*, 27–34.

Tamogami, S., Pakwal, R., & Agrawal, G. K. (2008). Interplant communication: Airborne methyl jasmonate is essentially converted into JA and JA-Ile activating jasmonate signaling pathway and VOCs emission. *Biochemical and Biophysical Research Communications, 376*, 723–727.

Tsukamoto, C., Shimada, S., Igita, K., Kudou, S., Kokubun, M., Okubo, K., et al. (1995). Factors affecting isoflavone content in soybean seeds: Changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. *Journal of Agricultural and Food Chemistry, 43*, 1184–1192.

Wasternack, C., & Parthier, B. (1997). Jasmonate-signalled plant gene expression. *Trends in Plant Science, 2*, 302–307.

Wheeler, E. L., & Ferrel, R. E. (1971). Method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry, 48*, 312–316.

Yu, O., & McGonigle, B. (2005). Metabolic engineering of isoflavone biosynthesis. *Advances in Agronomy, 86*, 147–190.

Zalewski, K., Nitkiewicz, B., Lahuta, L. B., Głowacka, K., Socha, A., & Amarowicz, R. (2010). Effect of jasmonic acid-methyl ester on the composition of carbohydrates and germination of yellow lupine (*Lupinus luteus* L.) seeds. *Journal of Plant Physiology, 167*, 967–973.