

Comparison of the Nutritional Compositions of Insect-Resistant and Glufosinate-Tolerant Rice and Conventional Rice

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Received: 7 June 2012 / Accepted: 8 October 2012 / Published Online: 31 March 2013
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Abstract The nutritional composition of transgenic rice (Agb0101) with *bar* and modified *cry1Ac1* genes grown with herbicides was compared with that of its non-transgenic counterpart. The analyzed components (proximates, amino acids, fatty acids, minerals, vitamins, trypsin inhibitors, and phytic acid) in the herbicide-treated Agb0101 brown rice were substantially equivalent to those of its non-transgenic counterpart.

Keywords compositional analysis · herbicide tolerance · insect resistance · substantial equivalence · transgenic rice

The insecticidal genes of *Bacillus thuringiensis* (Bt) are widely used for the production of insect-resistant plants (Maqbool et al., 2001; Bajaj and Mohanty, 2005), with *cry1Ab* and *cry1Ac* being the most commonly used Bt genes in transgenic crops. The genes encode the production of crystal (Cry) protein, which exhibits a toxic effect by penetrating the cell membrane and forming pores, resulting in injury to the midgut epithelial cells of some insects. The effect of Bt insecticidal proteins is highly specific to target insects while being harmless to plants and mammals (Bravo et al., 2007). In Korea, the Rural Development Administration (RDA) developed genetically modified rice (Agb0101) containing the modified *cry1Ac1* gene, which displayed high resistance to rice leaf folder under field conditions (Kim et al., 2009). In addition, to achieve herbicide-tolerance, the *bar* gene from *Streptomyces hygroscopicus* was also introduced into the rice genome. Phosphinothricin-*N*-acetyltransferase (PAT) encoded by the *bar* gene catalyzes acetylation of the free amino group of phosphinothricin,

thereby detoxifying the herbicide L-phosphinothricin (glufosinate) into an inactive compound (Oberdoerfer et al., 2005).

Assessing the safety of genetically modified (GM) plants is essential for commercialization and strictly required for the use of GM plants in food. This assessment is based on the ‘substantial equivalence’ concept formulated by the Organization for Economic Cooperation and Development (OECD) in 1993. Further details were added by the Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). Assessing substantial equivalence entails comparing the GM crop with its non-transgenic counterpart to identify possible differences. Substantial equivalence is not a safety assessment *per se*, but is a starting point from which to assess transgenic food safety.

Composition analysis is an important consideration in the evaluation of substantial equivalence. The OECD consensus documents have identified key food and feed nutrients and anti-nutrients required for the safety study of new rice varieties (OECD, 2004). In accordance with the guidelines, we analyzed nutrients and anti-nutritive components of Agb0101 grown in 2009 and confirmed that nutritional quality of Agb0101 was substantially equivalent to that of its non-transgenic counterpart (Park et al., 2012). The Codex guidelines have stated that trials should be conducted over a sufficient number of generations at multiple sites to allow accurate assessment of compositional characteristics. In the case of herbicide tolerance, comparisons involving the GM plants grown with herbicide applications may also need to be considered (Codex Alimentarius, 2009). Comparative compositional analyses have been previously reported for transgenic rice containing a *bar* gene (Li et al., 2008; Choi et al., 2012). However, herbicide applications to this transgenic rice have not been reported. Therefore, in this study we analyzed the nutritional composition and anti-nutrient content of Agb0101 grown under application of the herbicide glufosinate and then assessed whether the nutritional quality of Agb0101 is altered in the generations

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following herbicide treatment.

Seedlings of the GM rice and its parent cultivar (*Oryza sativa* L., cv. Nakdongbyeo) were planted in adjoining fields in 2010 under the same field management conditions. Two months after transplantation, glufosinate ammonium herbicide (Basta, Bayer Crop Science, Germany) was applied at 108 g active ingredient/ha as a single application. After harvest, the whole grain (rough rice) samples were dried and then manually hulled and ground to obtain a fine powder using a cyclone mixer mill (HMF-590, Hanil, Korea) and a mortar and pestle. The powder was used in compositional analyses, which were conducted according to experimental procedures described in our previous report (Park et al., 2012). The nutritional composition of herbicide-treated Agb0101 was then compared with that of its conventional counterpart. Equivalence tests were used to determine whether the differences in treatments exceeded the range of normal variation in the comparator. We used the two one-sided tests (TOST) procedure for equivalence testing. The statistical analysis was carried out using the SAS 9.2 software package (SAS Institute, USA). In the TOST procedure, the null hypothesis tested was “Treatment 1 is not equivalent to treatment 2” versus the alternative hypothesis “Treatment 1 is equivalent to treatment 2.” To test for equivalence, the 90% confidence intervals for the differences between the two treatments were constructed. The confidence intervals $100(1-2 \times \alpha)\%$ ($=90\%$), where $\alpha=0.05$, were calculated in pairs for the treatment differences. Thus, the 90% confidence interval method is equivalent to carrying out TOST at a 5% significance level. Equivalence boundaries were set to $\pm 20\%$ of the means according to the recommendation by the Nordic Council of Ministers (Nordic Council, 2000).

The main nutrient and amino acid compositions are described in Tables 1 and 2. Crude protein content was estimated by determining the total nitrogen using the Kjeldahl method (AOAC, 2005b), and crude fat was analyzed by the Soxhlet extraction method (AOAC, 2000a). Ash content was determined by gravimetric measurement of the sample residue after ignition in an oven at 600°C until a constant weight was reached (AOAC, 2005a). Total

dietary fiber was quantified as the weight of residue minus the weight of protein and ash after digestion of the sample with α -amylase, protease, and amyloglucosidase (Prosky et al., 1988). For the analysis of the sulfur-containing amino acids cysteine and methionine, samples were oxidized with performic acid followed by hydrolysis with hydrochloric acid. The remaining 15 amino acids were analyzed using an automatic amino acid analyzer directly after protein hydrolysis with hydrochloric acid (AOAC, 2005c). No significant differences were found in the mean values of protein, lipid, ash, dietary fiber, carbohydrate, and amino acids between Agb0101 and herbicide-treated Agb0101 compared with their non-transgenic counterpart. Although total dietary fiber levels were slightly high, the values of main nutrients and amino acids did not deviate considerably from ranges of commercial rice provided by the OECD (2004).

The individual fatty acid content was determined after lipid extraction and saponification with 0.5 N sodium hydroxide in methanol. The saponification mixture was methylated using 14% boron trifluoride/methanol, and the resulting methyl esters were extracted with pentane. The methyl esters of the fatty acids were analyzed by gas chromatography (AOCS, 1997b). Pentadecanoic acid was used as an internal standard. As shown in Table 3, the statistical analyses revealed that Agb0101, herbicide-treated Agb0101, and non-transgenic rice contained equivalent fatty acids. Although a consensus document published by the OECD was not available, the measured values of fatty acids fell within the ranges of values observed in the six typical Korean varieties as reported by Choe et al. (2002).

Levels of the minerals listed in Table 4 were determined by inductively coupled plasma optical emission spectrometry (Integra XL Inductively Coupled Plasma Optical Emission Spectrometer, GBC Co., Australia) according to AOAC method 999.11 (AOAC, 2000b). Extractions of vitamin B₁ and B₂ were performed according to the method of Sims and Shoemaker (1993) and Esteve et al. (2001). Vitamins were determined using a high-performance liquid chromatography method with fluorometric detection. Niacin and vitamin E were detected as described by

Table 1 Comparison and analysis of equivalence of the proximates measured in brown rice¹⁾

Component	<i>p</i> value ²⁾	Non-transgenic	Agb0101	Analysis of equivalence ³⁾	Agb0101 herbicide	Analysis of equivalence	OECD ⁴⁾
Protein	<0.05	7.08±0.27 ⁵⁾	7.09±0.27	yes	6.98±0.41	yes	7.1–8.3
Lipid	<0.05	2.19±0.26	1.97±0.19	yes	2.03±0.24	yes	1.6–2.8
Ash	<0.05	1.26±0.05	1.36±0.10	yes	1.39±0.05	yes	1.0–1.5
Total dietary fiber	<0.05	3.62±0.22	3.70±0.35	yes	3.91±0.26	yes	3.0
Carbohydrates ⁶⁾	<0.05	89.47±0.32	89.55±0.40	yes	89.39±0.58	yes	87.4–90.3

¹⁾The values of proximate were calculated as percentage of dry matter (sample contained 10% moisture).

²⁾On the basis of the TOST, confidence intervals $100(1-2 \times \alpha)\%$, where $\alpha=0.05$, were calculated for equivalence assessment. In the equivalence study, the null hypothesis tested was “Treatment 1 is not equivalent to treatment 2” versus the alternative hypothesis “Treatment 1 is equivalent to treatment 2.”

³⁾The criterion for equivalence (yes) is met when the 90% confidence interval of the difference does not exceed the 20% range of the reference (cv. Nakdongbyeo). If the 90% confidence interval of the difference exceeds the 20% range of the reference (cv. Nakdongbyeo), this is indicated by “no”.

⁴⁾Source: OECD data.

⁵⁾Each value is the mean \pm standard deviation ($n=10$).

⁶⁾Carbohydrates levels were estimated by the following formula: % carbohydrates = $100 - (\% \text{ protein} + \% \text{ lipid} + \% \text{ ash})$.

Table 2 Comparison and analysis of equivalence of the amino acids measured in brown rice¹⁾

Component	<i>p</i> value ²⁾	Non-transgenic	Agb0101	Analysis of Equivalence ³⁾	Agb0101 herbicide	Analysis of equivalence	OECD ⁴⁾
Alanine	<0.05	5.66±0.10 ⁵⁾	5.60±0.12	yes	5.62±0.29	yes	5.8
Arginine	< 0.05	8.53±0.62	7.74±0.57	yes	8.04±0.39	yes	8.5–10.5
Aspartic acid	< 0.05	9.24±0.15	9.24±0.22	yes	9.27±0.29	yes	9.0, 9.5
Cysteine	< 0.05	2.51±0.12	2.55±0.15	yes	2.61±0.10	yes	2.2–2.4
Glutamic acid	< 0.05	18.07±0.37	17.44±0.46	yes	17.54±0.49	yes	16.9, 17.6
Glycine	< 0.05	4.85±0.10	4.77±0.12	yes	4.83±0.19	yes	4.7, 4.8
Histidine	< 0.05	2.65±0.12	2.63±0.08	yes	2.67±0.11	yes	2.4, 2.6
Isoleucine	< 0.05	3.18±0.19	3.08±0.18	yes	3.04±0.19	yes	3.6–4.6
Leucine	< 0.05	7.81±0.27	7.63±0.20	yes	7.64±0.21	yes	8.3–8.9
Lysine	< 0.05	4.16±0.30	4.27±0.27	yes	4.17±0.24	yes	3.9, 4.3
Methionine	< 0.05	2.34±0.14	2.46±0.15	yes	2.56±0.23	yes	2.3, 2.5
Phenylalanine	< 0.05	4.69±0.12	4.60±0.16	yes	4.55±0.14	yes	5.0, 5.3
Proline	< 0.05	4.47±0.19	4.51±0.20	yes	4.53±0.19	yes	4.8, 5.1
Serine	< 0.05	5.22±0.16	5.06±0.17	yes	5.05±0.23	yes	4.8–5.8
Threonine	< 0.05	3.73±0.07	3.70±0.08	yes	3.71±0.12	yes	3.9–4.0
Tyrosine	< 0.05	3.99±0.10	3.86±0.21	yes	3.90±0.12	yes	3.8–4.6
Valine	< 0.05	4.67±0.19	4.56±0.28	yes	4.52±0.29	yes	5.0–6.6

¹⁾The values of amino acids were calculated as a percentage to total protein.

²⁾On the basis of the TOST, confidence intervals $100(1-2\times\alpha)\%$, where $\alpha=0.05$, were calculated for equivalence assessment. In the equivalence study, the null hypothesis tested is “Treatment 1 is not equivalent to treatment 2” versus the alternative hypothesis “Treatment 1 is equivalent to treatment 2.”

³⁾The criterion for equivalence (“yes”) is met when the 90% confidence interval of the difference does not exceed the 20% range of the reference (cv. Nakdongbyeo). If the 90% confidence interval of the difference exceeds the 20% range of the reference (cv. Nakdongbyeo), this is indicated by “no.”

⁴⁾Source: OECD data.

⁵⁾Each value is the mean \pm standard deviation (n=10).

Table 3 Comparison and analysis of equivalence of the fatty acids measured in brown rice¹⁾

Component	<i>p</i> value ²⁾	Non-transgenic	Agb0101	Analysis of equivalence ³⁾	Agb0101 herbicide	Analysis of equivalence
C14:0	< 0.05	0.31±0.03 ⁴⁾	0.35±0.05	yes	0.38±0.09	yes
C16:0	< 0.05	16.70±0.26	16.21±0.29	yes	16.28±0.24	yes
C18:0	< 0.05	0.75±0.12	0.84±0.13	yes	0.88±0.14	yes
C18:1	< 0.05	42.42±0.82	40.47±0.36	yes	40.32±0.50	yes
C18:2	< 0.05	36.36±0.54	38.24±0.49	yes	38.18±0.80	yes
C18:3	< 0.05	1.49±0.18	1.63±0.07	yes	1.62±0.07	yes
C20:0	< 0.05	0.66±0.04	0.67±0.09	yes	0.67±0.09	yes
C22:0	< 0.05	0.35±0.03	0.40±0.03	yes	0.41±0.08	yes
C24:0	< 0.05	0.90±0.09	1.03±0.15	yes	1.00±0.09	yes

¹⁾The values of fatty acids are indicated as a percentage of total fatty acids.

²⁾On the basis of the TOST, confidence intervals $100(1-2\times\alpha)\%$, where $\alpha=0.05$, were calculated for equivalence assessment. In the equivalence study, the null hypothesis tested was “Treatment 1 is not equivalent to treatment 2” versus the alternative hypothesis “Treatment 1 is equivalent to treatment 2.”

³⁾The criterion for equivalence (“yes”) is met when the 90% confidence interval of the difference does not exceed the 20% range of the reference (cv. Nakdongbyeo). If the 90% confidence interval of the difference exceeds the 20% range of the reference (cv. Nakdongbyeo), this is indicated by “no.”

⁴⁾Each value is the mean \pm standard deviation (n=10).

Kim et al. (2011; 2012). The levels of minerals and vitamins both in Agb0101 and herbicide-treated Agb0101 were very similar to those of their non-transgenic counterpart (Table 4). Their values were also statistically equivalent and, with the exception of copper and vitamin B₂, within the reference ranges reported by the OECD (2004). However, levels of both copper and vitamin B₂ were also

lower in the non-transgenic control than the range of values provided by the OECD, indicating that this is not an effect caused by either genetic modification or herbicide treatment.

Equivalence was demonstrated for anti-nutrients (Table 4). Trypsin inhibitor activity was determined using AOCS method Ba 12-75 (AOCS, 1997a). Phytic acid content was analyzed by ion

Table 4 Comparison and analysis of equivalence of the minerals, vitamins, and anti-nutrients measured in brown rice

Component	<i>p</i> value ¹⁾	Non-transgenic	Agb0101	Analysis of equivalence ²⁾	Agb0101 herbicide	Analysis of equivalence	OECD ³⁾
Minerals							
Copper, µg/g	<0.05	7.97±2.23 ⁴⁾	8.00±1.98	yes	7.56±1.78	yes	1–7
Iron, µg/g	<0.05	16.39±3.58	16.14±2.46	yes	16.24±2.30	yes	2–60
Sodium, µg/g	<0.05	194.02±42.04	205.26±53.35	yes	181.03±24.42	yes	20–400
Zinc, µg/g	<0.05	28.35±2.27	32.78±1.61	yes	31.93±1.70	yes	7–33
Calcium, mg/g	<0.05	0.16±0.04	0.16±0.01	yes	0.15±0.02	yes	0.1–0.6
Potassium, mg/g	<0.05	2.18±0.30	2.43±0.16	yes	2.73±0.31	yes	0.7–3.2
Magnesium, mg/g	<0.05	1.13±0.06	1.16±0.07	yes	1.15±0.05	yes	0.2–1.7
Phosphorus, mg/g	<0.05	3.00±0.14	3.20±0.21	yes	3.22±0.12	yes	2–5
Vitamins							
Vitamin B ₁ , µg/g	<0.05	3.81±0.31	4.18±0.35	yes	4.02±0.29	yes	2.9–6.1
Vitamin B ₂ , µg/g	<0.05	0.15±0.01	0.15±0.01	yes	0.15±0.01	yes	0.4–1.4
Niacin, µg/g	<0.05	50.69±1.77	49.63±1.22	yes	49.12±2.72	yes	35–53
Vitamin E, µg/g	<0.05	16.08±2.55	16.47±2.80	yes	16.92±3.29	yes	9–25
Anti-nutrients							
Trypsin inhibition, TIU/mg		<0.1	<0.1	yes	<0.1	yes	Not available
Phytic acid, %	<0.05	0.78±0.08	0.91±0.06	yes	0.87±0.06	yes	Not available

¹⁾On the basis of the TOST, confidence intervals $100(1-2\times\alpha)\%$, where $\alpha=0.05$, were calculated for equivalence assessment. In the equivalence study, the null hypothesis tested was “Treatment 1 is not equivalent to treatment 2” versus the alternative hypothesis “Treatment 1 is equivalent to treatment 2.”

²⁾The criterion for equivalence (“yes”) is met when the 90% confidence interval of the difference does not exceed the 20% range of the reference (cv. Nakdongbyeo). If the 90% confidence interval of the difference exceeds the 20% range of the reference (cv. Nakdongbyeo), this is indicated by “no.”

³⁾Source: OECD data.

⁴⁾Each value is the mean ± standard deviation (n =10).

exchange chromatography following AOAC 986.11 (AOAC, 2005d). Equivalence analysis showed that the two anti-nutritional factors were statistically equivalent. The inhibitory activity of trypsin inhibitors was very low in all of the rice samples compared to the 100–184 trypsin inhibitor units (TIU)/mg detected in soybean (Kakade et al., 1972).

In conclusion, the nutritional composition and anti-nutrient content of Agb0101 grown under herbicide application as studied here were substantially equivalent to those of the conventional rice counterpart. Comparison of nutritional composition is an important consideration in the safety assessment of food and feed products derived from GM crops for which no changes to the nutritional composition are intended. The results of this study confirm that the nutritional quality of Agb0101 with *bar* and modified *cry1Ac1* genes was unchanged by herbicide treatment and growing season expansion, when compared to that of its non-transgenic counterpart.

Acknowledgments This work was supported by grants from the National Academy of Agricultural Science (Code PJ006834) and the Next-Generation BioGreen 21 Program (SSAC, PJ0081842012), Rural Development Administration, Republic of Korea.

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