Comparative Evaluation of Nutritional Compositions Between Transgenic Rice Harboring the *CaMsrB2* Gene and the Conventional Counterpart

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Abstract As a part of a safety assessment of new transgenic crops, compositional equivalence studies between transgenic crops with non-transgenic comparators are almost universally required. This study was conducted to compare nutritional profiles of proximate composition, and fatty acid, amino acid, mineral, and vitamin contents, and anti-nutrients, between transgenic drought-tolerant Agb0103 rice harboring the pepper methionine sulfoxide reductase B2 gene *CaMsrB2* and the parental rice cultivar, 'Ilmi' as a non-transgenic control. Both transgenic and non-transgenic rice were grown and harvested in 2 different locations. Proximate compositions of moisture, starch, protein, lipid, and ash content of Agb0103 rice were similar to parental non-transgenic rice. There were no differences between transgenic and non-transgenic rice with respect to the whole nutritional composition, except for minor locality differences for a few nutritional components. Agb0103 rice with improved resistance to drought is nutritionally equivalent to the parental rice cultivar.

Keywords: transgenic rice, drought tolerance, food safety, nutritional composition, substantial equivalence

Introduction

Rice (*Oryza sativa* L.) is an important staple crop that serves as a primary source of calories for half of the world population. Production of rice is limited by many factors, including biotic and abiotic stresses (1). Genetically modified (GM) crops are becoming an increasingly important part of the common food supply, and genetic transformation of rice has demonstrated numerous important opportunities (2). Advances have been made in genetic engineering of rice through insertion of agronomically important traits, including plants with resistance against insect pests and disease, tolerance against herbicides, drought, heat, cold, and salt, and improved nutritional qualities.

Approximately half of total world rice production is from rainfed areas where drought is a major constraint frequently causing yield loss (3). Breeding for drought tolerance in rice remains a major challenge. Recently, the genetically modified rice Agb0103 containing the *CaMsrB2* gene, which displays resistance to drought, was developed by the Rural Development Administration (RDA) of Korea.

The pepper (*Capsicum annuum*) methionine sulfoxide reductase B2 gene *CaMsrB2* can play an important role as a novel defense regulator against oxidative stress and pathogen attack under drought stress conditions (4).

With increasing GM crop production worldwide and consumer concerns about the safety of GM foods, safety assessment of genetically modified (GM) plants has become essential for commercialization and is strictly required for use of GM plants for food production. Such assessments should be based on the concept of 'substantial equivalence', which was introduced by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) and developed by the Organisation for Economic Co-operation and Development (OECD). Research into unintended food safety risks for GM crops has primarily involved investigation of potential effects relating to crop composition (5,6). An important aspect in assessment of the safety of GM crops for human consumption is characterization of the nutritional composition in a host plant or in close relatives (7).

Substantial equivalent analysis has been studied for different

events of the transgenic crops such as corn, soybean, potato, wheat, and rice (8). The concept of substantial equivalence has been used internationally for risk assessment of genetically modified foods (9). An OECD consensus document (10) has identified several factors, including proximate composition, amino acids, fatty acids, minerals, vitamin contents, anti-nutritive components like phytic acid, and trypsin inhibitors in rice for compositional analysis.

Previous studies have shown no significant differences in the major nutritional components of transgenic tomatoes (11,12) and soybeans (13), compared with parental counterparts. Compositional analysis of transgenic rice has been reported as part of a safety assessment (14-18) with nutritional equivalence among transgenic and nontransgenic rice varieties.

Because of the possibility of unintended effects of the rice transgenic lines HV8 and HV23 containing the *CaMsrB2* gene, different vegetative and reproductive traits, and antioxidant properties of transgenic lines have been determined and found to be similar to nontransgenic counterparts (19). Recently, the transgenic line HV23 appeared to have better agronomic traits than HV8 under rain-fed paddy cultivation. However, there is a scarcity of information regarding nutrient levels in newly developed drought-tolerant rice harboring the *CaMsrB2* gene. Therefore, this study was carried out to investigate the nutritional composition of transgenic rice line HV23 containing the *CaMsrB2* gene from pepper (*Capsicum annum*), and to compare HV23 with the parental rice variety. Obtained data were analyzed for statistical significance.

Materials and Methods

Rice Drought-tolerant transgenic Agb0103 rice and the non-transgenic parent rice cv. Ilmi were obtained from the Rural Development Administration (RDA) in Suwon, Korea. The Agb0103 HV 23 line was originally developed based on insertion of the pepper *CaMsrB2* gene. Agb0103 rice and the parental rice were grown in adjoining experimental fields under the same environmental conditions and field management at the Rural Development Administration in Suwon, Gyeonggi, Korea and at Kyungpook National University in Gunwi, Gyeongbuk, Korea in 2012. Rough rice was dehulled using a rubber roll sheller (Satake rice machine-Type THU; Satake Engineering Co., Tokyo, Japan) to obtain brown rice that was ground through a 0.5 mm mesh screen using a cyclotec sample mill before analysis.

Proximate analysis Moisture, crude protein, and crude ash contents were determined following the oven-drying method, the Kjeldahl method, and the dry ashing method following AACC approved methods 44-15A, 46-13, and 08-01, respectively (20). Crude fat and crude fiber contents were determined following AOAC methods (21). The carbohydrate content was calculated as: % carbohydrates = 100% - (% crude protein + % crude fat + % crude ash). The starch content was

determined following AACC method 76-11 (20).

Fatty acid analysis The fatty acid content was determined after lipid extraction and saponification with 0.5 N NaOH in methanol. The saponification mixture was methylated using 14% boron trifluoride-methanol for 5 min. After cooling, phase separation was achieved with a saturated NaCl solution using 2 mL of isooctane as an extraction solvent, followed by drying on anhydrous Na₂SO₄, and resulting fatty acid methyl esters were analyzed using a gas chromatograph (6890 series; Hewlett-Packard, Palo Alto, CA, USA) equipped with a flame ionization detector.

Amino acid analysis A rice sample (0.3 g) was hydrolyzed with 15 mL of 6 N HCl at 105°C for 24 h, derivatized following the AccQ-Tag method (22), and the amino acid contents were determined using an automatic amino acid analyzer (Model 8000 series; JASCO, Tokyo, Japan).

Mineral analysis Approximately one gram of a rice sample was ignited in a muffle furnace at 450°C, and the resulting ash was dissolved in dilute HCl and filtered through filter paper. Mineral contents were determined using an inductively coupled plasma-atomic emission spectrometer (ICP-AES; Horiba Jobin-Yvon JY Activa, Longjumeau, France) following AOAC method 999.11 (21).

Vitamin analysis Vitamin B₁ and B₂ contents were analyzed following the method described by Martins-Junior *et al.* (23) with slight modification. Rice samples of 5 g were subjected to extraction using 40 mL of 75 mM ammonium formate and centrifuged at 1,500×g for 15 min. The supernatant was filtered through a 0.2 mm membrane filter and the vitamin B contents in the filtrate were determined using a HPLC (1200 Series; Agilent Technologies, Santa Clara, CA, USA). The vitamin E content was determined using an 1100 Series analytical HPLC system (Agilent Technologies) equipped with a Nova-Pak silica column (3.9×150 mm id) (Waters, Milford, MA, USA) following an AOAC method (21).

Anti-nutrient analysis Phytic acid was extracted from rice using 2.4% HCl and analyzed on a spectrophotometer at 500 nm after reaction with a 0.03% FeCl₃·6H₂O solution containing 0.3% sulfosalicylic acid following the method described by Bhandari and Kawabata (24). The trypsin inhibitor activity was determined in alkali solvent-extracted rice samples using a modification of AACC method 71-10 (20).

Statistical analysis All samples of transgenic and non-transgenic rice were analyzed in triplicate for each nutrient and results were expressed as mean ± standard error of the mean (SEM). Statistical significance was analyzed using the unpaired Student *t*-test method with SAS 9.2 software (SAS Institute, Cary, NC, USA) at *p* < 0.05.

Results and Discussion

Transgenic crops are required to pass a pre-market safety review conducted well in advance of commercialization. Consumer risk should be scrutinized in a transgenic foodstuff safety assessment. Compositional studies comparing transgenic crops with non-transgenic crops are almost universally required for a safety assessment of new transgenic crops by regulatory bodies (25). Following the guidelines of the Codex Alimentarius Commission, the concept of substantial equivalence, which stresses that an assessment of a novel food, in particular one that is genetically modified, should demonstrate that the food is as safe as its traditional counterpart, was used to assess the safety of newly developed drought-tolerant rice. Substantial equivalence was verified for proximate compositions, and amino acid, fatty acid, mineral, and vitamin contents.

Proximate composition The proximate compositions of drought-tolerant transgenic rice Agb0103 and non-transgenic control rice samples grown in 2 different places, Suwon and Gunwi, are shown in Table 1. Moisture, protein, fat, ash, fiber, starch, and total carbohydrate contents were determined. Although the carbohydrate content was not measured, the total carbohydrate content was calculated based on subtraction of the sum of protein, fat, and ash from 100. No

significant ($p>0.05$) differences were observed in levels of protein, lipid, fiber, ash, and total carbohydrates between Agb0103 rice and parental rice from both growth locations. Mean values for moisture, protein, fat, ash, and carbohydrate contents from rice samples analyzed in this study were within reference ranges of OECD consensus documents (10) for other commercial rice varieties, while the fiber content in transgenic rice was slightly higher than the reference range of 0.6 to 1.0% reported by OECD (10).

Fatty acid profile Individual fatty acid contents were analyzed using gas chromatography and expressed in % with respect to the total amount of fatty acids analyzed (Table 2). The two primary fatty acids in rice samples were oleic acid (C18:1) and linoleic acid (C18:2), followed by palmitic acid (C16:0), regardless of genotype or growth location (Table 2). No significant ($p>0.05$) differences were observed in fatty acid compositions between Agb0103 rice and the non-transgenic counterpart, except for the oleic acid content, which was significantly different ($p<0.05$). The fatty acid composition analyzed in this study was similar to reference values observed in 6 typical Korean varieties reported by Choe *et al.* (26). Levels of the very long chain gadoleic (eicosenoic), behenic, and lignoceric fatty acids were slightly higher than for reported values (26).

Table 1. Proximate compositions of parental rice and drought-tolerant transgenic Agb0103 rice

Component (%, d.b.)	Location 1 (Suwon)		Location 2 (Gunwi)		*Reference range
	Parental	Agb0103	Parental	Agb0103	
Moisture	9.89±0.40	9.26±0.44	11.16±0.45	11.99±0.51	14
Crude protein	7.32±0.06	7.27±0.02	7.20±0.03	7.08±0.06	7.1-8.3
Crude fat	2.55±0.01	2.31±0.02	2.70±0.13	2.44±0.04	1.6-2.8
Crude ash	1.38±0.01	1.33±0.07	1.21±0.03	1.23±0.03	1.0-1.5
Crude fiber	1.34±0.08	1.43±0.12	1.09±0.11	1.10±0.01	0.6-1.0
Total Carbohydrate	89.74±0.05	89.98±0.07	89.97±0.13	90.66±0.02	87.4-90.3
Starch	69.66±1.94	68.89±2.60	73.26±0.69	74.61±3.59	-

Values are means±SD ($n=3$). There was no significant difference between drought-tolerant transgenic rice and parental rice at $p<0.05$ based on *t*-test.

*Reference: OECD (10) data.

Table 2. Fatty acid compositions of parental rice and drought-tolerant transgenic Agb0103 rice

Fatty acid (%, d.b.)	Location 1 (Suwon)		Location 2 (Gunwi)		*Reference range
	Parental	Agb0103	Parental	Agb0103	
Myristic acid (C14:0)	0.20±0.00	0.21±0.01	0.17±0.02	0.20±0.01	0.39-3.71
Palmitic acid (C16:0)	17.05±0.18	17.60±0.27	17.03±0.13	17.59±0.24	16.9-40.0
Palmitoleic acid (C16:1)	0.14±0.01	0.14±0.02	0.13±0.01	0.14±0.01	0.08-0.39
Stearic acid (C18:0)	1.62±0.02	1.57±0.03	1.58±0.03	1.53±0.02	1.60-3.04
Oleic acid (C18:1)	42.01±0.93	40.82±0.81	41.41±1.03	40.01±0.76	29.7-47.0
Linoleic acid (C18:2)	35.39±0.54	35.67±0.32	35.97±0.33	36.55±0.12	5.79-47.7
Linolenic acid (C18:3)	1.14±0.04	1.21±0.08	1.18±0.08	1.27±0.05	1.0-2.63
Arachidic acid (C20:0)	0.62±0.01	0.60±0.01	0.57±0.02	0.55±0.01	0.4-0.71
Gadoleic acid (C20:1)	0.66±0.00	0.66±0.01	0.61±0.01	0.62±0.03	0-0.5
Behenic acid (C22:0)	0.38±0.05	0.47±0.02	0.39±0.04	0.45±0.01	0.28-0.30
Lignoceric acid (C24:0)	0.79±0.11	0.95±0.08	0.85±0.04	0.99±0.07	0.54-0.59

Values are relative contents of fatty acids as a percentage of total fatty acids. There was no significant difference between drought-tolerant transgenic rice and parental rice at $p<0.05$ based on the *t*-test.

*Reference: Choi *et al.* (27).

Table 3. Amino acid contents of parental rice and drought-tolerant transgenic Agb0103 rice

Amino acid (g/100 g)	Location 1 (Suwon)		Location 2 (Gunwi)		*Reference range (g/100 g)
	Parental	Agb0103	Parental	Agb0103	
Alanine	0.43±0.01	0.42±0.01	0.43±0.01	0.42±0.01	0.44-0.68
Arginine	0.54±0.01	0.54±0.02	0.55±0.01	0.54±0.01	0.44-0.80
Aspartic acid	0.68±0.01	0.67±0.01	0.67±0.01	0.64±0.01	0.58-0.94
Glutamic acid	1.39±0.01	1.41±0.01	1.38±0.02	1.33±0.02	1.59
Glycine	0.35±0.01	0.34±0.01	0.34±0.01	0.33±0.01	0.39-0.69
Histidine	0.16±0.01	0.16±0.01	0.17±0.01	0.16±0.01	0.10-0.20
Isoleucine	0.25±0.00	0.29±0.01	0.27±0.01	0.27±0.00	0.26-0.88
Leucine	0.68±0.00	0.67±0.01	0.68±0.01	0.66±0.01	0.32-0.69
Lysine	0.28±0.01	0.27±0.01	0.28±0.01	0.27±0.00	0.18-0.34
Methionine	0.12±0.01	0.13±0.01	0.12±0.01	0.12±0.00	0.14-0.42
Phenylalanine	0.34±0.01	0.38±0.01	0.35±0.01	0.35±0.01	0.29-0.44
Proline	0.26±0.01	0.25±0.00	0.24±0.01	0.23±0.01	0.10-0.38
Serine	0.37±0.01	0.35±0.01	0.36±0.01	0.35±0.01	0.26-0.56
Threonine	0.25±0.01	0.25±0.01	0.25±0.01	0.25±0.01	0.20-0.36
Tyrosine	0.24±0.01	0.23±0.01	0.24±0.01	0.27±0.01	0.13-0.71
Valine	0.35±0.00	0.40±0.01	0.37±0.01	0.38±0.01	0.26-0.58
Total amino acid	6.68	6.78	6.72	6.57	

Values are mean±SD ($n=3$). There was no significant difference between drought-tolerant transgenic rice and parental rice at $p<0.05$ based on the t -test.

*Reference: Choi *et al.* (27).

Table 4. Mineral contents of parental rice and drought-tolerant transgenic Agb0103 rice

Mineral	Location 1 (Suwon)		Location 2 (Gunwi)		*Reference range
	Parental	Agb0103	Parental	Agb0103	
Phosphorus, mg/g	3.29±0.20	3.06±0.18	3.12±0.08	2.87±0.12	2-5
Potassium, mg/g	2.76±0.12	2.52±0.19	2.71±0.12	2.52±0.09	0.7-3.2
Calcium, mg/g	0.11±0.01	0.12±0.01	0.11±0.01	0.11±0.01	0.1-0.6
Magnesium, mg/g	1.39±0.11	1.29±0.11	1.36±0.02	1.38±0.02	0.2-1.7
Sodium, µg/g	144.3±0.2*	179.7±2.1*	75.4±2.5	76.7±3.0	20-400
Copper, µg/g	4.4±0.1	3.3±0.1	2.3±0.2	2.3±0.1	1-7
Zinc, µg/g	26.6±0.1	25.3±0.1	20.3±0.1	20.9±0.1	7-33
Iron, µg/g	12.2±0.1	9.9±0.3	11.3±0.4	9.3±0.1	2-60

Values are mean±SD ($n=3$). *There was significant difference between drought-tolerant transgenic rice and parental rice at $p<0.05$ based on the t -test.

*Reference: OECD (10) data.

Amino acid profile Amino acid contents of rice samples, shown in Table 3, were determined using an automatic amino acid analyzer. Amino acid contents and profiles in Agb0103 rice were similar to controls at g/100 g. All analyzed amino acid levels, except alanine and glycine, were in agreement with reported reference ranges (27). Levels of alanine and glycine were slightly lower than the reference range reported by Choi *et al.* (27). However, the overall amino acid profile was similar to values previously reported (17).

Mineral levels Minerals are important for plant growth and development. The mineral content of Agb0103 rice, analyzed using inductively coupled plasma optical emission spectrometry, is shown in Table 4. A significant ($p<0.05$) difference was observed for the sodium content between Agb0103 rice and the non-transgenic counterpart grown in location 1 (Suwon). The sodium content of Agb0103 rice was increased by 1.24x, compared to the level of control rice. No significant ($p>0.05$) differences were observed in

other mineral contents between Agb0103 rice and the non-transgenic counterpart. Mineral contents of all rice samples were within the range of reported (OECD) values (10). Overall mineral contents of rice grown in location 1 (Suwon) were higher than in location 2 (Gunwi). Differences were attributed more to normal variability due to regional environment rather than to novel traits.

Vitamins Vitamin contents were determined using HPLC. Rice contains abundant fat-soluble vitamin E, and water-soluble vitamin B₁ (thiamin) and B₂ (riboflavin). Similar vitamin contents were observed between Agb0103 rice and the non-transgenic counterpart (Table 5). The vitamin B₂ content was comparatively lower than reported values (OECD) (10). The low level of vitamin B₂ reported herein was similar to the level recently reported for insect-tolerant GM rice (28). The vitamin E content measured in this study was also slightly lower than the range of reported levels (OECD) (10), but within the range of previously reported levels (27).

Table 5. Vitamin contents of parental rice and drought-tolerant transgenic Agb0103 rice

Vitamin	Location 1 (Suwon)		Location 2 (Gunwi)		*Reference range
	Parental	Agb0103	Parental	Agb0103	
Vit B ₁ (μg/g)	4.02±0.50	3.64±0.07	3.38±0.06	3.26±0.08	2.9-6.1
Vit B ₂ (μg/g)	0.22±0.04	0.11±0.02	0.11±0.02	0.23±0.06	0.4-1.4
Vit E (μg/g)	8.86±0.15	8.37±0.27	8.57±0.55	8.30±0.35	9-25

Values are mean±SD (n=3). There was no significant difference between drought-tolerant rice and parental rice at $p<0.05$ based on the *t*-test.

*Reference: OECD (10) data.

Table 6. Anti-nutrient contents of parental rice and drought-tolerant transgenic Agb0103 rice

	Location 1 (Suwon)		Location 2 (Gunwi)		*Reference range
	Parental	Agb0103	Parental	Agb0103	
Phytic acid (%)	0.87±0.06	0.92±0.09	0.98±0.05	0.83±0.06	0.7-1.4
Trypsin inhibitor (TIU/mg)	<0.1	<0.1	<0.1	<0.1	<0.1

Values are mean±SD (n=3). There was no significant difference between drought-tolerant rice and parental rice at $p<0.05$ based on the *t*-test.

TIU: Trypsin inhibitor unit.

*Reference: Choi *et al.* (27).

Anti-nutrients The contents of the anti-nutrients phytic acid and trypsin inhibitors were determined (Table 6). Phytic acid in food reduces the absorbability of nutritional metal ions, and trypsin inhibitors adversely affect nutritional properties by inhibition of proteinase activity. No significant ($p>0.05$) differences were observed in the phytic acid content between Agb0103 rice and non-transgenic rice. The inhibitory activity of trypsin inhibitors was low in all rice samples, in agreement with previous reports (10,17,29).

In conclusion, as foreign inserted genes might alter nutritional values of foods in unpredictable ways, food safety risk assessments are usually conducted using a comparative approach. In substantial equivalence studies of GM crops, targeted analyses of key compounds have been extensively conducted. Such analyses provide information regarding macro and micronutrients, and anti-nutrients (30). In this study, the nutritional composition of drought-tolerant transgenic rice Agb0103 was comparable with non-transgenic control rice. Values for nutritional and anti-nutritional components indicated that there were no differences between Agb0103 rice and the non-transgenic parental rice, except for minor locality differences in a few nutritional components. The nutritional components of transgenic drought-tolerant Agb0103 rice were within ranges of previously reported values for other commercial rice varieties.

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