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Effects of Biofertilizer on the Quality and Antioxidant Property of Rice (*Oryza sativa* L.)

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ABSTRACT The effect of biofertilizer in enhancing nutrient quality and antioxidant property of rice grain was investigated. The experiment was carried out in a randomized complete block design with 3 replications and 7 treatments namely : RF = N-P_2O_5-K_2O (11-5.5-4.8 kg 10 a^{-1}); half of the recommended fertilizer rate, HRF = N-P_2O_5-K_2O (5.5-2.75-2.4 kg 10 a^{-1}); HRF+Bio 250 = HRF combined with 250 kg Biofertilizer 10 a^{-1}; HRF+Bio 500 = HRF combined with 500 kg Biofertilizer 10 a^{-1}; Bio 250 = 250 kg Biofertilizer 10 a^{-1}; Bio 500 = 500 kg Biofertilizer 10 a^{-1}; and NF = No Fertilizer. Results showed that HRF+Bio 500 obtained a significantly higher protein content but a significantly lower amylose content compared with RF and NF treatments. Highest phytic acid content was recorded in NF treatment while the lowest was observed in HRF+500 treatment. The highest values in both electron donating ability and reducing power were obtained in HRF+Bio 500 treatment. All treatments obtained higher reducing power than that of the RF treatment and that NF treatment showed comparable values in both electron donating ability and reducing power with those of the treated plots. Highest antimutagenicity property was also observed in HRF+Bio 500 treatment followed by Bio 500 treatment. This study showed the possibility of using biofertilizer to enhance nutritional quality and antioxidant property of rice.

Keywords : biofertilizer, electron donating ability, phytic acid, rice

Grains contain unique phytochemicals that can combat oxidative stress in the body by helping maintain a balance between oxidants and antioxidants (Adom and Liu, 2002). These antioxidants are linked to reduce risk of cancer (Osawa, 1999; Slavin, 2000) and prevention of cardiovascular and age-related diseases (Jimenez and Calixto, 2005; Okai *et al.*, 2004). Thus, enhancing the production of these phytochemicals in rice grains can be investigated.

Brandt and Molgaard (2001) posed the question of whether organic agriculture enhances or reduces the nutritional value of plant foods. While it is still highly debatable whether organic farming management system can increase antioxidant levels in the plants compared to the conventional (high inorganic fertilizer input) farming system, much more impossible to determine the causes of increase in antioxidant level, proofs show that organic farming produce have higher antioxidant level than those produced using conventional methods (Benbrook, 2005). Studies show that organic farming system can increase antioxidant levels of marionberries and corn (Asami *et. al.*, 2003), strawberries (Wang and Lin, 2003), peach and pears (Carbonaro *et al.*, 2002) and vegetables (Ren *et al.*, 2001). Benbrook (2005) also noted that a small encouraging set of studies had focused on how farmers can increase average polyphenol and antioxidant levels through management system changes, such as adoption of organic farming methods.

Biofertilizer is a natural organic-based fertilizer that can possibly have a significant effect in enhancing the antioxidant property of rice. Its usage in combination with chemical fertilizer is gaining acceptance rice production. In this paper, the effect of biofertilizer on the nutritional quality, phytic acid content, antioxidant activity and antimutagenic property of rice grain was investigated.

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MATERIALS AND METHODS

The experiment was conducted at the Agricultural Research & Extension Services in Chilgok, Kyungpook, South Korea. Rice variety Junambyeo was provided by the Rural Development Administration (RDA), Gyeongbuk Province while biofertilizer was obtained from the Korean Forest Research Institute (KFRI). The experiment was laid out in a randomized complete block design with 7 treatments and 3 replicates. All other management practices in rice cultivation recommended by RDA were employed. The treatments were as follows : recommended fertilizer rate, RF = N-P_2O_5-K_2O (11-5.5-4.8 kg 10 a^{-1}); half of the recommended fertilizer rate, HRF = N-P_2O_5-K_2O (5.5-2.75-2.4 kg 10 a^{-1}); HRF+Bio 250 = HRF combined with 250 kg Biofertilizer 10 a^{-1}; HRF+ Bio 500 = HRF combined with 500 kg Biofertilizer 10 a^{-1}; Bio 250 = 250 kg Biofertilizer 10 a^{-1}; Bio 500 = 500 kg Biofertilizer 10 a^{-1}; and NF = No Fertilizer. All statistical analysis of data was done using SAS program (SAS Institute, Inc., Cary, NC, USA).

Measurement of rice quality

Protein and amylose contents were measured following the methods of Kawamura *et al.* (2003) and Villareal *et al.* (1994), respectively using whole grain analyzer (Foss Infratec 1241 Grain Analyzer, Sweden) while rice palatability was measured using Toyo taste meter (TOYO ME-90A, Japan). Alkali digestion value was determined by measuring the spreading degree (scale from 1 : not spreading to 7 : perfect spreading) of polished rice grains in 1.7% KOH solution incubated for 23 hours at 30°C (Little *et al.*, 1958).

Measurement of phytic acid content

Phytic acid was determined according to the method of Antonio *et al.* (1991). Extraction was done by mixing 2.5 g sample and 50 mL of 1.2% HCl-10% Na_2SO_4 solution in a shaker for 2 hours. Filtrate (10 mL) was added with 12 mL of $FeCl_3$ solution placed in a 100°C water bath for 75 mins. The sample was cooled for 1 hour in room temperature and then centrifuged for 15 mins at 3500 rpm. Filtered (4 mL) solution was added with 1 mL of wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ - 0.3% sulfosalicylic acid) and after 10 mins, absorbance at 500 nm was measured using a spectrophotometer. The results were then expressed as mg Fe bound per gram of sample extracted.

Measurement of electron donating ability by DPPH method

Free radical-scavenging activity of rice was assayed using a stable free radical, DPPH, according to the method of Blois (1958). The reaction mixture contained 0.5 mL of 0.5 mM DPPH and 0.1 mL of solution of bran extract and dimethyl sulfoxide at different concentrations (10~50 μg). The mixture was adjusted to 1.0 mL by adding 100 mM Tris-HCl buffer (pH 7.4). After standing for 20 mins at room temperature in the dark, absorbance reading at 517 nm was recorded.

Measurement of reducing power

The determination of reducing power was performed as described by Yen and Duh (1993). Various extracts (20, 50, and 100 mg mL^{-1}) were mixed with phosphate buffer (0.5 mL, 0.05 M, pH 6.6) and 1% potassium ferricyanide (5 mL), and incubated at 50°C for 20 mins. After addition of 0.5 mL of 10% trichloroacetic acid, the mixture was centrifuged at 5000 rpm for 10 minutes. The supernatant (0.5 mL) was mixed with distilled water (0.5 mL) and 0.1% of ferric chloride (0.1 mL). Absorbance reading at 700 nm was recorded.

Measurement of antimutagenicity by SOS-chromotest

The SOS-chromotest (EBPI, Ontario Canada) is based on the detection of damage to DNA as measured through the SOS DNA repair system. *E. coli* PQ37 strain was incubated overnight in Luria broth plus ampicillin (tryptone 10 g L^{-1} and yeast extract 5 g L^{-1} oxoid, NaCl 10 g L^{-1}, ampicillin 20 μg mL^{-1} Sigma-Aldrich) and then transferred (100 μL) into 5 mL of the same medium and incubated for 2 hours (OD_{520} = 0.3~0.4). One milliliter was added to 9 mL of fresh Luria broth without ampicillin and fractions of 600 μL were mixed with 20 μL of sample containing genotoxin (or supernatant of genotoxin-treated culture) and then incubated for 2 hours at 37°C. The activation of SOS DNA repair system was evaluated by induction of β-galactosidase, and correction was done on the basis of alkaline phosphatase (constitutive activity). Positive and negative controls were prepared in saline, with or without genotoxin, respectively. SOS induction factor (IF_{SOS}) was defined as the β-galactosidase to alkaline phosphatase ratio of the sample under analysis, divided by the same ratio of negative control. Both enzyme activities were detected colorimetrically according to details given in a previous study (Caldini *et al*., 2002).

RESULTS AND DISCUSSION

Chemical Properties of the Biofertilizer

The biofertilizer used in this experiment was prepared from aerobic decomposition of food waste residues and incorporated with a mixture of beneficial microorganisms. Chemical analysis of the biofertilizer was conducted by Cheogju Recycling Co., Ltd. and presented at Table 1. The analysis showed that the composition of the biofertilizer was well within the standard amount set by the government. Heavy metals such as As, Hg, Pb and Cd were present at even lower amount. Percent organic matter was very high while percent salinity was within the allowable limit.

Effect of biofertilizer on rice quality

The effects of biofertilizer and inorganic fertilizer on nutritional quality are presented in Table 2. Biofertilizer showed an inverse effect on protein and amylose contents of rice, that is, it significantly increased protein content but significantly decreased amylose content. Amylose Content is an important determinant of taste in rice. Cheaupun *et al.* (2005) reported that when cooked, rice with low amylose content always has a soft texture and sticky, rice with intermediate amylose content is rather soft and rice with high amylose content has a hard texture. Palatability and alkali digestion values did not show significant differences across treatments and are within the usual acceptable values. Chanseok *et al.* (2003) noted that it is difficult to control the taste of rice using the variable rate fertilizer application

Table 1. Chemical composition of the biofertilizer used.

Component	Amount	
	Standard	Biofertilizer
O.M. (%)	> 25	43.6
O.M.:N ratio	< 50	27.1
N (%)	-	1.6
Pb (mg/kg)	< 150	19.2
Cd (mg/kg)	< 5	0.6
Cu (mg/kg)	< 300	22.7
Cr (mg/kg)	< 300	18.2
As (mg/kg)	< 50	below detection limit
Hg (mg/kg)	< 2	below detection limit
Fe (mg/kg)	< 900	44.2
Ni (mg/kg)	< 50	7.8
Salinity (%)	< 1	0.9
Moisture (%)	< 50	35.2

O.M. : organic matter, standard : allowable contents as set by government regulations for biofertilizer registration.

Table 2. Effects of bio- and NPK-fertilizers on grain quality of rice.

Treatment	Protein (%)	Amylose (%)	Palatability (TOYO meter)	Alkaline digestion value (KOH 1~7)
RF	6.6 b	17.5 a	82.6 ab	4.4 ab
HRF	6.5 b	16.8 bc	82.5 ab	4.5 b
HRF + Bio 250	7.0 a	17.2 ab	83.5 ab	4.4 ab
HRF + Bio 500	7.2 a	16.6 c	85.1 a	4.1 a
Bio 250	6.7 b	17. 6a	80.1 b	4.3 ab
Bio 500	7.0 a	17. 6a	86.0 a	4.2 b
No fertilizer	6.6 b	17. 4a	84.9 ab	4.3 ab

RF = recommended fertilizer rate; HRF = half recommended fertilizer rate : Bio 250 = biofertilizer 250 kg 10 a^{-1} : Bio 500 = biofertilizer 500 kg 10 a^{-1} : DAT = days after transplanting. The same letters in each column are not significantly different at 5% level by DMRT.

because the taste of rice depends not only on protein but also on amylose, fatty acid and moisture content.

It is believed that rice quality is affected by the environmental conditions and cultural practices during growth aside from characteristics influenced by genetic control. There are varying conclusions on the effect of nitrogen on nutritional quality. RDA (2001) of Korea reported that although application of nitrogen fertilizer after heading stage increases yield, it, however, lowers the grain quality. Based on these findings, nitrogen fertilizer application after heading stage is discouraged in order to produce quality rice.

In a study conducted at the International Rice Research Institute, it was shown that late nitrogen fertilizer application improved the nutritional quality of rice grain (Perez *et al.*, 1996). Also, the study conducted by Leesawatwong *et al.* (2004) showed that added application of nitrogen fertilizer increased protein accumulation in rice grain. Results of this experiment are consistent with that reported above. The consistent supply of nitrogen all throughout the growing season of rice promoted protein storage and decreased amylose accumulation in the rice grain.

Effects of biofertilizer on phytic acid content

The highest phytic acid content (Fig. 1) was recorded in the NF treatment, which was statistically different from those obtained in the other treatments. On the other hand, the lowest phytic acid content, which was statistically similar with those obtained in all other treatments except NF, was recorded in HRF+Bio 500 treatment. The high phytic acid content obtained in NF treatment may be due to its vital role in seed or grain development and successful seedling growth (Lott *et al.*, 2000).

Considering more studies now show that phytic acid is a very good chelating agent that can significantly decrease absorption of micronutrients such as Zn, Fe and Beta carotene in the meal (Gillooly *et al.*, 1983; Hurell *et al.*, 2003; Cook *et al.*, 1997), this result indicates a better rice quality. Hurell *et al.* (2003) concluded that phytate degradation improved iron absorption from cereal porridges. Furthermore, Cook *et al.* (1997) suggested that modification in the milling and processing methods for cereal grains that reduce their phytic acid content is likely to improve their iron availability significantly.

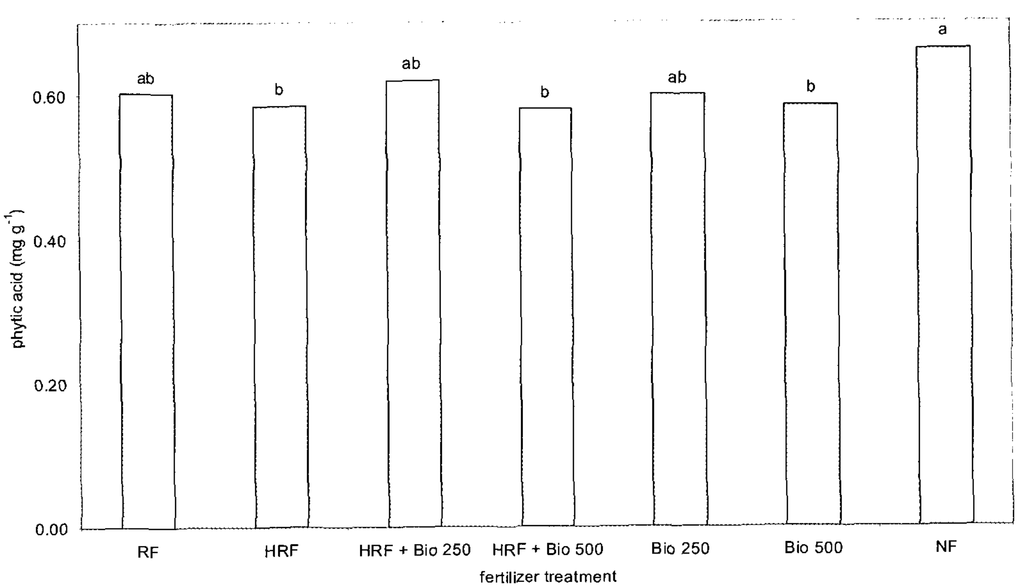

Fig. 1. Effects of bio- and NPK-fertilizers on phytic acid content of rice.
RF = recommended fertilizer rate; HRF = half recommended fertilizer rate; Bio 250 = biofertilizer 250 kg 10 a^{-1}; Bio 500 = biofertilizer 500 kg 10 a^{-1}; DAT = days after transplanting. The same letters in each column are not significantly different at 5% level by DMRT.

Effects of biofertilizer on antioxidant activity of rice grains

Results of the study showed that the electron donating ability (Table 3) of the harvested rice from different treatments have comparable values with those of the standards used namely : Vitamins E and C and Butylatedhydroxytoluene. In the case of reducing power (Table 4), all values at 100 mg mL^{-1} rice extracts from different treatments are higher than the standard Vitamin C. The highest values in both electron donating ability and reducing power were obtained in HRF+Bio 500 treatment. Surprisingly, all treatments obtained higher reducing power than that of the RF treatment while NF treatment showed comparable values in both electron donating ability and reducing power with those of the other treatments.

A growing number of studies regarding the effect of natural or organic agriculture on the antioxidant contents of crops are available. Studies have shown that organic farming system can possibly increase antioxidant levels of marionberries and corn (Asami *et al.*, 2003), strawberries

Table 3. Effects of bio- and NPK-fertilizers on electron donating ability of rice grains.

Treatment	Electron donating ability (%)		
	20 mg mL^{-1}	50 mg mL^{-1}	100 mg mL^{-1}
Vit. C		91.4 ± 1.8	
Vit E		89.7 ± 1.3	
Butylatedhydroxytoluene		90.2 ± 1.5	
RF	22.2 ± 5.6 a	51.0 ± 2.7 a	81.8 ± 2.6 a
HRF	22.5 ± 8.5 a	52.1 ± 7.0 a	83.3 ± 1.5 ab
HRF + Bio 250	25.5 ± 2.0 a	53.1 ± 3.4 a	84.6 ± 1.5 ab
HRF + Bio 500	28.3 ± 2.2 a	58.1 ± 8.4 a	87.2 ± 3.2 ab
Bio 250	21.2 ± 7.9 a	51.2 ± 7.4 a	84.5 ± 4.3 b
Bio 500	28.2 ± 1.4 a	56.9 ± 3.7 a	85.7 ± 1.8 ab
No fertilizer	25.8 ± 9.1 a	49.5 ± 2.4 a	84.7 ± 2.1 ab

RF = recommended fertilizer rate; HRF = half recommended fertilizer rate; Bio 250 = biofertilizer 250 kg 10 a^{-1}; Bio 500 = biofertilizer 500 kg 10 a^{-1}; DAT = days after transplanting. The same letters in each column are not significantly different at 5% level by DMRT.

Table 4. Effects of bio- and NPK-fertilizer on reducing power (optical density) of rice.

Treatment	Optical density (700 nm)		
	20 mg mL^{-1}	50 mg mL^{-1}	100 mg mL^{-1}
Vit. C	0.390 ± 0.076	0.441 ± 0.030	0.443 ± 0.037
RF	0.358 ± 0.048 a	0.474 ± 0.040 a	0.480 ± 0.001 a
HRF	0.371 ± 0.017 ab	0.466 ± 0.016 a	0.553 ± 0.006 a
HRF + Bio 250	0.324 ± 0.006 b	0.476 ± 0.004 a	0.579 ± 0.013 a
HRF + Bio 500	0.386 ± 0.030 a	0.505 ± 0.036 a	0.593 ± 0.094 a
Bio 250	0.364 ± 0.013 ab	0.496 ± 0.083 a	0.501 ± 0.044 a
Bio 500	0.346 ± 0.014 ab	0.432 ± 0.040 a	0.576 ± 0.052 a
No fertilizer	0.312 ± 0.013 b	0.437 ± 0.013 a	0.586 ± 0.006 a

RF = recommended fertilizer rate; HRF = half recommended fertilizer rate; Bio 250 = biofertilizer 250 kg 10 a^{-1}; Bio 500 = biofertilizer 500 kg 10 a^{-1}; DAT = days after transplanting. The same letters in each column are not significantly different at 5% level by DMRT.

Table 5. Effects of bio- and NPK-fertilizers on antimutagenicities of 70% ethanol extracts of rice-bran with mitomycin C using *E. coli* PQ 37 as an indicator cell.

Treatment	Antimutagenicity (%)		
	20 mg mL^{-1}	50 mg mL^{-1}	100 mg mL^{-1}
Positive control	100.0	100.0	100.0
Negative control	0.0	0.0	0.0
RF	78.8 ± 6.5 a	88.2 ± 2.1 b	96.7 ± 7.7 c
HRF	92.2 ± 9.6 a	102.9 ± 2.3 ab	115.4 ± 6.8 a
HRF + Bio 500	99.6 ± 9.6 a	106.7 ± 7.6 a	128.1 ± 5.0 ab
HRF + Bio 250	89.6 ± 1.6 a	90.9 ± 2.3 ab	96.3 ± 4.9 c
Bio 500	98.2 ± 0.3 a	105.0 ± 5.4 a	120.1 ± 4.8 bc
Bio 250	95.8 ± 8.8 a	104.7 ± 5.0 ab	104.9 ± 5.0 bc
No fertilizer	95.6 ± 1.9 a	104.0 ± 6.2 ab	113.6 ± 8.7 ab

RF = recommended fertilizer rate; HRF = half recommended fertilizer rate; Bio 250 = biofertilizer 250 kg 10 a^{-1}; Bio 500 = biofertilizer 500 kg 10 a^{-1}; DAT = days after transplanting. The same letters in each column are not significantly different at 5% level by DMRT.

(Wang and Lin, 2003), peach and pears (Carbonaro *et al.*, 2002) and vegetables (Ren *et al.*, 2001). There are few papers that discussed the antioxidant properties of grains including rice (Nam *et al.*, 2005; Jimenez and Calixto, 2005; Adom and Liu, 2002; Osawa, 1999; Okai *et al.*, 2004). Benbrook (2005) discussed elevating concentrations of antioxidants on vegetables, fruits, grains and dairy products through organic farming methods. Brandt and Molgaard (2001) noted that plants grown organically produced more secondary metabolites for the defense mechanism of the plants against pest infestation. Though the treatments applied in this experiment was not purely organic, the decrease in chemical fertilizer could have promoted production of secondary metabolites, thus, an increased antioxidant activity was observed in the reduced chemical fertilizer treatment and NF treatment. NF treatment obtained antioxidant and antimutagenic values comparable to that recorded in combined treatment of chemical fertilizer and biofertilizer and relatively better than that of the RF treatment. It could be that plants in NF treatment produced more secondary-metabolites (Brandt and Molgaard, 2001) that resulted to higher antioxidant and antimutagenic values.

Effects of biofertilizer on antimutagenicity of rice grains

Studies conducted by Ren *et al.* (2001) showed that cultivation practices could affect antimutagenic property in vegetables. The effect of biofertilizer treatment on the antimutagenic property of rice grains was investigated. Data (Table 5) showed that the highest antimutagenic activity of harvested rice tested against *E. coli* PQ37 strain was obtained in the HRF+Bio 500 treatment followed by Bio 500 treatment. On the other hand, the lowest antimutagenic activity was observed in RF treatment which was even lower than that obtained in the NF treatment. This result is in agreement with that observed in the antioxidant activities (Tables 3 and 4) as antimutagenicity of rice grains can be thought of as a direct effect of its antioxidant property.

In general, the results showed that using 500 kg Biofertilizer 10 a^{-1} could reduce chemical fertilizer to half the recommended application rate in rice production. Effects on the quality of rice showed that protein content significantly increased while antioxidant and antimutagenic activities of the rice grains were higher as compared to that of the recommended chemical fertilizer application rate.

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