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ARTICLE

Influence of Variety, Location, Growing Year, and Storage on the Total Phosphorus, Phytate-Phosphorus, and Phytate-Phosphorus to Total Phosphorus Ratio in Rice

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The influence of variety, location, growing year, and storage on the total phosphorus (total P), phytate-P, and phytate-P to total P ratio in Korean rice varieties was investigated. Experiment 1 investigated the influence of 9 rice varieties, 4 locations, and 2 growing years. In experiment 2, the effect of storage for 1, 6, and 12 months was examined. Results showed that locations, varieties, and their interactions had a significant effect on the total P and phytate-P contents in rice, whereas growing year did not. Location had a higher effect than variety. Rice grown at locations with higher precipitation levels and higher temperatures obtained lower total P, phytate-P, and phytate-P to total P ratio. Effect of variety and location showed the possibility of reducing phytate-P in rice. Storage of rice for up to 12 months did not affect the total P and phytate-P concentrations of rice.

KEYWORDS: Growing year; location; phytate; storage; total phosphorus; rice; variety

INTRODUCTION

Phytate, the salt of *myo*-inositol-1,2,3,4,5,6-hexaphosphoric acid, in major crops is a widely studied topic especially because there are two opposing effects on human nutrition. Phytate could be beneficial and harmful to human health (*1*, *2*). It has some antioxidant and anticancer functions and prevents coronary disease. On the other hand, it is also a strong chelator of mineral nutrients such as Ca, Zn, and Fe that could significantly decrease absorption of micronutrients in a meal. Thus, it may be reasonable to adjust phytate content in crops to a level in which medical and health functions of the food may be maintained and bioavailability of minerals is not much altered (*3*, *4*).

Breeding has been employed to produce crops with low phytate-P. Mutation breeding of rice, barley, maize, soybean, and wheat with low phytate content has been successfully done (*5*). Breeding programs require understanding of the major factors affecting total P and phytate accumulation and phytate-P to total P ratio in crops. Studies have shown that the important factors affecting P accumulation in rice, maize, wheat, barley, and triticale are genetics/variety and environment/locations and their interactions (*4*, *6*−*11*). Cropping season/growing year was also shown to have significant effects on the phytate-P in crops (*7*, *8*, *11*), but to a lesser extent than variety and locations. The available studies on the major crops revealed varying and contradictory results on the effect of variety, location, and growing year on P accumulation, indicating a crop dependency of these factors. Liu et al. (*4*) recommended that a comprehensive evaluation of rice germplasm be conducted for phytate content in multienvironments. Thus, the effects of the factors affecting phytate content in rice should be further explored.

Storage conditions can also be an important factor affecting total P and phytate-P contents in rice. Studies have shown that inorganic phosphorus level in wheat stored under high temperature and moisture increased with storage time due to phytate degradation by seed phytase (*12*). Investigations further revealed a higher loss in phytate content in barley wet aged at both elevated temperature (41 °C) and relative humidity (75%) than in barley exposed only to elevated temperature (*13*). On the other hand, a nonsignificant effect of storage at ambient temperature on the phytate-P content in wheat and cowpea flour was found (*8*).

There have been very few studies conducted regarding the effect of variety, location, growing year, and storage on the total P, phytate-P, and phytate-P to total P ratio of rice. Aside from Liu et al. (*4*), who investigated the effect of variety and location only, there has been no other study undertaken in rice. Likewise, to the best of our knowledge, studies dealing with the effect of storage and growing year on the phytate content of rice have not been conducted yet. Because rice is an important cereal crop that is consumed by more than half of the world's population (*14*), studies that would lead to better understanding of the factors affecting phytate-P level in rice are necessary. Such studies can be useful in identifying varieties with low phytate-P contents for breeding programs, modifying agronomic and storage practices, and cultivating suitable varieties at specific locations. Therefore, the objectives of this study were to assess the influence of (1) varieties, locations, and growing years and (2) storage time on the total P, phytate-P, and phytate-P to total P ratio in rice.

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Table 1. Ecological Classification and Meteorological Data during Cultivation Period (April to October) of the Growing Locations

growing location	classification	total precipitation (mm)	mean temperature (°C)	P_2O_5[a] (mg kg^{-1})
Daegu	plain area	1349.8 ± 240.3[b]	20.6 ± 0.60	129 ± 4.9
Andong	warm mountainous	1520.2 ± 272.7	20.4 ± 0.70	150 ± 4.3
Pohang	coastal area	1315.0 ± 56.6	18.5 ± 0.71	134 ± 6.5
Bonghwa	cold mountainous	1395.8 ± 164.9	16.2 ± 0.73	151 ± 7.1
$LSD_{0.05}$		136.2	3.2	5.50

[a] Measured before rice planting. [b] Values represent mean ± SD.

MATERIALS AND METHODS

Site Description and Cultural Management. Nine popular Korean rice varieties (Odae, Junghwa, Taebong, Hwayoung, Sula, Hwabong, Junam, Ilpum, Dongjin 1) were cultivated in four different locations (Daegu, E 128° 39′ 35″, N 35° 57′ 00″; Andong, E 128° 48′ 00″, N 36° 17′ 30″; Pohang, E 129° 21′ 30″, N 36° 02′ 32″; Bonghwa, E 128° 58′ 30″, N 36° 46′ 01″) in the southeastern part of Korea for 2 years (2003 and 2004). **Table 1** shows the soil P content at the start of experiment, ecological classification, and meteorological data for the duration of the experiment in these areas. The data for the two years were combined because there were no differences between precipitation levels and mean temperatures in each location for the two cropping seasons. Andong obtained the highest total precipitation level (1520.2 mm), which was similar with that of Bonghwa (1395.8 mm). Bonghwa, Daegu, and Pohang had similar precipitation levels (1315−1395.8 mm). Daegu and Andong (20.4−20.6 °C) recorded the highest mean temperature, whereas Bonghwa had the lowest (16.2 °C). Bonghwa registered the highest soil P content (151 mg/kg) and Andong, the lowest (129 mg/kg).

Rice varieties were germinated in late April and transplanted in late May. Each genotype was grown in three replicates of a six-row plot, 4 m long and 30 cm between rows. There were 28 hills for each row with 3 seedlings per hill. Cultivation conditions were kept as similar as possible. All experimental fields were fertilized with the same amount of P_2O_5 (57 kg/ha) during transplanting. Other management practices such as land preparation, weeding, and irrigation were employed according to the standard cultivation practices recommended by the Korean Rural Development Authority.

Sample Collection and Preparation. Rice samples were collected at maturity by harvesting 15 spikes from each replicate. Samples were dried at 60 °C for 48 h, milled, ground to pass through a 1.5 mm screen, and stored in sealed metal-sided bins until analysis. The total P and phytate-P contents of rice samples were analyzed within a month after harvest. For the analysis of the effect of storage, rice samples were stored in sealed metal-sided bins in a grain shed at ambient temperature and then analyzed for total P and phytate-P contents at 1, 6, and 12 months of storage.

Total P and Phytate-P Analysis. Analysis was done at Commonwealth Scientific and Industrial Research Organization (CSIRO) in Canberra, Australia. Total P was analyzed using the method described by Harris (*15*). Samples were dry-ashed, dissolved in concentrated HCl, and added with phosphomolybdate and aminonaphthol sulfonic acid reagents. The absorbance readings at 680 nm were then measured. Phytate-P content was determined following the method of Xu et al. (*16*).

Experimental Design and Statistical Analysis. The experiment was carried out in a randomized complete block design in a split-plot arrangement with three replications. Locations (L) represented main plots, whereas rice varieties (V) represented subplots. Data were analyzed using the GLM procedure of SAS (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Treatment Effects on Parameters Measured. The variety and location significantly affected the total P, phytate-P, and phytate-P to total P ratio in rice (**Table 2**). Their interactions were also significant except for Y × V and Y × L × V in phytate-P to total P ratio. Location had higher effect than variety, which could be attributed to the inherently different ecological classifications (plain field, warm mountainous, cold mountainous, and coastal area) and climatic conditions of the four growing areas. This result is in accordance with the previous paper that showed the significant effects of cultivar, location, and their interactions and the greater contribution of location to the variation in phytic acid content in rice (*4*).

Table 2. Mean Squared Error of Variety, Growing Area, and Growing Season for Total Phosphorus, Phytate-Phosphorus, and Phytate-Phosphorus to Total Phosphorus Ratio

source	total P[a] (g/kg)	phytate-P[a] (g/kg)	phytate-P to t otal P ratio[a] (%)
growing year (Y)	0.081 ns	0.075ns	0.078ns
location (L)	0.892**	0.866**	0.932**
Y × L	0.768**	0.762**	0.502*
variety (V)	0.552*	0.541*	0.568*
Y × V	0.515*	0.513*	0.085ns
L × V	0.530*	0.521*	0.533*
Y × L × V	0.519*	0.518*	0.074ns

[a] ns, not significant at $p < 0.05$; *, **, significant at $p < 0.05$ and $p < 0.01$, respectively.

Growing year did not affect the total P, phytate-P, and phytate-P to total P ratio in rice, which could be due to the nonsignificantly different meteorological data in the 2-year experiment. Various results have been reported on the effect of growing year on the phytate content of cereals. Studies revealed that yearly changes in phytate-P content in oats can be expected to be small unless the weather changes appreciably (*17*). On the other hand, growing year tended to affect phytate-P contents in wheat and barley (*8*, *9*, *11*, *13*). It appears that the current result could not provide a strong conclusion on the effect of growing year because the experiment was conducted for two years only with both years having similar weather conditions, and that the experiment should be repeated for a greater number of years to check the effect of varying weather conditions. However, the data showed that at similar weather conditions between cropping seasons, no significant effect of growing year on the total P and phytate-P of rice could be expected. Because the effect of growing year was not significant, the 2003 and 2004 data were combined.

Effect of Variety. The effect of variety on the total P, phytate-P, and phytate-P to total P ratio of rice is presented in **Table 3**. The result showed significant differences in total P, phytate-P, and phytate-P to total P ratio between varieties. Total P ranged from 5.3 g/kg for Hwabong to 8.6 g/kg for Odae, phytate-P from 4.0 g/kg for Junam to 5.9 g/kg for Odae, and phytate-P to total P ratio from 65.8% for Junghwa to 73.6% for Hwabong. The result illustrated the varietal dependency of total P and phytate-P contents in rice, which could be an important factor in selecting a more appropriate variety for breeding and production of rice. The result was also in agreement with that found in rice (*4*), wheat (*10*, *11*), oats (*17*), and triticale (*18*), but in contrast with that in barley (*11*, *13*) and wheat (*8*). Steiner et al. (*11*) noted that these differences might be due to factors such as analytical method, degree of fertilization, climatic conditions, or interactions between these factors.

In comparison with the world data on total P (0.31%) and phytate-P (0.90%) levels in rice (*19*), Korean rice varieties analyzed in this study exhibited higher total P (0.67%) and lower phytate-P (0.46%) contents. They also contain lower phytate-P

Table 3. Effect of Varieties Cultivated in Four Different Locations for Two Growing Years on the Total Phosphorus, Phytate-Phosphorus, and Phytate-Phosphorus to Total Phosphorus Ratio of Rice

rice variety	total P (g/kg)	phytate-P (g/kg)	phytate-P of total P (%)
Odae	8.5 ± 0.15[a]	5.9 ± 0.13	68.6 ± 1.31
Junghwa	7.9 ± 0.10	5.2 ± 0.16	65.8 ± 1.23
Taebong	6.8 ± 0.32	4.8 ± 0.25	70.6 ± 1.25
Hwayoung	6.8 ± 0.21	4.7 ± 0.24	69.1 ± 1.36
Sula	6.2 ± 0.21	4.3 ± 0.21	69.4 ± 1.22
Hwabong	5.3 ± 0.12	3.9 ± 0.16	73.6 ± 1.13
Junam	5.9 ± 0.15	4.0 ± 0.13	67.8 ± 1.11
Ilpum	6.0 ± 0.21	4.2 ± 0.21	70.0 ± 1.32
Dongjin 1	6.5 ± 0.20	4.4 ± 0.23	67.7 ± 1.22
mean	6.7 ± 1.00	4.6 ± 0.64	69.2 ± 2.18
$LSD_{0.05}$	0.7	0.3	3.4

[a] Values represent mean ± SD, n = 30.

Table 4. Effect of Location on the Total Phosphorus, Phytate-Phosphorus, and Phytate-Phosphorus to Total Phosphorus of Nine Rice Varieties Cultivated in Four Different Locations for Two Cropping Seasons

growing location	total P (g/kg)	phytate-P (g/kg)	phytate-P to total P (%)
Daegu	8.6 ± 0.1[a]	5.9 ± 0.5	69.5 ± 0.8
Andong	8.2 ± 0.4	5.6 ± 0.6	68.5 ± 0.7
Pohang	9.6 ± 0.4	7.0 ± 0.6	72.5 ± 0.8
Bonghwa	11.1 ± 0.5	8.0 ± 0.4	72.5 ± 0.8
$LSD_{0.05}$	0.37	0.14	3.31

[a] Values represent mean ± SD, n = 30.

levels (0.39−0.59%) than the japonica rice varieties (0.685−1.03%) (*4*). Moreover, the obtained value for phytate-P to total P ratio (69.2%) was similar to the value reported by Lott et al. (*19*), which was approximately 70%.

Effect of Location. There was a large variation in the total P, phytate-P, and phytate-P to total P ratio of the nine rice varieties grown in four different locations (**Table 4**). Similarly, significant effects of location were found in rice (*4*), wheat (*8*, *10*), barley (*9*), oats (*17*), and triticale (*18*). The significant effect of location could be attributed to the differences in soil P content, temperature, and precipitation levels during the cultivation period (**Table 1**). It has already been established that soil P content during cultivation positively affects the P content in crops (*20*−*22*). On the other hand, temperature and precipitation had varying effects on the P concentration in crops. Reports showed that the phytate-P content in soybean cultivated in a drained paddy field was higher than that in an upland field (*23*), wheat grown in irrigated conditions or locations with high precipitation levels had elevated phytate-P levels (*8*, *24*), and barley cultivated at higher temperature and precipitation levels had higher phosphorus and phytate contents (*13*). Futhermore, phytate-P content in oat grains decreased at high precipitation level during the grain filling stage and increased at high mean temperature (*25*).

The result showed that at similar precipitation level and mean temperature the P content in rice was positively affected. Daegu and Pohang recorded similar total precipitation levels and mean temperatures, but Pohang rice obtained total P and phytate-P (9.6 and 7.0 g/kg, respectively) higher than those of Daegu (8.6 and 5.9 g/kg, respectively) because of the higher soil P content in Pohang. However, at similar soil P content, the P content in rice was influenced more by the precipitation level and mean temperature. This was exhibited by the results in Andong and Bonghwa, which had similar soil P contents. Andong, which is a warm mountainous area and had the highest precipitation level (1520.2 mm) and high temperature (20.4 °C), obtained the lowest total P (8.2 g/kg), phytate-P (5.6 g/kg), and phytate-P to total P ratio (68.5%). On the other hand, Bonghwa, which is a cold mountainous area and had a total precipitation level (1395.8 mm) similar to that of Andong and the lowest temperature (16.2 °C) obtained the highest total P (11.1 g/kg), phytate-P (8.0 g/kg), and phytate-P to total P ratio (72.5%).

Table 5. Pearson's Correlation Coefficient between Growing Area, Growing Year, Total P, Phytate-P, and Ratio of Phytate-P to Total P

	location[a]	total P[a] (g/kg)	phytate-P[a] (g/kg)	phytate-P of total P[a] (%)
total P (g/kg)	0.765**			
phytate-P (g/kg)	0.633**	0.965***		
phytate-P of total P (%)	0.503*	0.569*	0.512*	
growing year	0.291[ns]	0.361[ns]	0.372[ns]	0.421[ns]

[a] ns, not significant at $p < 0.05$; *, **, ***, significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Table 6. Effect of Storage on the Total Phosphorus and Phytate-Phosphorus Contents in Nine Korean Rice Varieties Cultivated in Four Locations for Two Cropping Seasons

storage (months)	total P (g/kg)	phytate-P (g/kg)
1	8.0 ± 1.1[a]	5.6 ± 0.9
6	7.9 ± 1.0	5.5 ± 0.9
12	7.8 ± 0.9	5.3 ± 0.9
$LSD_{0.05}$	0.30	0.38

[a] Values represent mean ± SD, n = 30.

The result indicated the possibility of decreasing phytate-P in rice when cultivated in a warm mountainous area with high precipitation level and high temperature. Also, the result exhibited a negative relationship between phytate-P content and temperature and precipitation. This result was in agreement with the reported effect of precipitation level on phytate-P in oat (*24*), but in contrast with the generally observed effect of precipitation and temperature on P and phytate-P in wheat (*8*, *24*) and barley (*13*).

Correlation Analysis. **Table 5** shows the correlation analysis between location, growing year, total P, phytate-P, and phytate-P to total P ratio. Results showed a highly significant positive correlation (r = 0.965, $p < 0.001$) between phytate-P and total P content, which was similar to the high correlation (r = 0.93−0.97) found in wheat (*8*, *26*). Total P, phytate-P, and phytate-P to total P ratio were correlated with location (r = 0.765, $p < 0.01$; and r = 0.633, $p < 0.01$, respectively), which was in agreement with the significant effect of location on P contents in the rice samples analyzed. On the other hand, no parameters were correlated with the growing year, which could be due to the nonsignificant variations in temperature and precipitation levels in each location for the two cropping seasons.

Effect of Storage. The effect of storage on the total P and phytate-P concentration in rice is presented in **Table 6**. The total P and phytate-P concentrations did not decrease after dry storage for 12 months at ambient temperature. This result was similar with that of wheat stored at ambient temperature (*8*), but in contrast with that observed in wheat and barley stored at high temperature and moisture (*12*, *13*). The decrease in phytate content of grains stored at high temperature and moisture was attributed to phytate degradation by seed phytase (*12*, *13*), which is inactive in dry seeds (*27*). Thus, it could be inferred that the total P and phytate-P content stability in rice dry-stored at ambient temperature was due to the low phytase activity.

The results of this study revealed that variety and location significantly affected the total P, phytate-P, and phytate-P to total P ratio in rice, with location having a greater effect than variety.

A negative relationship between phytate-P content and temperature and precipitation was observed. Rice grown in locations with higher precipitation level and higher temperatures obtained the lowest total P, phytate-P, and phytate-P to total P ratio. Growing year did not affect the P accumulation in rice, which could be attributed to the similar climatic conditions in the 2-year experiment. Storage for 12 months did not change the total P and phytate-P in rice. The results could provide useful information for rice breeding and production purposes.

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