A Simplified Method for the Determination of the Amount of Mg and K Extracted from Rice Grains with a Hydrochloric Acid Solution

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Summary

A simplified method for selection for eating quality in early generations of rice involved the determination of the Mg/K ratio of brown rice by extraction with dilute hydrochloric acid (0.36 N) in several cultivars. Grains were extracted with hydrochloric acid for 60 minutes at room temperature, collected and washed immediately with distilled water. Germinability of grains after this procedure exceeded 80 %. Chemical analyses showed that 15-17 % of total K and 8-10 % of total Mg could be extracted by the proposed method.

Key Words: early generation, rice breeding, hydrochloric acid extraction, Mg/K ratio.

Introduction

Many components such as nitrogen, amylose, minerals and fatty acids in rice grains affect the eating quality of cooked rice. Kubo (1959) reported that "soft" rice which showed a high eating quality contained a large amount of Mg. Horino *et al.* (1983, 1984, 1994) and Okamoto (1994) reported that Mg and K contents or their ratio in brown rice were related to the stickiness of cooked rice. Cultivars, *e.g.* Koshihikari and Hinohikari, usually show a higher Mg/K ratio.

Lines in early generations of rice which are cultivated in a greenhouse for rapid generation advancement or in the field by direct planting, display a low growth rate and contain a few grains. In studies on selection in the early generations, breeding efficiency can be achieved provided that lines in the early generations which show a higher Mg/K ratio are selected rapidly using some nondestructive and simplified methods, to maintain a high level of germinability after chemical treatment.

Materials and Methods

Effect of HCl concentration and extraction time

Brown rice grains of 3 cultivars, *viz.* Nipponbare, Koshihikari and Takanari, harvested from 80 hills with 2 replications in a field of the Experimental Farm of Shiga Prefectural Agricultural Experiment Station in 1993 were tested. Approximately 2 g of grains were extracted with 100 ml of a 0.18-1.08 N HCl solution for

0.5-1.5 hours at room temperature (22 °C). Contents of extractable Mg and K were determined by atomic absorption and emission spectrophotometry. Dry weight of grains was measured after drying at 135 °C for 20 hours. After chemical treatment, the grains were soaked in water at 15°C for 72 hours and germinated on a moist filter paper in a culture dish at 30°C for 48 hours. Germination rate of grains was recorded, and the optimum pretreatment conditions were determined.

Effect of temperature

Brown rice grains of Nipponbare harvested at the Shiga Pref. Agr. Exp. Stn. in 1994 were tested. Approximately 2 g of grains were extracted with 100 ml of a 0.36 N HCl solution for 1 hour under 4 different temperature conditions, *viz.* 15, 20, 25 and 28 °C. Contents of extractable Mg and K were determined by atomic absorption and emission spectrophotometry. Dry weight of grains was measured at 135 °C for 20 hours. Germination rate of grains was recorded.

Relationship between T-Mg, T-K and Mg, K extracted from brown rice in some cultivars

Six cultivars harvested at the Shiga Pref. Agr. Exp. Stn. in 1994 were tested. Approximately 2 g of brown rice grains were extracted with 100 ml of a 0.36 N HCl solution for 1 hour at room temperature (22 °C). Dry weight of grains was measured at 135 °C for 20 hours. Nakajima *et al.* (1993) reported that the total amount of several minerals in brown rice powder was adequately extracted with 1 M hydrochloric acid, and Okamoto (1994) reported that the total amount of Mg and K in brown rice powder was adequately extracted with 1 % hydrochloric acid. In this paper, for the analyses of total Mg and total K, approximately 2 g of brown rice powder was added to a 0.36 N HCl solution, shaken for 1 hour and filtered with a filter paper (TOYOROSHI No. 6). Dry weight of powder was measured after drying at 135 °C for 2 hours. Contents of extractable Mg, K and T-Mg, T-K were determined by atomic absorption and emission spectrophotometry. The amount of Mg and K extracted from grains was compared with that of total Mg and K extracted from powder.

Results

Effect of HCl concentration and extraction time

Germination rate decreased with the increase of the HCl concentration or extraction time. As Table 1 and

Fig. 1 show, germination rate over 80 % was observed in samples extracted for 60 minutes using less than 0.36 N HCl. As Table 3 shows, analytical variation using 0.36 N HCl was remarkably smaller than that by 0.18 N

Fig. 1 Effect of HCl concentration and extraction time on the germinability of Nipponbare grains.
○ : 0.18N, △ : 0.36N, ● : 0.54N.

Table 1. Effect of concentration of HCl on germination rate and amount of extractable Mg, K¹⁾

Cultivar	Concentration of HCl(N)	Germination rate %	K (mg/100g)	Mg (mg/100g)
Koshihikari	0	98(100)		
	0.36	83(85)	43.2	12.0
	0.72	65(66)	42.0	12.8
	1.08	49(50)	46.3	15.2
Nipponbare	0	99(100)		
	0.36	88(89)	34.5	10.1
	0.72	74(75)	35.2	11.4
	1.08	65(66)	33.1	11.5
Takanari	0	76(100)		
	0.36	69(91)	44.2	15.0
	0.72	55(72)	46.7	18.7
	1.08	51(67)	45.0	20.0

¹⁾ Extracted at room temperature (22 °C) for 60 mins.
Cultivars tested were harvested in 1993, with 3 replications.

Table 2. Relationship between the amount of T-Mg, T-K extracted from brown rice powder and the amount of Mg, K extracted from brown rice grains¹⁾

Cultivar	K(mg/100g)		Mg(mg/100g)		Mg/K Chemical equivalent ratio	
	Powder ²⁾	Grains ²⁾	Powder ²⁾	Grains ²⁾	Powder	Grains(Calculated) ³⁾
Koshihikari	300(100) ^c	47.6(15.9) ^{cd}	135(100) ^a	11.5(8.5) ^b	1.45	0.78(1.40)
Nipponbare	285(100) ^d	45.1(15.8) ^{de}	122(100) ^b	10.1(8.3) ^d	1.38	0.72(1.30)
Kinuhikari	294(100) ^c	49.8(16.9) ^{bc}	122(100) ^b	10.7(8.9) ^c	1.34	0.69(1.24)
Hanaechizen	341(100) ^a	58.7(17.2) ^a	133(100) ^a	11.6(8.7) ^b	1.26	0.64(1.15)
Kokorozukushi	308(100) ^b	52.7(17.1) ^b	118(100) ^b	11.3(9.6) ^{bc}	1.24	0.69(1.24)
Hinohikari	249(100) ^e	42.0(16.9) ^e	131(100) ^a	13.3(10.2) ^a	1.69	1.02(1.83)

¹⁾ Extracted with 0.36 N HCl at room temperature (22 °C) for 60 mins. Three replications for powder and 5 for grains.

²⁾ Values followed by a common letter within a row are not significantly different at the 5 % level according to Duncan's Multiple Range Test.

³⁾ Calculation was as follows; 16 % of extractable K within total K, 9 % of Mg, shown in rows of relative K and Mg contents in grains.

HCl extraction.

Effect of temperature

The amount of extractable Mg increased in parallel to that of extractable K at higher temperatures. Germination rate, however, decreased at higher temperatures as shown in Fig. 2. The proposed procedures are considered to be suitable when the room temperature is less than 22 °C.

Relationship between T-Mg, T-K and Mg, K extracted from brown rice in some cultivars

Table 2 shows that the amount of Mg and K extracted from grains was 15-17 % for T-K and 8-10 % for T-Mg of that extracted from powder, respectively. Comparison of T-Mg/T-K ratios in 6 cultivars, *viz.* Koshihikari, Nipponbare, Kinuhikari and Hinohikari, revealed relatively higher values, over 1.30, with Hinohikari showing the highest value. Comparison of extractable Mg/K ratios revealed that the cultivars tested except for Hinohikari showed relatively similar ratios and the order of cultivars did not change.

The results of analytical variation are presented in Table 3. For both elements, although grains showed

Fig. 2 Effect of temperature during extraction on amount of Mg, K and germinability of Nipponbare grains.
○ : K, □ : Mg, ● : Germination rate.

Table 3. Variation in chemical analyses¹⁾

Cultivar	K analyses (CV %) ²⁾			Mg analyses (CV %) ²⁾		
	Powder	Grains	Grains	Powder	Grains	Grains
Koshihikari	0.57	2.65	8.55 ³⁾	0.60	4.00	10.58 ³⁾
Nipponbare	1.16	2.38	9.30 ³⁾	0.39	1.91	11.22 ³⁾
Kinuhikari	0.83	2.01	—	0.77	4.75	—
Hanaechizen	1.00	5.44	—	0.71	2.30	—
Kokorozukushi	3.01	6.18	—	0.80	4.03	—
Hinohikari	0.19	5.83	—	3.12	3.95	—

¹⁾ Extracted with 0.36 N HCl at room temperature (22 °C) for 60 mins.

Three replications for powder, 5 for grains.

²⁾ CV denotes coefficient of variation.

³⁾ Extracted with 0.18 N HCl.

obviously a higher variation, the coefficients of variation were relatively low, less than 6 %.

Discussion

Based on the present method, over 80 % of grains harvested from lines in early generations with limited growth still retained a high level of germinability after the chemical treatment. Extraction of T-K ranging from 15 to 17 % and of T-Mg in the range of 8-10 % was rapid. Horino *et al.* (1984) reported that minerals of brown rice could be analysed from the bran part and the Mg/K ratio of brown rice was similar to that of bran. By this method, the Mg and K contents of rice bran were analysed without milling. The amount of sample, 2 g, was sufficient to determine the Mg/K ratios because of the relatively smaller variation in the analyses. Provided that the variation of the Mg/K ratio and extraction rates among grains of 1 panicle remain small,

analysis of 1 grain may be possible. Further experiments should be carried out to confirm this assumption.

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