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Comparison and Evaluation on the Chemical Constituents of Progeny in T-DNA Inserted Rice

Yang Qin*, Suk-Man Kim*, Gynheung An**, and Jae-Keun Sohn*†

*Department of Agronomy, Kyungpook National University, Daegu, 702-701, Korea
**National Research Laboratory of Plant Functional Genomics Division of Molecular Life Sciences, Pohang University of Science and Technology (POSTECH), Pohang, 790-784, Korea

ABSTRACT With the development of diverse agricultures worldwide, biofortified rice noted for its preferable marketability and palatability plays an important role in the world's agricultural economics and rice breeding programs. In this report, several M_5 of T-DNA inserted lines derived from the donor cultivars, 'Hwayong' and 'Dongjin', were selected for high or low protein, high lipid and low amylose content, respectively. The coefficients and ranges of variation for the chemical constituents between M_4 and M_5 T-DNA inserted lines were evaluated in comparison with those of the donor varieties. Results indicated that T-DNA insertion might be an effective way to generate useful variations for chemical composition of rice grains which could be used for the development of biofortified rice cultivars.

Keywords : rice, chemical constituents, T-DNA inserted

Rice is the most important cereal crop in the developing world and it is the staple food for over half the world's population. With the consumption of boiled rice decreasing, however, the maintenance of its consumption is being pursued through the development of new products and the improvement of traditional grain products in order to maintain total rice production and consumption. Efforts to develop various biofortified rice cultivars and food stuffs have been in the demand for improving rice marketability and palatability (Choi, 2002). In recent years, biofortified rice has become a favorite food of customers. These include high amylose and high protein rice varieties for the processing of rice noodles and bread; indica scented rice for rice cakes and saccharified rice beverage; chalky kernel and large size rice for brewing and popping (Lee *et al.*, 1996); and high fiber and high amylose rice for healthy nutrition (Kang *et al.*, 2003; Kang *et al.*, 2005).

Knowledge gained in the past decades has indicated that conventional rice breeding, mutation breeding and collections from weedy rice were effective in developing biofortified cultivars (Hirochika *et al.*, 2004). Among them, mutation breeding was still the most universal method. Following physical and chemical mutagenesis systems, recent studies have indicated that the somaclonal variation can be treated as a third mutagenesis system, named biological mutagenesis. In this study, the M_4 and M_5 generations engineered from T-DNA inserted lines were separated according to grain chemical constituents and appearances. This was done in order to enhance biofortified rice development using T-DNA insertional mutagenesis.

MATERIALS AND METHODS

Plant materials

Fifty-six M_3 T-DNA inserted rice lines developed from the co-cultivating of scutellum-drived embryonic calli derived from the two cultivars, 'Hwayong' and 'Dongjin' and Agrobacterium tumefaciens LBA4404 carrying the binary- tagging vector were obtained from POTECH (Jeon *et al.*, 2000) in 2004. T-DNA inserted rice lines were sown during the rice-growing season from 2005 to 2006 on the experimental fields at Kyungpook National University, Gunwie, Korea. Twenty plants per line were planted at the spacing of 30 cm × 15 cm. About 30-day-old seedlings of transgenic lines were transplanted, with donor varieties as the control for each ten T-DNA inserted lines. Field management was conducted according to the normal cultivation practices recommended by rural development administration (RDA) with an

†Corresponding author: (Phone) +82-53-950-5711
(E-mail) Jhsohn@knu.ac.kr <Received August 13, 2007>

application for fertilizer at the rate of 110 kg N ha^{-1}, 45 kg P_2O_5 ha^{-1}, and 57 kg K_2O ha^{-1}.

Seed selection

The seeds of three or five plants were separately sampled for each transgenic line during harvest in 2005, with each plant as one line. After the seed harvest, a total of fifteen M_4 of T-DNA lines were selected by the agronomic traits and grain qualities, consisting of two lines of high protein, or low lipid content, three lines of low protein, or high lipid content and five lines of low amylose content (Fig. 1). As a result, a new transgenic rice population as mentioned above was constructed and sown in 2006. Five plants for each line were sampled during harvest, except one line of low lipid content with high sterility which was harvested as a bulk.

Trait evaluation

The agronomic traits consisting of heading date, culm length, panicle length and the number of panicles per hill were evaluated for the selected T-DNA inserted lines and five plants of each donor cultivars. The culm length was the distance from soil to the first node below the panicle, whereas the panicle length was the distance from the first node below the panicle to the top of the panicle. After harvest, the panicles per hill were counted as the number of panicles.

After threshing, the rice grains were dried and stored at room temperature for at least 3 months before analysis. The paddy rice was milled into brown rice using a small miller. Then, grain shape and chemical constituents were evaluated separately. The damaged grains, red grains, green grains and broken grains were removed from the brown rice before test. Lipid, protein and amylose content were tested by a NIR (Near-Infrared Spectroscopy) spectrophotometer (FOSS 6500) with three replications. Grain length, width and thickness were determined using a vernier caliper for the two replicates with 10 brown grains as one replicate for each line. One hundred grain weight was measured for the two replicates of 100 grains for each line.

RESULTS

Mean variations of chemical constituents for T-DNA inserted lines

The increased currents of mean values were shown on the lipid and protein content of T-DNA inserted lines in comparison with donor varieties; whereas an opposite trend on the amylose content (Table 1). The high coefficients of variation and wide ranges of values completely explained relatively wide variations on lipid, protein and amylose content occurred in the M_4 and M_5 T-DNA inserted lines. Compared to the M_4 generation, M_5 generation showed a distinct decrease in the lipid and protein content. Coefficients of variation between two generations of T-DNA inserted lines showed that the lipid content of transgenic lines derived from 'Hwayong' had a highly significant variation of 38.3%, whereas the protein content of transgenic lines derived from 'Dongjin' had a small variation of 4.9%. The correlation coefficients between amylose and lipid, or protein content were positively and negatively significant ($r = 0.64^*$, $r = -0.64^*$), respectively, indicating that T-DNA inserted had few effect on the relationships among the three chemical constituents of rice grains.

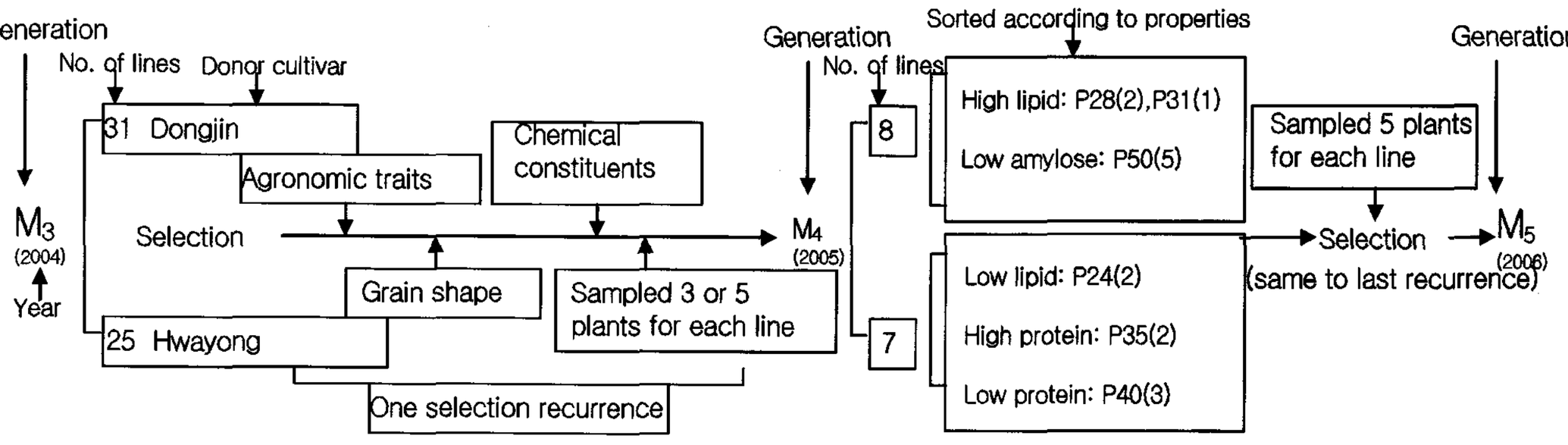

Fig. 1. Selection process of T-DNA inserted lines from M_3 to M_5 generation.

Table 1. Mean values and variations of chemical constituents for the M_4 and M_5 T-DNA inserted lines.

Varieties and lines	Lipid (%)			Protein (%)			Amylose (%)		
	Mean±SD	Range	CV (%)	Mean±SD	Range	CV (%)	Mean±SD	Range	CV (%)
Dongjin (2005)	1.92±0.05	-	5.4	7.21±0.04	-	1.1	19.41±0.20	-	2.1
M_4 T-DNA inserted lines	2.46±0.22	1.75~3.33	25.4	6.78±0.18	6.35~7.69	7.4	18.07±1.12	15.67~22.19	17.6
Dongjin (2006)	2.05±0.04	-	4.8	6.01±0.14	-	5.2	21.15±0.23	-	2.4
M_5 T-DNA inserted lines	2.32±0.06	1.91~3.04	16.8	6.35±0.07	5.65~7.34	7.1	20.49±0.43	17.67~25.79	13.4
Hwayong (2005)	1.89±0.03	-	3.7	6.92±0.09	-	3.4	20.36±0.38	-	2.7
M_4 T-DNA inserted lines	1.93±0.09	1.62~2.31	12.7	7.81±0.74	6.20~10.91	25.1	18.60±0.30	17.06~19.40	4.3
Hwayong (2006)	1.89±0.02	-	2.2	6.07±0.05	-	1.7	23.01±0.14	-	1.4
M_5 T-DNA inserted lines	1.70±0.06	1.22~2.02	20.1	6.35±0.13	5.29~8.05	11.1	21.04±0.40	13.08~23.37	10.5

Properties of the elite T-DNA inserted lines

Physicochemical characteristics of the elite lines are shown in Table 2. Lipid contents of the three M_5 T-DNA inserted lines were higher by 38.5% and 50.7% than that of variety 'Dongjin'. The M_5 lines with high protein and low amylose content relative to each donor variety were revealed an increased variation of 18.5-20.9% and a decreased variation of 15.0-19.3%; whereas a small decreased variation of 4.1-5.1% was evaluated for the lines of low protein content. Comparing to the coefficients of variation of two donor cultivars between two years, the transgenic lines showed small variations between M_4 and M_5, except that the two lines of high protein content showed significant coefficients of variation with a range of 19.3-23.7% between two generations.

The agronomic traits, grain shape and weight for the elite lines with variation of physicochemical traits were evaluated as shown on table 3. The culm length and panicle length were sharp reduction on the M_5 generation comparing to those of M_4 generation of P28, a mutant line with high lipid content. The M_5 of P40 with low protein content, as against the M_4, showed an increase on culm length, but a decrease on panicle length. The numbers of panicles per hill were markedly increased on M_5 of P35 and P40, two lines with high or low protein content, in contrast with those of M_4 lines and the donor cultivar of 'Hwayong'. As a mutant line

Table 2. The variations of chemical constituents between elite transgenic lines and each donor cultivar

Properties	M_4 of elite T-DNA inserted lines (2005)	Mean±SD[†]	M_5 of elite T-DNA inserted lines (2006)	Mean±SD	CV between M_4 and M_5[‡] (%)
Lipid content (%)	Dongjin[§]	1.92±0.10	Dongjin	2.05±0.09	3.78
	P28-2	3.33±0.04	P28-2-2	3.04±0.02	5.26
	P31-2	3.14±0.09	P31-2-1	3.09±0.09	0.93
Amylose content (%)	Dongjin	19.41±0.36	Dongjin	21.15±0.45	4.95
	P50-1	15.72±0.07	P50-1-1	17.07±0.48	3.88
	P50-5	15.67±0.09	P50-5-5	17.98±0.38	7.93
Protein content (%)	Hwayong[§]	6.92±0.17	Hwayong	6.07±0.10	7.56
	P35-2	10.28±0.06	P35-2-3	7.34±0.09	19.27
	P35-3	10.91±0.23	P35-3-1	7.19±0.11	23.73
	P40-1	6.20±0.10	P40-1-2	5.79±0.20	3.95
	P40-2	6.31±0.06	P40-2-1	5.76±0.07	5.26

[†]SD means standard deviation
[‡]CV indicates coefficient of variation
[§]Dongjin and Hwayong are the donor varieties of transgenic lines

Table 3. Comparison on the agronomic traits and grain shape of the M_4, M_5 elite transgenic lines in comparison with each donor cultivar

Donor varieties/ T-DNA inserted lines		Heading date (m/d)	Culm length (cm)	Panicle length (cm)	Number of panicles/hill	Length (mm)	Width (mm)	Thickness (mm)	Length /width	100-grain weight (g)
Dongjin		8/13-8/20	78.7±1.1	20.8±0.5	15±0.8	5.06±0.01	2.89±0.07	2.18±0.05	1.75±0.04	2.25±0.16
P28 (high lipid)	M_4	8/16	73.4±5.6	20.2±3.2	11±2.0	4.77±0.07	2.53±0.11	1.81±0.04	1.89±0.06	1.48±0.06
	M_5	8/19	56.2±2.3	15.8±1.7	10±2.1	4.84±0.14	2.50±0.09	1.73±0.09	1.94±0.05	1.28±0.08
P50 (low amylose)	M_4	8/21	76.5±1.1	22.3±3.4	14±3.2	4.90±0.21	2.79±0.09	1.89±0.09	1.76±0.09	2.12±0.05
	M_5	8/21	82.6±5.8	20.6±2.1	13±1.4	5.14±0.24	2.72±0.02	1.94±0.04	1.89±0.03	1.91±0.07
Hwayong		8/13-8/15	75.6±2.7	19.6±1.0	16±1.2	5.08±0.01	2.96±0.02	2.15±0.02	1.72±0.01	2.37±0.32
P35 (high protein)	M_4	8/16	46.4±2.0	17.4±0.8	12±3.0	4.69±0.32	2.70±0.18	1.94±0.18	1.74±0.06	1.89±0.19
	M_5	8/20	50.0±2.6	17.1±1.3	26±1.9	4.61±0.18	2.61±0.19	1.93±0.06	1.77±0.04	1.25±0.12
P40 (low protein)	M_4	8/15	67.2±2.5	19.1±1.5	11±2.0	5.29±0.26	3.02±0.10	1.97±0.01	1.75±0.03	2.27±0.10
	M_5	8/19	79.1±6.1	17.6±2.3	18±1.2	5.11±0.05	2.80±0.12	1.94±0.03	1.83±0.08	1.64±0.17

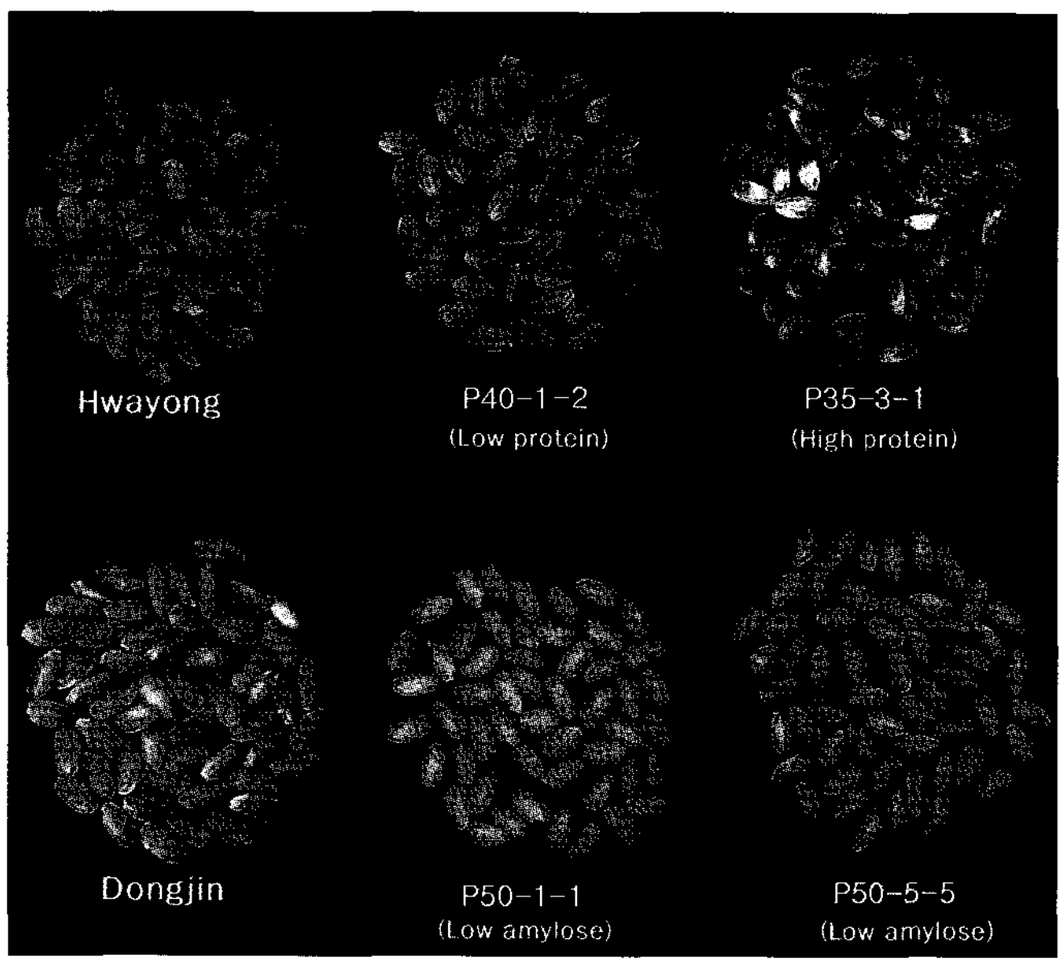

Fig. 2. Comparison on the appearance characteristics of the elite lines with high and low protein and low amylose and each donor cultivars.

with high protein content, P35 behaved in a stable trend between two generations of M_4 and M_5 on the culm length and panicle length, which were far below those of donor variety.

The significant decreases were observed on the grain thickness and weight of elite lines in compared with those of donor varieties. The variation in grain weight ranged from 34.0% to 38.7% in the lines with high lipid and protein content, but 10.6% in the lines of low amylose content. In addition, the transparency of brown rice was significantly declined on the elite lines with high or low protein and low amylose content, contrast with that of each donor variety (Fig. 2).

DISCUSSION

In the present study, significant variations of protein content were observed on the T-DNA inserted lines with high protein content between two years, indicating that the heredity of two generations was unstable. The reason for the low heritability of the transgenic descent might be considered as followed: First, the external environments, such as the temperature during the period of grain filling and fertilizers in the soil, either have some effects on the inserted T-DNA so as to interfere with self-expression, or to initiate the malfunction on gene expression of protein. Second, the inserted T-DNA was unstable to inherit over generations. The T-DNA either was a functional transposon which can move and activate the gene expression associated with protein synthesis, such like the *Ac/Ds* and *En/Spm* transposon systems (Parinov *et al.*, 1999; Tissier *et al.*, 1999), or keeps silencing of gene expression in the later generations.

Furthermore, the comparison on donor cultivars and T-DNA inserted lines for agronomic traits and grain shape revealed that plant height, grain weight and thickness were distinctly reduced in the transgenic lines of high protein and lipid content, but not in those of low amylose content.

Yoshida *et al.* (2002) mapped a QTL affecting protein content on the chromosome 1 beside semidwarf gene (*sd-1*) indicated that protein content may be controlled either by independent genetic effects or by secondary effects of sink-source balance to some extent. Moreover, the high protein lines tended to have lower yields, in view of the greater energy requirements for protein synthesis relative to starch synthesis (Juliano, 2003). However, it is interesting to note that T-DNA inserted promoted gene expression on the chromosomal region influencing protein content, but it seemed to have little effects or changes on genetic linkage of protein and culm length in this study. Thereby, T-DNA inserted lines with high protein as a kind of effective germplasms should be applied in the gene pyramiding and MAS breeding systems.

In addition, there is little probability that the mutated lines used in this study were *Tos17* inserted lines, even though we can not exclude the possibility completely. However, only 5% to 10% of the mutations identified in a tissue culture derived *Tos17* library were found to be caused by insertion of the retroelement (Hirochika 2001). Generally speaking, T-DNA integrates into gene and intergenic regions with comparable frequency and seems to prefer regions surrounding the ATG and stop codons, contrasting with *Tos17* insertions which exhibit a clear preference for coding sequences. The activity of the retrotransposon copies existing in the cultivars determined the occurrence of new *Tos17* inserts. But T-DNA lines derived from the donor variety of 'Dongjin' in the present study, which harbored an average of four new copies (Guiderdoni *et al.*, 2007). It indicates that the variations of *Tos17* inserted were not predominant.

Recently, a significant trend in rice mutation breeding is to lower protein content, even though to some extent an increase in protein content has been a major international breeding objective. Research was indicated that for proteins which are accommodated by two types of bodies, only one of them could be digested by human digestive organs. In order to reduce the uptake of the excess protein, low-protein rice was recommended for the patients with kidney troubles (Kumamaru *et al.*, 1997). In our study, the phenotypes of the elite lines with high lipid and low protein showed relatively stable expression, but those of the elite lines with high protein and low amylose declined the expression degree or expression dosage. Generally, the inheritance across environments and generations is considered valuable for breeders. In conclusion, for quantitative traits such as protein and lipid content, however, the successive selection and progeny test should be processed in a breeding system.

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REFERENCES

Choi, H. C. 2002. Current status and perspectives in varietal improvement of rice cultivars for high-quality and value-added products. Korean J. Crop Sci. 47(S) : 15-32.

Guiderdoni, E., G. An, S. M. Yu, Y. Hsing, and C. Y. Wu. 2007. Rice functional genomics. Chapter 9: T-DNA Insertion Mutants as a Resource for Rice Functional Genomics. pp. 208-213. Springer Science +Business Media, LLC.

Hirochika, H. 2001. Contribution of the *Tos17* retrotransposon to rice functional genomics. Curr Opin Plant Biol. 4 : 118-122.

Hirochika, H., E. Guiderdoni, G. H. An, Y. I. Hsing, M. Y. Eun, C. D. Han, N. Upadhyaya, S. Ramachandran, Q. F. Zhang, A. Pereira, V. Sundaresan, and H. Leung. 2004. Rice mutant resources for gene discovery. Plant Molecular Biology. 54 : 325-334.

Jeon, J. S., S. C. Lee, K. H. Jung, S. H. Jun, D. H. Jeong, J. W. Lee, C. H. Kim, S. H. Jang, S. Y. Lee, K. Y. Yang, J. M. Nam, K. S. An, M. J. Han, R. J. Sung, H. S. Choi, J. H. Yu, J. H. Choi, S. Y. Cho, S. S. Cha, S. I. Kim, and G. H. An. 2000. T-DNA insertional mutagenesis for functional genomics in rice. The Plant Journal. 22(6) : 561-570.

Juliano, B. O. 2003. Rice chemistry and quality, 3rd ed., pp. 25-39, Philippine rice research institute, Manila.

Kang, H. J., I. K. Hwang, K. S. Kim, and H. C. Choi. 2003. Comparative structure and physicochemical properties of Ilpumbyeo, a high-quality japonica rice, and its mutant, Suweon 464. J. Agric. Food Chem. 51 : 6598-6603.

Kang, H. G., S. H. Park, M. Matsuoka, and G. H. An. 2005. White-core endosperm floury endosperm-4 in rice is generated by knockout mutations in the C_4-type pyruvate orthophosphate dikinase gene (*OsPPDKB*). The Plant Journal. 42 : 901-911.

Kumamaru, H., H. Sato, and H. Satoh. 1997. High-lysine mutants of rice, Oryza Sativa L.. Plant breeding. 116 : 245-249.

Lee, S. Y., Y. T. Lee, H. J. Kang, H. S. Kim, Y. D. Kim, and H. T. Shin. 1996. Agronomic characteristics and physicochemical properties of white-center endosperm mutants in

rice. Korean J. Breed. 28(4) : 399-408.

Parinov, S., M. Sevugan, Y. De, W. C. Yang, M. Kumaran, and V. Sundaresan. 1999. Analysis of flanking sequences from dissociation insertion lines: a databasefor reverse genetics in *Arabidopsis*. Plant Cell. 11 : 2263-2270.

Tissier, A. F., S. Marillonnet, V. Klimyuk, K. Patel, M. A. Torres, G. Murphy, and J. D. G. Jones. 1999. Multiple independent defective *Suppressor-mutator* transposon insertions in *Arabidopsis*: a tool for functional genomics. Plant Cell. 11 : 1841-1852.

Yoshida, S., M. Ikegami, J. Kuze, K. Sawada, Z. Hashimoto, T. Ishii, C. Nakamura, and O. Kamihima. 2002. QTL analysis for plant and grain characters of sake-brewing rice using a doubled haploid population. Breeding Science. 52 : 309-317.