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Genetic Mapping of Giant Embryo Mutant and Varietal Variation of Rice Embryo

BY

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UNDER THE DIRECTION OF PROFESSOR HEE-JONG KOH
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF SEOUL NATIONAL UNIVERSITY

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GILEUNG LEE

GENERAL ABSTRACT

Rice embryo provides many essential nutrients for human health as well as other valuable industrial materials, and the embryo size is also positively correlated with the contents of such salutary compounds as γ-aminobutyric acid (GABA), α-tocopherol and vitamins. Thus controlling embryo size is becoming more and more important in the field of rice breeding. We obtained three giant embryo mutants derived from Hwacheongbyeo (Korean *japonica* cultivar) by *N-methyl-N-nitrosourea* (MNU). These three mutants, embryo size of all of which were larger than that of wild type (Hwacheongbyeo), were named according to different embryo size as *ge-m*, *ge* and *ge-s*, from the smallest to the largest, respectively. The result of allelism test among wild type, ge-*m*, *ge* and *ge-s* revealed that the embryo size of *ge* and *ge-s* was controlled by the same gene (*GIANT EMBRYO*, *GE*). Through *GE* locus sequencing of three mutants, we found that each of *ge* and *ge-s* had a point mutation in *GE* locus, causing amino acid

substitution in the coding region, but *ge-m* had no sequence alteration in the locus. We performed genetic mapping of the gene controlling *ge-m* phenotype and identified that the gene is located in chromosome 3 between S03p28781 and RM15758 markers.

In spite of high nutritional value, most of embryos, in general, are detached from grain and lost during milling process. Therefore, increasing unstripped embryo rate (UER) is important during milling process. We conducted comparative analysis on grain and embryo traits among 49 rice varieties in order to select desirable candidates of embryo rice and to collect basic data for developing high UER variety. Correlation analysis among traits collected showed that embryo dent, which in turn is expected to affect UER was positively correlated with grain width($r=0.53^{**}$) and grain area($r=0.34^{*}$), while negative correlation with grain length to width ratio($r=-0.38^{**}$), revealing that the attached shape of embryo was influenced by the shape of grain. Likewise, it was noted that the embryo dent was mainly affected by grain width, not by grain length, and embryo dent affected to the position of embryonic shoot and radicle. Daerip 1, Jinbu, Jinbo, Heugseol, Obong, Unkwang, Cheongnam, Koshihikari, Cheonghaejinmi and Boramchan showed high embryo dent. These data and the varieties identified by this study could be useful in selecting promising varieties with high UER and developing new variety of higher UER.

Key words: rice, giant embryo mutant, embryo size, mapping, unstrapped embryo rate (UER), embryo dent

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CHAPTER Ⅱ

VARIETAL VARIATION OF RICE EMBRYO

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LIST OF ABBREVIATIONS

BSA	Bulked segregant analysis
CAPS	Cleaved amplified polymorphic sequence
CTAB	Cetyltrimethylammonium bromide
dCAPS	Derived cleaved amplified polymorphic sequence
ED	Embryo dent
EER	Embryo to endosperm ratio
EL	Embryo length
EMW	Endosperm weight
ENW	Embryo weight
GA	Grain area
GABA	γ −aminobutyric acid
MNU	N−merhyl−N−nitrosourea
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
SAS	Statistical analysis system
SSR	Simple sequence repeat
STS	Sequence tagged site
UER	Unstripped embryo rate

CHAPTER Ⅰ.

Genetic analysis of giant embryo mutants

ABSTRACT

Rice embryo provides many essential nutrients for human health as well as other valuable industrial material, and the embryo size is also positively correlated with the contents of such salutary compounds as γ-aminobutyric acid (GABA), α-tocopherol and vitamins. Thus controlling embryo size is becoming more and more important in the field of rice breeding. We obtained three giant embryo mutants derived from Hwacheongbyeo (Korean *japonica* cultivar) by treatment of chemical mutagen, *N-methyl-N-nitrosourea* (MNU). These three mutants, embryo size of all of which were larger than that of wild type (Hwacheongbyeo), were named according to different embryo size as *ge-m*, *ge* and *ge-s*, from the smallest to the largest, respectively. The result of allelism test among wild type, *ge-m*, *ge* and *ge-s* revealed that the embryo size of *ge* and *ge-s* was controlled by the same gene (*GIANT*

EMBRYO, *GE*). Results of *GE* locus sequencing in three mutants revealed that each of *ge* and *ge-s* had a point mutation, causing amino acid substitution in the coding region, but *ge-m* had no sequence alteration in the locus.

In this study, we identified that *ge*, *ge-s* were allelic to *GE* and that *ge-m* gene is located in short arm of chromosome 3 between two markers, S03p28781 and RM15758.

INTRODUCTION

Rice is one of the most important food crops in the world, especially in Asian countries. In the past few decades, elevating yield was the most important objective in rice research. Currently, this goal has been achieved in some degree, however, emphasis has been changed to improve the grain quality, due to the increasing local and global demand for high quality of rice (Fitzgerald et al. 2009). Although rice embryo accounts for only 2~3% of total seed, it contains protein, fat, vitamins and embryonic metabolites such as γ-aminobutyric acid (GABA), α-tocopherol and γ-oryzanol, whereas endosperm contains most of the starch which is energy source for human beings. Previous studies using various embryo mutants (Nagato et al. 1989; Kitano et al. 1993; Hong et al, 1995) showed that embryogenesis is controlled by complex regulatory processes and endosperm development affects to embryo size regulation (Hong et al. 1996). Thus, it is important to understand how embryo size is regulated because it dictates the composition of rice nutrients. The *giant embryo (ge)* mutant was first reported by Satoh and Omura (1981). Single recessive *GE* gene was mapped between RZ395 and RG678 on chromosome 7 (Koh et al. 1996). This *GE* gene, encoding for cytochrom p450 protein was cloned by Cahoon et al. (2003). Park et al. (2009) reported a new allele of *GE*

gene, *ge*^{t}, derived by anther culture. Histological observation revealed that the phenotype of *giant embryo* mutant, enlarged embryo, was caused by increase of scutellum size, not by increase of embryonic shoot and radical size (Kitano et al. 1993). The enlarged embryo size showed increase of embryonic nutrients in rice (Koh et al. 1993; Park et al. 2009; Zhang et al. 2005) and in maize (Moose et al. 2004). Zhang et al. (2012) reported that evolutionarily conserved mechanisms for regulation of embryo size were existed in rice and maize. Other giant embryo mutant, *goliath (go)*, was reported by Taramino et al. (2003) and was cloned by Sakai and Taramino (2006). Kawakatsu et al. (2009) also reported a new allele of *GO* gene, *plastochron3 (pla3)*, and isolated GO gene by positional cloning in chromosome 3. This gene encodes glutamate carboxypeptidase regulating various developmental processes including plastochron. Also each three QTLs for embryo length and embryo width were detected by Dong et al. (2003). In maize, 10 QTLs for embryo to endosperm ratio (EER) were identified by Yang et al. (2012) and *ZmGE2* gene, homologs of *OsGE*, was cloned by Zhang et al. (2012). In arabidopsis, Kpndou et al. (2008) reported that *RETARDED GROWTH OF EMBRYO1 (RGE1)* gene control embryo growth through positively regulation of other genes including gene encoding cytochrome p450 protein.

In this study, characterization of three giant embryo mutants (Kim et al. 1991) and genetic mapping of *ge-m* which regulates embryo size were performed.

MATERIALS AND METHODS

1. Plant materials

Wild type (Korean *japonica* cv. Hwacheongbyeo) and three giant embryo mutants, *ge-m*, *ge* and *ge-s* (Kim et al. 1991) were used in this study. These mutants were derived from Hwacheongbyeo by treatment of *N-methyl-N-nitrosourea* (MNU). *ge-s* is allelic to *GIANT EMBRYO (GE)*. Plant materials were grown at the Experimental Farm of Seoul National University in Suwon, Korea.

2. Phenotypes examination

For the phenotypes examination of mutants, several agronomical traits from plant to grain were measured in wild type and three giant embryo mutants. Including heading date, culm length, panicle length, tiller number, panicle number, spikelet per panicle, spikelet fertility, 1000-grains weight, grain length, grain width, grain length to width ratio, embryo length and EER, a total of 13 traits were measured. These were analyzed using Statistical Analysis System (SAS) program. Husked seeds were used in measuring grain and embryo dimension including 1000-grains weight, grain length, grain width, grain length to width ratio, embryo length and EER. For measuring EER, 200 uniform grains (13% water content) were dissected into embryo and endosperm and each parts was weighed,

respectively, and EER was calculated.

3. DNA extraction and PCR amplification

Genomic DNAs were extracted from fresh young leaves according to the modified CTAB method (Murray and Thompson 1980). PCR amplification was performed in a total 20ul reaction mixture containing 2ul of genomic DNA (20ng/ul), 1ul of 10X buffer (Mg^{2+}), 1ul of each primer (2.5uM) and 0.5U of Taq DNA polymerase. Amplification was carried out in a PTC220 dual 96-well thermocycler (MJ Research, USA). The PCR conditions were 5min at 95℃ and 35cycles(30 sec at 94℃, 30sec at 56℃, 1min 72℃), followed by final extension for 7min at 72℃. For detection of polymorphisms, the PCR products were electrophoresed on 3% agarose gel.

4. Primers

Primers were developed based on available rice genome sequence data (http://www.ncbi.nlm.nih.gov; http://www.gramene.org). CAPS and dCAPS markers were designed with dCAPS Finder 2.0 (http://helix.wustl.edu/dcaps/dcaps.html) and STS markers were designed by *in silico* approach (Primer3 software version 0.4.0; http://frodo.wi.mit.edu/primer3).

5. γ-aminobutyric acid (GABA) content analysis

50mg of finely ground brown rice powder was homogenized with 1ml of 25% acetonitrile /0.01N HCL, and then centrifuged at 13,000 rpm for 3min. After filtered with 0.2um syringe filter, the supernatant was derivatized by using EZ:Faast kit (Phenomenex, Torrance, USA) followed by detection through gas chromatography. A GC-2010 gas chromatograph (GC-2010, Shimadzu, Japan) equipped with a flame ionization detector (FID) was used with a ZB-5(30m x 0.25mm, 0.25um) capillary column. Each sample was tested four times.

6. Histochemical analysis

For characterization of embryonic phenotypes, a standard paraffin embedding method was performed with slight modifications. All seed samples which were harvested at the stage of 20 days after pollination (DAP) were fixed in FAA (formaldehyde 3.7% : acetic acid 5% : ethanol 50%) for 1day at 4℃ and then dehydrated for 2 hours each in a graded ethanol solution series(70%, 85%, 95% and 100%). At the final step, rice embryo samples were dehydrated for 1day. After that, dehydrated samples were cleared for 2hours each in a clearing solution series consisting of 75% ethanol / 25% histo-clear, 50% ethanol / 50% histo-clear, 25% ethanol / 75% histo-

clear, followed by clearing with 100% histo-clear for 1 day. For paraffin infiltration, samples were soaked for 2 hours each in the histo-clear/paraffin solution series consisting of 75% histo-clear / 25% paraffin, 50% histo-clear / 50% paraffin, 25% histo-clear / 75% paraffin, and 100% paraffin at 55℃ for 1day. The paraffin infiltrated samples were embedded in tissue path embedding ring and then cut by HM 340 E Rotary Microtome (MICROM Lab, Germany) into 6um sections. The sections were transferred to gelatin (sodium salicylate 1g, egg white 50ml, glycerol 50ml) coated microscope slide glass and dried at 40℃ for 1day. The sections were deparaffinized with 100% xylene for 30 min and followed by hydrated with 50% xylene / 50% ethanol for 30min, followed by soaking for 2min in sterile water twice. The samples were then stained in safranin O solution (1% safranin O : 30% ethanol=1:4) for 2min. After washing with sterile water twice, Samples were dehydrated for 2 min each in a graded ethanol solution series (30%, 50%, 70%, 85% and 95%). Finally, the samples were cleared by soaking in 100% xylene and mounted in canada balsam. The anatomical structure of rice embryo was observed with optical microscopy at magnification of 100X and 600X.

7. Sequencing alignment and co-segregation analysis

Two overlapping DNA fragments including *GIANT EMBRYO (GE)* gene (Cahoon et al. 2003) were amplified by PCR. PCR products were sequenced with an ABI Prism 3730 XL DNA Analyzer (PE Applied Biosystems, USA) via TA cloning. The results of sequencing were aligned using CodonCode Aligner software (CodonCode Corperation, USA).

For co-segregation analysis, two sets of primers, CAPSge-F (5'-CCCTACATCCAGTCCATCGT-3') / CAPSge-R (5'-ATCGCCCACATGTTCACC-3') and dCAPSgesX-F (5'-ACCCGCCGGGCCCACTCCTGTCGT-3') / dCAPSgesX-R(5'-GTCCTCCCCCTCCGAGAAG-3'), were designed and used for PCR amplification. PCR products amplified by CAPSge and dCAPSgesX primer set were digested with *Sph I* and *Xcm I*, respectively, at 37℃ for 2 hours. After digestion, digests were electrophoresed in a 3% agarose gel.

8. Genetic analysis and mapping

F_1 and F_2 seeds derived from reciprocal crosses among Hwacheongbyeo (normal embryo size), and three mutants (enlarged embryo size) were used for genetic analysis. F_2 seeds of each cross combination were classified according to embryo size, respectively.

For genetic mapping of *ge-m* gene, F_2 population was developed from a cross between *ge-m* and Hangangchal 1 (a Korean *tongil-type*, similar to *indica*) for genetic mapping of *ge-m* gene. Phenotype of F_2 plant was determined through F_3 seed segregation ratio. The 20 *ge-m* type plants and 20 wild-type plants in the *ge-m*/Hangangchal 1 F_2 population were selected and used in bulked segregant analysis (BSA) (Michelmore et al. 1991). Two bulked sample of each ten plants genomic DNA were employed for genotyping. The 66 STS markers (Chin et al. 2007), 3 SSR markers (Akagi et al. 1996; Temnykh et al. 2001; International Rice Genome Sequencing Project 2005) and 7 newly developed STS markers were used for genetic mapping. Newly designed primers are listed in (Table 1.)

Table 1. PCR-based molecular markers designed for fine mapping of the *ge-m* gene

Name	Type	Sequence (Forward / Reverse)	Product size (bp)	Physical position (NCBI)
S03p28125	STS	GGTAATCATCAGCGTTGTGAGA / TTAGCTTTGCCCCATGTCTACT	149	28124834
S03p28566	STS	GAATTGGGCTTATCCCTGGT / TTGCATATGAGCTTAATTTATCCAG	159	28565785
S03p28769	STS	GGGAGGTTTGTGGGATTCTT / TTAATAACCTCGCAGCAGCA	187	28769263
S03p28781	STS	CCGTGTCAATTGGTACTTGG / TATCCGGCCTCTGCAATAAG	177	28780630
CS03p28875	CAPS	GAGCGCACATGGTGGTAAT / TCGCTTCACCATGTTAGGTG	314	28875349
S03p28946	STS	GCAACATCTACGTTTGCAAGTT / GTTAGTATAGCAGGGGCACAGG	254	28945638
S03p28957	STS	AAGTTGCAACGAAAAAGACAAA / CACCTACGGCCTAGCTTTGA	245	28957211
S03p29096	STS	GAAAAACTCGGAAGAAAAGCAA / GGTTTAAATTGGGTTCAGCAA	137	29084637

NCBI (http://www.ncbi.nlm.nih.gov/)

RESULTS

Characterization of three giant embryo mutants

We obtained three giant embryo mutants derived from Hwacheongbyeo (Korean *japonica* cultivar) by treatment of chemical mutagen, *N-methyl-N-nitrosourea* (MNU). All of these three mutants had enlarged embryo phenotype compared to wild type but presented a difference in the degree of embryo size. Mutants named according to different embryo size as *ge-m*, *ge* and *ge-s* (Fig.1). The embryo of *ge-m* is smaller than that of *ge,* and the *ge-s* had large embryo compared to *ge*. Analysis of embryonic traits among wild type and three mutants showed significant differences in embryo length and EER (Table 2). The more embryo size was large, the more embryo length and EER were increased. Also, several agronomic traits showed significant difference among wild type and three mutants (Table 3). The *ge-m* was 10 cm shorter than wild type and other mutants in culm length and has slightly small grain length. Panicle length of *ge* and *ge-m* slightly shorter than wild type, whereas *ge-s* taller than wild type.

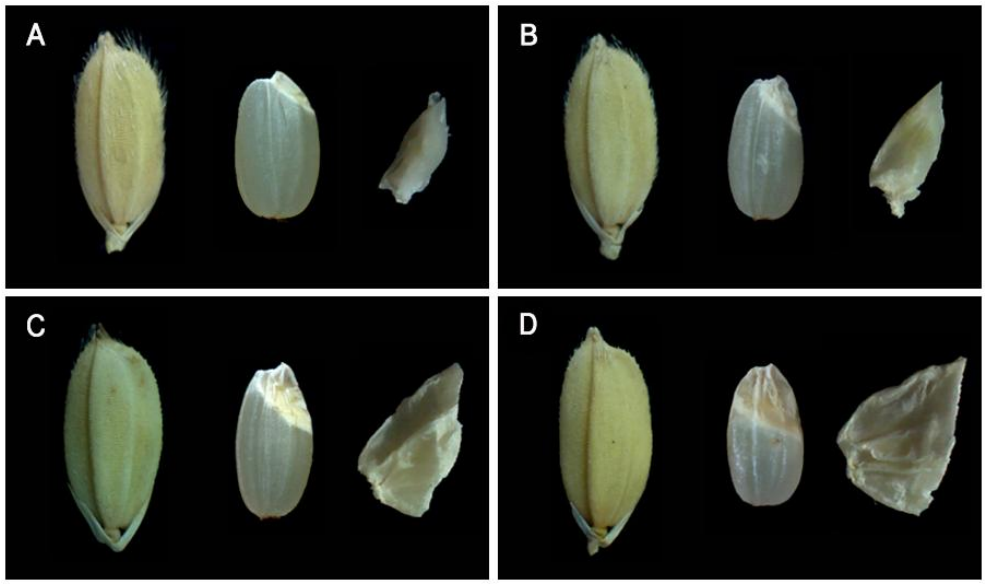


Figure 1. Grain phenotype of wild type and thee giant embryo mutants. from left : seed, brown rice and dissected embryo of (A) wild type (Hwacheongbyeo), (B) *ge−m*, (C) *ge*, (D) *ge−s*.

Table 2. Embryonic traits of wild type, *ge−m*, *ge* and *ge−s*

	EL* (mm)	EMW (g)	ENW (g)	EER (EMW/ENW)
Wild type	2.0±0.11d[a]	0.13	4.13	0.03
ge−m	2.2±0.08c	0.17	3.56	0.05
ge	2.7±0.12b	0.32	3.21	0.10
ge−s	3.3±0.30a	0.45	2.82	0.16

EL=embryo length, EMW=embryo weight, ENW=endosperm weight.

* denote a difference at the 5% significance level.

[a] Means followed by the same letter are not significantly different at the 5% level, as verified by an DUNCAN test.

Table 3. Agronomic traits of wild type, *ge-m*, *ge* and *ge-s*.

	HD (date)	CL*[a] (cm)	PL* (cm)	PN[ns] (No.)	SPP[ns] (No.)	SF[ns] (%)	1000-grains weight(g)	GL* (mm)	GW[ns] (mm)	LWR* (GL/GW)
Wild type	Aug. 20	86.4±2.3a[b]	18.5±0.6ab	12.4±1.8	94.7±4.2	93.3±1.9	21.3	4.9±0.2bc	2.8±0.1	1.7±0.1bc
ge-m	Aug. 19	77.0±2.6b	18.7±1.2a	12.3±2.2	101.0±1.0	91.1±6.5	18.7	4.8±0.2c	2.8±0.1	1.7±0.1c
ge	Aug. 23	88.9±3.7a	17.6±1.5b	14.2±2.5	100.3±12.2	92.4±4.4	17.6	5.0±0.2ab	2.8±0.1	1.8±0.1ab
ge-s	Aug. 19	89.0±2.6a	19.1±0.9a	13.3±2.2	94.3±5.5	94.3±1.0	16.4	5.2±0.2a	2.8±0.1	1.9±0.1a

HD=heading date, CL=culm length, PL=panicle length, PN=panicle number per plant, SPP=spikelet per panicle, SF=spikelet fertility, GL=grain length, GW=grain width, LWR=grain length to width ratio

[a] * and ns denote a difference among wild type (Hwacheongbyeo) and three mutants at the 5% significance level and at a non-significant level, respectively.

[b] Means followed by the same letter are not significantly different at the 5% level, as verified by an DUNCAN test.

Comparison of GABA contents

γ-aminobutyric acid (GABA) is well known bioactive plant component in rice embryo which is effective in sleeplessness, depression, blood pressure regulating and recovery of alcohol-related symptoms (Omori et al. 1987; Elliott and Hobbiger 1959; Oh et al. 2003). GABA content was analyzed in Hwacheongbyeo (wild type), *ge-m*, *ge* and *ge-s*. Finely ground brown rice powder was used for analysis. The result showed that the GABA content was increased in grain of mutants compared to wild type (Fig. 2). Furthermore, it displayed a positive correlation between embryo size and GABA content.

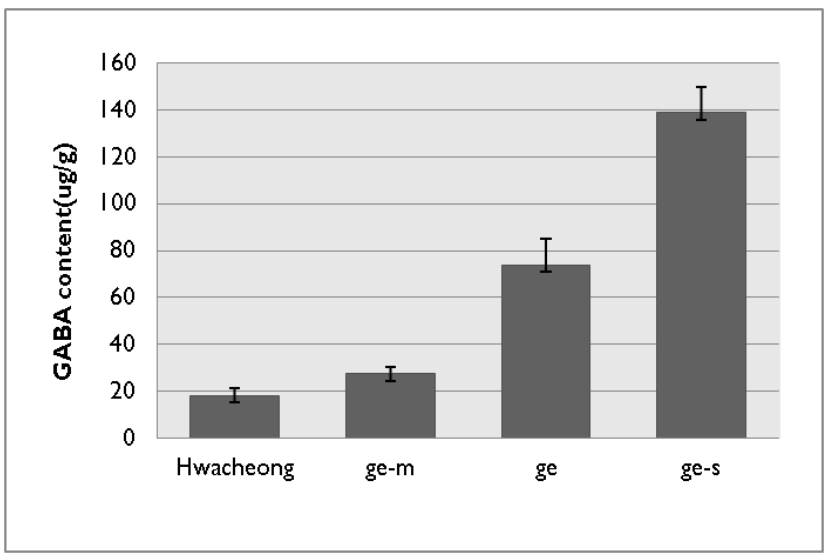


Figure 2. Comparison of GABA content among wild type, *ge-m*, *ge*, and *ge-s*.

Histological analysis

Longitudinal sectioning of embryo was carried out to observe changes at histological level. Previous study using giant embryo mutants revealed that giant embryo phenotype was due to only the enlargement of scutellum, while the size of shoot and radical was unchanged (Kitano et al. 1993). Similar to previous studies, the most noticeable change was enlarged cells in scutellum of mutants compared to wild type (Fig. 3). In all of the three mutants, enlarged scutellum phenotype was observed, but the size of enlarged cell was different in proportion to embryo size. No significant change was observed in shoot and radical. It was also noted that *ge* was found to be almost similar to histological morphology of *ge-s* which is allelic to *GIANT EMBRYO (GE)*. This suggests that the mutant phenotype of *ge* might be affected by *GE* gene, which encode cytochrome p450 or other gene related to cell division and expansion.

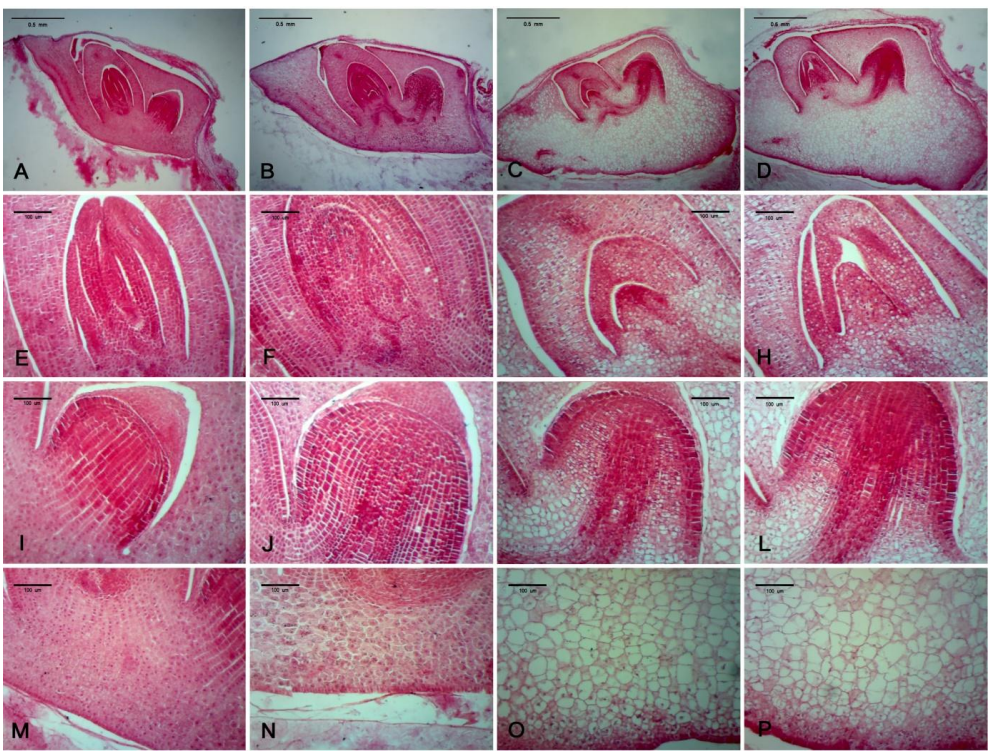


Figure 3. A longitudinal section of embryo. (A–D) Longitudinal morphology of whole embryo at 20 DAP. (E–H) Close–up of the shoot. No change was detected in shoot. (I –L) Close–up of the radical. No change was detected in radical. (M–P) Close–up of the scutellum. The cell size of scutellum was enlarged. (A, E, I, M)= Wild type (Hwacheongbyeo), (B, F, J, N)= *ge–m*, (C, G, K, O)= *ge*, (D, H, L, P= *ge–s*.(Bar = 0.5mm in (A–D), 100um in (E–P)

Genetic analysis of three giant embryo mutants

For allelism test, reciprocal crosses were performed among wild type (Hwacheongbyeo), *ge-m*, *ge* and *ge-s*. F_2 seeds derived from 12 cross combination were used for analysis of segregation ratio (Table 4). 6 F_2 seed populations which were derived from crosses between wild type and mutants were fitted the expected ratio of 3:1. Therefore, each of three mutants was controlled by single recessive gene. Two F_2 seed populations derived from crosses between *ge* and *ge-s* were also fitted the expected ratio of 3:1, but all of the seeds were mutant type, *ge* type with *ge-s* type at 3:1 ratio. This result presented that *ge* was allelic to *ge-s*. In other words, the two mutants were controlled by *GIANT EMBRYO (GE)* gene. Interestingly, four F_2 seed populations which were derived from crosses between *ge-m* and other two mutants included wild type seeds and were fitted the expected ratio of 9:3:1 (Fig. 4). This suggests that *ge-m* was controlled by a gene which is different with the gene controlling *ge* and *ge-s* phenotype. With these data, it was noted that *ge* and *ge-s* were allelic to *GE* gene and that *ge-m* phenotype affected by a single recessive gene differed with *GE* gene.

Table 4. Chi-square test of F_2 seed segregation ratio

Cross combination	Segregation					Expected Ratio	*x2*	p-value
	Normal	*ge-m*	*ge*	*ge-s*	Total			
Wild type[a] / *ge-m*	299	99	-	-	398	3:1	0	1
ge-m / Wild type	260	100	-	-	360	3:1	1.34	0.25
Wild type / *ge*	348	-	134	-	582	3:1	1.87	0.17
ge / Wild type	269	-	83	-	352	3:1	0.31	0.58
Wild type / *ge-s*	246	-	-	76	322	3:1	0.27	0.61
ge-s / Wild type	265	-	-	84	349	3:1	0.12	0.73
ge-m / *ge*	177	64	71	-	312	9:3:4	1.16	0.56
ge / *ge-m*	145	48	63	-	256	9:3:4	0.02	0.99
ge-m / *ge-s*	178	60	-	78	316	9:3:4	0.02	0.99
ge-s / *ge-m*	160	51	-	63	274	9:3:4	0.67	0.72
ge / *ge-s*[b]	-	-	-	-	-	-	-	-
ge-s / *ge*	-	-	296	117	413	3:1	2.27	0.13

[a] Wild type = Hwacheongbyeo

[b] *ge* / *ge-s* cross combination excepted from genetic analysis owing to poor ripening

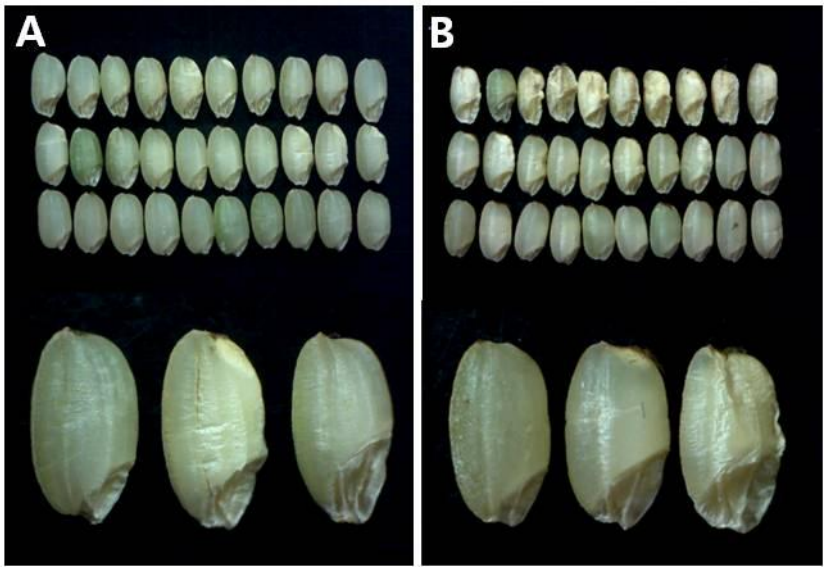


Figure 4. F_2 seed phenotype segregated from *ge−m* / *ge* and *ge−m* / *ge−s*. (A) F_2 seeds phenotype derived from *ge−m* / *ge* cross combination, (b) F_2 seeds phenotype derived from *ge−m* / *ge−s* cross combination.

GIANT EMBRYO (GE) gene sequence alignment

GIANT EMBRYO (GE) gene was sequenced in *ge−m*, *ge*, *ge−s* and wild type (Hwacheongbyeo), and alignment analysis was carried out in order to confirm the result of allelism test. This analysis identified one point mutation each in *ge* and *ge−s* and further confirmed that enlarged embryo phenotype was controlled by *GE* gene in *ge* and *ge−s* (Fig. 5). In the *GE* ORF region, *ge* had a guanine to adenine non synonymous nucleotide substitution causing arginine to histidine amino acid change and *ge−s* had a guanine to

thimine non synonymous nucleotide substitution causing tryptophan to leucine amino acid change. Unlike the two mutants, sequence of *ge-m* was same with wild type in *GE* locus. In addition, sequence of *GOLIATH (GO)* gene, other gene causing giant embryo phenotype, was found to be the same with wild type in *ge-m* (data not shown). In conclusion, we demonstrated that two mutants, *ge* and *ge-s*, were allelic to *GE* gene and that another *ge-m* phenotype was controlled not by *GE* and *GO* gene but other gene.

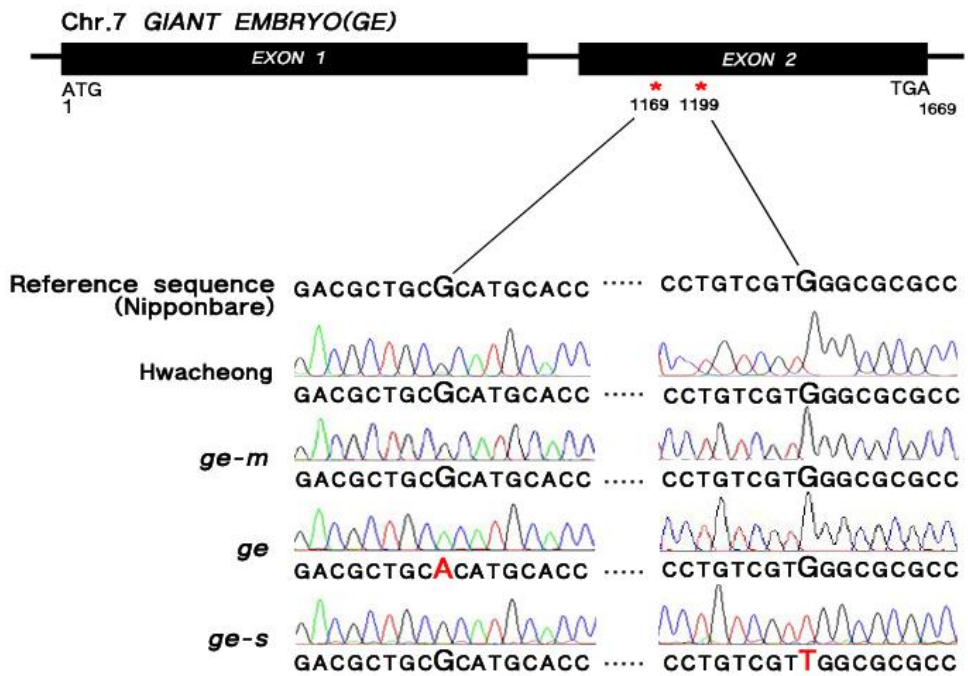


Figure 5. Mutation points of *ge* and *ge-s* in *GE* locus. Red star denote two mutation points. One is G1169A and the other is G1199T causing R390H and W340L, respectively.

Co-segregation test of *ge* and *ge-s* alleles

To confirm the association between enlarged embryo phenotype and point mutations in *GE* gene, CAPS and dCAPS marker were developed. For this analysis, two F_2 seed populations derived from wild type / *ge* and wild type / *ge-s* were used for genomic DNA extraction. First, CAPS primer set consisting of CAPSge-F and CAPSge-R was used to confirm the correlation between enlarged embryo phenotype and *ge* point mutation in *GE* gene. 7 wild type seeds and 7 mutant type seeds were selected from F_2 seed population derived from wild type / *ge,* and PCR was performed. After digestion with *Sph I* , only wild type PCR product was cut into 112bp size. A clear co-segregation observed between wild type and *ge* type (Fig. 6A). Second, dCAPS primer set consisting of dCAPSgesX-F and dCAPSgesX-R was used for co-segregation analysis in F_2 seed population derived from wild type / *ge-s*. Same process was performed except digestion with *Xcm I* . The enzyme only cut the wild type PCR product into 160bp. Co-segregation between wild type and *ge-s* type was clearly distinguished (Fig. 6B).

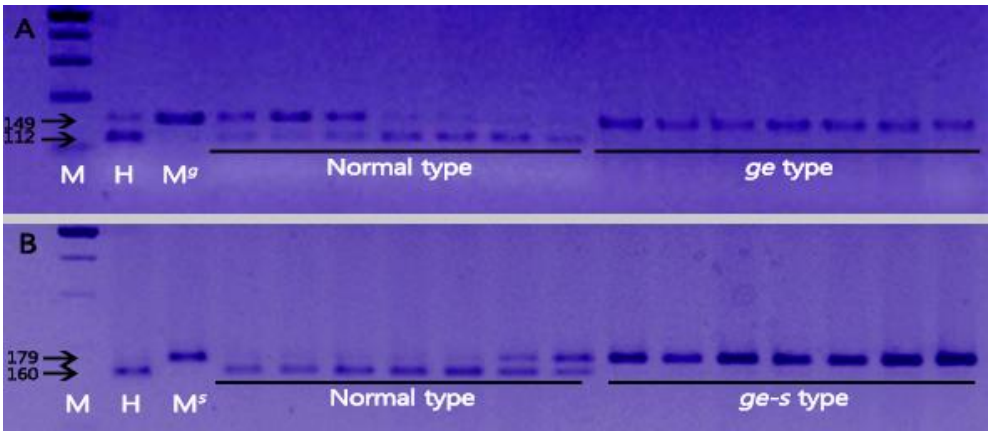
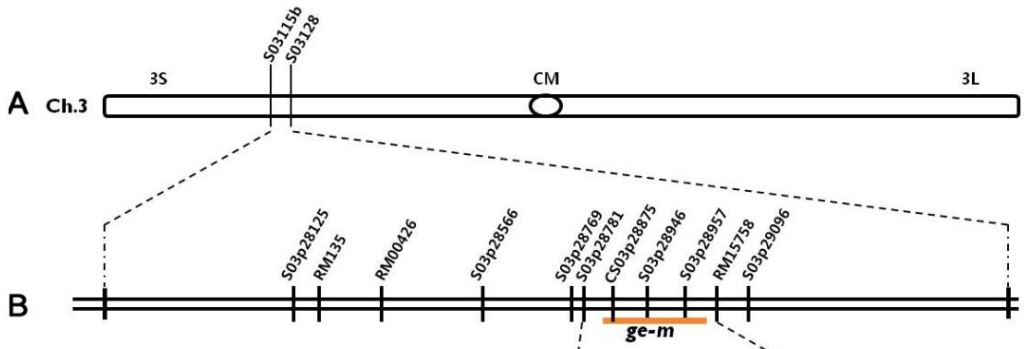


Figure 6. Co-segregation test in *ge* and *ge-s* mutation point. (A) CAPS analysis between wild type and *ge* type seeds from F_2 seed population (wild type / *ge*). (B) dCAPS analysis between wild type and *ge-s* type seeds from F_2 seed population (wild type / *ge-s*). M= ladder marker, H= wild type (Hwacheongbyeo), M^g= *ge*, M^s= *ge-s*.

Genetic mapping of *ge-m* mutant

149 F_2 plants and 857 F_3 plants derived from *ge-m* / Hangangchal 1 were used to map *ge-m* gene. First, bulked segregant analysis (BSA) was conducted on 4 bulks, two wild type bulks and two mutant type bulks, using a total of 66 STS markers and sub-species specific STS markers which cover all rice chromosomes. Results showed that the two markers, S03115b and S03128, on short arm of chromosome 3 were closely linked to *ge-m* (Fig. 7A). In order to narrow down the candidate region, 7 additional STS

markers and one CAPS marker were designed by *in silico* approach (Fig. 7B). With the use of additional markers for screening recombinants, the region of candidate gene was narrowed down to 255.3kb (Fig. 7C). Based on Rice Genome Annotation Project database (http://rice.plantbiology.msu.edu/), a total of 44 genes involved in the candidate region of 255.3kb were identified and were listed in (Table 5.)



C

Plant No.	Phenotype	S03p28566	S03p28769	S03p28781	CS03p28875	S03p28946	S03p28957	RM15758
66(2)-23	N	3	1	1	1	1	1	1
66(4)-19	M	2	2	2	2	2	2	3
F3-222	M	3	3	3	2	2	2	2
F3-247	M	3	3	3	2	2	2	2
F3-564	M	3	2	2	2	2	2	2
F3-666	H	2	2	3	3	3	3	3

Figure 7. Genetic and physical maps of the *ge-m* gene. (A) Candidate region was on the short arm of chromosome 3 between S03115b and S03128. (B) Candidate region was narrowed down between S03p28781 and RM15758. (C) Graphical genotype of key recombinants nearby loci of the *ge-m* gene. N= Normal type, M= Mutant type (*ge-m*), H= hetero

Table 5. The list of genes in the candidate region

Locus Name	Gene Product
LOC_Os03g49190	oleosin, putative, expressed
LOC_Os03g49200	WD domain, G−beta repeat domain containing protein, expressed
LOC_Os03g49210	BRCA1 C Terminus domain containing protein, expressed
LOC_Os03g49220	adenylosuccinate synthetase, chloroplast precursor, putative, expressed
LOC_Os03g49230	acetyltransferase, GNAT family, putative, expressed
LOC_Os03g49240	expressed protein
LOC_Os03g49250	OsFBO16 − F−box and other domain containing protein, expressed
LOC_Os03g49260	lipoxygenase, putative, expressed
LOC_Os03g49270	THION36 − Plant thionin family protein precursor, expressed
LOC_Os03g49280	THION37 − Plant thionin family protein precursor, putative, expressed
LOC_Os03g49290	hypothetical protein
LOC_Os03g49300	Plant thionin family protein precursor, putative, expressed
LOC_Os03g49310	THION39 − Plant thionin family protein precursor, expressed
LOC_Os03g49330	retrotransposon protein, putative, Ty3−gypsy subclass
LOC_Os03g49340	transposon protein, putative, Mariner sub−class, expressed
LOC_Os03g49350	lipozygenase protein, putative, expressed
LOC_Os03g49360	expressed protein
LOC_Os03g49380	lipoxygenase, putative, expressed
LOC_Os03g49400	ethylene−insensitive protein, putative, expressed
LOC_Os03g49410	expressed protein
LOC_Os03g49420	HEAT repeat family protein, putative, expressed
LOC_Os03g49430	pre−mRNA−splicing factor, putative, expressed
LOC_Os03g49440	phosphatase, putative, expressed
LOC_Os03g49450	expressed protein
LOC_Os03g49464	tetratricopeptide−like helical, putative, expressed
LOC_Os03g49480	elongation of fatty acids protein 2, putative, expressed
LOC_Os03g49485	expressed protein
LOC_Os03g49490	expressed protein
LOC_Os03g49500	ethylene receptor, putative, expressed
LOC_Os03g49510	phosphatidylinositol−4−phosphate 5−kinase, putative, expressed
LOC_Os03g49520	pinin/SDK/memA protein, putative, expressed
LOC_Os03g49524	anthocyanidin 3−O−glucosyltransferase, putative, expressed
LOC_Os03g49530	transposon protein, putative, unclassified

LOC_Os03g49540	transposon protein, putative, unclassified, expressed
LOC_Os03g49550	glucosyltransferase, putative, expressed
LOC_Os03g49560	expressed protein
LOC_Os03g49570	domain of unknown function, DUF250 domain containing protein, expressed
LOC_Os03g49580	eukaryotic peptide chain release factor subunit 1-1, putative, expressed
LOC_Os03g49590	expressed protein
LOC_Os03g49600	Os3bglu7 - beta-glucosidase, exo-beta-glucanse, expressed
LOC_Os03g49610	Os3bglu8 - beta-glucosidase, exo-beta-glucansase, highsimilaritytoOs3bglu7,expressed
LOC_Os03g49620	BRASSINOSTEROID INSENSITIVE 1 -associatedreceptorkinase1precursor,putative,expressed
LOC_Os03g49630	expressed protein
LOC_Os03g49640	STE_MEKK_ste11_MAP3K.14 - STE kinases include homologs to sterile7, sterile11 and sterile20 from yeast, expressed

Rice Genome Annotation Project database (http://rice.plantbiology.msu.edu)

DISCUSSION

Rice endosperm composed mainly of starch is in charge of diet in the world, especially Asian countries. Rice embryo contains many essential nutrients such as γ−aminobutyric acid (GABA), α−tocopherol, proteins and vitamins, and embryo size determines nutrient composition in rice, thus controlling factors that affect embryo size could help improve rice quality. In spite of the importance of embryo size in rice, the knowledge of gene and pathway regulating embryo size was limited.

In this study, we carried out characterization of the three giant embryo mutants named according to different embryo size as *ge−m*, *ge* and *ge−s*. These mutants had enlarged embryo with different size. GABA is one of the major embryonic bioactive components in rice and GABA content was high in giant embryo rather than in normal embryo. Results revealed that GABA content of three mutants increased in proportion with respect to embryo size. The positive correlation between embryo size and many embryonic nutrients indicated that these three mutants could be used as potential donor for improving nutrient composition of rice

But, enlarged embryo size caused loss of starch yield because embryo size was negatively correlated with endosperm (Hong et al. 1996). The three mutants also showed decrease in endosperm size,

increased EER, and it cause decrease of 1000-grain weight compared to wild type. It means that the energy source was used for synthesis of embryo-specific metabolites instead of starch and finally enlarged embryo size cause reduce starch yield. Therefore this should be considered when regulating embryo size for controlling nutrients composition of rice. Histological analysis revealed that all of three mutants showed enlarged cell size of scutellum in embryo, but degree of variation among three mutants differed. Analysis of variance among agronomical traits collected showed significant differences among mutants. *ge-m* expressed different phenotype such as short culm length and small grain length. It implied that the gene or regulating mechanism might be different between *ge-m* and other two mutants. In previous study, two genes causing enlarged embryo phenotype were cloned in rice. The *GIANT EMBRYO (GE)* and *GOLATH (GO)* encoded cytochrome p450 protein (Cahoon et al. 2003) in chromosome 7 and glutamate carboxypeptidase (Kawakatsu et al. 2009) in chromosome 3, respectively. Koh et al. (1996) mapped *ge-s* in chromosome 7 using *ge-s*. Through the allelism test, we identified that *ge* and *ge-s* were controlled by a same single recessive gene, *GE* in chromosome 7. Sequence alignment analysis identified that two mutants, *ge* and *ge-s*, had a non synonymous point mutation each in different nucleotide positions in exon 2 of *GE* gene. Another mutant,

ge−m, also showed segregation ratio of 3:1 but no point mutation was detected on both *GE* and *GO* gene, suggesting that *ge−m* is a novel single recessive gene controlling embryo size. To narrow down candidate gene of *ge−m,* fine mapping was performed. The candidate region was narrowed down to 255.3kb between two markers, S03p28781 and RM15758, in chromosome 3. In the candidate region, we were able to identify 44 genes using Rice Genome Annotation Project database (http://rice.plantbiology.msu.edu). 8 out of the 44 genes were strongly expressed in embryo or seed as compared to other organs. These 8 genes might be the candidate for *ge−m*.

In conclusion, we were able to identify two novel alleles associated with *GE* gene and selected 8 genes for *ge−m* candidate. Fine mapping of *ge−m* gene is still under progress. The new discovery of *ge−m* gene will be helpful to better understand how to regulate embryo size and cell growth. Moreover, these new variations related to embryo size will be greatly helpful to follow increasing global demand for high quality of rice.

CHAPTER Ⅱ.
Varietal variation of rice embryo

ABSTRACT

Embryo has many nutrients and bioactive components, like protein, lipid, vitamins, tocopherol and γ-aminobutyric acid (GABA). Thus the interest in embryo is getting higher in research and market. In general, most of embryos are detached from grain and lost during milling, but depending on the varieties, the degree of unstripped embryo rate (UER) varies.

In this study, we conducted comparative analysis on grain and embryo traits among 49 rice varieties in order to select desirable candidates of embryo rice and to collect basic data for developing high UER variety. Correlation among various traits showed that embryo dent, which in turn is expected to affect UER, was positively correlated with grain width ($r=0.53^{**}$) and grain area($r=0.34^{*}$), while negatively correlated with grain length to width ratio($r=-0.38^{**}$), revealing that the attached shape of embryo

was influenced by the shape of grain. Likewise, it was noted that the embryo dent was mainly affected by grain width, not by grain length, and embryo dent affect to the position of embryonic shoot and radicle. Daerip 1, Jinbu, Jinbo, Heugseol, Obong, Unkwang, Cheongnam, Koshihikari, Cheonghaejinmi and Boramchan showed high embryo dent. The data and varieties identified by this study could be useful in selecting promising varieties with high UER and developing new variety of higher UER.

INTRODUCTION

Rice is important staple crop for many countries, especially in Asia. Generally, consumers prefer polished rice by milling process. Recently, as the nutritional value of brown rice was reported, there has been an increasing demand for brown rice. Brown rice is produced by removing only the outermost layer, the hull, and has more nutrient and bioactive components including dietary fiber, vitamins, γ−aminobutyric acid (GABA) and tocopherol (Son et al. 1996; Choe et al. 2002). Rice embryo (germ) which contains protein, oil, vitamins and trace minerals plays important role in the nutritional value of brown rice (Saunders 1985). Previous studies showed that embryo size is correlated with the increasing amounts of nutrients (Koh et al. 1993; ZHANG et al. 2005; Choi et al. 2006). However, some studies revealed that the fiber of bran in brown rice and giant embryo rice have a bad effect on taste and texture, so most of consumers preferred polished rice produced by milling process. Although milling process has a lot of advantages like improvement of taste and texture, good appearance of grain, it could however result to significant loss of embryo and decreased nutritional value (Kim and Jeon 1996). Therefore unstripped embryo rate (UER) is very important for maintenance of high

nutritional value in polished rice. UER is influenced by milling process, water content and harvest time, and so on. Milling process is one of the most important factors in improvement of UER and many studies on optimized milling condition, optical abrasive and friction milling ratio and developing of milling machine were reported (정종훈 and 엄천일 2002; Kim et al 2009). Water content influence not only UER but also milled rice recovery so if water content is too high or too low, it lead to reduce UER (Ha et al. 2002). Transplanting time and Harvest time also has to be considered because it influences ripening condition and water content of maturation of grain, respectively (Ko et al. 1998; Kim et al. 2001). Furthermore, many comparative milling test among varieties showed that UER is affected by varieties (최경미 et al. 2011; 오성환 et al. 2012; 하기용 2009). The difference of UER among varieties implies that UER is regulated by genetically inheritable characters such as adhered shape of embryo and structural difference of adhesive layer.

This study was conducted to analyze various traits for grain and embryo among 49 varieties. Data generated from this study will be beneficial for improving the selection of rice varieties for higher UER and for breeding variety of higher UER.

MATERIALS AND METHODS

1. Varieties

A total 49 varieties (38 *Japonica* varieties, 4 *Tongil* type varieties and 7 *Indica* varieties) which have various grain shape were used in this study. (Table 6)

2. Measurement of grain and embryo traits

Six traits such as grain width, grain length, grain length to width ratio, grain area, embryo length, embryo dent were measured to analyze variation of grain and embryo among 49 varieties. Five uniform seeds were dehulled and cut longitudinally for measurement. Each of traits was measured in image magnified by 40X using optical microscopy. Grain area was measure by area formula of ellipse, [¼ π x GW x GL]. Fig. 8 shows measurement method of each of traits.

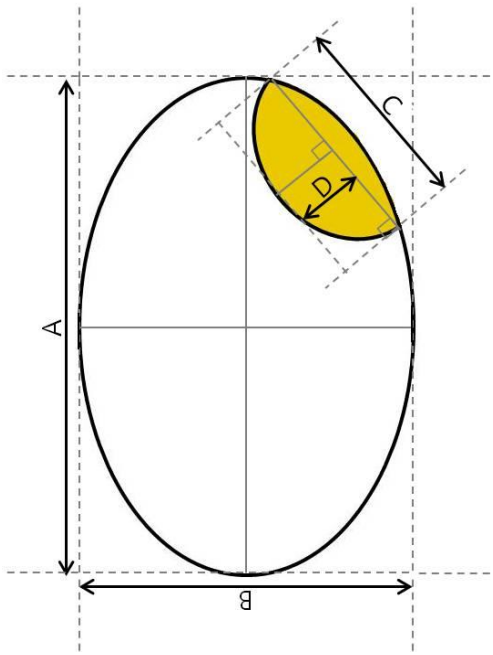


Figure 8. Measurement method of grain and embryo traits. (A)=Grain width, (B)=Grain length, (C)=Embryo length, (D)=Embryo dent.

3. Statistical analysis

An analysis of variance (ANOVA), Tukey test and correlation analysis for 49 varieties were performed using the Statistical analysis system (SAS).

4. Histological analysis of embryo

Matured seeds were used for paraffin embedding and section. Paraffin embedding and section were performed using previously described method(chapter Ⅰ MATERIALS AND METHODS).

RESULTS

Grain and embryo traits

Grain and embryo traits of 49 varieties were described in Table 6. Analyses of variances revealed significant differences among all the embryo and grain traits collected in each variety. Grain length and grain width were ranged from_4.66(Baegjinju1) to 6.86(IR64) mm and from 2.13(IR56, IR72) to 3.34(Darip1) mm, respectively. Grain length to width ratio showed short and round grains in *Japonica* varieties while long and slender grains in *Indica* varieties. *Tongil* type and *Indica* type varieties, except Hangangchal 1, showed low embryo dent compared with *Japonica* varieties. Nevertheless Seonong 17 and Keunnum, giant embryo varieties, were japonica variety, these showed very low embryo dent. Darip 1, Jinbu, Jinbo, Heugsul, Obong, Unkwang, Cheongnam, Koshihikari, Cheonghajinmi and Boramchan showed high embryo dent. Fig. 9 shows histological morphology of embryo among high embryo dent variety (Obong), low embryo dent variety (Samkwang) and giant embryo variety (Seonong 17). We could not find any difference of embryo adhered layer among varieties, but the position of embryonic shoot and radicle body showed significant difference among those varieties. Embryonic shoot and radicle body was deeply embedded in

endosperm. In contrast, those of Samkwang and Seonong 17 were located near the grain surface and Seonong 17, a giant embryo variety, showed extended embryo length rather than embryo dent.

Table 6. Grain and embryo traits of 49 varieties

Vriety	type	GW (mm)	GL (mm)	LWR (GL/GW)	GA (mm^2)	EL (mm)	ED		
							(mm)	rank	Tukey gouping
Daerip 1	Japonica	3.34±0.16	6.24±0.17	1.87±0.09	16.37±0.96	2.27±0.08	0.59±0.10	1	A[a]
Jinbu	Japonica	3.09±0.06	5.35±0.11	1.73±0.06	12.98±0.27	2.07±0.06	0.55±0.08	2	AB
Jinbo	Japonica	3.05±0.07	5.45±0.08	1.79±0.05	13.08±0.35	2.11±0.14	0.51±0.03	3	ABC
Heugseol	Japonica	2.70±0.15	5.41±0.12	2.00±0.15	11.46±0.53	1.93±0.12	0.49±0.07	4	ABCD
Obong	Japonica	2.90±0.03	4.89±0.19	1.69±0.06	11.13±0.48	2.02±0.08	0.48±0.04	5	ABCD
Unkwang	Japonica	2.98±0.03	5.01±0.11	1.68±0.05	11.73±0.23	2.10±0.13	0.48±0.09	5	ABCD
Cheongnam	Japonica	3.02±0.03	5.19±0.22	1.72±0.08	12.33±0.47	2.09±0.17	0.48±0.03	5	ABCD
Koshihikari	Japonica	2.83±0.08	5.09±0.15	1.80±0.04	11.30±0.62	1.88±0.10	0.47±0.04	8	ABCD
Cheonghaejinmi	Japonica	2.95±0.05	5.23±0.23	1.77±0.05	12.14±0.72	2.01±0.13	0.47±0.06	8	ABCD
Boramchan	Japonica	3.02±0.09	4.97±0.12	1.65±0.02	11.80±0.62	2.05±0.09	0.47±0.06	8	ABCD

Ilpum	Japonica	2.96±0.09	5.07±0.15	1.71±0.08	11.79±0.42	2.16±0.08	0.46±0.06	11	ABCD
Hangangchal 1	Tongil	2.73±0.09	4.97±0.17	1.82±0.06	10.69±0.64	1.63±0.07	0.46±0.03	11	ABCD
Boseogchal	Japonica	2.83±0.08	5.00±0.13	1.77±0.06	11.12±0.46	2.13±0.07	0.46±0.05	11	ABCD
Milkyqueen	Japonica	2.92±0.10	5.01±0.15	1.72±0.02	11.48±0.72	1.90±0.05	0.46±0.08	11	ABCD
Gopum	Japonica	2.88±0.09	4.88±0.10	1.69±0.06	11.04±0.39	2.03±0.09	0.45±0.05	15	ABCD
Hanmaeum	Japonica	3.13±0.04	5.90±0.11	1.88±0.05	14.53±0.23	2.12±0.13	0.45±0.07	15	ABCD
Nampyeong	Japonica	2.80±0.09	4.84±0.15	1.73±0.10	10.64±0.33	2.01±0.03	0.44±0.10	17	ABCD
Jinmi	Japonica	2.87±0.10	5.01±0.16	1.75±0.06	11.29±0.61	2.09±0.13	0.44±0.08	17	ABCDE
Baegjinju 1	Japonica	2.92±0.06	4.66±0.06	1.60±0.03	10.67±0.28	1.79±0.18	0.44±0.10	17	ABCDE
Younghojinmi	Japonica	3.04±0.05	4.88±0.14	1.61±0.05	11.67±0.44	2.01±0.10	0.43±0.06	20	ABCDE
Dongjin 1	Japonica	2.91±0.12	5.02±0.14	1.73±0.06	11.46±0.72	2.04±0.11	0.43±0.06	20	ABCDE
Junam	Japonica	2.88±0.09	4.94±0.16	1.72±0.07	11.18±0.55	2.08±0.15	0.42±0.02	22	ABCDE
Ilmi	Japonica	2.75±0.09	4.94±0.15	1.80±0.06	10.670.57	1.99±0.12	0.42±0.06	22	ABCDE
Youngan	Japonica	2.88±0.13	4.98±0.22	1.73±0.08	11.26±0.84	2.11±0.10	0.42±0.04	22	ABCDE

Odae	Japonica	2.91±0.06	4.94±0.02	1.70±0.04	11.31±0.22	2.08±0.10	0.42±0.06	22	BCDE
Sindonjin	Japonica	3.03±0.15	5.49±0.23	1.81±0.11	13.05±0.92	2.21±0.07	0.41±0.07	26	BCDE
Hwayoung	Japonica	2.91±0.08	5.01±0.31	1.72±0.10	11.46±0.89	2.02±0.12	0.41±0.04	26	BCDE
Nipponbare	Japonica	2.90±0.06	5.03±0.17	1.73±0.04	11.48±0.58	1.95±0.15	0.41±0.05	26	BCDE
Hongjinju	Japonica	2.91±0.08	4.93±0.09	1.69±0.06	11.25±0.36	1.99±0.07	0.41±0.06	26	BCDE
Mihyang	Japonica	2.78±0.05	4.99±0.15	1.79±0.03	10.90±0.50	1.97±0.07	0.41±0.04	26	BCDE
Hitomebore	Japonica	2.89±0.13	5.10±0.14	1.76±0.10	11.58±0.60	2.02±0.07	0.40±0.05	31	BCDE
IR24	Indica	2.24±0.10	6.83±0.21	3.05±0.08	12.03±0.83	1.68±0.05	0.40±0.06	31	BCDE
Samkwang	Japonica	2.78±0.14	4.90±0.20	1.76±0.05	10.70±0.95	2.07±0.11	0.39±0.10	33	BCDE
Chilbo	Japonica	2.72±0.15	5.13±0.28	1.89±0.09	10.98±1.08	2.04±0.18	0.39±0.05	33	BCDE
Chucheong	Japonica	2.80±0.09	4.74±0.21	1.69±0.03	10.44±0.79	2.00±0.12	0.38±0.13	35	CDE
Hopum	Japonica	2.82±0.09	4.85±0.07	1.72±0.05	10.74±0.41	1.96±0.04	0.38±0.07	35	CDE
Hwacheong	Japonica	2.81±0.06	4.77±0.06	1.70±0.05	10.54±0.21	1.97±0.14	0.38±0.03	35	CDE
IR36	Indica	2.27±0.07	6.65±0.13	2.93±0.15	11.86±0.21	1.64±0.07	0.37±0.08	38	CDE

IR64	Indica	2.14±0.06	6.86±0.12	3.21±0.10	11.54±0.37	1.58±0.11	0.37±0.05	38	CDE
IR56	Indica	2.13±0.04	6.78±0.22	3.18±0.15	11.32±0.28	1.76±0.06	0.36±0.05	40	CDE
Milyang23	Tongil	2.52±0.05	6.01±0.13	2.38±0.04	11.90±0.44	1.85±0.09	0.36±0.04	40	CDE
Dasan 2	Tongil	2.74±0.09	6.05±0.14	2.21±0.08	13.02±0.55	1.87±0.08	0.36±0.05	40	CDE
IR8	Indica	2.66±0.09	6.70±0.23	2.52±0.15	13.98±0.49	2.01±0.09	0.35±0.09	43	CDE
IR72	Indica	2.13±0.06	5.57±0.25	2.62±0.17	9.32±0.37	1.64±0.10	0.35±0.06	43	CDE
Dasan	Tongil	2.73±0.13	5.96±0.11	2.18±0.13	12.77±0.59	1.81±0.09	0.35±0.03	43	CDE
Segyejinmi	Indica	2.64±0.09	5.87±0.38	2.22±0.13	12.17±1.04	1.79±0.08	0.35±0.04	43	CDE
Seonong 17	Japonica	2.82±0.09	4.68±0.11	1.66±0.02	10.38±0.55	2.32±0.07	0.35±0.10	43	DE
Seogeum	Japonica	2.87±0.07	5.08±0.07	1.77±0.03	11.45±0.41	2.03±0.12	0.32±0.07	48	DE
Keunnun	Japonica	3.03±0.08	5.00±0.17	1.65±0.05	11.89±0.64	2.53±0.10	0.27±0.08	49	E

GW=grain width, GL=grain length, LWR=grain length to width ratio, GA=grain area, EL=embryo length, ED=embryo dent.

[a] menas followed by the same letter are not significantly different at the 5% level, as verified by an Tukey test.

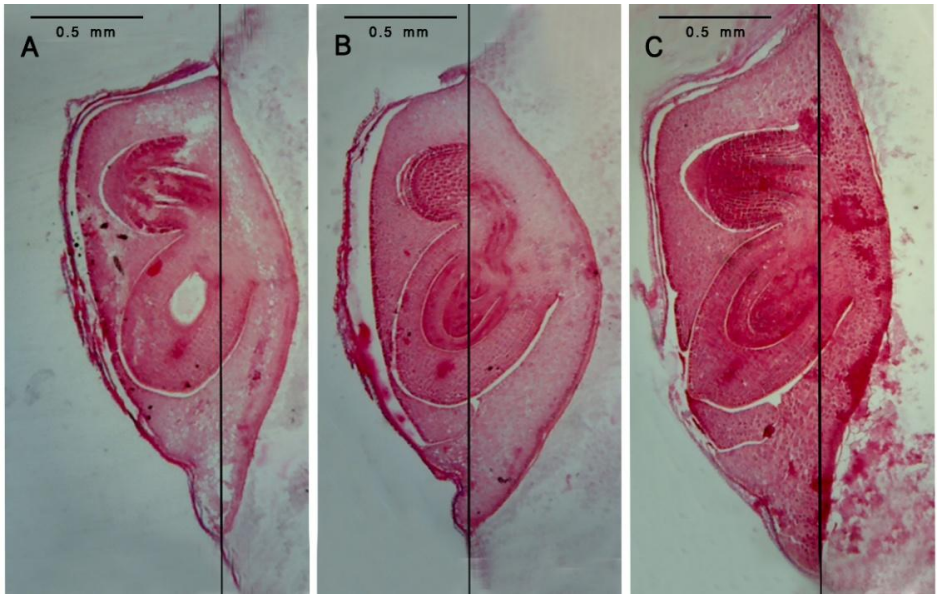


Figure 9. Histological morphology of embryo. (A) Samkwang (low embryo dent variety), (B)Obong (high embryo dent variety), (C)Seonong 17 (giant embryo rice variety)

Correlation analysis among grain and embryo traits

Table 7 shows the result of correlation analysis among grain and embryo traits. It was identified that embryo dent was influenced by grain width($r=0.53^{**}$) and grain area($r=0.34^{*}$). Grain width was highly positively correlated with embryo length($r=0.76^{**}$), embryo dent($r=0.53^{**}$) and grain area($r=0.38^{**}$), but was negatively correlated with grain length($r=-0.58^{**}$). There is no significant correlation between grain length and embryo dent but grain length showed highly positive correlation with grain area($r=0.52^{**}$) while negatively correlated with embryo length($r=-0.46^{**}$).

Table 7. Correlation analysis among grain and embryo traits

	GW[a]	GL	LWR	GA	EL
GL	-0.58**	-			
LWR	-0.87**	0.90**	-		
GA	0.38**	0.52**	0.11ns	-	
EL	0.76**	-0.46**	-0.65**	0.26ns	-
ED	0.53**	-0.18ns	-0.38**	0.34*	0.17ns

**; significant at P < 0.01, *; significant at P < 0.05, ns; non-significant

[a] Refer to Table 1 for abbreviations

DISCUSSION

Most of embryos are detached from grain and lost during milling, but after researches which embryo has high nutritional value, the interest in embryo is increasing in research and market. However, it is not easy to elevate unstripped embryo rate (UER) during milling process, because UER is affected by many factors such as milling machine, water content, harvest time and variety. To find other key factor which could increase UER, we evaluated total 6 grain and embryo traits among 49 rice varieties. All of traits measured showed wide variations among varieties. Among 6 traits, we focused on embryo dent which is related to adhered shape of embryo. Through correlation analysis among traits, we were able to identify correlation among embryo dent with grain width, grain length to width ratio and grain area, indicating that overall grain shape plays important role in increasing embryo dent. Especially, embryo dent was related by grain width, not by grain length. In histological analysis, high embryo dent variety showed that embryonic shoot and radicle body located in inside of grain compared with low embryo dent variety. Darip 1, Jinbu, Jinbo, Heugsul, Obong, Unkwang, Cheongnam, Koshihikari, Cheonghajinmi and Boramchan were selected for high embryo dent variety. Interestingly, Obong and Unkwang were reported promising

candidate of embryo rice variety in milling test(하기용 2009; Choi et al. 2011). Enlarged embryo of giant embryo rice were contained various nutrients and bioactive components more than normal rice embryo (Koh et al. 1993; Choi et al. 2006), but giant embryo varieties showed very low UER (Choi et al. 2011). In our data, Seonong 17 and Keunnun were ranked last and third from last, respectively, in embryo dent. Through all these results, we could expect that embryo dent is the factor affecting UER and that the data of varietal variation of embryo dent will help to select or develop promising high UER variety.

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초록

거대 배아 돌연변이 벼의 유전자 지도 작성과 벼 배아의 품종간 변이 조사

벼의 배아는 GABA, 토코페롤, 비타민 지질 등과 같은 기능성물질이나 산업적으로 이용가치가 높은 물질들을 다량 함유하고 있으며 그 크기 증가함에 따라 함유물질의 양이 증가하는 것으로 알려져 있다. 따라서 배아크기의 조절은 육종적으로 매우 중요한 의의를 갖는다. 이 연구에서 사용된 세가지 거대배아 돌연변이체는 자포니카 품종인 화청벼에 화학적 돌연변이 유도물질인 *N-methyl-N-nitrosourea* (MNU)를 처리하여 얻었으며 세 돌연변이체의 배아는 모본인 화청벼의 배아에 비해 크기가 증가한 형태를 나타낸다. 또한 세 돌연변이체 사이에도 크기의 차이가 있었으며 크기순서에 따라 *ge-m*, *ge*, *ge-s*라 이름 붙여졌다. *ge-m*은 화청벼 보다 약간 큰 배아크기를 보였고 *ge-s*는 종자의 절반 정도가 배아일 정도로 매우 큰 배아를 갖고 있었다. 이들의 유전자 차원에서의 차이를 알아보기 위해 교배를 통한 대립검정을 수행하였다. 이를 통해 *ge*와 *ge-s*는 같은 유전자에 의해 배아크기가 조절되는 것이 확인되었다. 배아크기를 조절한다고 알려진 *GIANT EMBRYO(GE)* 유전자의 sequence 비교한 결과 *ge*와 *ge-s*에서 아미노산 치환을 일으키는 점 돌연변이가 각각 하나씩 발견되었으나 *ge-m*은 화청벼와 차이를 보이지 않았다. *ge-m*의 거대배아형질을 조절하는 유전자를 찾기 위해 유전자 지도작성을 진행하였고 그 결과 이유전자는 3번 염색체의 S03p28781과 RM15758의 두 분

자마커 사이에 존재하는 것으로 밝혀졌다.

배아는 높은 영양적 가치를 지녔지만 도정과정을 거치면서 대부분 손실된다. 따라서 배아의 크기를 조절하는 연구와 함께 도정과정에서 일어나는 배아의 손실을 줄이는 연구 또한 매우 중요하다 할 수 있겠다. 배아잔존율을 높이기 위한 연구에 이용될 수 있는 기초자료를 확보하기 위해 종자형태가 다양한 49개 품종에 대해 종자 및 배아의 형태적 특성에 대해 조사를 실시하였다. 상관분석에서 배아부착과 밀접하게 연관되어 있을 것으로 생각되는 배아함몰도의 경우 종자 폭($r=0.53^{**}$) 그리고 종자넓이($r=0.34^{**}$)와 양의 상관을 나타냈으며 종자장폭비($r=-38^{**}$)와는 음의 상관을 보였다. 이러한 결과를 통해 배아의 크기나 부착형태는 종자의 전반적인 형태와 관련이 깊다는 것을 확인 할 수 있었고 특히 배아함몰도의 경우 종자 길이보다는 종자 폭에 의해 영향을 받는다는 것을 알 수 있었다. 또한 파라핀 절편을 통해 배 형태를 관찰하였을 때, 배아함몰도가 높은 품종에서 배아의 유근과 근초가 배유에 더 깊숙히 매몰되어 있는 것을 확인 할 수 있었다. 대립벼1, 진부, 진보, 흑설, 오봉, 운광, 청남, 고시히카리, 청해진미, 보람찬 등이 배아함몰도가 높은 품종으로 확인되었으며 선발된 품종과 조사자료는 앞으로 배아미 후보품종 선반 혹은 배아잔존율이 높은 배아미 품종육성 연구에 많은 도움이 될 것으로 기대된다.

주요어: 벼, 거대배아 돌연변이체, 배아크기, 유전자지도작성, 배아잔존율, 배아함몰도

학번: 2011-21215

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