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THESIS FOR THE DEGREE OF MASTER OF SCIENCE

**Fine Mapping and candidate gene analysis of
Small Round Grain Mutant dep2-3 in Rice**

BY

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AUGUST, 2012

**MAJOR IN CROP SCIENCE AND BIOTECHNOLOGY
DEPARTMENT OF PLANT SCIENCE
THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY**

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**UNDER THE DIRECTION OF DR. HEE-JONG KOH
SUBMITTED TO THE FACULTY OF THE GRADUATE
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ABSTRACT

Grain size is one of the most important trait determining yield in cereal crops apart from number of grains per panicle, number of panicles per plant and 1000 seed weight. Plant architecture is another very important factor influencing yield by affecting the amount plant surface area directly exposed to the sun light. Erect panicle is important morphological characteristic which helps in enhancing the yield by allowing sun light to fall directly on leaves unlike curved panicle which blocks sunlight and consequently reduce photosynthesis. Here we report a new allele *dep2-3* (dense and erect panicle) which has pleiotropic effects on grain size/shape and plant architecture. A small round grain and erect panicle mutant was obtained by treating *Hwachong* rice (*japonica*) with MNU (N-methyl-N-nitrosourea) chemical mutagen. Through map based cloning using STS (Sequence-Tagged Sites) and SS-STS (Sub-species Specific Sequence-Tagged Site) markers we identified the mutated gene on chromosome 7 and found that it is a new allele of previously reported *dep2* in rice. Two alleles of *dep2* namely *dep2-1* and *dep2-2* have been reported in previous study, therefore we named this new allele as *dep2-3*. *dep2-3* mutant manifested characteristics like reduced grain size and plant height, dense and erect panicle and relatively erect plant compared to

the wild type. When we crossed the mutant with its parent (*Hwachong*), F1 panicle and grain characteristics showed intermediate phenotype, therefore, we concluded that wild type allele of this gene shows incomplete dominance, which is not reported in the previous study. Scanning electron microscopy (SEM) result shows that increase in width of mutant grain, which changes its shape, is due to increase in width of glume cells. Phenotypic examination shows that dense and erect panicle phenotype is result of reduction in length of rachis, primary and secondary branch.

Keywords: Rice, mapping, grain size, glume, dep2(dense and erect panicle2), splicing.

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LIST OF ABBREVIATIONS

Dep	Dense and erect panicle
MNU	N-methyl-N-nitrosourea
SAS	Statistical analysis system
PCR	Polymerase chain reaction
TBE	Tris-borate-EDTA
EtBr	Ethidium bromide
BSA	Bulked segregant analysis
STS	Sequence tagged site
CAPS	Cleaved amplified polymorphic sequence
Kbp	Kilo base pair
<i>E.coli</i>	<i>Escherichia coli</i>
BAC	Bacterial artificial chromosome

Introduction

Rice is the staple food for most of the human population and is the second largest produced cereal crop after maize. To increase the productivity of rice in order to meet global food requirement, we need a comprehensive understanding of the factors which control the yield and optimize them through molecular breeding and tools of biotechnology. Amid various concerns regarding environmental protection (Byrnes 1990), unlike first green revolution, the second green revolution has to be achieved without use of chemical fertilizers. To achieve such a goal, human knowledge about basic biological mechanisms has to improve further. To start with, two very important factors determining yield of rice crop are grain size and plant architecture (Sakamoto, Morinaka et al. 2005). Change in grain size directly affects the yield, whereas, plant architecture indirectly affects yield by affecting amount of surface area directly exposed to sun light (Kong, Wang et al. 2006). In last decade since the advent of modern genomics technology many QTLs related with grain size and shape have been identified in rice. GS3 which is related with grain size (Fan, Xing et al. 2006), GW2 which is related with grain size and filling (Song, Huang et al. 2007), GW5 is also related with grain size (Weng, Gu et al. 2008) and GIF1 which is related with grain filling (Wang, Wang et al. 2008) are the four grain size related genes which have been cloned in rice so far (Miura, Ashikari et al. 2011). GS3 is the major QTL for grain length and weight and minor QTL for grain width and thickness located on chromosome 3. With the LOD value of 129.2 for grain length, GS3 is supposed to be one of the most important QTLs discovered in rice so far. Function of GS3 protein could not be related to glume cell size/division yet however, study by (Mao, Sun et al. 2010) linked the differential domain functions of GS3 protein to natural variation of grain size in rice. Grain size is function of glume size and

grain filling, moreover, glume size is determined by the glume cell size, shape and division. (Xing and Zhang 2010). The brief explanation as to how grain size and shape is controlled is as follows; longitudinal division of glume cells or longitudinal increase in glume cell size enhances grain length. Latitudinal division of glume cell or latitudinal increase in glume cell size enhances grain width. The ratio of grain length and width defines grain shape which is important quality standard in rice. (Shomura, Izawa et al. 2008) also reported that deletion in *qSW5* (QTL for seed width on chromosome 5), increases the sink size by increasing the cell number in the outer glume surface of rice flower. The natural variation in this locus reveals that this trait was selected by humans during domestication of rice. Therefore, understanding basic mechanisms involved in regulation of grain size and shape also enhances our knowledge about how the crops which we grow today were domesticated thousands of years ago.

It has been long known that plant architecture is also a major factor affecting yield of crop plants. Dense and erect panicle is one of the desirable characteristics breeders are exploiting to breed higher yielding cultivars especially in Northern China. There are several reports on genetic and molecular mechanisms controlling panicle architecture in rice such as DEP1 (dense and erect panicle 1) (Huang, Qian et al. 2009), EP (erect pose panicle) (Wang, Nakazaki et al. 2009), EP2 (erect panicle2) (Zhu, Tang et al. 2010), EP3 (erect panicle3) (Piao, Jiang et al. 2009), and dense and erect panicle2 (Li, Liu et al. 2010). DEP1 locus is again-of-function mutation causing truncation of a phosphatidylethanolamine-binding protein-like domain protein. The effect of this allele is to enhance meristematic activity, resulting in a reduced length of the inflorescence internode, an increased number of grains per panicle. EP is also governed by single dominant gene. Although both EP and DEP1 reduce the grain size but greatly enhance the yield by increasing the number of grain per

panicle owing to increase in number of secondary branches in case of EP and number of primary and secondary branches both in case of DEP1. Another study by (Zhou, Zhu et al. 2009) reported qPE9-1 QTL (on chromosome 9) related with erect panicle and plant architecture is said to be related with domestication of rice. It is proposed in this study that the panicle erectness trait resulted from a natural random loss-of-function mutation for the qPE9-1 gene and has subsequently been the target of artificial selection during japonica rice breeding. qPE9-1 also affects grain length and plant architecture. All previous reports and current study show a strong correlation between grain size and plant architecture.

Here, we report a new allele *dep2-3* of previously reported locus DEP2 in rice. Previous study reported two allelic mutant *dep2-1* and *dep2-2*, therefore we named new allele as *dep2-3*. We found that wild type allele DEP2-1 shows incomplete dominance which is not reported in the previous study. *dep 2-3* negatively affects grain size and also affects the panicle and overall plant architecture.

Materials and Methods

I. Plant materials

The dense and erect panicle2-3 (*dep 2-3*) mutant was obtained by treating Korean *japonica* cultivar Hwachong waxy with MNU (N-methyl-N-nitrosourea) chemical mutagen. The mutant was selected in M2 generation and was fixed through selfing in successive generations. The pedigree record of *dep2-3* mutant is *Hwachong(wx)M-B-33-2-2-2-1-B-B-1-1-B*. To genetically map the mutated gene, mapping population was developed by crossing mutant (*dep2-3; japonica* background) with Milyang23 (*indica* type cultivar). F₂ and F₃ population were grown in green house and field respectively and these populations were used to fine map the candidate gene. Mutant parent was also crossed with its wild type parent (Hwachong waxy *japonica* cultivar) and F₂ population from this cross was grown in the green house along with mapping population at experimental farm of Seoul National University, Suwon, Republic of Korea during 2010-2011.

II. Phenotypic evaluation

To distinguish wild and mutant phenotype, grain size characteristics like grain length, grain width and grain thickness were measured using digimatic capilar (Mitutoyo, Japan) and analyzed using Statistical Analysis System (SAS) program. Other agronomics characteristics like culm length, panicle length, number of panicles per plant, number of spikelets per panicle, spikelet fertility and 1000 grain weight was measured and statistically analyzed.

III. Histological analysis

A. Scanning electron microscopic observation

For scanning electron microscopy (SEM), samples of rice spikelet at the mature stage were

fixed overnight at 4° in formalin:glacial acetic acid:70% ethanol (FAA) at 1:1:18. After dehydration in a graded ethanol series and substitution with isoamyl acetate, the samples were critical-point dried, sputter coated with gold, and observed under a scanning electron microscope SUPRA 55VP (Carl Zeiss, Germany).

B. Paraffin sectioning of peduncles

Paraffin-embedded peduncle tissue sections were prepared following the methods described by (Piao, Jiang et al. 2009) with slight modifications. Panicles were harvested at ripening stage. The peduncle were cut 1 cm in length from the panicle node and fixed in FAA solution (50% ethanol, 5% acetic acid, 3.7% formaldehyde) and stored at 4°C for 1 day. The fixed peduncles were dehydrated by soaking for 2h each in an ethanol solution series 70, 85, and 95% followed by incubation in 95% ethanol overnight. Finally, the peduncles were soaked in 100% ethanol for 2h. Peduncle samples were cleared by soaking for 2h in the clearing solution series consisting of 75% ethanol/25% xylene, 50% ethanol/50% xylene, 25% ethanol/75% xylene, followed by soaking in 100% xylene solution overnight. For paraffin infiltration, the cleaned peduncle samples were soaked for 2h in the solution series of 75% xylene/25% paraffin, 50% xylene/50% paraffin, 25% xylene/75% paraffin, and 100% paraffin followed by 100% paraffin at 55°C overnight. The infiltrated sample was embedded in a paraffin block and then cut into 9µm sections using a microtome (MICROM Lab, Walldorf, Germany) and mounted on a Superfrost-plus glass slides (Fisher Scientific, Pittsburgh, PA, USA) and dried at 42°C for 1 day. The sections were deparaffinized with 100% xylene for 45 min followed by hydration by soaking for 2 min each in 50% ethanol/ 50% xylene, 100% ethanol, and sterile water. Peduncle samples were stained in 1% safranin/30% ethanol for 30s followed by washing with sterile water

two times. Samples were soaked for 2 min each in 30% ethanol, 50% ethanol, 70% ethanol, 85% ethanol, and 95% ethanol. Finally the sections were cleared by soaking twice in 100% xylene for 10 min and mounted in Canada balsam. The cross sections of peduncle were observed by optical microscopy at 56X magnification.

IV. Genetic mapping

A total of 980 F₂ and F₃ plants from the cross between dep2-3 and Milyang 23 were used for fine mapping. Genomic DNA samples were extracted from rice leaves using the CTAB method (Second and McCouch 1994) Eight mutant and eight wild-type F₂ plants were selected and an equal amount of DNA from each of the eight plants were pooled into a single sample for bulked-segregant analysis (BSA) (Michelmore, Paran et al. 1991). After BSA, fine mapping was conducted with the neighboring STS markers, which were developed by designing primers based on the differences in DNA sequences between the indica and japonica rice subspecies (Chin, Kim et al. 2007). 9 STS markers were developed additionally for fine mapping of the dep2-3 allele based on the available rice genome sequence data at <http://www.ncbi.nlm.nih.gov/>, <http://www.gramene.org> and <http://www.rgp.dna.affrc.go.jp/>. Primers of these STS markers were designed *in silico* using Primer3 software version 0.4.0, <http://frodo.wi.mit.edu/>. Primer sequences are listed in table1. PCR was performed in a reaction volume of 20µl containing 40 ng of template DNA, 0.2µM of each primer, 200µM of each dNTP, 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin and 0.5 U of Taq DNA polymerase. Amplifications were carried out in a PTC100 96U Thermocycler (MJ Research, Reno, NV, USA) in the following sequence: 5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C, and 5 min at 72°C for a 10 min extension. PCR products were separated in 3.0% agarose/0.5X TBE gels and visualized by ethidium bromide staining.

V. Sequence analysis of the candidate genes.

The full-length genomic DNA sequences of candidate genes were determined by dividing the genes into several overlapping segments. Specific PCR primers for each segment were used to amplify genomic DNA from wild type Hwachong wax and dep2-3. PCR products were purified using a PCR purification kit (Bioneer, Deajeon, Korea) for TA cloning. Purified PCR product was introduced into the pGEM-T Easy Vector (Promega, Madison, WI, USA) and transformed into the E. coli strain DH5 α . Sequencing of genomic inserts was performed using an ABI Prism 3730 XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). T7 and SP6 primers were used. Sequence alignment was performed with the BLAST network services in National Center for Biotechnology Information (NCBI) <http://blast.ncbi.nlm.nih.gov/> and the European Bioinformatics Institute (<http://www.ebi.ac.uk>). The results of sequencing were aligned with wild type parent using CodonCode Aligner software (CodonCode Corporation, USA).

VI. CAPS maker analysis

For CAPS (cleaved amplified polymorphic sequences) analysis, PCR amplification with the primer set Nru1CAPS-F (5'-GAGGAGAGCTTGGGGTTCTT-3') and Nru1CAPS-R (5'-GTGCCCTTGCTGAACCAC-3') were used. Suitable restriction enzyme for co-segregation analysis was searched using the dCAPS finder 2.0 program (<http://helix.wustl.edu/dcaps/dcaps.html>) and primers were designed *in silico* using Primer 3.0 software version 0.4.0, <http://frodo.wi.mit.edu/>. After PCR, each product (5 μ l) was digested with Nru1 in a total volume of 15 μ l at 37°C for 2h. After digestion, 5 μ l of each digest was visualized on a 2.5% agarose/0.5X TBE gel.

Table1. STS markers used in fine mapping of the dep2-3 allele.

Marker	Forward primer (5'-3')	Reverse primer (5'-3')	Product Size (bp)
S02458	CAAGCTCTCTATGCATGGCTAA	GCTGTTCCATGAGATACTACTACAAA	140
S02468	ACGGCGAGAAGGATAAGGAT	GCTAGCCTACTACTCCGTTGG	194
S02471	CGGGTCGTTGTCTTCTTCAT	TGTGGCGGCTAATCTGATCT	179
S02497	CCGCAAGATGATGGTAACCT	TCGGATCCTCGGATGTAAAG	201
S02519	TTCCTGTCAATATGCGGTATCA	AATATCGAACGAAGCACATCC	138
S02535	GCGTTGTCAGCTGTCTCTT	TCGGGCATCACTAGATACCA	195
S02541	CTTTGCACTCTCAGCCCAT	CAAGTTTGGTGGTGCATTTG	159
S02548	CCCTACGAGGAGGAGATGAG	AGGTAACCGAGCAGCAACAT	213
S02552	CCCAGAACTGGATGATGGTT	TGCCCATACGGAGAGAAAAG	186

Results

I. Characterization of the *dep2-3* mutant

- Grain size

Dep2-3 mutant showed small round grain phenotype compared to wild type (Figure1). The average grain length of wild type was 5.73mm whereas that of mutant type was 4.83mm with statistically significant difference at 1% probability table4. Unlike grain length, width of mutant grain was higher compared with wild type, the average grain width of wild type was 3.29mm whereas that of mutant type was 3.47mm. Grain thickness was also higher in case of mutant type (2.20mm) compared to wild type (2.08mm).

- Grain Shape

Grain shape is defined as ratio of grain length to grain width. The standard criteria used to categorize shape of mutant and wild type grain is shown in table2. The ratio of grain length to width was 1.74 in case of wild type whereas that of mutant type was 1.39. This categorizes wild type grain into semi round category and mutant type grain into round shape category.

Table2. Standard basis used to characterize grain shape of wild type and *dep2-3*.

Type	Standard L/W
Round	<1.5
Semi-round	1.5-1.99
Half spindle-shaped	2.00-2.49
Spindle-shaped	2.50-2.00
Long spindle-shaped	>3

- Plant phenotype

The plant height in case of mutant was observed to be shorter than that of wild type fig1. Culm length in case of wild type was 87cm compared with mutant type 72.5cm with statistically significant difference at 1% probability table4. Apart from height, the overall architecture of the plant was erect (especially leaves) compared to wild type figure1.

- Agronomic characteristics

Due to reduction in grain length, the 1000 seed weight was significantly reduced in case of mutant (21g) compared to wild type (23.3g). Reduction in panicle length was also observed with the average length of wild type panicle being 19cm compared to 14.10cm that of mutant type. Also, decrease in the spikelet fertility was observed in case of mutant. However, there was no significant difference found between wild type and mutant type in case of number of panicles per plant (table4)

Table4. Statistical analysis of agronomic and grain size/shape characteristics of wild type and *dep2-3* mutant. Table also shows the statistical analysis of wild v/s F₁ and mutant v/s F₁. Significant difference between wild v/s F₁ and mutant v/s F₁ helps in inferring that wild type allele DEP2-3 shows incomplete dominance.

Traits	CL (cm)	PL (cm)	PN (No.)	SF (%)	1000 GW (g)	Grain Width (mm)	GL (mm)	GT (mm)	LWR
Wild-type	87.00±4.92	19.00±0.94	9.50±2.12	96.61±0.95	23.30±0.02	3.29±0.09	5.73±0.22	2.08±0.06	1.74±0.09
Mutant	72.50±3.85	14.10±1.45	8.50±1.5	90.45±2.45	21.00±0.03	3.47±0.13	4.83±0.14	2.20±0.1	1.39±0.06
F₁	86.00±2.2	16.62±0.98	9.30±1.5	96.00±1.0	21.93±0.02	3.39±0.12	5.47±0.23	2.15±0.06	1.61±0.08
Difference									
(Wild v/s	**	**	NS	**	**	**	**	**	**
Mutant)									
(Wild v/s	NS	**	NS	NS	**	**	**	**	**
F₁)									
(Mutant v/s	**	**	NS	**	*	*	**	*	**
F₁)									

CL=Culm Length, PL= Panicle Length, PN= Panicle Number per plant, SF= Spikelet Fertility, GW=Grain Weight, GL= Grain Length, GT= Grain Thickness, LWR= Length Width Ratio



Figure1. Phenotypic characterization of wild type (*Hwachong waxy japonica* cultivar) and *dep2-3* mutant. A. Grain of wild type and *dep2-3* mutant with and without hull. B. Phenotype of the plant.

II. Histological analysis

- Scanning electron microscopic (SEM) observation of the glume surface

As mentioned earlier, the change in glume cell size directly affects the grain size. Therefore, to observe change in glume cell size we did scanning electron microscopic observation of outer glume surface with 1000X magnification. A clear increase in the cell width was observed in case of mutant compared to wild type figure3. This increase in cell width explains increase in the grain width in case of mutant.

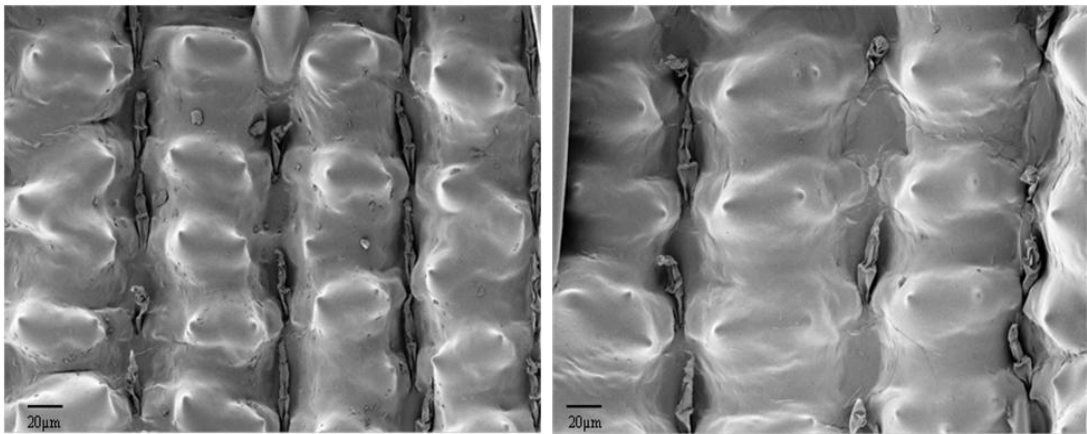


Figure3. Scanning electron microscopic view of the surface of rice glumes. Figure on left is glume surface of wild type and on the right is glume surface of *dep2-3* mutant. As seen the cell width in case of *dep2-3* mutant has increased which explains change in grain width

- Paraffin sectioning of paduncles

To observe the changes at histological level due to mutation in *dep2* locus, we performed paraffin sectioning of the peduncle (Figure2). Very distinct difference could be observed in case of number of vascular bundles. The number of inner large vascular bundle was significantly higher in case of wild type compared to mutant type whereas, number of outer small vascular bundles was significantly higher in case of mutant compared to wild type. The total number of vascular bundle was found to be higher in case of mutant type compared to wild type table3. This result shows that in the mutant type, there is more growth in outer layer of peduncle compared to inner layer which indicates that the erectness of the panicle.

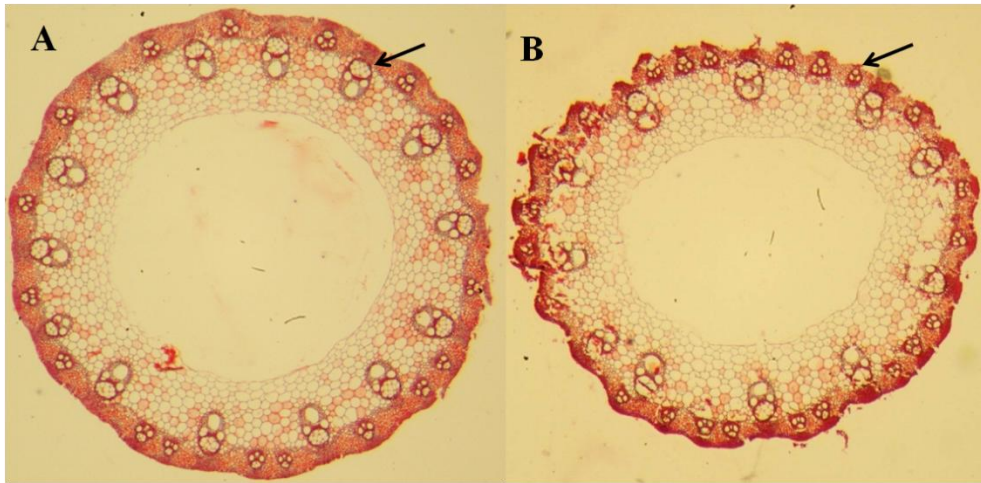


Figure2. Cross section of the penduncle. A. Wild type; arrow showing inner large vascular bundle. B. *dep2-3* mutant; arrow showing small inner vascular bundle. Number of inner vascular bundle is higher in case of wild type whereas, number of outer vascular bundle is higher in case of *dep2-3* mutant. Total number of vascular bundles is higher in case of *dep2-3* mutant.

Table3. Statistical analysis of the number of vascular bundles in wild type and *dep2-3* mutant.

Characteristics	Outer (small) Vascular bundle	Inner (large) Vascular bundle	Total
Wild	18.1±0.33	13.9±0.33	32.0±0.67
Mutant	22.8±0.67	10.9±0.67	33.89±0.33
Difference (wildv/s mutant)	**	**	**

III. Genetic control of mutated trait

To know whether *dep2-3* behaves as recessive or dominant allele, we crossed mutant with its original parent (Hwachong wax *japonica* cultivar). Interestingly, F₁ plant showed intermediate characteristics with semi erect panicle (intermediate of wild and mutant type) and grain characteristics like length, width, thickness, grain shape and 1000 seed weight that of intermediate between wild and mutant type with statistically significant difference (table 4, figure 4,5). Through this data we concluded that wild type allele of the *dep2* locus shows incomplete dominance while mutant allele behaves as recessive type. We also measured grain length and width of all F₃ plants and checked the frequency distribution pattern. Both grain length and length to width ratio showed normal distribution (figure 6).



Figure4. Grain morphology of wild type, *dep2-3* mutant and F₁. F₁ showed intermediate phenotype.



Figure5. F₁ Showing intermediate panicle phenotype

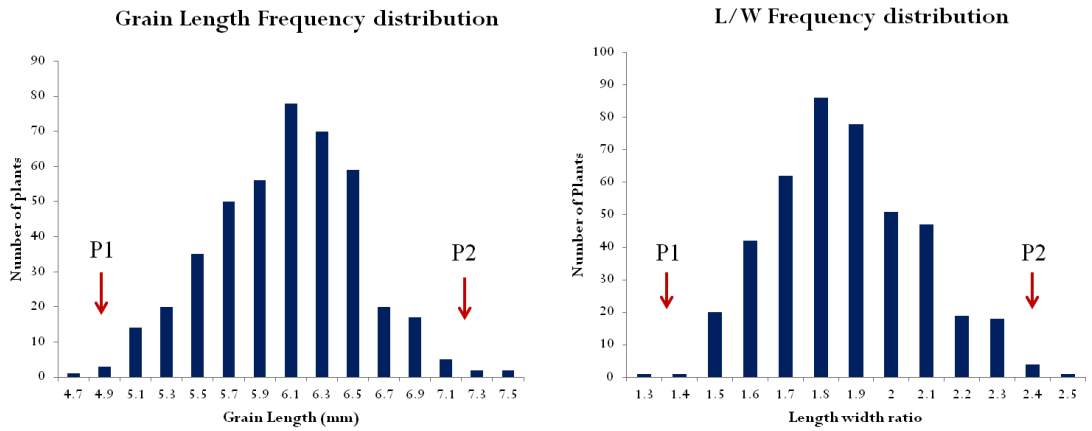


Figure6. Frequency distribution curve of grain length and length to width ratio. Both curve showed normal distribution.

IV. Fine mapping and candidate gene analysis

To genetically map the mutation we performed bulked segregant analysis (BSA) using STS and sub-species specific STS markers. Among total 80 markers which covered all chromosomes, two markers, S07080 and S07094 on long arm of chromosome 7 demonstrated significant polymorphism between bulked DNA samples of wild and mutant type plants. To further narrow down the candidate region additional 9 STS markers were designed table1. Through additional markers, we narrowed down the candidate region to 168.75kbp between marker S02535 and S02552 (figure7). Through rice genome annotation database (<http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/>) we found that this candidate region of 168.75kbp had a total of 26 genes. Among 26 genes the LOC_Os07g42410 was reported to be related with erect panicle phenotype in

maize and rice (*dep2*). After sequencing this gene we found a point mutation (G to A) at the 5' splicing site of first intron (figure 7). This mutation leads to the splicing error.

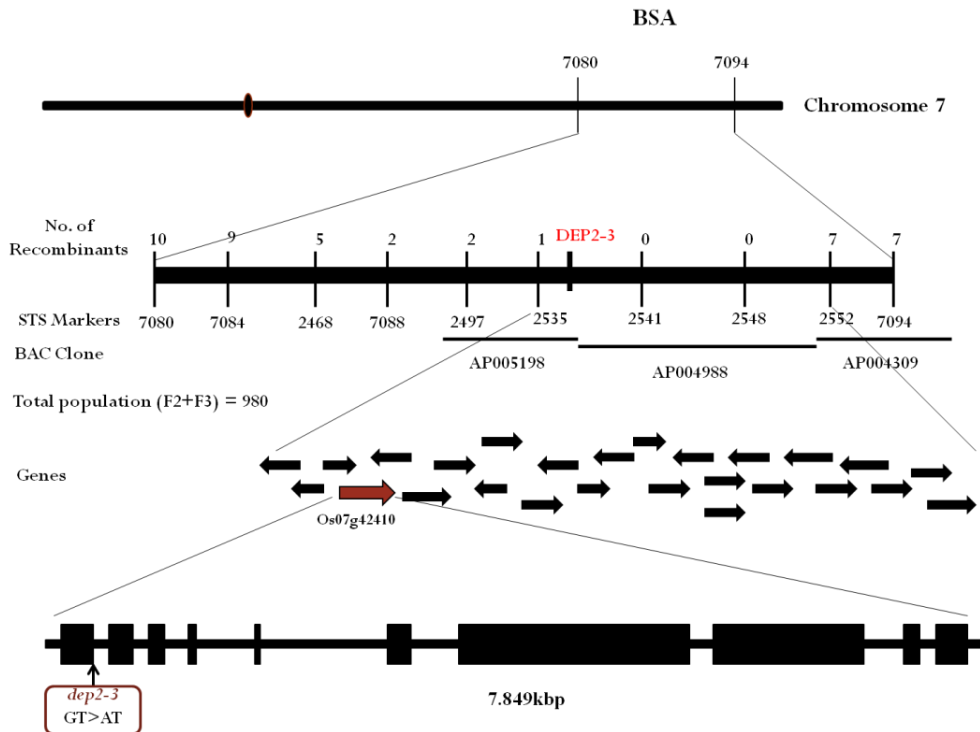


Figure7. Genetic mapping of *dep2-3* mutant. Location of *dep2-3* could be found on BAC clone AP005198. Sequencing result of LOC_Os07g42410 revealed point mutation (G to A) at the 5' end splicing site of first intron.

In case of eukaryotes, m RNA has introns and exons. Introns are spliced off before translation of m rna. Intron has a specific sequence GU at its 5' end and AG at its 3' end. In *dep2-3* mutant this G has been mutated to A. Spliceosome

which binds at this site and helps in splicing off intron will not be able to do so after mutation. This leads to the splicing error. We analysed the sequence and found that this error might also lead to frameshift mutation.

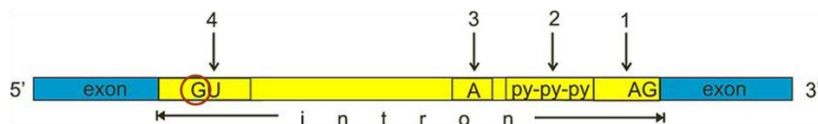


Figure 9. The position of mutation at 5' end of the splicing site.

To confirm the mutation, CAPS marker with Nru1 restriction enzyme site (TCGCGA) was designed. 7 mutant type and 7 wild type plants were selected from F3 population and PCR was performed using CAPSNru1 marker which, after digestion with Nru1, will cut the mutant sequence. A clear co-segregation could be observed between wild type and mutant type plants from F3 population (figure8).

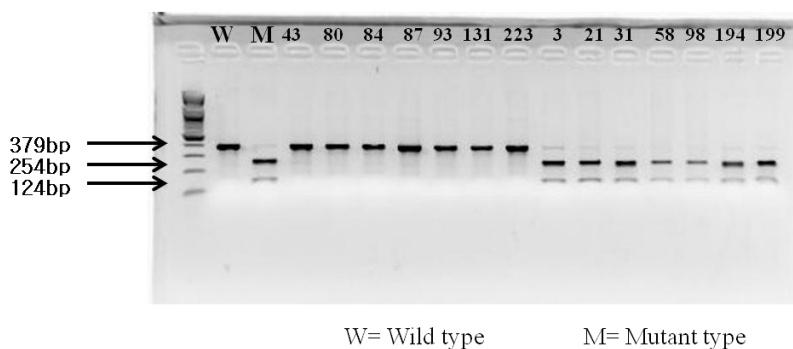


Figure8. Co-segregation test between wild and mutant type plants from F₃ generation using CAPS marker with Nru1 restriction enzyme

Discussion

One common phenomenon which could be distinctly observed in almost all previous and also in current study in erect panicle mutants is that, all erect panicle mutants manifest small grain phenotype and slight reduction in plant height. This observation clearly shows that all these genes EP2, EP3, DEP1, qPE9-1 and DEP2 directly or indirectly affects cell size/shape and/or cell division which changes plant and panicle architecture. Previous study on *dep2* (Li, Liu et al. 2010) reported that the expression level of cell cycle related genes like *CycB1;1*, *CycB2;1*, *CycB2;2*, *CycD3;1*, and *CDKB2;1* was reduced in case of mutant compared to the wild type while cell size remained unchanged. However, in present study we also observed the change in cell shape in case of mutant glume (figure3), this indicates that *dep2* has pleiotropic effect on the cell division and cell shape. Genes affecting plant architecture also tend to affect the grain yield by affecting glume size. Since the overall architecture of plant is shaped by cell division, cell size and its shape, the genes which affect plant height and panicle characteristics also have a substantial effect on the grain size. Therefore it is very important to understand the mechanism involved in plant architecture at molecular level.

(Yan, Zou et al. 2011) in a very interesting study tried to find the relationship between four genes related with grain size and shape in rice; *GS3*, *GW2*, *qSW5/GW5* and *GIF1*. Since the ultimate aim of discovering various genes related with grain size is to exploit them in the breeding program, specially by

pyramiding all alleles which positively contribute to grain size in to one variety, it is important to understand how these alleles interact with one another when introgressed in to one background genome. It is now known that qSW5 and GW2 positively regulate the expression of GS3 and GIF1 expression was found to be positively regulated by qSW5 but negatively by GW2 and GS3. Studying the relationship between these genes and genes related with erect panicle and plant architecture will further enhance our understanding of basic mechanisms involved with yield related traits in crops.

Erect panicle phenotype is the result of decrease in length of rachis, primary and secondary branch, this might be due to decrease in cell size or due decreased cell division, moreover, as mentioned earlier, this also affects grain size negatively. However decrease in grain size does not always lead to reduction in grain yield, according to the report by Qian et al (Huang, Qian et al. 2009), *dep1* enhances the grain yield by increasing number of grains per panicle. Therefore to achieve maximum yield there is need to optimize the plant architecture, grain size, shape and number.

In the histological analysis of peduncle, higher number of vascular bundle in case of mutant, (specifically small, outer vascular bundle) indicates more growth in outer cell layers. This makes the stem and panicle stronger and is responsible for the erectness. Further proteomic study has to be done to know if there is any relation of *dep2* protein with plant hormones like auxin, cytokinin or brassinosteroids. Since plant hormones affect the cell division and shape and growth, there might be some common link between *dep2* protein and hormone

pathway.

First erect panicle variety named Guihuahuang was released in China in 1960's, since then breeders have developed many erect panicle varieties which have contributed to increase in rice production in China. Identification of genes related with erect panicle and their alleles in various *japonica* and *indica* cultivars will further strengthen the rice breeding programs.

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초록

벼 종자크기 유전자의 지도 작성 및 분리 연구

종자 크기는 이삭 당 종자 수, 개체 당 이삭 수 그리고 천립중을 비롯하여 식량작물의 수량을 결정하는 가장 중요한 형질중의 하나이다. 또한 초형은 햇빛에 직접적으로 노출되는 표면적의 양에 영향을 줌으로써 수량에 영향을 주는 매우 중요한 요소이다. Erect panicle은 햇빛을 막아 광합성을 감소시키는 curved panicle과는 달리 햇빛이 직접 앞에 도달하도록 하여 수량을 증대시키는데 도움이 되는 중요한 표현형적 특성이다. 우리는 종자의 크기와 형태, 그리고 초형을 조절하는 다면발현효과를 갖는 새로운 대립유전자인 *dep2-3* (dense and erect panicle) 에 대해 밝혀내었다. 화청벼 (*japonica*) 에 화학적 돌연변이 유도물질인 MNU (N-methyl-N-nitrosourea) 를 처리하여 small round grain과 erect panicle을 나타내는 돌연변이체를 획득하였다. STS (Sequence-Tagged Sites)와 SS-STS (Sub-species Specific Sequence-Tagged Sites) marker를 이용한 map based cloning을 통해 7번 염색체에서 mutation을 발견하였고, 이것이 벼에서 이전에 보고된 *dep2* 유전자의 새로운 대립유전자임을 밝혀내었다. 이전에 보고된 *dep2*의 두 대립유전자가 각각 *dep2-1* 과 *dep2-2* 로 명명되었기 때문에, 우리는 새로운 대립유전자를 *dep2-3*라고 명

명하였다. dep2-3 는 종자 크기와 식물체의 키가 작고, dense and erect panicle 을 가지며 야생형에 비해서 전체적인 초형도 erect한 형태를 보이는 특징을 갖고 있다. 이 돌연변이체와 야생형 (화청) 벼를 교배한 결과 F₁의 이삭과 종자가 중간 표현형을 보였다. 따라서 우리는 이 유전자의 야생형 대립유전자가 불완전우성을 보인다는 결론을 내렸고, 이것은 이전의 연구에서 보고된 바가 없었다. 주사전자현미경 (SEM) 관찰 결과 돌연변이체 종자의 폭이 증가하였고, 그에 따라 종자의 형태가 변화하였고, 이것은 glume cell 의 폭이 증가했기 때문이라는 것을 확인할 수 있었다. 표현형 관찰 결과 dense and erect panicle 표현형은 이삭에서 rachis의 길이, 그리고 primary 와 secondary branch 의 길이가 감소한 결과라는 것을 알 수 있었다.

주요어 벼, 유전자 지도, 종자크기, glume, dep2, splicing.

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