THESIS FOR DEGREE OF MASTER OF SCIENCE

Regulatory Roles of *OsERF101* in Grain Size and Tiller Number in Rice

벼의 OsERF101 전사인자의 벼 종자 크기와 분얼수 조절기작 연구

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MAJOR IN CROP SCIENCE AND BIOTECHNOLOGY

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THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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ABSTRACT

Regulation of grain size and tiller number is important for improving rice yield. However, many parts of the genetic and molecular mechanism controlling grain size and tiller number are still unknown. Here, we show that *OsERF101* transcription factor is positively regulate both grain size and tiller number. The T-DNA insertion knock out mutants of *OsERF101*(*oserf101*) show short grains and less tillers, but overexpression lines of *OsERF101*(*OsERF101*-OX) show long grains and many tillers. Grain weight and panicle number is also low in knock out mutant and upregulated in overexpression line. To elucidate the mechanism of regulation in grain length, we made images of surface of grain hull by using scanning electron microscope. As a results of analyzing these images, we can expect that *OsERF101* regulate grain length by regulating grain epidermal cell size. To clarify the exact molecular mechanism of regulating grain length, we harvested RNA samples from developing panicles and requested RNA sequencing. As a results of analysis, we estimated target genes of *OsERF101*. Expression levels of *SRS5*, *PGL1*, *DGL1* in *oserf101*

line were lower than those of wild type(WT). Knock out mutant of *SRS5* shows shorter grain, overexpression line of *PGL1* had longer grain phenotype. *DGL1* knock out mutant have shorter grain hull. So we can suppose that these genes are target of *OsERF101*. While we checked the tiller number of *oserf101*, WT and *OsERF101*-OX as they grow up, we could find that their gap of tiller number was bigger and bigger. Flowering time can affect duration of tillering stage, so we checked their flowering time. And we could find that their flowering times are not significantly different. From these data, we could assume that *OsERF101* regulate tiller number. Knock out mutant of *OsHAK5*, gene of coding potassium transporter, has similar phenotypes of tiller number with *oserf101*. Expression level of *OsHAK5* was upregulated in *OsERF101*-OX than WT. So we performed chromatin immunoprecipitation assay, and the result of assay seems like that *OsERF101* bind 3' UTR region. In conclusion, our results suggest that *OsERF101* regulate grain length and tiller number by modulating expression of *SRS5*, *PGL1*, *DGL1*, and *OsHAK5*.

Keywords: rice, grain size, tiller number, *OsERF101*, transcription factor, overexpression, *SRS5*, *PGL1*, *DGL1*, *OsHAK5*

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ABBREVIATION

ERF	Ethylene Response Factor
SEM	Scanning Electron microscopy
DAG	Days After germination
WT	Wild type
UBQ	Ubiquitin
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
HAK	High-Affinity K+
ChIP	Chromatin Immunoprecipitation
NLD	Natural Long Day

INTRODUCTION

As the world population very rapidly increase, the importance of production of rice(*Oryza sativa*), one of the major crops in the world, be improved. [1] The yield of rice consists of three components, panicle number per plant, grain number per panicle and grain weight. Grain weight is determined by grain size. Proliferation or expansion of epidermal cells in grain hull affect grain size. [2, 3] Growth of these cells vigorously occur at young panicle stage before heading date. There are many genes associated with regulating of grain size phenotypes but molecular mechanisms of these genes are not elucidated absolutely yet. For examples of these reported genes, *GRAIN WIDTH AND WEIGHT2* (*GW2*), [4] *GRAIN SIZE ON CHROMOSOME5* (*GW5*/*GSE5*), [5, 6] *GRAIN WIDTH ON CHROMOSOME8* (*GW8*/*OsSPL16*), [7] *GRAIN SIZE ON CHROMOSOME3* (*GS3*), [8, 9] *GRAIN SHAPE ON CHROMOSOME9* (*GS9*), [10] *OsMKKK10-OsMKK4-OsMPK6*, [11, 12, 13] and *Mitogen-activated protein Kinase Phosphatase1* (*MKP1*) [14, 13] are genes that control grain size by regulating cell proliferation. In addition, *GRAIN SIZE ON CHROMOSOME2* (*GS2*/*OsGRF4*), [15, 16, 17] *GSK3*/*SHAGGY-like kinase5* (*OsGSK5*), [18, 19, 20] *GRAIN LENGTH AND WEIGHT ON CHROMOSOME7* (*GLW7*/*SPL13*), [21] *GRAIN LENGTH ON CHROMOSOME7* (*GL7*/*GW7*/*SLG7*), [22, 23, 24] *POSITIVE REGULATOR OF GRAIN LENGTH1*/*2* (*PGL1*/*PGL2*), [25, 26] and *ANTAGONIST OF PGL1*

(*APG*) [25, 26] are affect grain size by controlling cell expansion. *OsLG3*, a member of ERF transcription factor subfamily control grain length by regulating cell proliferation. [27]

Panicle number is affected by tiller number. [28] Tillers arise from the axillary bud apical meristem (AM). [28] They are developed in the axils of leaves during the vegetative stage in rice. [28] Developmental stages of tillers are divided into two stages, formation of axillary bud in leaf sheath and outgrowth of axillary bud. [28] In rice, *Oryza sativa homeobox1* (*OSH1*), which is preferentially expressed in the AM, is required for the initiation of AM formation and the maintenance of undifferentiated cell fate. [29] *LAX PANICLE1* (*LAX1*) also plays an essential role in AM formation by genetically functioning together with *LAX PANICLE2* (*LAX2*) and *MONOCULM1* (*MOC1*). [30] *TILLERS ABSENT1* (*TAB1/OsWUS*) encodes a transcriptional regulator containing a homeodomain WUS box and an ethylene-responsive element-binding factor (EAR) motif and is expressed in the pre-meristem zone. Mutation of *TAB1* causes reduction in *OSH1* expression in the pre-meristematic region and defects in AM formation. [28] Another factor involved in AM formation is a *LEAFY* orthologue, *RICE FLORICULA/LEAFY* (*RFL*), also called *ABERRANT PANICLE ORGANIZATION 2* (*APO2*). *RFL* is preferentially expressed in the axils of leaves at the juvenile vegetative stage and maintains AM specification by promoting expression of *LAX1* and *CUP SHAPED COTYLEDON* (*CUC*) genes. [31] *Hairy Leaf 6 (HL6)*, an AP2/ERF family transcription factor, is

overexpressed, tiller number is downregulated. [32]

The APETALA2/Ethylene Response Factor (AP2/ERF) family can be divided into four subfamilies depending on the number of AP2/ERF domains and their amino acid similarity, such as APETALA2 (AP2), Related to ABI3/VP1 (RAV), Dehydration Responsive Element Binding protein (DREB), and Ethylene Response Factor (ERF) subfamilies. [33, 34] DREB and ERF subfamilies have a single AP2/ERF domain that can bind to both the C–Repeat/Dehydration Responsive Element (CRT/DRE) and the Ethylene Response Element (ERE) in the promoter regions of their target genes. ERFs regulate the expression of abiotic stress-related genes in various plant species including rice, [35] Arabidopsis, [36] wheat (*Triticum aestivum*), [37] maize (*Zea mays*), [38] soybean (*Glycine max*), [39] and tobacco (*Nicotiana tabacum*). [40] Previous studies showed that the rice *ETHYLENE RESPONSE FACTOR 101* (*OsERF101*) plays an important role in enhancing tolerance to drought stress in reproductive tissues [41] and positively regulating leaf senescence. [42] In this study, we reveal that OsERF101 regulate grain size and tiller number and presume target genes.

MATERIALS AND METHODS

Plant materials and growth conditions

The T-DNA insertion *oserf101* (PFG_2D-00368.L) mutants were obtained from Crop Biotech Institute at Kyung Hee University, Korea. The overexpressed *OsERF101*-OX1, 2 mutants were generated by *Agrobacterium tumefaciens* (strain LBA4404)-mediated transformation to introduce pMDC32-*OsERF101* vector in “Dongjin” calli. The WT japonica cultivar “Dongjin”, and *oserf101*, *OsERF101*-OX1, 2 mutants were grown in paddy field and greenhouse under natural long days (>14 h light / dark) in Suwon, South Korea (37N latitude).

Scanning Electron Microscopy

The samples were first fixed in 2.5% glutaraldehyde in 0.1-M phosphate buffer, pH 7.2, then rinsed three times in distilled water, dehydrated gradually in an ethanol series (30%, 50%, 70%, 80%, and 95%), and rinsed three times in 100% ethanol. The samples were finally dried using a critical point dryer (EM CPD300, Leica, Wetzlar, Germany). Images were obtained using a scanning electron microscope (SIGMA, Carl Zeiss, Germany).

Reverse Transcription and Quantitative Real-Time PCR (RT-qPCR) Analysis

Total RNA was extracted from rice leaf tissues, young panicle and shoot base with the MG Total RNA Extraction Kit (Macrogen, Korea). First-strand cDNA was synthesized with 2 μg of total RNA in a 25 μl volume using M-MLV reverse transcriptase and oligo(dT)15 primer, and diluted with 75 μl water. The 20 μl of qPCR mixture was prepared including 2 μl of the first-strand cDNA mixture, 10 μl of 2X GoTaq PCR Mix (Roche), and 1 μl of 10 pM qRT primer pairs. qPCR was conducted on the LightCycler 2.0 instrument (Roche Diagnostics). The qPCR conditions were 95 ℃ for 2 min, followed by 40 cycles at 95 ℃ for 5 s, 59 ℃ for 15 s, and 72 ℃ for 10 s. *OsGAPDH* (Os04g0486600) was used as an internal control. Primers used for qRT-PCR analysis are listed in Table 1.

Chromatin Immunoprecipitation (ChIP) Assays

For the ChIP assay, the Ubi : OsERF101-Myc and Ubi : Myc were transfected into rice protoplasts as previously described. [43] Protoplasts were then subjected to cross - linking with 1% formaldehyde for 30 min under vacuum. Then, nuclei were isolated and lysed, and chromatin complexes were isolated and sonicated, as described. [44] DNA was sonicated using a BIORUPTORII (COSMO BIO). Anti-Myc monoclonal antibody (Abcam, Cambridge, UK) and protein A agarose beads (Merck Millipore) were used for immunoprecipitation. DNA recovered from agarose beads was purified using the DNeasy Plant Mini

Kit (Qiagen).

Observation of Phenotype

For observation of phenotype about grain length and width, randomly chosen 10 seeds of WT, *oserf101*, *OsERF101*-OX1, 2 were used. For measuring cell length of grain epidermis, scanning electron microscopy images of grain hull were used. ImageJ program was used to measure grain length, width, and grain epidermal cell length.

Table1. Information of primers used in this study.

Marker	Forward primer (5'→3')	Reverse primer(5'→3')
A. Primers for verification of transgenic plants		
PFG 2D-00368.L	GATAAGGGACCCGAAGAAGG	TTGCATGCATTTTCATCTCC
B. Primers used for gene cloning		
OsERF101	ATGGTCACCGCGCTAGCC	TCACGACGACGAATCCTTCT
C. Primers used for qRT-PCR		
OsERF101	GTTCAAGGGGACCAAGGCCAA	GGCTACTACCCCTCGTCGTC
OsHAK5	CCCAATCAGCAGGCAGAAGA	AATCCAATGAGCCCGCTTGA
OsUBQ5	ACCACTTCGACCGCCACTACT	ACGCCTAAGCCTGCTGGTT
OsGAPDH	AAGCCAGCATCCTATGATCAGATT	CGTAACCCAGAATACCCTTGAGTTT
C. Primers used for ChIP assay		
*OsHAK5*_ChIP_A	TGTTCTCAGTGCTGAGTGAGG	ATCTCCATGCATGTTCTGTGGT
*OsHAK5*_ChIP_B	GAACCCCAATCCACATGCTC	AATCGGTGGTATACGCTCTTCT

RESULTS

Mutation of *OsERF101* Causes Short Grain

We checked mutation of T-DNA insertion line *oserf101*. *oserf101* was inserted T-DNA in second exon region (Figure 1A). semi-qRT PCR results show that *oserf101* are knock out mutant line (Figure 1B). To verify the overexpression of *OsERF101*-OX1, 2, we performed qRT PCR. The result shows that the OsERF101-OX1, 2 are overexpress *OsERF101* (Figure 1C). We observed that grain length of the mutant and overexpression lines are shorter and longer than WT, respectively (Figure 1D, E). However, grain width of these lines were non-significantly different (Figure 1F, G). To confirm that these phenotypes were caused by *OsERF101*, we checked expression level of this gene in young panicle of WT before heading time (1 to 10 cm of young panicle) that the growth of grain hull is vigorously occur. In these results, we could find that the expression level of *OsERF101* was largely upregulated in 6 cm of young panicle (YP6) (Figure 1H). Therefore, we could presume that this grain length phenotypes are induced by *OsERF101*. In order for the growth of grain hulls, which affect grain length, leads to increase of yield, it must affect uncovered grain length. So we confirmed uncovered grain length (Figure 2A, B), and width (Figure 2C, D). As these results, uncovered grains showed similar phenotypes with covered grains.

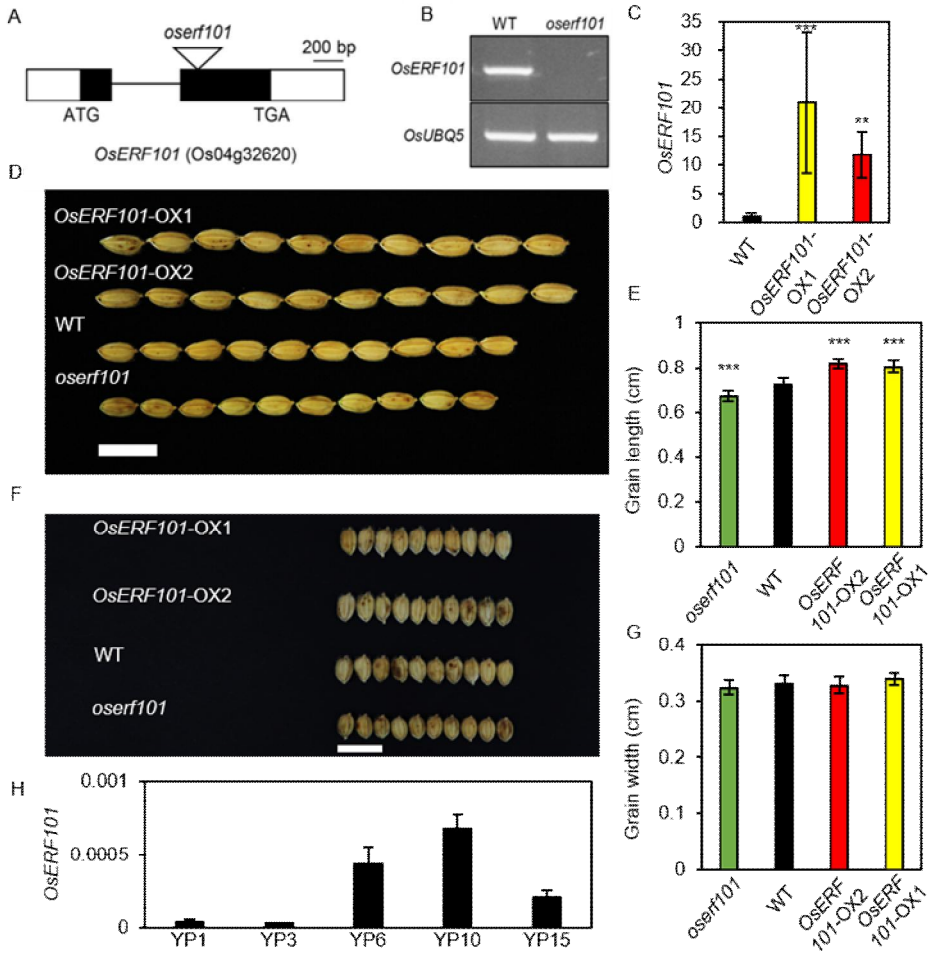

Figure 1. Expressional profile and phenotypes of *OsERF101*

(A) Schematic diagram of *oserf101* mutant. The black box and white boxes stand for exon and untranslated regions, respectively. The triangle represent for the T-DNA insertion position in second exon region of *OsERF101*. (B) Mutation of *OsERF101* was confirmed by semi-quantitative RT-PCR. Total RNA was isolated from second leaves of a 3-week-old plant. *OsUBQ5* (Os01g22490) was used as a loading control. (C) *OsERF101* transcript levels of overexpression lines were confirmed by qRT-PCR. Total RNA was isolated from second leaves of a 2-week-old plant. (D), (E) Grain length phenotype of *oserf101*, *OsERF101*-OX1, 2 compared with WT (bar = 1 cm). Ten biological

replicates were used (n = 10). (F), (G) Grain width phenotype of *oserf101*, *OsERF101*-OX1, 2 compared with WT (bar = 1 cm). Ten biological replicates were used (n = 10). (H) Relative *OsERF101* gene expression in young panicles of 1 cm (YP1) to 15 cm (YP15) in WT. Three biological replicates were used (n = 3). Asterisks indicate a significant difference (Student's t-test, *P < 0.05, **P < 0.01, ***P < 0.001).

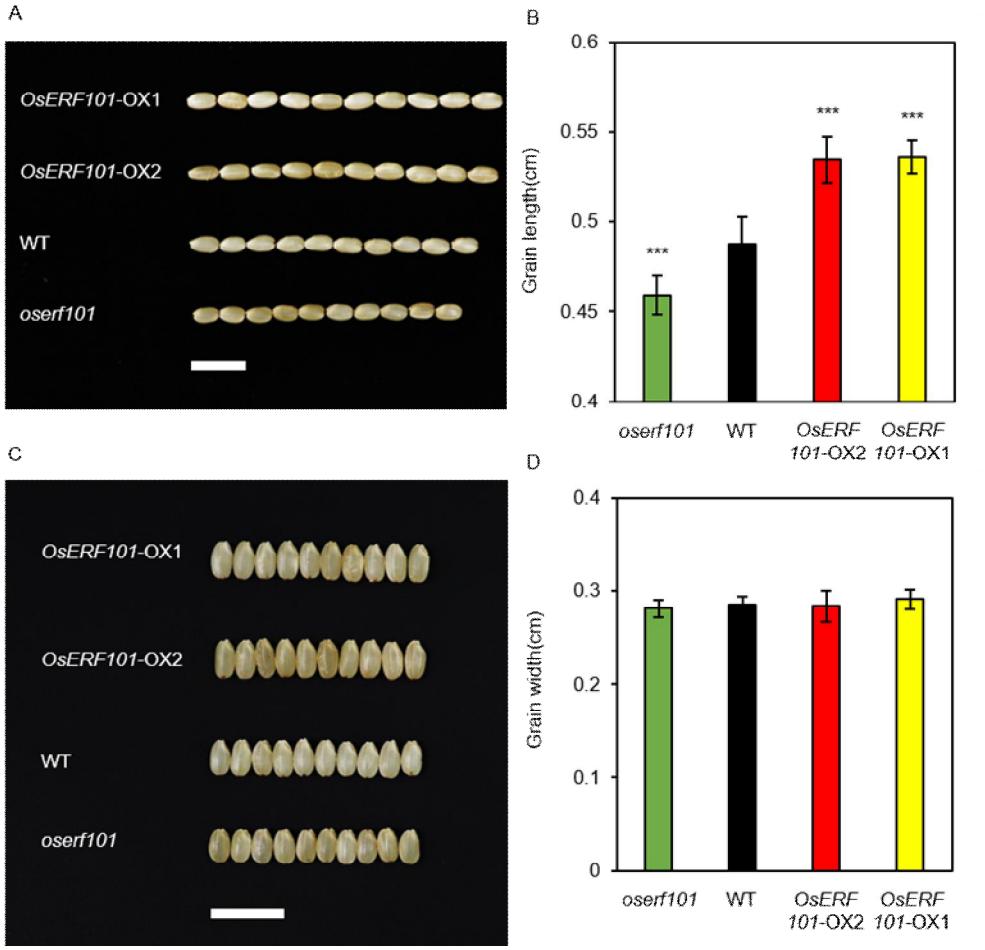

Figure 2. Length and width phenotype of uncovered grain

(A), (B) Uncovered grain length phenotype of *oserf101*, *OsERF101*-OX1, 2 compared with WT (bar = 1 cm). (C), (D) Grain width phenotype of *oserf101*, *OsERF101*-OX1, 2 compared with WT (bar = 1 cm). Ten biological replicates were used (n = 10). Asterisks indicate a significant difference (Student's t-test, ***P < 0.001).

OsERF101 Affects Grain Epidermal Cell Length.

To find out the mechanism that *OsERF101* regulates grain length, we take images of grain epidermal cells of each lines by scanning electron microscopy (SEM) (Figure 3A, B, C, D). To count cell number in longitudinal direction, we connected SEM images in longitudinal direction. In these results, we could find that those cell numbers in longitudinal direction were non-significantly different (Figure 3E). In case of cell size, they showed obvious different (Figure 3F). In these results, we could presume that *OsERF101* controls grain length by affecting grain epidermal cell length.

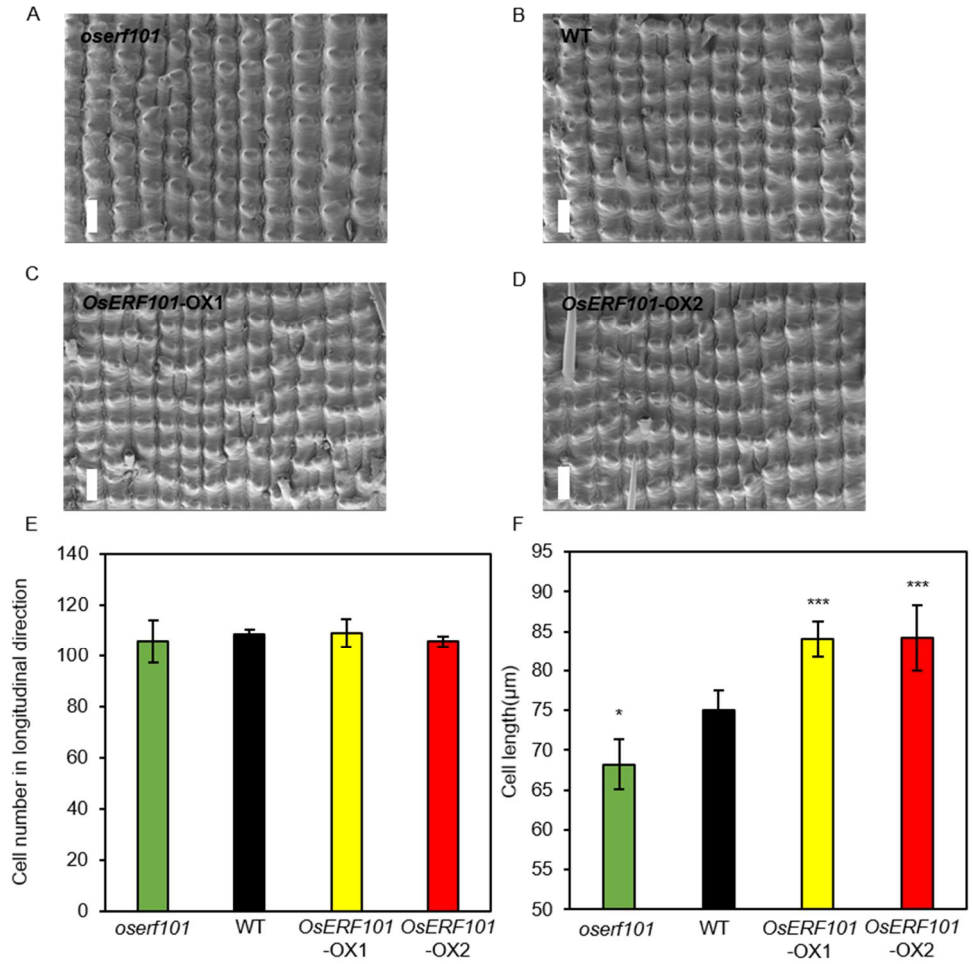

Figure 3. Grain epidermal cell number and length

(A), (B), (C), (D) SEM images of grain epidermal cells of *oserf101* (A), WT (B), *OsERF101*-OX1 (C), and *OsERF101*-OX2 (D). (E) Grain epidermal cell number in longitudinal direction of each lines. Three biological replicates were used (n = 3) (F) Grain epidermal cell length of each line. Twenty biological replicates were used (n = 20) Asterisks indicate a significant difference (Student's t-test, *P < 0.05, ***P < 0.001). Scale bars = 100 μm

OsERF101 Can Regulate Other Genes Related Grain Length

To elucidate molecular mechanisms of regulation of grain length by *OsERF101*, we compared the transcriptomes from 10 cm of young panicles, which express *OsERF101* maximally, of *oserf101* and WT plants using transcriptome deep sequencing (RNA-sequencing). The threshold for significantly differentially expressed genes (DEGs) was set at a (log2 scale)-fold change (FC) value of more than 1 or less than −1 and adjusted P <0.05. Using these criteria, we identified 812 DEGs downregulated and 673 DEGs upregulated in *oserf101* compared to the WT. These DEGs are shown in the volcano plots, which illustrate the asymmetry between upregulated (brown) and downregulated (blue) DEGs (Figure 4A). Especially, among 812 genes downregulated in *oserf101*, we found *Small and Round Seed 5* (*SRS5*), *POSITIVE REGULATOR OF GRAIN LENGTH 1* (*PGL1*) and *Dwarf and Gladius Leaf 1* (*DGL1*). These genes were reported about grain length and cell elongation. [45, 25, 46] Therefore, these results suggest that *OsERF101* can regulate grain length by affecting these genes.

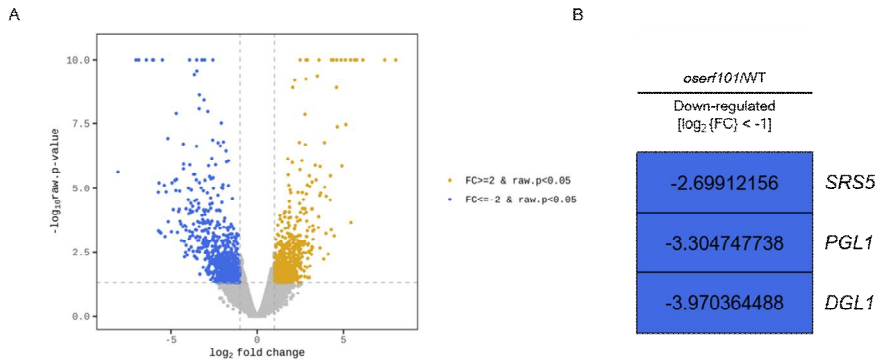

Figure 4. DEG analysis between *oserf101* and WT

(A) Volcano plots comparing the transcriptomes of *oserf101* and WT. X-axis and Y-axis represent log2 FC and −log10(P-value), respectively. The blue and brown dots represent downregulated DEGs with log2(FC) less than −1 and upregulated DEGs with log2(FC) >1, respectively. The gray dots represent no significant difference in transcriptomes. (B) Table of DEGs downregulated in *oserf101*.

Mutation of *OsERF101* Causes Less Tiller Number

As tiller buds begin to develop, expression level of *OsERF101* in shoot base of WT plant was increased (Figure 5A). At mature stage, *oserf101* plants show less tiller number and *OsERF101*-OX1, 2 plants show more tiller number (Figure 5B, C). We checked their tiller numbers by 15 days period, and we could find that their gaps of tiller numbers were getting bigger (Figure 5D). The tiller number can be affected by flowering time, we checked their heading date under natural long-day condition (Figure 5E). In these results, their flowering times were non-significantly different. Thus, *OsERF101* seems to regulate tiller number. In addition, *oserf101* and *OsERF101*-OX1, 2 showed similar phenotype in seedling stage. Tiller buds of each lines on 20 DAG

revealed obvious developmental difference (Figure 6A). Like mature stage, increase speed of tiller number of oserf101 and OsERF101-OX1, 2 were slower and faster than WT in seedling stage, respectively (Figure 6B). In these results, we could presume that *OsERF101* affects tiller development in both mature stage and seedling stage.

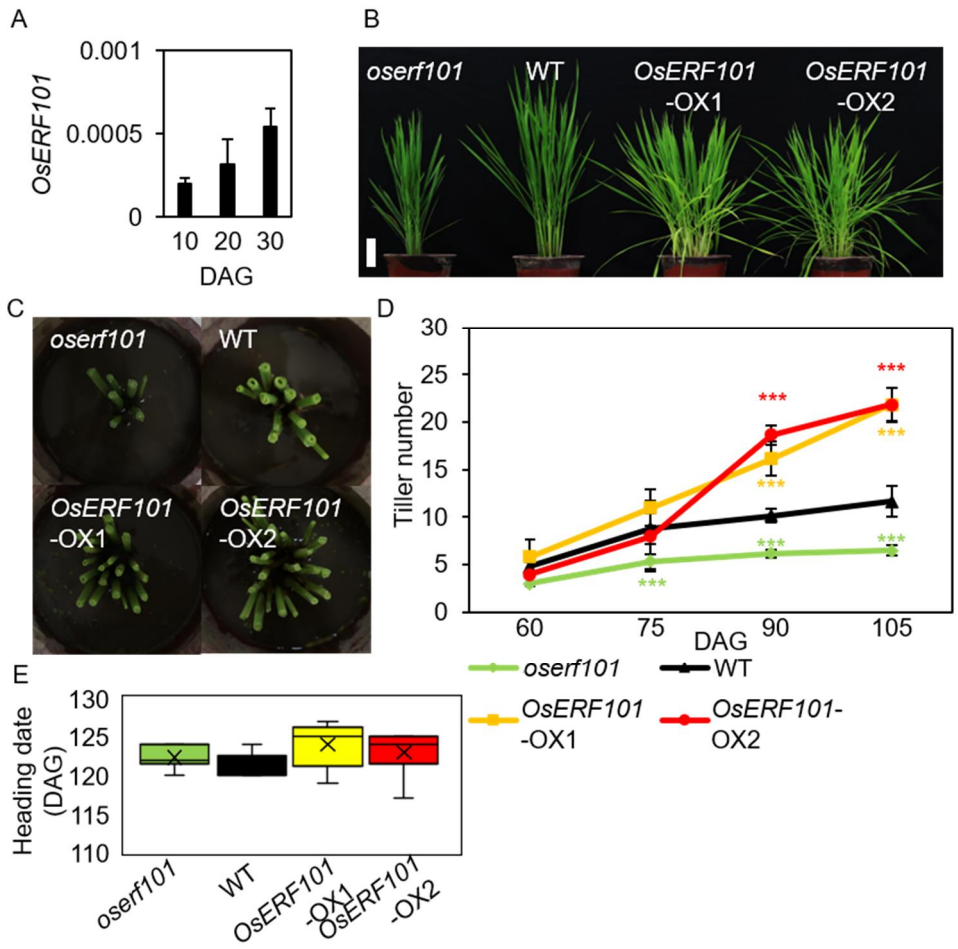

Figure 5. Phenotypes of *oserf101*, *OsERF101*-OX1, 2 about tiller number
(A) Expression level of *OsERF101* by 10 days period in shoot bases of WT seedling. Four biological replicates were used (n = 4). (B) Tiller phenotype of 105 DAG plant of each lines. Scale bar = 10 cm. (C) Cross section of tillers of same plant with (B). (D) Tiller numbers by period in tillering stage. Six biological replicates were used (n = 6). (E) Days to heading under natural long-day condition. The date at which the first panicles emerged were scored as the number of days after germination (DAG) to heading date. Six biological replicates were used (n = 6). Asterisks indicate a significant difference (Student's t-test, ***P < 0.001).

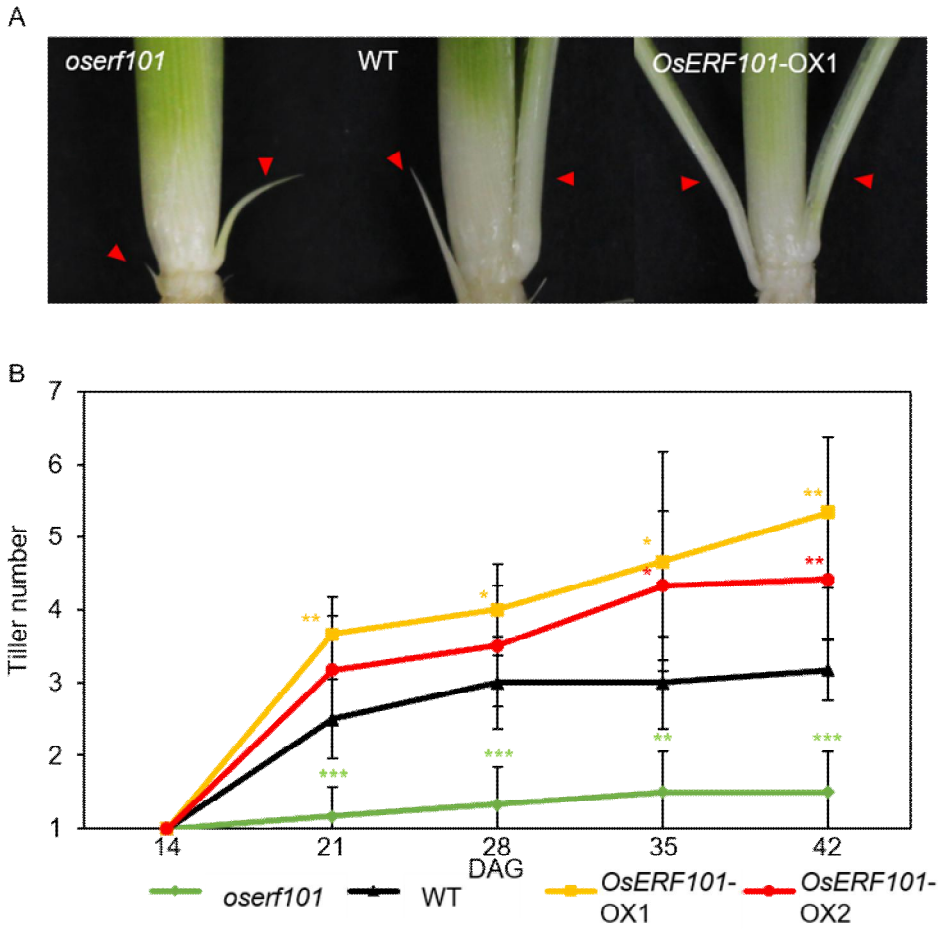

Figure 6. Tiller number phenotype in seedling stage

(A) Tiller buds of 20 DAG plants of *oserf101*, WT, *OsERF101*-OX1, 2 (B) Tiller number measured by a week period in seedling stage. Six biological replicates were used (n = 6). Asterisks indicate a significant difference (Student's t-test, *P < 0.05, **P < 0.01, ***P < 0.001). DAG, days after germination.

OsERF101 Can Directly Regulate *OsHAK5*

In previous study, it was reported that the inactivation of *OsHAK5*, a rice potassium transporter gene, decrease tiller number. So we checked expression level of *OsHAK5* in shoot base of WT and *OsERF101*-OX1 (Figure 7A). In these results, we could find that expression level of *OsHAK5* is upregulated in *OsERF101*-OX1. It suggests that the possibility of regulation of *OsHAK5* by *OsERF101*. We researched binding motifs of ERF family, and we could find 2 core sequence of DRE motif(CCGAC) in 3'-UTR of *OsHAK5* (Figure 7B). To verify the direct binding to *OsHAK5*, we performed chromatin immunoprecipitation assay. But, we couldn't repeat assay, only tried once. In these results, *OsERF101* seems to bind B region of Figure 7B and directly regulate *OsHAK5* (Figure 7C).

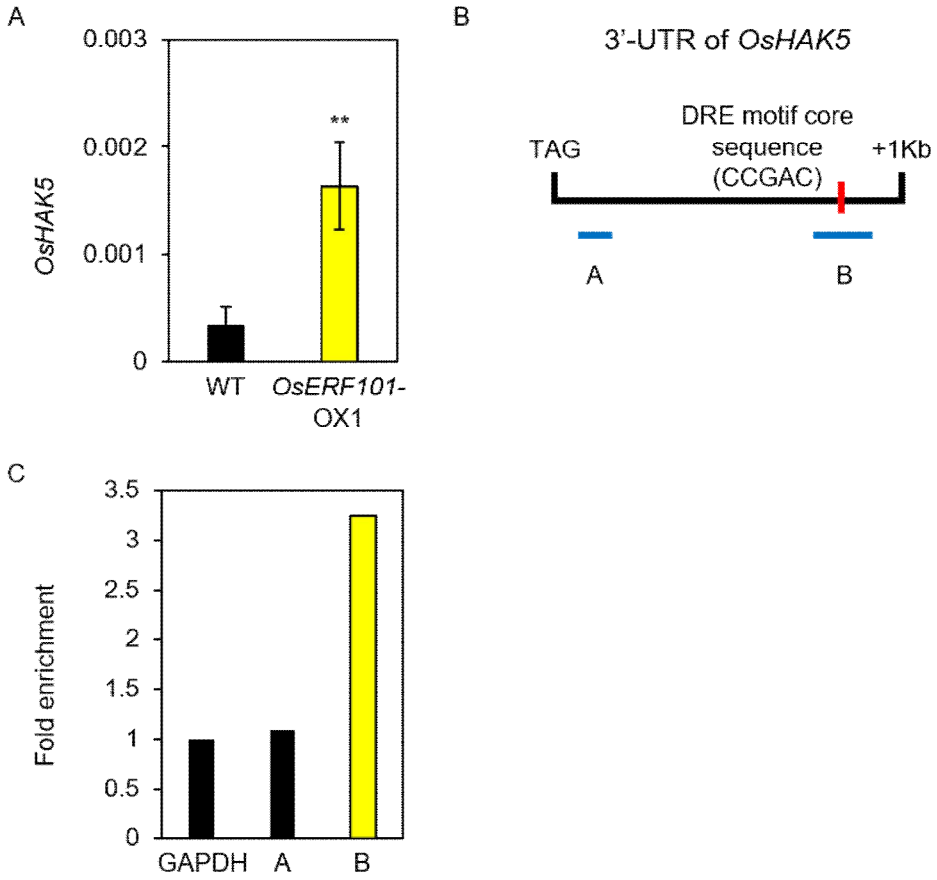

Figure 7. Possibility of direct regulation to *OsHAK5* of *OsERF101*

(A) Expression level of *OsHAK5* at shoot base part in WT and *OsERF101*-OX1. Three biological replicates were used (n = 3). Asterisks indicate a significant difference (Student's t-test, **P < 0.01). (B) Positions of fragments used for the ChIP assay (blue horizontal lines) in the 3'-UTR region of *OsHAK5*. Red bar indicates the position of the core sequence of DRE binding motif. (C) *OsERF101* binding affinity assays to the 3'-UTR region of *OsHAK5* in planta examined by ChIP assays. *OsERF101*-Myc was transiently expressed in protoplasts isolated from 10-day-old WT seedlings. Fold enrichment of the 3′-UTR fragments was measured by immunoprecipitation

with an anti-Myc antibody (see Methods). The *OsGAPDH* gene was used as a negative control.

Overexpression of *OsERF101* Increase Panicle Number and Grain Weight

Based on the results so far, we could presume that the overexpression of *OsERF101* increases yield by upregulating panicle number and grain weight. Thus, we examined several agronomic traits grown in the paddy field under NLD conditions. We evaluated the plant height, number of panicles per plant, 500-grain weight, panicle length. Unfortunately, we couldn't check exact yield. In these results, plant height of both *oserf101* and *OsERF101*-OX1, 2 were lower than WT (Figure 8A). But panicle number of *oserf101* and *OsERF101*-OX1, 2 were less and more than WT as expected (Figure 8B). In 500-grain weight, mutation of *OsERF101* decreases it and overexpression makes it increased (Figure 8C). In addition, panicle length of *oserf101* was reduced (Figure 8D). As these results, we can expect that overexpression of *OsERF101* makes yield increased.

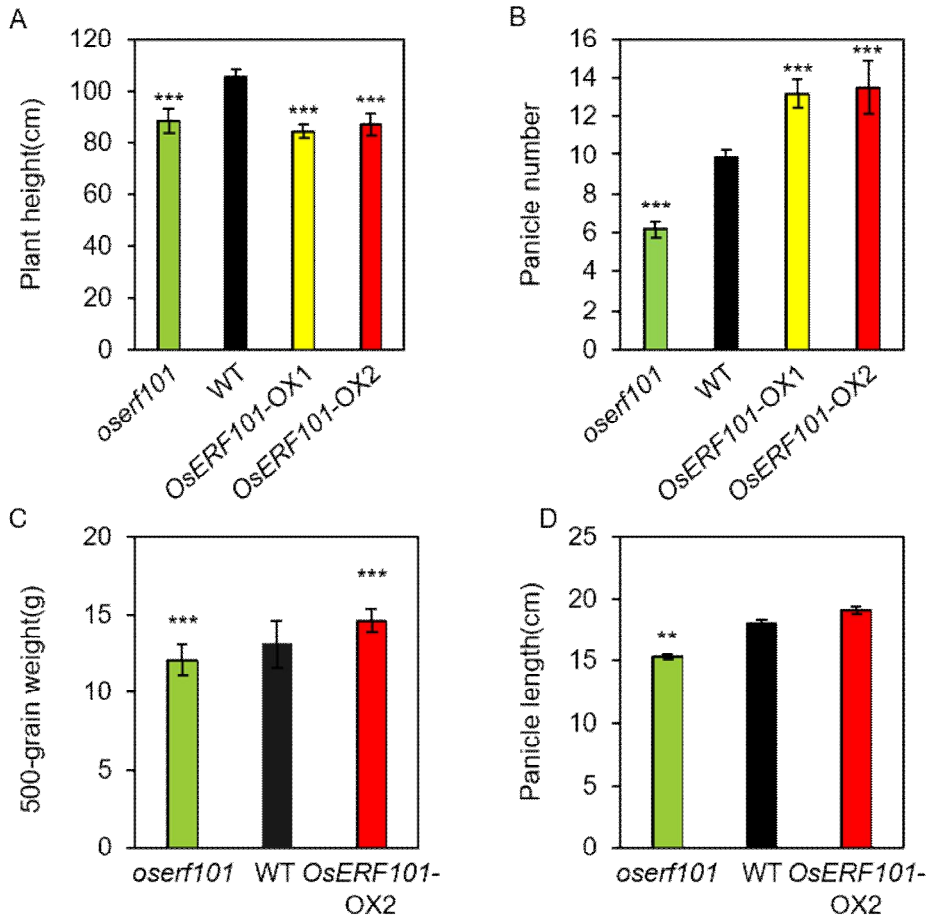

Figure 8 Agronomic traits measured in the *oserf101*, *OsERF101*-OX1, 2, WT Agronomic traits of *oserf101*, *OsERF101*-OX1, 2 were compared between with the WT plants after harvest in the autumn. (A) Plant height. Eighteen biological replicates were used (n = 18). (B) Panicle number per plant. Six biological replicates were used (n = 6). (C) 500-grain weight. Six biological replicates were used (n = 6). (D) Panicle length. Six biological replicates were used (n = 6). Asterisks indicate a significant difference (Student's t-test, **P < 0.01, ***P < 0.001).

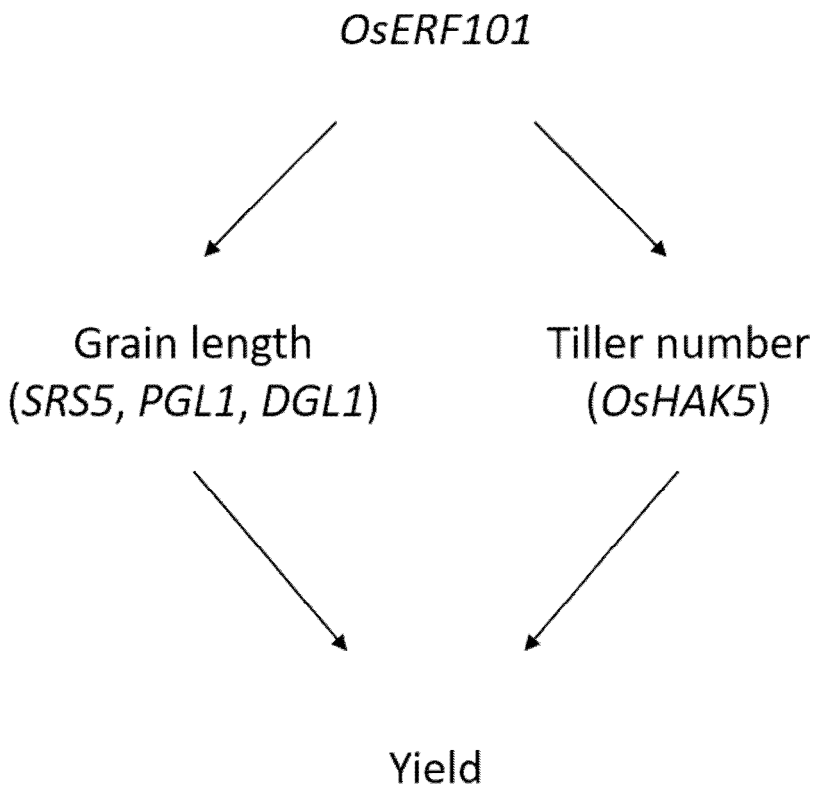

Figure 9. Tentative model of *OsERF101* roles in yield regulation
Arrows represent the tentative regulatory mechanism of *OsERF101*.

DISCUSSION

Grain size is critical determinant of grain yield, but the genetic and molecular mechanisms of grain size control in rice are still unclear. In this study, we identify *OsERF101* as a regulator of grain size. The *oserf101* mutant produced short grains in comparison to the wild type. By contrast, overexpression of *OsERF101* caused long grains. Thus, *OsERF101* is a positive regulator of grain size. Cellular analyses support that *OsERF101* controls grain size by regulating cell expansion. DEG analyses show that *OsERF101* affects *SRS5*, *PGL1*, *DGL1* which control grain length or cell size. These results suggest that *OsERF101* influences the grain size.

Tiller number is another element of yield. In this study, we also find that the *OsERF101* controls tiller number. *oserf101* showed less tiller number, and *OsERF101*-OX1, 2 produced more tiller. We tracked their growth, and we could find that tiller development were positively regulated by *OsERF101* in both mature and seedling stages. *OsHAK5*, rice potassium transporter, produce more tiller when it overexpressed, and it upregulated in *OsERF101*-OX1. In first try of ChIP assay of 3'-UTR of *OsHAK5*, *OsERF101* show the possibility of binding to 3'-UTR of *OsHAK5*.

Mutation of *OsERF101* caused low 500-grain weight, less panicle number, and short panicle. Overexpression of *OsERF101* made 500-grain weight and panicle number increased. Although we couldn't checked exact yield, these

data's suggest that *OsERF101* upregulate yield.

Thus, we can presume tentative model of *OsERF101* roles in yield regulation (Figure 10). *OsERF101* positively regulate grain length and tiller number by controlling related genes. These factors are elements which can improve yield. For further study, we need to verify these molecular mechanism by additional binding assay. And we must check exact yield of overexpression lines and mutant line in paddy field. Based on this study, the molecular and phenotypical facts about *OsERF101* could be used in molecular breeding for improving yield.

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초 록

본 연구에서 *OsERF101* 전사 인자가 종자 크기와 분얼수를 모두 조절한다는 것을 밝혀내었다. 종자 크기와 분얼의 수는 벼의 수확량을 결정하는 중요한 요인이다. 그러나 이 같은 표현형을 조절하는 유전자에 대해서는 아직 많은 연구가 필요하다. *OsERF101* 의 T-DNA 삽입 돌연변이체는 짧은 종자와 더 적은 분얼을 나타냈고 *OsERF101* 의 과발현체는 긴 종자와 많은 분얼수를 나타냈다. 종자 길이 조절 기작을 밝히기 위해 주사전자현미경을 이용하여 종자 표면을 촬영하였다. 촬영한 이미지를 분석한 결과, *OsERF101* 이 종자 표피 세포 크기를 조절하여 곡물 길이를 조절함을 알 수 있었다. 종 길이를 조절하는 분자적 기작을 파악하기 위해 우리는 발달 중인 이삭에서 RNA 를 추출하여 전사체 분석을 진행하였다. 분석 결과 돌연변이체에서 *SRS5*, *PGL1*, *DGL1* 의 발현량이 야생형보다 낮은 것을 확인하였다. 기존 연구에 따르면 *SRS5* 돌연변이체는 짧은 종자 표현형을 나타냈고, *PGL1* 과발현체는 더 긴 종자 표현형을 나타냈다. DGL1 돌연변이체는 종자 길이가 더 짧은 표현형이 보고되었다. 따라서 우리는 이 유전자들이 *OsERF101* 의 목표라고 가정할 수 있었다. 돌연변이체, 야생형 및 과발현체의 분얼수를 확인하였을 때 성장하면서 그 차이가 점점 더 커짐을 알 수 있었다. 개화 시기는 분얼 형성 시기의 지속 기간에 영향을 미칠 수 있으므로 개화 시기를 확인하였다. 그 결과 우리는 그들의 개화 시기가 크게 다르지 않다는 것을 발견할 수 있었다. 이 결과에서 *OsERF101* 이 분얼수를 조절한다고 가정할 수 있었다. *OsHAK5* 는 칼륨 수송 단백질을 암호화하는 유전자로 그 돌연변이체의 표현형과 *OsERF101* 돌연변이체의 분얼수 표현형이 유사했다. *OsHAK5* 의 발현량이 *OsERF101* 과발현체에서 증가했기 때문에 염색질 면역침강법을 시행한 결과 *OsERF101* 이 3' 비번역 부위에 결합하는 것으로 나타났다. *OsERF101* 돌연변이체와 과발현체의 농업 형질을 측정했을 때, 돌연변이체에서 이삭의 수, 종자 무게, 이삭의 길이가 감소했고, 과발현체에서 이삭의 수, 종자 무게가 증가하는 것을 알 수 있었다. 결론적으로, 우리의 결과는

*OsERF101*이 *SRS5*, *PGL1*, *DGL1* 및 *OsHAK5*의 발현을 조절함으로써 종자 길이와 분얼수를 조절하고 이를 통해 농업 형질에 영향을 준다는 점을 나타냈다. 이는 본 연구 결과가 향후 수확량 증가를 위한 분자적 육종에 활용될 수 있음을 시사한다.