



저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:



저작자표시. 귀하는 원저작자를 표시하여야 합니다.



비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.



변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 [이용허락규약\(Legal Code\)](#)을 이해하기 쉽게 요약한 것입니다.

[Disclaimer](#)

Master's Thesis of Science in Agriculture

**Overexpression of *OsNAC17* Enhances Drought
Tolerance in Rice**

벼 *OsNac17* 전사인자의 가뭄저항성 증진에 관한 연구

August 2017

Tae Hwan Kim

**Department of International Agricultural Technology
Graduate School of International Agricultural Technology
Seoul National University**

Overexpression of *OsNAC17* Enhances Drought Tolerance in Rice

A thesis

**submitted in partial fulfillment of the requirements to the faculty
of Graduate School of International Agricultural Technology
for the Degree of Master of Science in Agriculture**

By

Tae Hwan Kim

Supervised by

Prof. Ju-Kon Kim

**Major of International Agricultural Technology
Department of International Agricultural Technology
Graduate School of International Agricultural Technology
Seoul National University**

August, 2017

Approved as a qualified thesis for the Degree of Master of Science in Agriculture
by the committee members

Chairman Choonkyun Jung, Ph.D.

Member Jin-Ho Kang, Ph.D.

Member Ju-Kon Kim, Ph.D.

Abstract

Drought stress reduces crop production yields. Plant specific NAC transcription factors in rice are known to play an essential roles in stress resistance transcriptional regulation. However, It is still remains how each NAC genes involve transcriptional regulation in response to drought stress in rice. Here, we show that the rice (*Oryza stiva L japonica*) *NAM*, *AFTF* and *CUC* transcription factor *OsNAC17*, which is predominantly induced by abiotic stress in leaf, contributes to the drought tolerance in transgenic rice plants. We generate transgenic plants overexpressing *OsNAC17* using constitutive (*PGDI*) promoter. Ectopic overexpression of *OsNAC17* improved drought resistance phenotype at the vegetative stage. Agronomic traits such as grain yield, grain filling rate, and total grain weight improved by 22~64% over wild type plants under drought conditions during the reproductive stage. DEG profiling experiment identified 119 up-regulated genes by more than twofold ($P < 0.01$). Differentially expressed genes include *UDP-glycosyltransferase family protein, similar to 2-alkenal reductase (NADPH-dependent oxireductase)*, *similar to retinol dehydrogenase 12*, *Lipoxygenase*, and *NB-ARC domain containing protein* related in cell death. *OsNAC17* acts as a transcriptional activator in transcriptional activation assay, which has an activation domain in C-terminal region. Furthermore, it

was proved that *OsNAC17* is localized in the nucleus. These result suggest that the overexpression of *OsNAC17* improve drought tolerance by regulating ROS related enzymes and by reducing stomatal density.

.....
keywords : NAC transcription factors, drought tolerance, ROS,
transcriptional activation, Stomatal density

***Student Number* : 2015-22419**

Contents

Abstract	i
Contents	vi
List of Tables	viii
List of Figures	xii
Introduction	1
Materials and Methods	4
1. Vector construction and rice transformation	4
2. RNA isolation and real-time RT-PCR	5
3. Abiotic stress treatment	5
4. Drought tolerance assay	6
5. Characterization of agronomic traits	6
6. Subcellular localization	7
7. Transcriptional activation assay	7
8. Visualization of stomatal density	8
9. RNA sequencing and transcriptome analysis	8
Results	9
1. Structure and phylogenetic analysis of <i>OsNAC17</i>	9
2. <i>OsNAC17</i> is induced under abiotic stress	10
3. <i>OsNAC17</i> is a transcription factor that localized in nucleus and acts as a transcriptional activator	10
4. Overexpression of <i>OsNAC17</i> improves drought tolerance in vegetative stage	12
5. Constitutive overexpression of <i>OsNAC17</i> increases rice grain yield under	

field drought conditions	13
6. Identification of DEG involved in the <i>OsNAC17</i> -mediated drought tolerance pathway	14
7. Production of <i>OsNAC17</i> K/O lines using CRISPR/cas9 system	15
Discussion	42
References	46
Abstract in Korean	50
Acknowledgement	52

List of Tables

Table 1. . List of genes up-regulated(> 2 fold) in <i>PGD1:OsNAC17</i> plants ...	37
Table 2. List of primer sequences used in this study	40

List of Figures

Figure 1. NAC domain phylogenetic tree and protein sequence alignment and stress induced expression pattern of <i>OsNAC17</i>	17
Figure 2. Transcriptional activation assay of <i>OsNAC17</i>	20
Figure 3. Subcellular localization of <i>OsNAC17</i>	22
Figure 4. Enhanced tolerance of <i>PGD1:OsNAC17</i> plants against of drought stress	24
Figure 5. Agronomic traits of <i>PGD1:OsNAC17</i> plants grown in the field under both normal and drought conditions	26
Figure 6. Transcriptomic analysis of the differentially expressed genes in the <i>PGD1:OsNAC17</i> plants	28
Figure 8. Validation of the up-regulated genes in <i>PGD1:OsNAC17</i> plants ..	30
Figure 9. Vector constructions used in this research	33
Figure 10. Production of <i>Osnac17</i> k/o lines using CRISPR/cas9 system ..	35

Introduction

Water deficits in arable soils during cultivation are considered to profoundly affect the production yield of crops less than a maximum yield under normal condition. Adverse environmental stresses hampering plant development change the expression pattern of a variety of genes that trigger stress responsive transcriptional regulation in many plant species(Saibo, Lourenço et al. 2009). To overcome drought stress, plants being sessile activates stress responsive genes that regulate the expression of stress responsive genes by binding to specific *cis*-elements in promoter region through the plant specific drought-responsive transcription factors(Xiong, Schumaker et al. 2002, Bartels and Sunkar 2005). In plants, *NAC*, *AP2/ERF*, *bZIP* and *MYB* transcription factor families have been identified to enhance drought tolerance adaptive by using strategies, including developmental and physiochemical changes(Shinozaki, Yamaguchi-Shinozaki et al. 2003, Xiang, Tang et al. 2008, Jeong, Kim et al. 2010, Jung, Chung et al. 2017).

Plants response to abiotic stress have been widely studied in comprehension of the physiological and molecular mechanisms (Wang, Vinocur et al. 2003, Chaves, Flexas et al. 2009). Plants under drought condition experience a physiological moisture deficiency reducing photosynthesis and hinder plant cell growth(Griffin, Ranney et al. 2004, Wassmann, Jagadish et al. 2009). This result in reduction of chlorophyll concentration and total soluble proteins, with

reduction in photosynthetic primary products(Wassmann, Jagadish et al. 2009). In rice, water deficit condition around the reproductive stage may lead to reduce spikelet sterility under water deficit(O'toole and Namuco 1983) caused reduced grain yield. In perspectives of molecular mechanisms, the upstream regulatory networks under drought condition, including transcription factors, protein kinases, proteins phosphatases and other signaling molecules have been uncovered(Shinozaki, Yamaguchi-Shinozaki et al. 2003, Sreenivasulu, Sopory et al. 2007, Chaves, Flexas et al. 2009, Nakashima, Yamaguchi-Shinozaki et al. 2014). However, the details of the regulatory networks are still extensively unknown.

Increasing attention on the roles of transcription factor for adverse environmental stress has been focused on the function of plant specific *NAC* genes. *NAM*, *ATAF*, *CUC* transcription factor family is a plant specific transcription factor super family and is identified 151 non-redundant *NAC* genes in rice(Nuruzzaman, Manimekalai et al. 2010). *NAC* transcription factor members are highly conserved at the N-terminal *NAC* binding domain and have a highly variable C-terminal domain that plays a major role in the transcriptional regulator(Olsen, Ernst et al. 2005). Main function of *NAC* transcription factors are associated with biological processes, such as shoot apical meristem development, secondary wall formation, cell division, lateral root formation, and induction to a variety of biotic and abiotic stress (Xie, Frugis et al. 2000, Mitsuda, Seki et al. 2005, Hu, Dai et al. 2006, Kim, Kim et al. 2006, Nikovics, Blein et al. 2006, Jeong, Kim et al. 2010). Recently,

the stress-responsive *NAC TFs* was reported that overexpressed transgenic plants had improved tolerance to drought, high salinity in a variety of crop plants.

In this work, we report an analysis of the rice *NAC* domain family, *OsNAC17*, in their responses to stress and characterized them through physiological and morphological peculiarities. Transcriptional activation activity of *OsNAC17* was examined and transcriptional activation domains were analyzed. Their responses to drought stress were evaluated using transgenic rice during vegetative stage and reproductive stage. Constitutive *PGD1* promoters to generate overexpression of *OsNAC17*, respectively, facilitate the understanding on the effects of this gene on drought tolerance. *PGD1:OsNAC17* plants under drought condition improve grain yield. Furthermore, the transgenic rice overexpression of *OsNAC17* show improved tolerance to drought stress by protecting dehydration.

Materials and methods

1. Vector Construction and rice transformation

For the overexpression experiments, the full-length cDNAs of *OsNAC17* were amplified using *OsNAC17* primers (S1 Table) from cDNA of rice (*O. sativa* cv. Nakdong), before being cloned into the pENTR/D (Invitrogen, CA, USA). *OsNAC17* cDNAs were then inserted into a rice transformation vector containing the *PGD1* promoter using the Gateway system (Invitrogen, Carlsbad, CA, USA). For protein-DNA interaction experiment, the full-length cDNAs of *OsNAC17* eliminated stop codon were amplified using *OsNAC17* w/o stop codon primers (S1 Table). We introduced *OsNAC17* to pE3n and pE3c vector, before being cloned into the rice transformation vector containing the *GOS2* promoter using the Gateway system (Invitrogen, Carlsbad, CA, USA).

CRISPR/cas9 system was used to generate knock-out transgenic plants. The 5' end of the transcript containing a 20-bp target sequence (5'-CGAAGATGGGGGAGAAGGAG-3') was inserted to before gRNA scaffold that express using OsU3 promoter. The vector construction of *OsNAC17*-targeting CRISPR/Cas9 system is provided in Figure 9. All of the constructs was introduced into rice (*O. sativa* cv. *Nakdong*, and *Donjin*) by *Agrobacterium tumefaciens* (strain *LBA4404*)-mediated transformation, as previously described (Park, Bang et al. 2012).

2. RNA isolation and real-time PCR

Total RNA was isolated from rice plant with the RNeasy plant mini kit (Gene all) following the manufacturer's manual. First strand cDNA was synthesized from 3 μ g of total RNA using a RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Burlington, Canada). Real-time PCR analysis was performed using the EvaGreen™ Mix (SolGent, Deajeon, Korea) with a Mx3000P Real-Time PCR system (Stratagene, CA, USA), according to the manufacturer's manuals. *OsUbi1* gene (AK121590) was used as an internal control and three biological replicated were analyzed. Values are the means \pm SD (standard deviation) of three independent experiments. All primer sequences are listed in Table 2.

3. Abiotic stress treatment

To analyze the expression patterns of the *OsNAC17* genes subjected to low temperature, ABA, drought and high salinity, nontransgenic plants (*O. sativa* cv. *NACkdong*) were grown in soil for 2 weeks under normal condition. Abiotic stress adjustment treated under high salinity (200mM), drought, ABA treatment (100 μ M), and low temperature (4°C), based on *Sejune Oh et al.*, 2012. All the treatment transferred to the distilled water to stabilize mRNA level.

4. Drought tolerance assay

Four single copy *PGDI:OsNAC17*(#15, 16, 19, 23) T3 plant lines isolated by Taq-man probe PCR were grown in soil for 4 weeks with non-transgenic plants (*O. sativa* cv. *NACkdong*) under normal greenhouse condition. After 6 days of drought treatment, the plants were re-watered. Morphological symptoms were visualized by imaging *PGDI::OsNAC17* and NT plants at the indicated time points using a NEX-5N camera (Sony, Tokyo, Japan). Survival Rate were calculated based on arithmetic analysis (independent line survival number/ independent line on totally planted number X 100). For Jip test, drought conditions were treated for 12days and data collected over a time-course by measuring every day at 25 under dark condition.

5. Characterization of agronomic traits

To compare agronomic trait under normal condition, four independent T3 homozygous line of *PGDI:OsNAC17* with NT plants were planted in a rice paddy field at Kyoungpook National University, Gunwi, Korea in 2016. Three biological replicates using four different plots were designed and fifteen seedling of each independent line were transplanted into the plots at 25 days after sowing. To compare agronomic trait under drought condition, Independent plant were planted 15cm white pot in green house designed for drought treatment in flowering stage at Kyoungpook National University, Gunwi, Korea in 2016. Three biological replicates using four different plots

were designed and six seedling of each independent line were transplanted into the plots at 25 days after sowing.

6. Subcellular localization

For transient expression of *OsNAC17*-GFP in rice protoplasts, the predicted *OsNAC17* coding regions without the stop codon were cloned to the pHBT vector (GenBank accession No. EF090408) between the 35S promoter and the GFP coding sequencing using the BamH1 and Not1 restriction sites. All primer sequences are listed in Table S1. The constructs, *35S:OsNAC17-GFP* was transformed into rice protoplasts using PEG (polyethylene glycol)-mediated transformation. Isolation and PEG-mediated transformation were performed followed as previously described

7. Transcriptional activation assay

For transactivation assay, the open reading frame of *OsNAC17*, *OsNAC17ΔN*, *OsNAC17ΔC*, *OsNAC17ΔC263*, and *OsNAC17ΔC310* generated by PCR was fused in frame with the yeast *GAL4* DNA binding domain in the vector p*GBKT7*. p*GADT7-BD* used as a negative control by the Gateway Recombination Cloning method (Invitrogen). The fused gene was expressed in yeast strain *Y2H Gold* (Invitrogen). The transformed yeast strain was plated on SD/*Trp*- medium and cultured for 3 d, and colony-lift filter assay (X-gal assay) was performed as described by the manufacturer's manual (Invitrogen).

8. Visualization of stomatal density

Leaves of 1-month-old *PGDI:OsNAC17* and NT plants under drought or normal condition were detached and directly fixed by 2.5% glutaraldehyde (Jinjie Li et al., 2016). The stomatal pictures were obtained using a scanning electron microscopy, and the percentage of stomatal partially opening, opening and closing were calculated. The second fully expanded leaves were applied to measure stomatal imaging.

9. RNA sequencing and transcriptome analysis

For Rice RNA-*seq* analysis, whole plants from 2 week-old rice were collected from transgenic and non-transgenic plants under normal condition. Total RNA was isolated using the Plant RNeasy mini kit (Qiagen, Valencia, USA). The quality, quantity, and purity of the total RNA was assessed using a Thermo Scientific Nanodrop 2000 and an Agilent Bioanalyzer 2100. RNA-*seq* libraries were prepared using the mRNA-*seq* 4 samples prep kit (Illumina, San Diego, USA) according to the manufacture's protocol and sequenced (Macrogen, Seoul, Korea) using the Illumina HiSeq2000 (Illumina, San Diego, USA).

Result

1. Structure and phylogenetic analysis of *OsNAC17*

Plant-specific *NAC* transcription factors are 151 non redundant genes in rice, which is greater than that 117 members in Arabidopsis. Nuruzzaman, Manimekalai et al. (2010) suggests that *NAC* transcription factors can identify as two major groups and sixteen subgroups classified its known function and DND-binding domain. *OsNAC17* located in NAM/CUC3, Group B. Expression patterns with abiotic treatment have a difference as diverse subgroup. Up regulation of a variety of genes belonging to the NAM/CUC3, and *SNAC* subgroups was common in response to various abiotic stresses. The phylogenetic relationship of *OsNAC17*, NAM/CUC3 and *SNAC* was constructed based on full-length of ORF in silico using CRISTAL W program. The phylogenetic tree show that higher bootstrap values among the NAM/CUC3 (Figure 1. A). Phylogenic analysis of the amino acid sequences of the corresponding four NAM/CUC3 genes and two *SNAC* genes show highly conserved five subdomains and WVLCR conserved DNA-binding domain (figure 1. B). Interestingly, Sequence similarity on the members of each subgroup were found to be closely relate with response to stress. But *OsNAC17* with subgroup NAM/CUC3 have no research result for response to abiotic stress (Figure 1. B, C). It means that

studying the function of *OsNAC17* is worthwhile to elicit novel drought tolerance mechanism.

2. *OsNAC17* is induced under abiotic stress.

To enable the overexpression of the *OsNAC17* genes in rice, full-length of cDNA were isolated from *O. sativa* cv. *Nakdong*. We extracted RNAs from leaves of 14-d-old rice seedling that subjected to drought, high salinity, low temperature and ABA treatment. Overall induction a series of abiotic stress treatment was observed in leaf than root, but relative ABA level was induced similarly 10 times in whole plants (Figure. 1 C, D). The expression pattern especially, drought and high salinity was induced as time course in leaf (Figure. 1 D). This result indicates that *OsNAC17* is induced stress condition have putatively important role in leaf.

3. *OsNAC17* is a transcription factor that localized in nucleus and acts as a transcriptional activator.

Transcription factors are distinctively localized in nucleus where they work DNA binding and transcriptional regulation roles. A variety of transcriptional regulators do activation or repression through domain motif which do not share conserved motif as compared to the binding domains(Roberts 2000). Most of the studies have proven that *NAC* proteins function as transcriptional activators to trigger related gene expression(Duval, Hsieh et al. 2002, Zheng,

Chen et al. 2009). However, some of *NAC* transcription factors have transcription repression domain(Hao, Song et al. 2010).

To determine whether *OsNAC17* had transcriptional activator activity, the yeast strain Y2H Gold using the full length cDNA sequences of *OsNAC17* fused to the DNA-binding domain of GAL4 produced the same transactivation activity (Fig. 2 A). All yeast transformants grew well in SD/Trp- medium. On SD/Trp-A200- X- α -gal medium, transformants carrying *pGBKT7:OsNAC17*, *OsNAC17*, *OsNAC17 Δ N*, *OsNAC17 Δ C263*, and *OsNAC17 Δ C310* grow and showed β -galactosidase activity, whereas transformants carrying *pGBKT7:BD* empty vector and *OsNAC17 Δ C* did not. Serial deletion of *OsNAC17* in the C-terminal region from 1aa to 310aa did not affect the activation, but deletion of *OsNAC17* C-terminal region did (Fig. 2 B). These results indicate that the *OsNAC17* protein is a transcriptional activator and have a transcriptional activation domain in predicted C-terminus region from 310aa to 358aa. We next determined the subcellular localization of *OsNAC17* using a rice protoplast transient expression system (Figure 3. A). We transformed *OsNAC17-GFP* construct under the control of the cauliflower mosaic virus (*CaMV*) 35S promoter into rice protoplast cells using PEG-mediated method. A green fluorescence signal of transformed cells carrying *OsNAC17-GFP* was observed in the nuclei, while the control *OsNF-YA7-mCherry* protein was located in the nucleus (Figure 3. B). This result demonstrates that *OsNAC17* is transcription factor localized in nucleus.

4. Overexpression of *OsNAC17* improves drought tolerance in vegetative stage

To test the impact of *OsNAC17* overexpression on drought tolerance, the full length of cDNA of *OsNAC17* under *PGDI* constitutive gene promoter was inserted into japonica cultivar *Nakdong* (SH Park et al, 2012). Of four single copies, independent T3 transgenic plants were selected in drought tolerance assay at vegetative stage. *OsNAC17* expression in whole plants was clearly overexpressed in all the transgenic lines but not in the NT control (Figure 4. B). For visualization whether the *PGDI:OsNAC17* improve drought stress at vegetative stage, 1-month-old transgenic plants and NT controls were treated under drought stress for six days (Figure 4. A). The NT plants started drying visual symptom can recognize of drought-induced damage, such as leaf rolling and wilting. After 7days, Plants was re-watering since soil moisture contents were similar both transgenic and NT (Figure 4. D). Even though the transgenic plants and NT plants has a drought-induced damage, the transgenic plant recovered more quickly than the NT plants. After re-watering stage, number of survived plants were calculated relatively. All transgenic line was recovered over 80 % but NT was recovered only 18 % (Figure 4. C). The To further verify this stress tolerance phenotype, chlorophyll fluorescence measurement measured by the *Fv/Fm* values of the

two transgenic and NT control plants, all at the vegetative stage for 9 days (Figure 4. E). F_v/F_m is calculated maximum fluorescence by minimum fluorescence. Two transgenic plants were also higher in both compared with the NT controls. These results indicate that the overexpression of *OsNAC17* in transgenic rice primarily increases their tolerance to drought condition.

5. Constitutive overexpression of *OsNAC17* increases rice grain yield under field drought conditions

We measured the agronomic traits of the transgenic and non-transgenic plants under normal and field drought conditions a cultivating seasons (2016). Four independent T4 lines of *PGDI:OsNAC17* plants were transplanted to a paddy field and grown to maturity. 45 plants per transgenic line from three replicates scored yield component. The grain yield of the *PGDI:OsNAC17* plants remained similar to that of the *NT* controls under normal field conditions. This result indicates that overexpression line did not affect to pleiotropism in transgenic rice. In the *PGDI:OsNAC17* plants under drought conditions in filed trial, however, total grain weight was increased by 6% to 19% compared with the *NT* controls, which was due to increased numbers of filled grains and column length (Figure 5). These morphological changes prompted us to assess the yield matters of the *PGDI:OsNAC17* plants grown under drought condition at the reproductive stage.

6. Identification of DEG involved in the *OsNAC17*-mediated drought tolerance pathway

RNA-sequencing analysis was performed to further give an account the roles of *OsNAC17* on the improved drought tolerance. As a transcription factor, *OsNAC17* may regulate the transcription of downstream genes in response to drought stress. We performed an RNA-sequencing transcriptome analysis of 2-week-old non-transgenic plants and homozygous T3 *PGD1::OsNAC17* to identify downstream genes which are regulated by *OsNAC17*. Among them, 117 genes were significantly upregulated (Fold changes > 2 ; P<0.001) in transgenic plants (Figure 7A). We then classified gene ontology using PANTHER (<http://pantherdb.org/>) *in silico* analysis. Gene ontology analysis for up-regulated genes showed higher expression in response to stress and metabolic process while evenly regulate in diverse process (Figure 7. B). We hypothesized that these transcriptomic changes means that primary target genes-mediated *OsNAC17* may contribute to the response to stress. These genes were enriched in a number of stress-related genes including programmed cell death such as NB-ARC domain containing protein, and ROS-scavenging enzyme such as NAD(P)-binding domain containing protein, UDP-gulucoronosyl, and Similar to retinol dehydrogenase 12 and lipoxygenase (Table 1.).

There were 22 genes related to stress responsive genes and metabolic process-involving 17 genes were significantly upregulated (Fold changes > 2; P< 0.001) in *PGD1::OsNAC17*. Most of these genes contain *NAC* recognition core sequence(CACG) in their promoter region, suggesting that they could be direct target genes. We selected eleven candidate genes, which might involve *OsNAC17*-mediated drought tolerance, such as similar to phenylalanine ammonia-lyase, Chitinase7, similar to PDR-type ABC transporter2, lipoxygenase, NB-ARC domain containing protein, Ankyrin repeat domain containing protein, UDP-glycosyltransferase family protein, Concavalin A-like lectin.gluconase, similar to retinol dehydrogenase 12, similar to 2-alkenal reductase and transferase family protein. Those candidate genes were performed qRT-PCR for validation (Figure 8). The results were consistent with the RNA-sequencing results, demonstrated that the transcription of these genes may be positively regulated by *OsNAC17*. These data suggest that the overexpression of many stress-responsive genes was increased to enhance the drought tolerance of the transgenic plants.

7. Production of *Osnac17* k/o lines using CRISPR/cas9 system

Recent advances in genome editing technologies, Type 2 clustered, regularly interspaced, short palindromic repeats (CRISPR), disrupt to endogenous target gene using single nuclease, a CRISPR-associated protein Cas9(Shan, Wang et al. 2013). Cas9 can form a complex with single-guide RNA (sgRNA)

which guides Cas9 to cleave target DNA. gRNA protospacer was substituted with *OsNAC17* protospacer located in 1st exon. To confirm whether CRISPR:Cas9 induce genome editing in target DNA site, Genomic DNA from leaf of agrobacterium-mediated 32 transgenic T0 plants were extracted using Geneall kit. Then, PCR and sequencing assay on target site were carried out. 12 plants used to agrobacterium-mediated transformation have a genetic changes induced by CRISPR/Cas9 in rice (Figure 10. A). Tendency of deletion each transgenic showed 3 sequence deletion which is translated to *asparagine* and 1 sequence deletion which can break *OsNAC17* protein structure (Figure 10. B). These data demonstrate that targeted genome editing technology using CRISPR/Cas9 system in rice make us to enable basic biotechnological research.

Figure 1. NAC domain Phylogenetic tree and protein sequence alignment and stress induced expression pattern of *OsNAC17*

A. Phylogenetic tree of highly conserved NAC transcription factors in arabidopsis and rice. Blue circular box ; SNAC family, blue circular box ; NAM/CUC1 family B. Protein alignment of NAC protein sequences. Analysis for conserved NAC proteins were aligned using the ClustalW program. C. Quantitative RT-PCR analysis of *OsNAC17* under high salinity (200mM), drought, ABA treatment (100μM), and low temperature (4°C). NT plants were grown in soil for 2 weeks in leaf D. Quantitative RT-PCR analysis of *OsNAC17* under high salinity (200mM), drought, ABA treatment (100μM), and low temperature (4°C). NT plants were grown in soil for 2 weeks in root

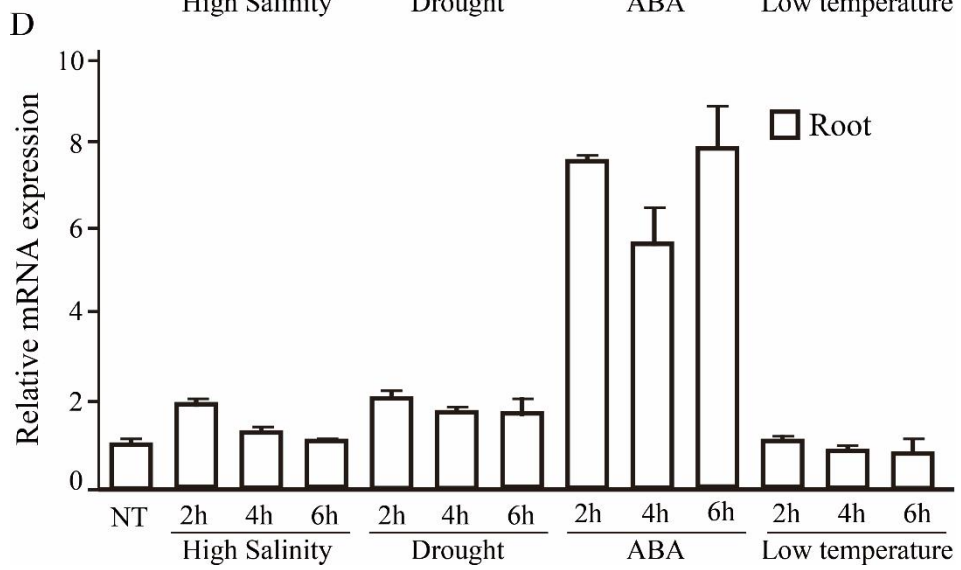
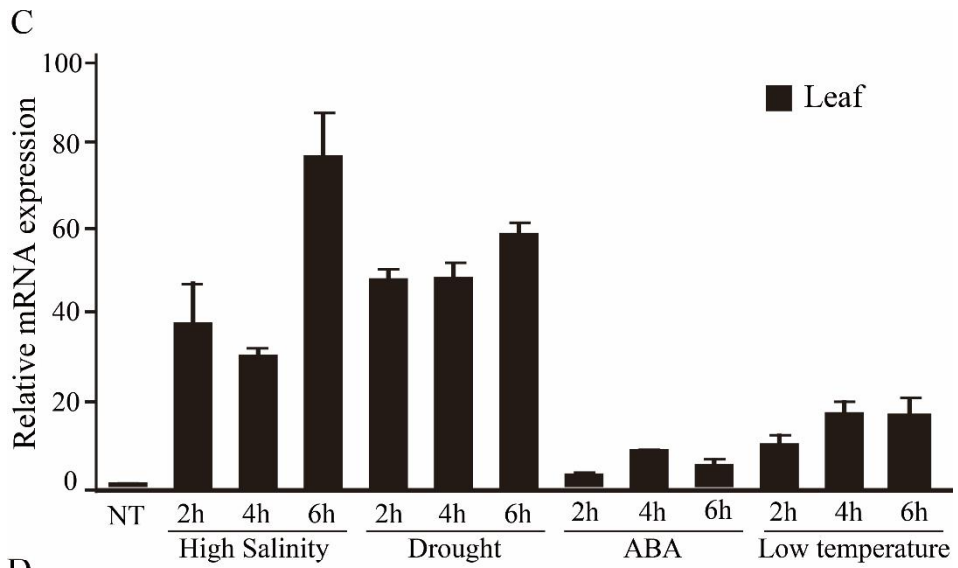
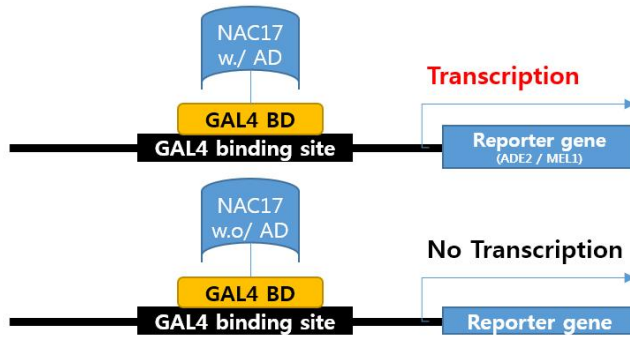


Figure 2. Transcriptional activation assay of *OsNAC17*

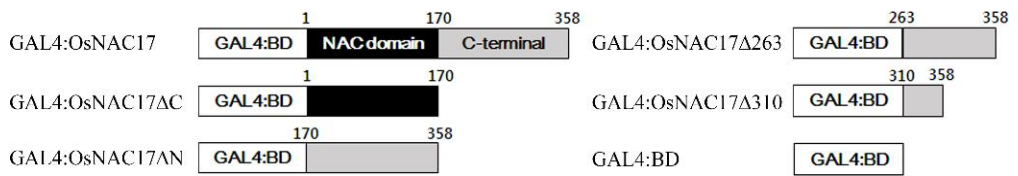
A. Principle of transcriptional activation assay using yeast two hybrid system.

B. Schematic structure on the serial deletion of *OsNAC17*. Yeast Y2H Gold cells carrying *GAL4:OsNAC17*, *GAC4:OsNAC17ΔC*, *GAL4:OsNAC17ΔN*, *GAL4:OsNAC17ΔC263*, *GAL4:OsNAC17ΔC310*, and *pGBKT7*(as a negative control) were transformed. C. Yeast Y2H Gold cells carrying serial deletions were streaked on *SD/Trp⁻* plates (*left*), *SD/Trp⁻A200-* plates supplemented with x-α-gal (*middle*) for 3 days at 28°C.

A



B



C

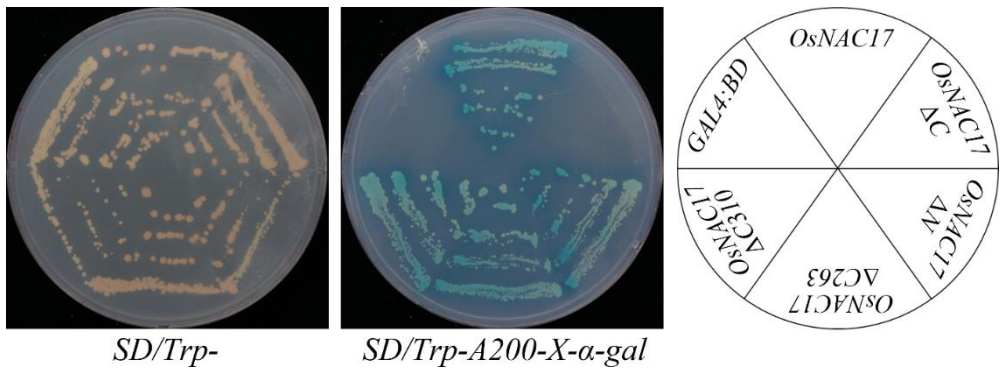
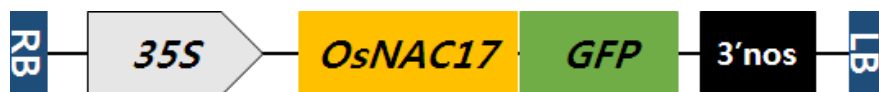


Figure 3. Subcellular localization of OsNAC17

A. Schematic structure of the *OsNAC17-GFP* fusion to the pHBT cloning vector (GenBank accession No. EF090408). RB, right border., 35S, Califlower mosaic virus 35S constitutive promoter., nos, terminator. B. Confocal images of *OsNAC17-GFP* in rice protoplast with *OsNF-YA7-mCherry* protein(nucleus targeting proteins). Scale bars, 10 μ m

A



B

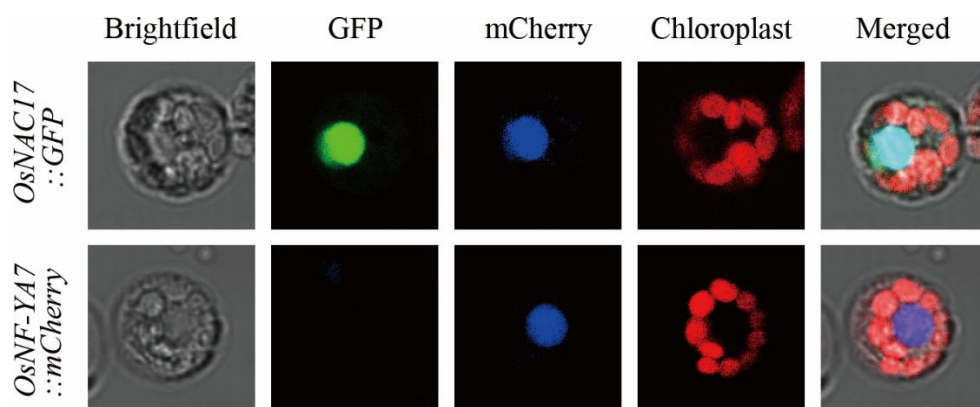


Figure 4. Enhanced tolerance of *PGD1:OsNAC17* plants against of drought stress

A. The appearance of transgenic plants during drought stress. Four independent homozygous T4 lines of *PGD1:OsNAC17* plants and NT controls were grown for a month, subjected to 6 d of drought stress, and followed by 7d of re-watering in the greenhouse. B. Quantitative RT-PCR of *OsNAC17* in leaves and roots for relative overexpression. C. Survival rate for drought stress treatment. After re-watering, Number of survived plants in both the transgenic and NT plants were calculated relatively. D. Soil-moisture contents for drought stress treatment. Soil-moisture were measured using Moisture Content BF 202, Japan E. Jip-tests of *PGD1:OsNAC17* plants. Chlorophyll fluorescence (F_v/F_m) of two-month-old plants exposed to drought stress was measured in the dark chamber. Each data point represents the mean $n \pm SD$ of triplicate experiments ($n = 20$)

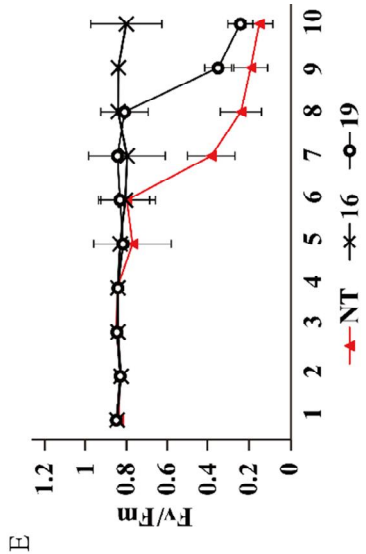
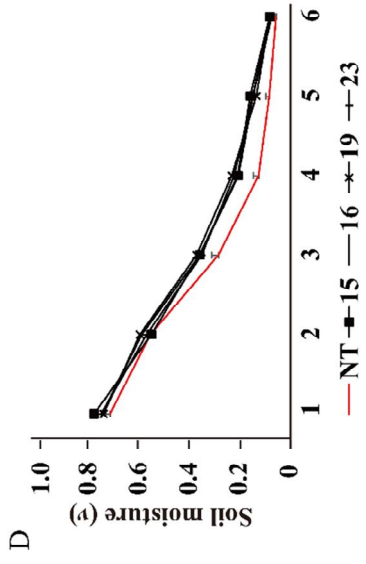
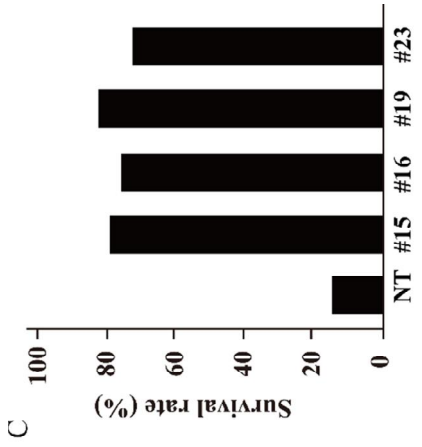
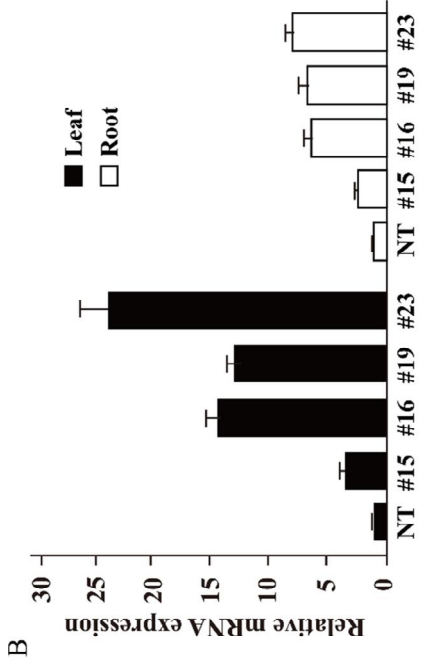
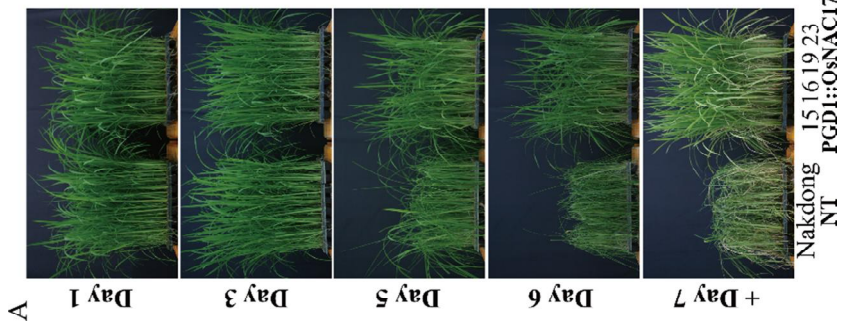
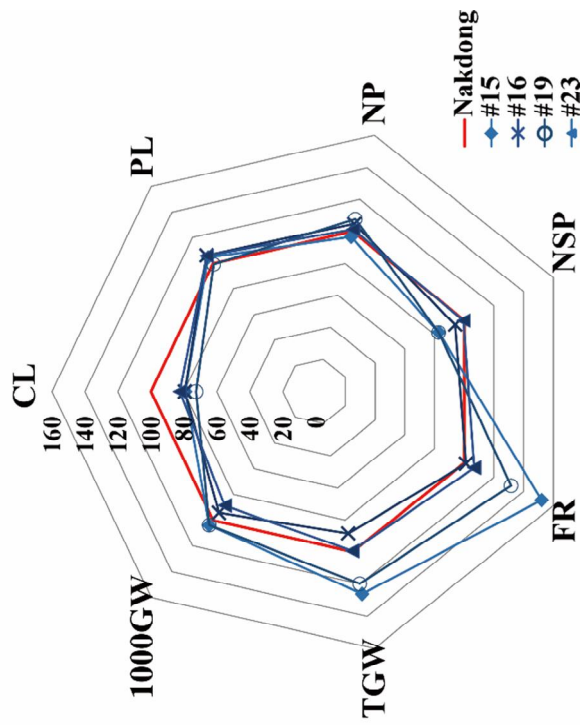


Figure 5. Agronomic traits of *PGD1:OsNAC17* plants grown in the field under both normal and drought conditions.

Each plot of the agronomic traits of four independent T3 lines of *PGD1:OsNAC17* plants and corresponding NT controls under both normal(A) and drought conditions(B) were drawn using Microsoft Excel.

n_≥18 for each line (CL, Colum length; PL, Panicle length; NP, Number of panicles; NSP, Number of spikelet per panicle; FR, Filling rate; TGW, Total grain weight; 1000GW, 1000 grain weight

Drought



Normal

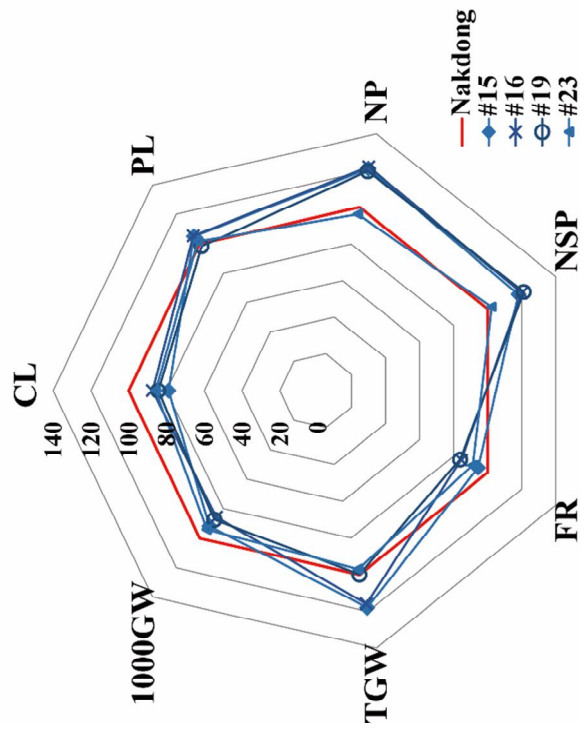
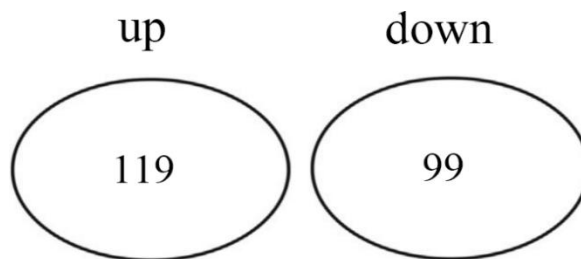


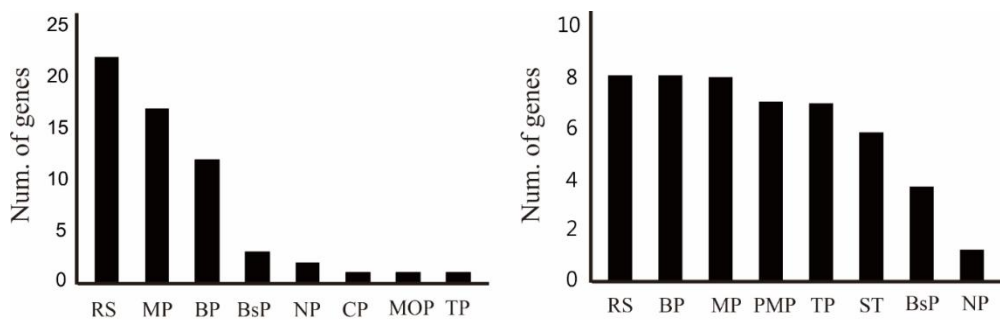
Figure 6. Transcriptomic analysis of the differentially expressed genes in the *PGDI:OsNAC17* plants

A. Venn diagrams of *OsNAC17*-regulated genes. Up-(fold change > 2) and down-regulated genes (fold change > 2) in two-week-old *PGDI::OsNAC17* transgenic plants compared to NT plants. B. Gene ontology analysis on the up- and down-regulated genes classified with biological process.

A



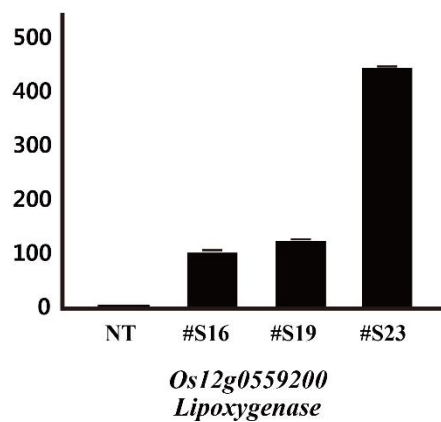
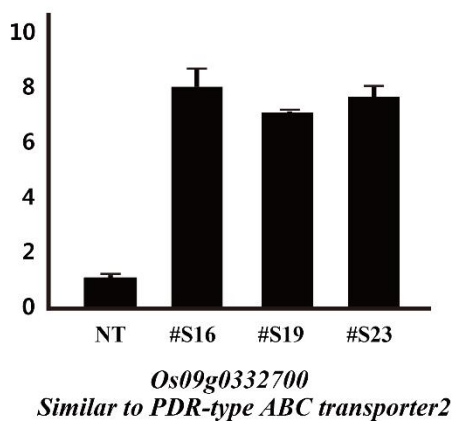
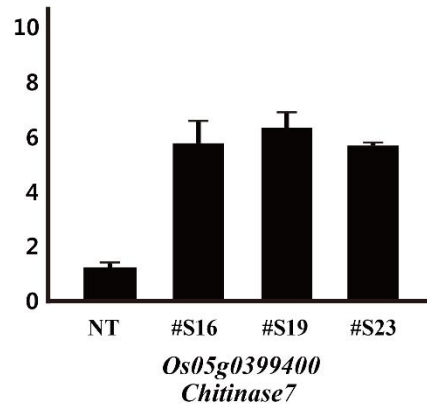
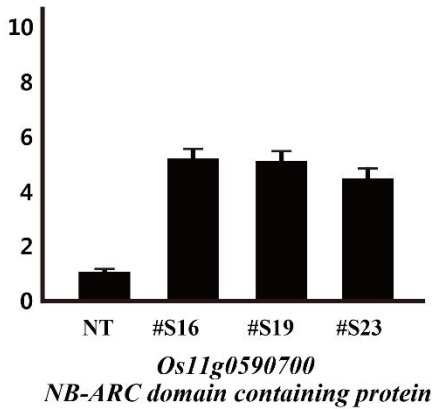
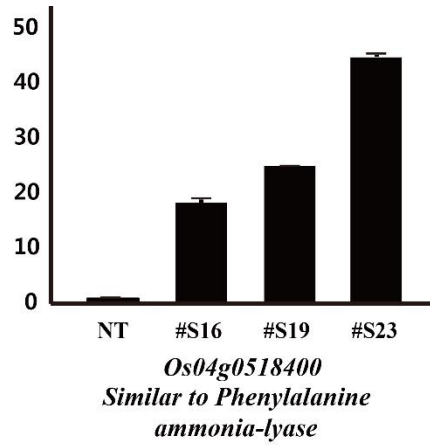
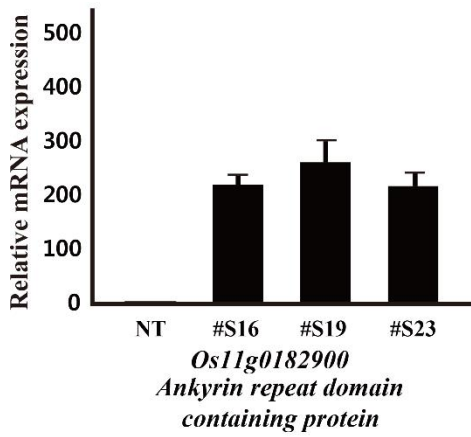
B

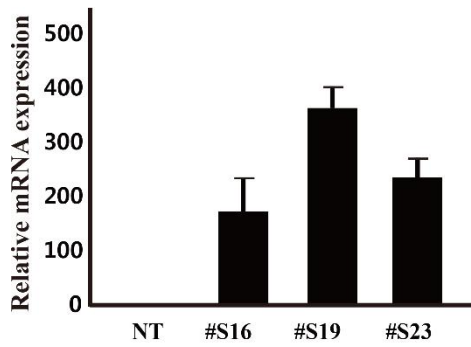


RS : response to stress, MP : metabolic process, BP : Biological process, BsP : Biosynthetic Process, NP : Nucleic acid metabolic process, CP : Catabolic process, MOP : Multicellular organismal process, TP : Transport, PMP : Protein modification process

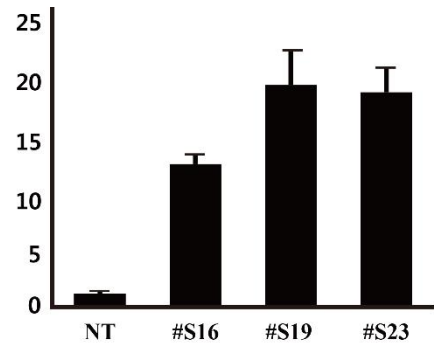
Figure 8. Validation of the up-regulated genes in *PGD1:OsNAC17* plants

Validation of *OsNAC17*-mediated stress responsive genes. Quantitative RT-PCR of *OsNAC17*-mediated drought regulatory genes in two-week-old NT and *PGD1::OsNAC17* whole plants. *Ubiquitin1* was used as an internal control. Data are shown as the mean \pm SE of three biological replicates.

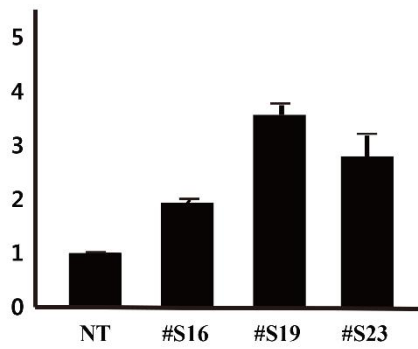




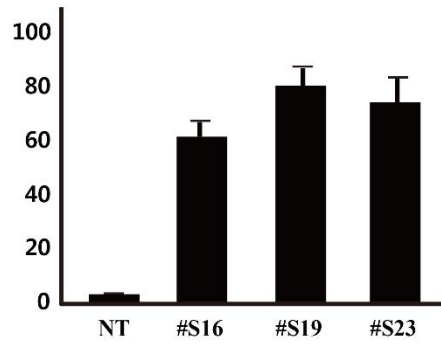
Os08g0124100
Concavalin A-like lectin/glucanase



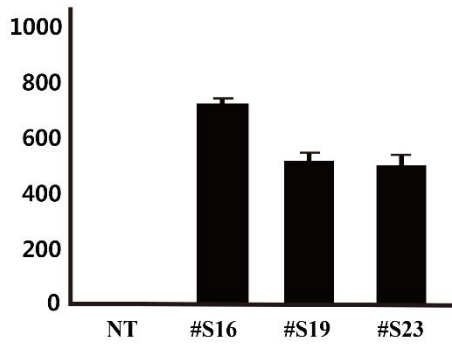
Os07g00490100
UDP-glycosyltransferase family protein



Os11g0181700
Similar to retinol dehydrogenase 12



Os12g0226400
Similar to 2-alkenal reductase

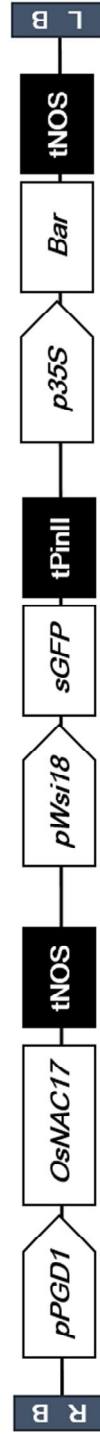


Os12g0458100
Transferase family protein

Figure 9. Vector construction used in this research and for further study

A. Overexpression of *OsNAC17* under the *PGD1* promoters driving constitutive expression in whole plant body. B. Knockout of *OsNAC17* construction using CRISPR/Cas9 system. C. Translational fusion of myc gene to confirm the protein-DNA interaction using chromatin immunoprecipitation assay. (*pGOS2*; 'Go/G1 Switch2' constitutive promoter, *pPGD1*; 'Phosphogluconate dehydrogenase 1' constitutive promoter, *p35S*; 'CMV 35S' constitutive promoter, *pWis18*; 'water stress inducible 18' promoter, tNOS; 'nopaline synthase' terminator, tPINII; terminatr

A. *pPGD1::OsNAC17*



B. *CRISPR/Cas9 Osnac17*



C. *pGOS2::OsNAC17_myc*

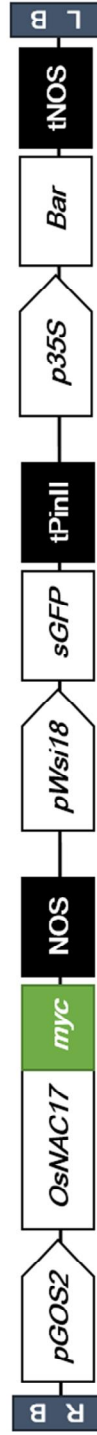
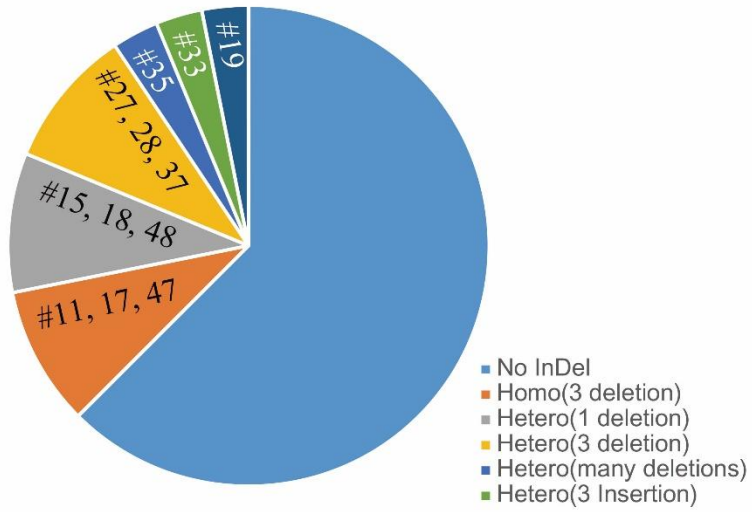


Figure 10. Production of *Osnac17* k/o lines using CRISPR/cas9 system

A. Pie graph of KO_*nac17* To plants classified by genotype homo- and hetero using PCR and Sequencing assay. B. Sequencing pattern for CRISPR/Cas9 mediated InDel mutagenesis

A



B

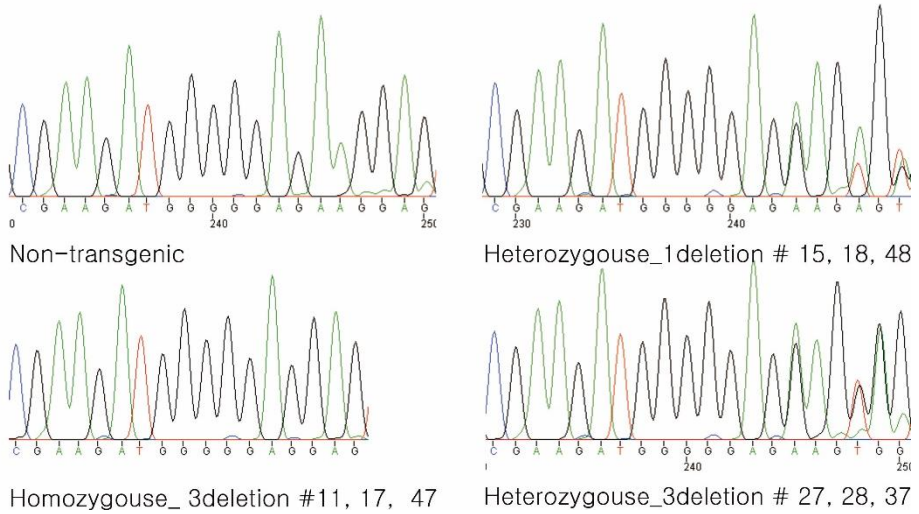


Table 1. . List of genes up-regulated (> 2 fold) in *PGD1:OsNAC17* plants

Gene ID	Description	log	P-value
Response to stress			
Os11g0558500	Ribosome-inactivating protein domain containing protein.	4.2	0.0087
Os11g0615700	Proteasome subunit alpha type 5	2.8	0.031
Os06g0286700	Similar to Piz-t.	2.5	0.0237
Os05g0399400	Chitinase 9.	2.7	0.0375
Os09g0357400	Disease resistance protein domain containing protein.	2.5	0.0116
Os11g0691100	Similar to Protein kinase.	75.4	0.0009
Os11g0694100	Similar to Protein kinase domain containing protein, expressed.	3.2	0.0089
Os11g0213000	Similar to Protein kinase domain containing protein, expressed.	2.4	0.0432
Os12g0458100	Transferase family protein.	33.7	0.037
Os01g0607100	Bifunctional inhibitor/plant lipid transfer protein	3.9	0.0172
Os11g0182900	Ankyrin repeat domain containing protein.	42.9	0.012
Programmed cell death			
Os08g0260800	Similar to NB-ARC domain containing protein.	2.4	0.0213
Os11g0590400	Similar to NB-ARC domain containing protein.	3.9	0.0268
Os11g0590700	NB-ARC domain containing protein.	6.3	0.0064
Os11g0606900	Similar to NB-ARC domain containing protein, expressed.	3.3	0.0052
Os11g0673600	Similar to NB-ARC domain containing protein, expressed.	2.1	0.0437
Os11g0673900	Similar to NB-ARC domain containing protein.	3.8	0.0101
Os11g0676800	Similar to NB-ARC domain containing protein.	2.4	0.0175
Os11g0686500	NB-ARC domain containing protein.	3.1	0.0058
Os12g0281600	NB-ARC domain containing protein.	3.1	0.0043
Reactive Oxygen Species biosynthesis			
Os04g0107200	NAD(P)-binding domain containing protein.	2.1	0.0116
Os11g0181700	Similar to retinol dehydrogenase 12.	2.6	0.0075
Os02g0100100	Thioredoxin domain domain containing protein.	2.3	0.0412
Os11g0186900	Similar to 1-aminocyclopropane-1-carboxylate oxidase	2.1	0.0404
Os12g0226400	Similar to 2-alkenal reductase.	4.4	0.0049
Os12g0183100	Similar to Branched-chain alpha keto-acid dehydrogenase.	2.1	0.0302
Os06g0560000	Ferroportin1 family protein.	2.3	0.0211

Os04g0518400	Similar to Phenylalanine ammonia-lyase.	5.3	0.0132
Os07g0655201	Similar to BLE2 protein.	4.0	0.0118
Os06g0230100	Similar to Cellulose synthase-like A1.	3.8	
Os12g0559200	Lipoxygenase (EC 1.13.11.12).	12.3	0.0302
Os12g0559934	Similar to Lipoxygenase.	10.5	0.021
ABA-mediated stress response			
Os01g0192900	1-aminocyclopropane-1-carboxylate synthase family protein.	2.6	0.011
Os01g0901600	Similar to 4-coumarate--CoA ligase-like 6.	2.5	0.0253
Os09g0332700	Similar to PDR-type ABC transporter 2.	4.2	0.0015
Soluble sugar pathway			
Os07g0490100	UDP-glucuronosyl/UDP-glucosyltransferase family protein.	4.0	0.0184
Os08g0124100	Concanavalin A-like lectin/glucanase	6.1	0.0126
Os04g0131850	Similar to UDP-glucose:sterol glucosyltransferase.	2.8	0.0074
Os07g0421300	Similar to Alpha glucosidase-like protein.	2.0	0.0138

Table 2. List of primer sequences used in this study

PCR primers	5' to 3'	Strand
OsNAC17:GFP	CGGGATCC ATGTCTGAGGTGTCCGT	Forward
OsNAC17:GFP	TATAGCGGCC GTTCCAGAAGTCGTCG	Reverse
Crispr:OsNAC17	TATGGCCGAGGTGGAGGAGACCACGGATCATCTGCA	Forward
Crispr:OsNAC17	TCTCCTCCACCTCGGCCATAGTTTATAGAGCTAGAAATAGC	Reverse
OsNAC17 Promoter	CACCCACTCCTGAAGTCCTACCAAG	Forward
OsNAC17 Promoter	TGGGAACGAGAGAATGGACTTGG	Reverse
OsNAC17qRT- PCR	GGCGAAGATGGGGGAGAA	Forward
OsNAC17qRT- PCR	CTGATGGACCCTTCCTTC	Reverse
myc:OsNAC17	CGGGATCC ATGTCTGAGGTGTCCGT	Forward
myc:OsNAC17	TATAGCGGCCGCGTTCCAGAAGTC TCGAGGCCCA	Reverse
Os11g0181700	CTGCAGTTCGCAACGAATCA	Forward
Os11g0181700	ACCATCACTTCTCTGTGCG	Reverse
Os12g0226400	TCTGGGCACCTGGATTTCAA	Forward
Os12g0226400	ACCACATGCCAGAACACCAG	Reverse
Os04g0518400	CTCCCACCGGAGGACCAA	Forward
Os04g0518400	AGCAGAGTGTTGATGCGGAC	Reverse
Os08g0124100	CGTCGAGTACGACAACGTCT	Forward
Os08g0124100	GAGAAGCCAACGGAGACCAG	Reverse
Os11g0182900	ACGTGGGCATCATCGTTTTG	Forward
Os11g0182900	AGTTGTAGATGGAAGCGACGG	Reverse
Os11g0590400	CCCAACCGTCCAGTCATTC	Forward
Os11g0590400	AGCAAGGCTGGTTGGTTTCT	Reverse
Os11g0590700	CTATCGTACAGCATCGAGGA	Forward
Os11g0590700	AGTCCAAGATACGAGGGTC	Reverse
Os07g0490100	GTGAGCTGCCGTTCCAC	Forward
Os07g0490100	CCAGCATTGTCAGCTTGGG	Reverse
Os12g0458100	GACTAAGGCGTCATGTTCC	Forward
Os12g0458100	CCCACGTGCATTCACAACAA	Reverse
Os09g0332700	GTGTTCTACAGGGAACGAGCA	Forward
Os09g0332700	GGCAGCTGTCCACTCAAATC	Reverse

Discussion

1. *OsNAC17* is a stress-inducible NAC domain transcription factor

NAC transcription factor play an important roles in many regulatory and developmental processes. They have been extensively investigated in *Arabidopsis* and rice. In this study, sequence alignment showed that *OsNAC17* contains a highly conserved five *NAC* subdomain located in the N-terminal region (Figure 1. A). Subgroup E could be involved in DNA binding for their promoter region be subjected to transcriptional regulation. Subcellular localization analysis revealed that *OsNAC17* localized in the nucleus (Figure 3. B), and transcriptional activation assay explain that *OsNAC17* act as a transcriptional activator having activation domain in C-terminal region (Figure 2. B) These results are consistent with the previous research on the other *NAC* genes functions(Lu, Ying et al. 2012), but another genes interacting with *miR164* (Nikovics, Blein et al. 2006).

Expression pattern analysis showed that the *OsNAC17* gene was highly induced by drought, high salinity and exogenous Abscic acid in leaf. (Figure 1. A). This results are consistent with Jeong, Kim et al. (2010) classified by stress inducible *NAC* transcription factors using microarray research. For spatio-temporal visualization, we fused *OsNAC17-GUS*, *OsNAC17-GFP* to 35S constitutive promoter. In histochemical GUS assay will be done as transgenic plants is produced. Taken together, these analysis combined with

our experimental results, significantly indicating that *OsNAC17* is a stress-responsive *NAC* transcription factor, and might play an important role in response to various environmental stresses,

2. *OsNAC17* confers drought tolerance of transgenic rice

The prior to expression pattern under drought stress in non-transgenic plants imply that *OsNAC17* gene may play an important role in the transcriptional regulation of plant response to drought stress. To clarify the function of *OsNAC17* in drought stress during whole life time, we generate transgenic plant, *PGD1:: OsNAC17* constitutively overexpressing in rice, and performed drought tolerance experiment. The transgenic plants showed significantly improved drought tolerance compared with WT plants (Figure 4). The stomatal density relative water loss in leaf reduced slightly. Although, These data provided evidence that *OsNAC17* can confer the drought tolerance, abiotic stress regulating mechanisms is still missing. To clarify the transcriptionally regulating mechanisms, we generate knock out plant and myc-tagging line for further study (Figure 9). Because of somatical variation, we will reproduce until T3 generation. The plants control the abiotic stresses through ABA-dependent and ABA-independent pathways (Agarwal and Jha 2010). Our data for stress inducible expression pattern was slightly induced to exogenous ABA compared with other abiotic stresses (Figure 1 C, D). Our data demonstrated that *OsNAC17* have a potential to regulate through the

ABA-independent pathway. For another, we evaluate agronomic traits transgenic plants compared with WT. Grain yield under water deficit condition at reproductive stage increased compared with WT. These results, combined with water deficit field trials, led us to prove that *OsNAC17* confer drought tolerance in ABA-independent signaling pathway in response to abiotic stresses.

3. *OsNAC17* regulates stress-responsive gene expression

As a transcription factor, *OsNAC17* regulate the downstream genes in response to drought stress. Our RNA sequencing data demonstrated that overexpression of *OsNAC17* could change the relative expression level its downstream 119 genes were significantly up-regulated in transgenic plants. Up-regulated genes enrich to response to abiotic or biotic. NB-ARC proteins involving programmed cell death was significantly up-regulated. Reactive oxygen species mediated abiotic stress induce programmed cell death, necrosis, and adaptation to stress(Petrov, Hille et al. 2015). Also, genes related to ROS was significantly up- and down- regulated such as NAD(P)-binding domain containing protein, UDP-glycosyltransferase. These data provide hypothesis that *OsNAC17* can enhance drought tolerance by promoting reactive oxygen species and induce programmed cell death. In addition to, Gens related to biotic stress, such as Piz-t, Typical p-type R2, Ankyrin repeat domain was up-regulated(Maverakis, Kim et al. 2015) (Table

1). To prove this hypothesis, we will compare with knock out transgenic plant, OX and WT. *NAC* TFs mainly recognize the consensus *cis*-acting *NAC* recognition sequence ‘CACG’ in their promoters, suggesting that downstream gene might be direct target genes of *OsNAC17*. We made a candidate genes direct targeting their promoter regions using in silico analysis (Table 1). Taken together, *OsNAC17* triggered many stress-related genes could be considered as direct or indirect downstream genes. Further works still need to uncover the *OsNAC17* related signal pathways during abiotic stress. Therefore, We generate *myc*-tagging line using *GOS2::OsNAC17-myc* construct into *pSB11* vector. This transgenic plant made us facilitate to find primary targeting downstream genes of *OsNAC17* that may involved in stress response.

In conclusion, our results represented that *OsNAC17* is a stress responsive *NAC* transcription factor in plants. Overexpression of *OsNAC17* in rice confer enhanced drought tolerance. This study suggest that *OsNAC17* functions as a positive regulator of water deficit stress and may have a possibility for the genetic improvement of environmental stress tolerance in crop plants.

Discussion

Agarwal, P. K. and B. Jha (2010). "Transcription factors in plants and ABA dependent and independent abiotic stress signalling." Biologia Plantarum **54**(2): 201-212.

Bartels, D. and R. Sunkar (2005). "Drought and salt tolerance in plants." Critical reviews in plant sciences **24**(1): 23-58.

Chaves, M. M., J. Flexas and C. Pinheiro (2009). "Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell." Annals of Botany **103**(4): 551-560.

Duval, M., T.-F. Hsieh, S. Y. Kim and T. L. Thomas (2002). "Molecular characterization of AtNAM: a member of the Arabidopsis NAC domain superfamily." Plant molecular biology **50**(2): 237-248.

Griffin, J. J., T. G. Ranney and D. M. Pharr (2004). "Heat and drought influence photosynthesis, water relations, and soluble carbohydrates of two ecotypes of redbud (*Cercis canadensis*)." Journal of the American Society for Horticultural Science **129**(4): 497-502.

Hao, Y.-J., Q.-X. Song, H.-W. Chen, H.-F. Zou, W. Wei, X.-S. Kang, B. Ma, W.-K. Zhang, J.-S. Zhang and S.-Y. Chen (2010). "Plant NAC-type transcription factor proteins contain a NARD domain for repression of transcriptional activation." Planta **232**(5): 1033-1043.

Hu, H., M. Dai, J. Yao, B. Xiao, X. Li, Q. Zhang and L. Xiong (2006). "Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice." Proceedings of the National Academy of Sciences **103**(35): 12987-12992.

Jeong, J. S., Y. S. Kim, K. H. Baek, H. Jung, S.-H. Ha, Y. Do Choi, M. Kim, C. Reuzeau and J.-K. Kim (2010). "Root-specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions." Plant Physiology **153**(1): 185-197.

Jung, H., P. J. Chung, S.-H. Park, M. C. F. R. Redillas, Y. S. Kim, J.-W. Suh and J.-K. Kim (2017). "Overexpression of OsERF48 causes regulation of OsCML16, a

calmodulin-like protein gene that enhances root growth and drought tolerance." Plant Biotechnology Journal: n/a-n/a.

Kim, Y.-S., S.-G. Kim, J.-E. Park, H.-Y. Park, M.-H. Lim, N.-H. Chua and C.-M. Park (2006). "A membrane-bound NAC transcription factor regulates cell division in Arabidopsis." The Plant Cell **18**(11): 3132-3144.

Lu, M., S. Ying, D.-F. Zhang, Y.-S. Shi, Y.-C. Song, T.-Y. Wang and Y. Li (2012). "A maize stress-responsive NAC transcription factor, ZmSNAC1, confers enhanced tolerance to dehydration in transgenic Arabidopsis." Plant cell reports **31**(9): 1701-1711.

Maverakis, E., K. Kim, M. Shimoda, M. E. Gershwin, F. Patel, R. Wilken, S. Raychaudhuri, L. R. Ruhaak and C. B. Lebrilla (2015). "Glycans In The Immune system and The Altered Glycan Theory of Autoimmunity: A Critical Review." Journal of autoimmunity **0**: 1-13.

Mitsuda, N., M. Seki, K. Shinozaki and M. Ohme-Takagi (2005). "The NAC transcription factors NST1 and NST2 of Arabidopsis regulate secondary wall thickenings and are required for anther dehiscence." The Plant Cell **17**(11): 2993-3006.

Nakashima, K., K. Yamaguchi-Shinozaki and K. Shinozaki (2014). "The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat." Abiotic Stress: Molecular Genetics and Genomics: 25.

Nikovics, K., T. Blein, A. Peaucelle, T. Ishida, H. Morin, M. Aida and P. Laufs (2006). "The balance between the MIR164A and CUC2 genes controls leaf margin serration in Arabidopsis." The Plant Cell **18**(11): 2929-2945.

Nuruzzaman, M., R. Manimekalai, A. M. Sharoni, K. Satoh, H. Kondoh, H. Ooka and S. Kikuchi (2010). "Genome-wide analysis of NAC transcription factor family in rice." Gene **465**(1): 30-44.

O'toole, J. and O. Namuco (1983). "Role of panicle exertion in water stress induced sterility." Crop Science **23**(6): 1093-1097.

Olsen, A. N., H. A. Ernst, L. L. Leggio and K. Skriver (2005). "NAC transcription factors: structurally distinct, functionally diverse." Trends in plant science **10**(2): 79-87.

Park, S.-H., S. W. Bang, J. S. Jeong, H. Jung, M. C. F. R. Redillas, H. I. Kim, K. H. Lee, Y. S. Kim and J.-K. Kim (2012). "Analysis of the APX, PGD1 and R1G1B constitutive gene promoters in various organs over three homozygous generations of transgenic rice plants." Planta **235**(6): 1397-1408.

Petrov, V., J. Hille, B. Mueller-Roeber and T. S. Gechev (2015). "ROS-mediated abiotic stress-induced programmed cell death in plants." Frontiers in Plant Science **6**(69).

Roberts, S. (2000). "Mechanisms of action of transcription activation and repression domains." Cellular and Molecular Life Sciences CMLS **57**(8-9): 1149-1160.

Saibo, N. J. M., T. Lourenço and M. M. Oliveira (2009). "Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses." Annals of Botany **103**(4): 609-623.

Shan, Q., Y. Wang, J. Li, Y. Zhang, K. Chen, Z. Liang, K. Zhang, J. Liu, J. J. Xi and J.-L. Qiu (2013). "Targeted genome modification of crop plants using a CRISPR-Cas system." Nature biotechnology **31**(8): 686.

Shinozaki, K., K. Yamaguchi-Shinozaki and M. Seki (2003). "Regulatory network of gene expression in the drought and cold stress responses." Current opinion in plant biology **6**(5): 410-417.

Sreenivasulu, N., S. Sopory and P. K. Kishor (2007). "Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches." Gene **388**(1): 1-13.

Wang, W., B. Vinocur and A. Altman (2003). "Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance." Planta **218**(1): 1-14.

Wassmann, R., S. Jagadish, K. Sumfleth, H. Pathak, G. Howell, A. Ismail, R. Serraj, E. Redona, R. Singh and S. Heuer (2009). "Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation." Advances in Agronomy **102**: 91-133.

Xiang, Y., N. Tang, H. Du, H. Ye and L. Xiong (2008). "Characterization of OsZIP23 as a key player of the basic leucine zipper transcription factor family for conferring abscisic acid sensitivity and salinity and drought tolerance in rice."

Plant physiology **148**(4): 1938-1952.

Xie, Q., G. Frugis, D. Colgan and N.-H. Chua (2000). "Arabidopsis NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development." Genes & development **14**(23): 3024-3036.

Xiong, L., K. S. Schumaker and J.-K. Zhu (2002). "Cell signaling during cold, drought, and salt stress." The plant cell **14**(suppl 1): S165-S183.

Zheng, X., B. Chen, G. Lu and B. Han (2009). "Overexpression of a NAC transcription factor enhances rice drought and salt tolerance." Biochemical and biophysical research communications **379**(4): 985-989.

벼 *OsNAC17* 전사인자의 가뭄저항성 증진에 관한 연구

김 태 환

서울대학교 국제농업기술대학원

국제농업기술학과

지도교수 김 주 곤

가뭄은 작물의 생산성을 감소시키는 중요한 인자로 작용한다. 식물 특이적 전사인자 NAC 단백질은 외생적 스트레스에 반응하는 전사적 조절에 중요한 역할을 하는 것으로 보고되었다. 그러나 아직까지 벼에 존재하는 각각의 NAC 유전자에 대한 연구결과가 부족하다. 본 연구에서는, 벼 유래의 NAM, ATAF 그리고 CUC 전사인자 중의 하나인 *OsNAC17*의 분자생물학적 특성을 규명하였고, 과발현체에서의 가뭄저항성 증진에 기여한다는 것을 밝혀냈다. *OsNAC17*이 전사적 활성을 확인하기 위해 효모를 이용하여 실험을 한 결과 C-terminal 지역에 활성도메인을 가지고 있는 것을 확인하였다. 또한 세포내에서의 발현부위를 확인하기 위해 원형질체로 GFP와 결합시킨 *OsNAC17*-GFP를 제작하여 공초점현미경으로 확인한 결과 핵 내부에서 타켓팅하는 전사인자임을 확인하였다. 가뭄저항성의 기능을 밝혀내기 위하여

항시발현 프로모터인 PGD1을 이용하여 OsNAC17의 과발현체를 제작하였다. PGD1:OsNAC17은 영양생장기간 중에 가뭄처리를 하였을 때 저항성이 증가되는 표현형을 보였으며 생식생장기간 동안 총알곡중량이 일반 종자와 비교하였을 때 22~64%까지 증가하는 것을 보여주었다. OsNAC17에 의해 조절되는 하위유전자를 분석하기 위하여 RNA sequencing을 수행하였고 총 119개의 유전자의 발현량이 증가하였다. 발현량이 증가한 유전자 중에서, 활성산소종의 대사와 관련한 유전자인 UDP-glucosyltransferase family protein, similar to 2-alkenal reductase (NADPH-dependent oxireductase), similar to retinol dehydrogenase 12, Lipoxygenase와 세포괴사에 관여하는 NB-ARC domain containing protein이 있었다.

본 연구의 결과를 통해 OsNAC17이 가뭄 저항성에 관여하는 전사인자이며 그 과발현체에서의 가뭄저항성을 증진시키는 것을 밝혀냈다.

주요어 : NAC transcription factors, drought tolerance, ROS, transcriptional activation, subcellular localization

학번 : 2015-22419