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# Regulation of rice responses to submergence by WRKY transcription factors

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## Abstract

Responses of rice to submergence have been extensively studied, but the molecular network behind the tolerance to this stress is still incomplete. Transcription factors (TFs) are important players in gene transcription regulation during stresses. Here, we analyzed expression of *WRKY* genes and morphological and anatomical changes in different rice cultivars under submergence. When they were submerged for 48 h, changes in root number, fresh and dry masses, and aerenchyma development were observed. Although accumulations of *WRKY* transcripts were observed in both shoots and roots, root tissues showed higher accumulation with a peak already after 6 h under submergence. Especially transcriptions of *OsWRKY11* and *OsWRKY56* were high, more than 100-fold in comparison with controls. The *WRKY* promoter analysis showed that some *cis*-regulatory elements could be characterized as stress-responsive elements and linked to oxygen depletion. In the promoter of *OsWRKY62*, two *cis*-regulatory elements were found: ARE and GC-motif. These elements are known to be involved in oxygen deficiency responses. In addition, the W-box *cis*-regulatory element, the target of WRKY transcription factors, was found in *OsWRKY11*, *OsWRKY56*, and *OsWRKY62*, suggesting a feedback control acting on the upregulation of WRKY transcription factors. Genes involved in the submergence stress and resulting aerenchyma development had a W-box in their promoter regions, which also suggested regulation by WRKYs. Overall, the results support the role of WRKY transcription factors in rice submergence tolerance and unveil their action in other tolerance mechanisms.

*Additional key words*: abiotic stress, *cis*-regulatory elements, gene expression, *Oryza sativa*, oxygen deficiency.

## Introduction

In a climate change scenario, oscillations in water availability (floods and droughts) become a problem in agricultural areas around the world. Rice is one of the most important crops worldwide, but many rice producing regions are flooding prone (Bailey-Serres *et al.* 2012). Submergence is a type of flooding stress where the plant is immersed partially/completely in water, leading to limiting (hypoxia) or complete absence of oxygen (anoxia), which can promote changes in gene expression (Tamang *et al.* 2015, Loreti *et al.* 2016).

The molecular mechanisms behind submergence has been studied extensively (Xu *et al.* 2006). Rice genotype FR13A, which presents the *Submergence 1* (*SUB1*) locus, is able to survive two weeks under submergence. *SUB1* encodes three genes belonging to the ethylene-response factor (ERF) subgroup VII, named *SUB1A*, *SUB1B*,

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*Abbreviations*: ABA - abscisic acid; CL - coleoptile length; CREs - *cis*-regulatory elements; ERF - ethylene response factor; LN - leaf number; MeJA - methyl jasmonate; QTL- quantitative trait locus; RDM - root dry mass; RFM - root fresh mass; RL - root length; RN - root number; RT-qPCR - reverse transcription quantitative polymerase chain reaction; SA - salicylic acid; SAM - S-adenosyl-L-methionine; SDM - shoot dry mass; SFM - shoot fresh mass; SL - shoot length; TFs - transcription factors.

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and *SUB1C*. Although *SUB1B* and *SUB1C* are quite invariable, *SUB1A* can appear as two alleles, *SUB1A-1* and *SUB1A-2* where only *SUB1A-1* confers submergence tolerance. To confer tolerance, *SUB1A* acts on a quiescence strategy where, under submergence, content of gibberellin (GA) limiting shoot elongation is repressed (Fukao and Bailey-Seres 2008). Additionally, a novel quantitative trait locus (QTL) has been recently reported in *SUB1* lines as a candidate for further improvement of submergence tolerance in *SUB1* lines, *qSUB8.1*, derived from *Ciherang-Sub1* genotype. It is a major QTL in chromosome 8 and contributes by 27.5 % of phenotypic variance (Gonzaga *et al.* 2017). Furthermore, *SNORKEL 1* (*SK1*) and *SNORKEL 2* (*SK2*), which also encode ERFs, can induce tolerance in rice plants (Hattori *et al.* 2009). When submerged, ethylene accumulation induces *SK1* and *SK2*, followed by induction of GA, providing a rapid internode elongation, *i.e.*, strategy conferring adaptation to flooding (Hattori *et al.* 2009).

These findings highlight relevance of transcription factors (TFs) in submergence events, demonstrating their fundamental role in the molecular responses of stressed plants. TFs regulate the expression of many other genes by binding to specific DNA sequences called *cis*-regulatory elements (CREs) in the promoter region of genes. These can initiate the signal cascade, either by regulating different stress-related genes or regulating their own promoter, therefore amplifying the signal (Banerjee and Roy Choudhury 2015). A transcriptomic analysis in *Arabidopsis thaliana* under hypoxic conditions show up-regulated TFs belonging to the most representative families including WRKY in both shoot and root tissues (Hsu *et al.* 2011).

WRKY TFs contain a specific signature with a highly conserved WRKY domain and have about 60 amino acid residues, WRKYGQK sequence at the N-terminus, and a zinc finger motif $C_2H_2$- or $C_2HC$-type at the C-terminus (Rushton *et al.* 2010, Bakshi and Oelmüller 2014). WRKYs bind with a high affinity to a DNA sequence containing a CRE called W-box (C/TTGACT/C) (Fukushima *et al.* 2016, Liu *et al.* 2016). Phukan *et al.* (2016) reviewed many biological functions of plant WRKY TFs, such as plant growth and development, synthesis of secondary metabolites, bacterial or fungal resistance, and tolerance to different abiotic stresses. Thus, considering the importance of WRKY TFs, this work aimed to investigate the morphological changes and the expression profile of different *WRKY* genes in rice plants under submergence.

## Material and methods

**Plants and growth conditions:** Pre-germinated seedlings of *japonica* (cv. Nipponbare) and *indica* (cvs. Epagri 108 and BR IRGA 409) rice (*Oryza sativa* L.) genotypes were grown in pots containing soil under a 16 h-photo-period, irradiance of 48 μmol $m^{-2}$ $s^{-1}$, a temperature of about 25 °C, and a relative humidity of 80 % for 14 d. They were watered with a complete nutrient solution as described by Yoshida (1976). Subsequently, plants were completely submerged (Fig. 1 Suppl.) for 6, 12, 24, and 48 h (Liu *et al.* 2005) in order to simulate flooding stress. The experiment consisted of three replicates in a completely random design, where each replicate comprised 20 seedlings. Leaves and roots were collected and stored at -80 °C until extraction of total RNA.

Also, morphological traits known to be involved in response to flooding stress were analyzed 6, 12, and 48 h after stress application. Measured traits included: shoot length (SL), root length (RL), coleoptile length (CL), leaf number (LN), root number (RN), shoot fresh and dry masses (SFM, SDM) and root fresh and dry masses (RFM, RDM). For dry mass analyses, shoots and roots were kept in an oven (80 °C) for 24 h (Krzyzanowski *et al.* 1999).

**Root anatomy:** Samples of root maturation regions of seedlings grown under control conditions and 48 h after submergence were collected and fixed in Karnovsky (1965) solution modified by adding a phosphate buffer (pH 7.2), then dehydrated in an ethanol series and embedded in plastic resin (*Historesin*®, *Leica*, Nussloch/Heidelberg, Germany). The blocks were then sectioned (5 μm thick) using a rotary microtome. The sections were stained with 0.05 % (m/v) toluidine blue in citrate-phosphate buffer, pH 4.5 (Sakai 1973) and mounted in *Entellan*® synthetic resin (*Merck*, Darmstadt, Germany). Photomicrografhs were taken with a *Leica®DM LB* microscope equipped with a *Leica DC 300F* camera for digital images. An estimation of the root radius was used to infer aerenchyma differences using *ImageJ* software (Schneider *et al.* 2012).

**RNA extraction and cDNA synthesis:** Total RNA was extracted from 2 g of fresh root or shoot tissues following the protocol described by *PureLink*® plant RNA reagent (*Invitrogen*, Carlsbad, CA, USA). Samples were treated with *DNAse I* (*Invitrogen*). The quantity of the RNA was assessed by spectrophotometry and the quality by agarose gel electrophoresis. Each sample (2 μg) was reverse-transcribed into cDNA using the commercial kit *SuperScript*® *III* first-strand system for RT-PCR (*Invitrogen*).

**Real-time quantitative PCR analysis:** The real-time quantitative RT-PCR experiment was performed according to *MIQE* guidelines (Bustin *et al.* 2009) using oligonucleotide pairs for five WRKY rice genes.

Oligonucleotides were designed from sequences deposited in the rice annotation project, *MSU* – (http://rice. plantbiology.msu.edu/home_overview.shtml) using *Primer Express*® (*Applied Biosystems*, Foster City, CA, USA) (Table 1 Suppl.). The criteria used for primer selection were amplicon size between 50 and 150 bp, cytosine and guanine (CG) content between 40 and 60 %, and melting temperature ranging from 60 to 65 °C according to *Applied Biosystems* recommendations. Assay was conducted in triplicate in *Applied Biosystems 7500* fast real-time PCR system using *SYBR™ Green PCR Master Mix* (*Invitrogen*). The quantification was performed according to ΔΔCt method (Livak and Schmittgen 2001), where *elongation factor α* (*EF1-α*) was used as endogenous reference gene (Jain *et al.* 2006).

**Search of CRE pattern in WRKY promoters:** For CRE analysis, WRKY promoters of cv. Nipponbare were analyzed. The putative promoters (1.5 kb upstream of transcription start site) were obtained from the *Rice Annotation Project Database* (*RAP-DB*; http://rapdb.dna.affrc.go.jp), the CRE information was obtained from the *PlantCare* database (http://bioinformatics.psb.ugent.be/webtools/plantcare/ html) (Lescot *et al.* 2002), and the analysis was performed considering just CREs with matrix score ≥ 5 (Bazzini *et al.* 2009).

W-box is a binding site of WRKY proteins in target gene promoter regions (Fukushima *et al.* 2016, Liu *et al.* 2016). For W-box sequence search, promoters of 33 genes described with involvement in submergence stress and aerenchyma development were analyzed. The W-box information was obtained from the *PlantCare* database as mentioned above.

**Statistical analysis:** Analysis of variance (*ANOVA*) was performed using the *SAS v. 9.3* software. Means were separated the by Tukey's honest significant difference (HSD) test at $P \leq 0.05$.

## Results and discussion

To verify the effects of submergence on rice growth and development, morphological traits were analyzed. The analyses of variance (Table 2 Suppl.) suggests that most traits did not differ ($P \leq 0.05$), indicating that all genotypes showed a similar performance under both treatments. Intersection was verified for RFM and RDM and the effects were available through regressions to elucidate the genotype performance in each level of treatment (Fig. 1*A,B*). According to relative performance, a stalling in shoot growth was observed for all genotypes, and no differences were observed between treatments and the control. This stalling suggests a similar mechanism to Sub1, *i.e.*, submergence tolerance through quiescence strategy (Fig. 1, Table 1).

Concerning the shoot biomass accumulation, significant differences between control and 48-h submergence were not observed (Table 1). However, Nipponbare and BR IRGA 409 were able to slightly increase shoot biomass by 19 and 39 %, respectively. On the other hand, Epagri 108 showed a slight reduction of 10 % in shoot biomass (Table 1). When partially submerged, a shoot biomass reduction has been reported in rice plants, due to the reduction of leaf area (Kato *et al.* 2014).

Table 1. Growth characteristics in rice cultivars under control conditions and 48 h after submergence: shoot length (SL), root length (RL), root number (RN) leaf number (LN), coleoptile length (CL), shoot fresh mass (SFM), and shoot dry mass (SDM). Means followed by different letters differ according to Tukey test ($P \leq 0.05$), $n = 3$.

| Conditions | Genotype | SL [cm] | RL [cm] | RN | LN | CL [cm] | SFM [mg] | SDM [mg] |
|---|---|---|---|---|---|---|---|---|
| Control | Nipponbare | 10.59A | 4.28A | 8A | 3A | 0.60A | 0.54A | 0.09A |
| | Epagri 108 | 9.29A | 4.39A | 6B | 3A | 0.62A | 0.50A | 0.09A |
| | BR IRGA 409 | 11.03A | 3.86A | 7A | 4A | 0.58A | 0.50A | 0.07A |
| Submerged | Nipponbare | 11.46A | 3.86A | 7A | 3A | 0.54A | 0.82A | 0.11A |
| | Epagri 108 | 9.32A | 4.71A | 4B | 3A | 0.74A | 0.69A | 0.08A |
| | BR IRGA 409 | 12.58A | 3.46A | 7A | 3A | 0.59A | 0.76A | 0.10A |

As a morphological adaptation mechanism to flooded conditions, constitutive and/or induced aerenchyma formation is often observed. It can transfer oxygen from shoots to roots (Jackson and Ismail 2015) to maintain root growth and dry matter production (Suralta and Yamauchi 2008). Aerenchyma formation in response to complete submergence for 48 h was studied in rice roots by light microscopy (Fig. 2*A-F*). Nipponbare did not show aerenchyma formation when submerged for 48 h, presenting a decrease of 12 % in the aerenchyma radius when compared to the control conditions, and did not show increases in root growth and root dry mass (Fig. 1*B*). Epagri 108 was also not able to develop aerenchyma until 48 h and showed a 10 % decrease in the

aerenchyma radius when compared to control conditions. However, it did show a small increase in root growth but no changes in root biomass (Fig. 1*B*)

Rice as well as maize, wheat and barley develop lysigenous aerenchyma in root cortex through induced root cell death and lysis (reviewed in Yukiyoshi and Karahara 2014). Studies regarding the aerenchyma are important because this tissue development is positively correlated with submergence tolerance. Oat and triticale are tolerant crops and develop more aerenchyma compared to less tolerant crops such as wheat and barley (Setter and Waters 2003). In agreement with the above reports, the studied genotypes were able to develop aerenchyma in the first hours of the submergence stress suggesting that these changes should be further investigated.

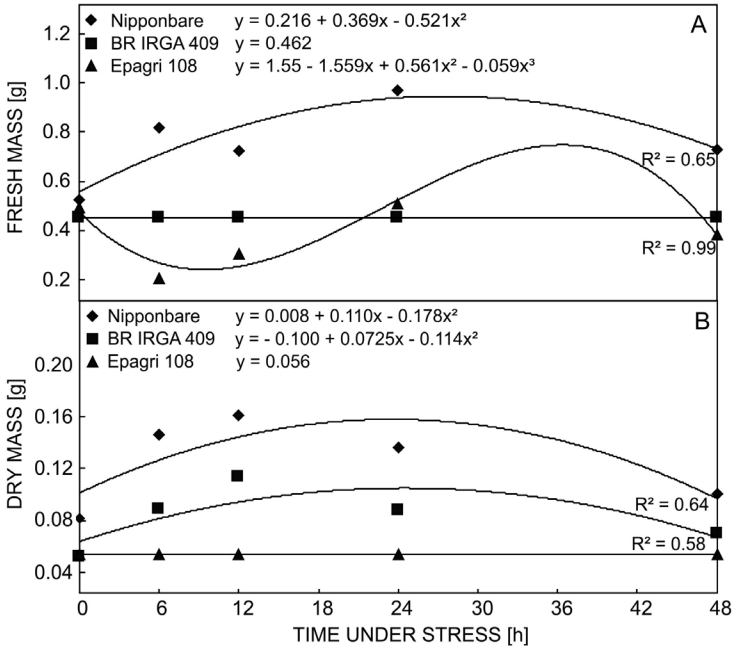


Fig. 1. Changes in root fresh and dry masses in cvs. Nipponbare, Epagri 108, and BR IRGA 409 during submergence stress. Regression coefficients ($R^2$) are also shown.

In order to verify the involvement of WRKY TFs in response to submergence, the expressions of five *WRKY* genes in shoots and roots of rice cultivars Nipponbare, Epagri 108, and BR IRGA 409 under submergence stress were analyzed by real-time RT-qPCR (Figs. 3 and 4). In general, the analyzed *WRKYs* responded to submergence stress by increasing transcript abundance. Although morphological changes after 2 d under complete submergence were not observed (Table 1), transcript abundance analysis can be informative, since the expression of genes related to ethylene and oxygen depletion can start within 2 h of submergence (Dawood *et al.* 2014). Transcription of *OsWRKY11* in shoots showed 18- and 12-fold increases when BR IRGA 409 plants were submerged for 12 and 24 h, respectively. Under the same conditions, a 6-fold increase was observed in Epagri 108 whereas the transcription was not altered in Nipponbare. Transcription of *OsWRKY17* in shoots showed a 13-fold increase in BR IRGA 409 at 48 h after submergence, but an overall suppression of *OsWRKY17* was detected in Epagri 108 and Nipponbare. For *OsWRKY56,* a 27-fold increase was detected in BR IRGA 409 at 48 h after submergence. However, a small change in expression at 12 h was observed in Nipponbare and at 6 and 24 h in Epagri 108. *OsWRKY62* and *OsWRKY73* showed opposite profiles, *i.e.*, a maximum increase of 17-fold in BR IRGA 409 at 24 h and 17-fold in Epagri 108 at 12 h after submergence, respectively. Later (at 48 h), both *OsWRKY62* and *OsWRKY73* were down-regulated (Fig. 3).

Transcriptomic analyses showed that roots are more severely affected by submergence and low oxygen than shoots (Mustroph *et al.* 2009, Hsu *et al.* 2011). In this sense, root appears to be more sensitive than shoot tissue as it can be observed by the amount of accumulated transcripts (compare Figs. 3 and 4). A peak of well pronounced responses at 6 h after submergence was observed in roots (Fig. 4). The relative expressions of *OsWRKY11* and *OsWRKY56* showed 147- and 125-fold increases, respectively, in BR IRGA 409 at 6 h after submergence. However, transcription of *OsWRKY56* in Nipponbare and Epagri 108 was suppressed under submergence. *OsWRKY17*, *OsWRKY62*, and *OsWRKY73* showed a similar behavior after 6 h of submergence,

reaching 45-, 32- and 17-fold increase, respectively, in BR IRGA 409, but they were repressed in Nipponbare and Epagri 108.

This is one of few reports showing a transcript analysis of WRKY TFs during submergence responses in rice. Upregulations of *OsWRKY1*, *5*, *19*, *28*, *68* and *77* were detected by microarray and upregulation of *OsWRKY24* was also detected by RT-qPCR during submergence stress in a mutant for *alcohol dehydrogenase 1* (*ADH1*) (Mohanty *et al.* 2016). It was suggested that WRKYs can act as negative regulators of coleoptile growth towards maintaining metabolites necessary for cell survival in the rice mutant.

Transcriptomic analyses in other species indicated that genes encoding WRKY TFs are usually up-regulated during submergence. This trend is observed in submerged *A. thaliana* shoots and roots after 7 h (Hsu *et al.* 2011), in roots after 2 h (Klok *et al.* 2002), and in roots after 7 d (Mustroph *et al.* 2009). Similar trend was observed in shoots and roots of poplar after 168 h (Kreuzwieser *et al.* 2009) and in shoots of *Brachypodium distachyon* after 48 h of submergence (Rivera-Contreras *et al.* 2016). In maize, the involvement of WRKYs during submergence events was also detected and the up-regulation of TFs, including WRKYs at 24, 48, and 72 h has been reported (Campbell *et al.* 2016). The overexpression of the sunflower *HaWRKY76* in *A. thaliana* promotes tolerance to submergence and drought by preserving sugar reserves during submergence and avoiding oxygen reactive species accumulation after recovery (Raineri *et al.* 2015).

To understand the regulation of *WRKY* genes and to study the similarities among the *WRKY* promoters, a CRE analysis was performed for 1.5 kb putative promoters of Nipponbare *OsWRKY11*, *17*, *56*, *62* and *73*. From

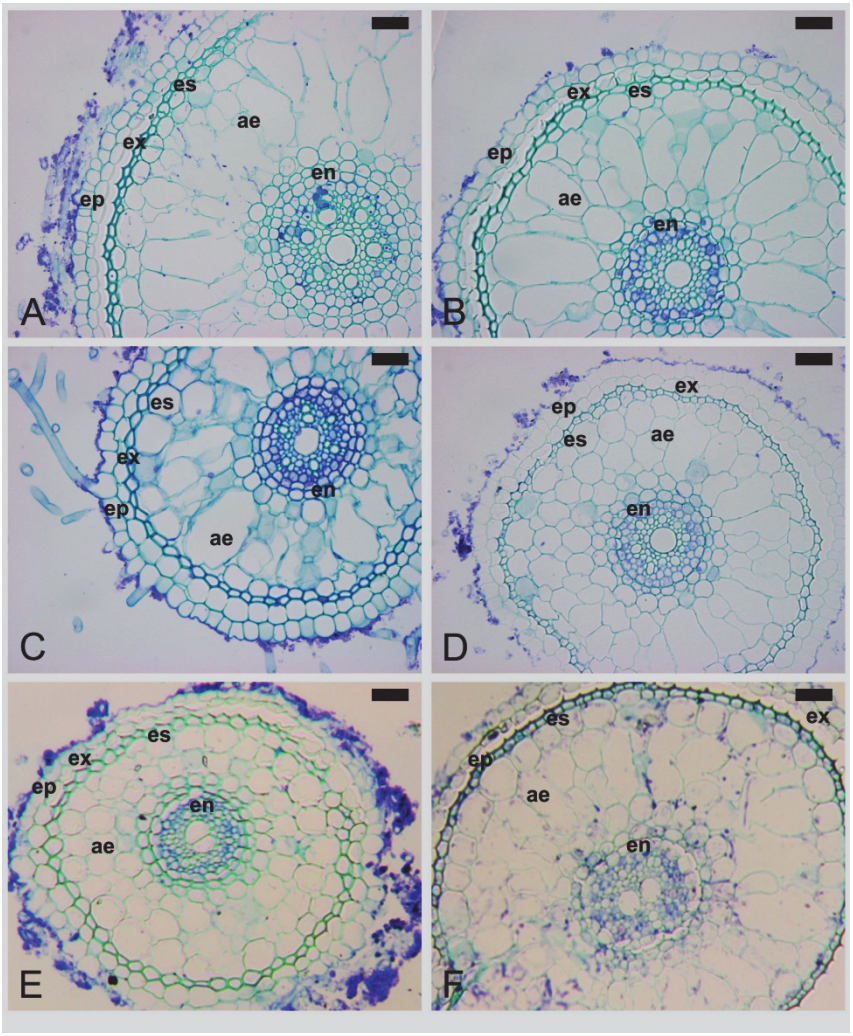


Fig. 2. Transverse sections of rice roots grown under control conditions or submergence for 48 h. Root transverse section of Nipponbare under control (*A*) and submergence (*B*), of Epagri 108 under control (*C*) and submergence (*D*), and of BR IRGA 409 under control (*E*) and submergence (*F*) conditions (ep - epidermis, ex - exodermis, ae - aerenchyma, en - endodermis, es - sclerenchyma; *bar* = 30 μm).

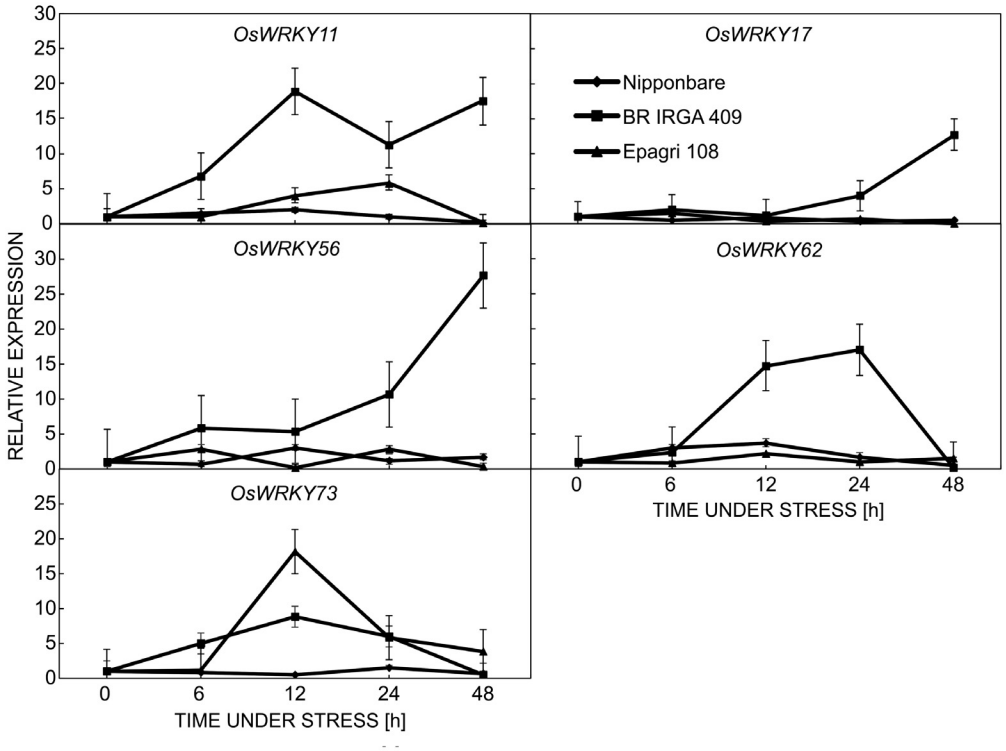


Fig. 3. Relative expression of *WRKY* genes in shoot tissue of Nipponbare, Epagri 108, and BR IRGA 409 submerged for 0, 6, 12, 24, and 48 h. Means ± SDs, $n = 3$.

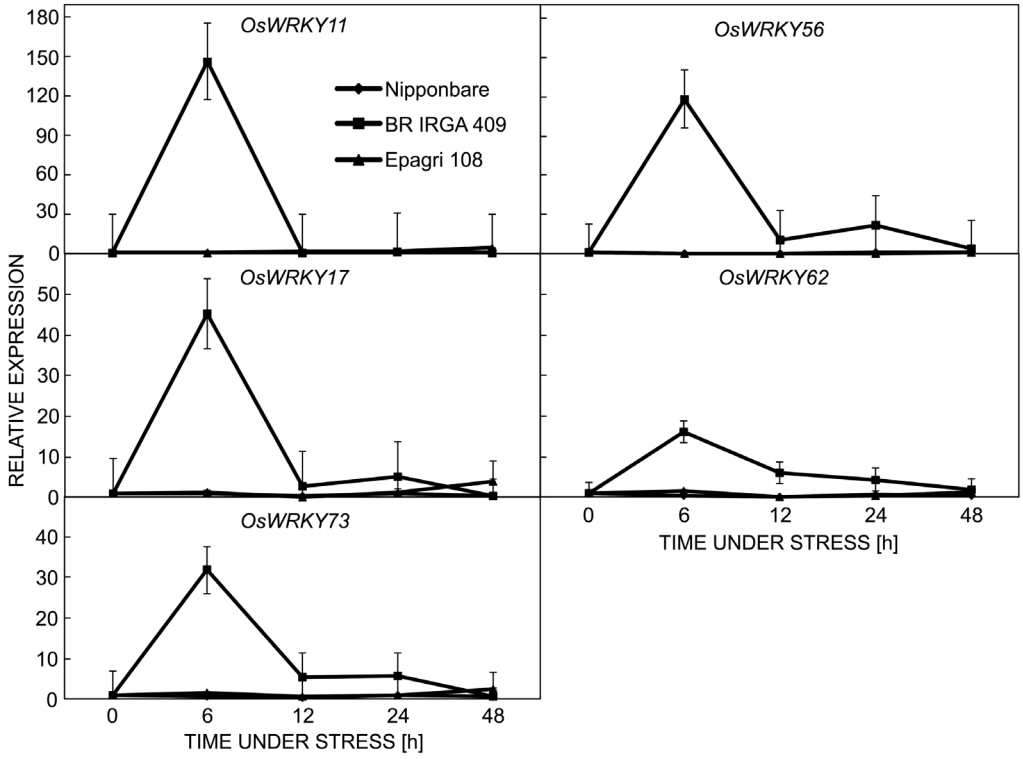


Fig. 4. Relative expression of *WRKY* genes in root tissue of Nipponbare, Epagri 108, and BR IRGA 409 submerged for 0, 6, 12, 24, and 48 h. Means ± SDs, $n = 3$.

70 different CREs found (Table 3 Suppl.), WRKY promoters share more than 75 % similarity suggesting that they are regulated in a similar way (Fig. 5). Eight CREs, including two common CREs (CAAT-BOX and TATA-BOX), O2-site and Skn-1_motif (involved in plant development), G-Box and GT1-motif (light responsive elements) and Unnamed_4 (Fig. 5) were found in all analyzed promoters and may be a key for submergence stress responses. Putative promoters of *OsWRKY11* and *OsWRKY62* seem to be more similar since they share 23 CREs (Fig. 5).

Many CREs in *OsWRKY11*, *OsWRKY56, OsWRKY62*, and *OsWRKY73* were detected to be involved in responses to abiotic stresses. Interestingly, two CREs found in the *OsWRKY62* promoter are known to be involved in response to oxygen deficiency and anoxic conditions, anaerobic response element (ARE) and GC-motif, respectively. It demonstrates the role of *OsWRKY62* in submergence stress through oxygen depletion. Under low oxygen content, pyruvate is fermented to ethanol catalyzed by two cytoplasmic enzymes, pyruvate decarboxylase and alcohol dehydrogenase (Magneschi and Perata 2009). In *A. thaliana* protoplasts under low oxygen content, MYB2 TF activates the transcription of *alcohol dehydrogenase 1* (*ADH1*) by binding to its promoter GT-motif, which is a component of the ARE, demonstrating the association of this CRE and genes responding to anaerobic stress (Hoeren *et al.* 1998).

W-box elements were detected in the promoter of *OsADH1*, suggesting its regulation by WRKYs and therefore supporting the role of this TFs in submergence responses (Table 4 Suppl. and Mohanty *et al.* 2016). In rice, *ADH1* is repressed by WRKYs, causing suppression of coleoptile elongation under submergence, in order to maintain metabolites for cell viability. This response plays a major role in the physiological process leading to cell survival (Mohanty *et al.* 2016). Taking into account the stalling of Nipponbare coleoptile growth at 48 h after submergence (Table 1), changes in *OsWRKY62* transcript accumulation at 12 h (Fig. 3), and presence of MYB and ARE element binding sites (Table 3 Suppl.), *OsWRKY62* could repress *ADH1* expression, which inhibited coleoptile growth to maintain cell viability.

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Fig. 5. Venn diagram of *cis*-regulatory elements shared in promoters (1.5 kb upstream) of *WRKY* genes in rice cv. Nipponbare.

Hormonal changes affect cell growth during the stress, thus the analysis of the CREs found in WRKY promoters, should reveal many CREs characterized in hormone responses (Table 3 Suppl.). In fact, CREs for gibberellic acid responses were detected, such as GARE-motif in *OsWRKY11*, *OsWRKY62*, *OsWRKY71*, and *OsWRKY56*; P-box in *OsWRKY11* and *OsWRKY73*, and TCA-element in *OsWRKY73*, *OsWRKY62*, and *OsWRKY71*. Interestingly, *OsWRKY11* and *OsWRKY62*, which had two gibberellin CREs, did not show changes in their regulation of shoot growth when Epagri 108 and Nipponbare were compared (Table 1). This could indicate a possible involvement in submergence tolerance. Also, a copy of ethylene response element (ERE) was found in *OsWRKY73*. This result, together with the upregulation of *OsWRKY73* in BR IRGA 409 roots (Fig. 4), suggested a role in the signaling pathway linked to aerenchyma development during submergence.

Thus, the W-box, a target of WRKY proteins that are known to mediate signaling by binding themselves to the

target gene promoter regions (Fukushima *et al.* 2016, Liu *et al.* 2016), were found in *OsWRKY11*, *OsWRKY56*, and *OsWRKY62* promoters (Table 3 Suppl.). The presence of a W-box sequence in *WRKY* promoters suggested self-regulation as a mechanism of amplifying the amount of *WRKY* transcripts in response to stress. Therefore, the particular role of each WRKY in submergence stress response could be demonstrated by the analysis of CREs, and each WRKY might display different roles towards stress resistance.

It was demonstrated that WRKY TFs (and other TFs) are abundant during rice aerenchyma formation, indicating their role in that process (Yoo *et al.* 2015). A role of WRKY TFs in aerenchyma development in rice plants under submergence stress was verified. The promoter analysis in 33 genes associated with aerenchyma formation was performed (Table 4 Suppl.) and it showed that 13 genes presented a W-box element in their promoters. W-box elements are target of WRKY TFs (Fukushima *et al.* 2016, Liu *et al.* 2016), which suggests their possible regulation of aerenchyma development.

Ethylene stimulates aerenchyma development under low oxygen content by promoting cell death (Drew *et al.* 2000, Yukiyoshi and Karahara 2014). Ethylene biosynthesis involves a sequence of enzymatic reactions, such that methionine conversion to S-adenosyl-L-methionine (SAM) by SAM synthetase, which is by 1-aminocyclopropane-1-carboxylate synthase (ACS) converted to 1-amino-cyclopropane-1-carboxylic acid (ACC) and finally ACC is converted to ethylene by 1-aminocyclopropane-1-carboxylase oxidase (ACO) (Wang *et al.* 2002, Van de Poel and Straeten 2014). Therefore, promoters of *OsACS1*, *OsACS2*, *OsACS3*, *OsACO1*, and *OsACO2* were analyzed (Table 4 Suppl.). W-box elements were found in *OsACS3* and *OsACO1*, suggesting a possible involvement of WRKYs in the regulation of ethylene biosynthesis. Furthermore, the promoter of *OsWRKY73* displayed an ethylene responsive element (ERE) (Table 4 Suppl.) and it was upregulated in BR IRGA 409 roots at 6 h after submergence (Fig. 4). These results and the decrease of RFM (Fig. 1*A*) indicated an association of aerenchyma development and ethylene production during oxygen depletion, therefore regulation of *OsACS3* and *OsACO1* by binding the corresponding gene to their promoter W-boxes.

## Conclusion

In the last few years the major rice producing countries suffered from floods (Jackson and Ismail 2015), so the need to develop cultivars able to survive under submergence becomes a challenge. Molecular studies, such as gene identification and their interactions under flooding and other stresses, open up opportunities for genetic manipulation of important pathways for the improvement of rice plants (Bailey-Serres and Voesenek 2008). An example is the use of overexpressed WRKY lines, which can induce tolerance since they act as positive or negative regulators in stress signaling pathways (Rushton *et al.* 2010). WRKY TFs are exhaustively studied in many abiotic and biotic stresses as well as developmental processes in plants (Phukan *et al.* 2016). However, submergence studies are still scarce. Here, we supported the role of WRKY TFs under submergence (Fig. 2 Suppl.). New studies regarding the WRKY TFs should be performed with the aim of deciphering the molecular network involved in submergence tolerance and how these genes are regulated. Breeding programs for major crop improvement could benefit from the understanding of the molecular basis of this response mechanism.

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