

# Effect of Rice *AUXIN BINDING PROTEIN57* (*OsABP57*) Overexpression in Response to Flooding

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**Abstract:** This study reports the effects of *Oryza sativa* *AUXIN BINDING PROTEIN57* (*OsABP57*) overexpression towards flooding in rice. *OsABP57* was previously reported to activate plasma membrane H<sup>+</sup>-ATPase. Earlier studies address the ability of transgenic *OsABP57* overexpression in enduring drought and salinity stresses but none on the flooding. In this study, complete submergence analysis was carried out and several morphophysiological parameters were analyzed such as plant height, root architecture and relative chlorophyll content. Results showed that there are no differences between *OsABP57* overexpression rice compared to MR219 control rice in terms of chlorophyll content and plant height after 1-3 weeks of flooding treatments. Root analysis, however, found that transgenic rice *OsABP57* produced more adventitious roots compared to MR219 rice under normal condition, which may be due to the role of the gene that encodes for auxin binding protein. The semi-quantitative polymerase chain reaction (PCR) on *Oryza sativa* pyruvate decarboxylase (*OsPDC*) gene after two weeks of flooding treatment showed an increase of expression in *OsABP57* transgenic compared to MR219. Overall, the overexpression of *OsABP57* did not show any significant difference in terms of morphophysiological analysis between the transgenic line and MR219, yet, there is an increase of *OsPDC* gene in the transgenic background which may need further experimental analysis in the future to map the network between auxin and hypoxia core genes.

**Key words:** *Oryza sativa*, flooding, *OsABP57*, MR219, hypoxia.

## 1. Introduction

Due to the growing number of population, future food security and sustainability have become the major concerns of researchers all over the world. Rice, as the staple food for majority of Asians is highly affected by climatic uncertainties, rendering scientific community to focus their limelight towards rice sustainability in challenges concerning abiotic and biotic stresses aside from boosting its yield [1].

Recently, some of the plant response mechanisms towards abiotic stresses including drought, salinity, cold, heat and flooding have been extensively studied.

These studies elaborated the signaling complexity of abiotic stresses network through observation made on gene interactions, cell signaling and protein functions [2]. Abiotic stress is characterized to affect certain activities in plant cell such as protein translocation in the endoplasmic reticulum and disruption of homeostasis in chloroplasts [3].

Among drought, salinity and heat stresses, flooding poses slight change on the crop condition due to water excess whereby this eventually introduces low oxygen state condition (hypoxic) leading to plant energy crisis. Unlike drought, salinity and heat, those stresses deal with water deficit and osmotic adjustment. Generally, submerging crops in water will reduce the absorption of carbon dioxide and oxygen to plants,

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which can result in situations such as hypoxia (lack of oxygen) and anoxia (no oxygen). In addition, full submergence may impart damage on plant's photosynthesis, resulting in inhibition and reduction of the photosynthetic rate [4]. The mechanisms of flood resistance are divided into two: quiescent mechanism which enables survival through saving energy, and avoidance mechanism where the growth of shoots is stimulated beyond the water level. Both mechanisms entail the role of ethylene via the ethylene group VII transcription factors (*ERF VII*), which are *SUBMERGENCE 1 (SUB1)* and *SNORKEL1/2*, respectively [5, 6].

When plants are in a state of oxygen deprivation, several genes will be activated and expressed to trigger signals and also to begin the synthesis of anaerobic polypeptides (ANPs). These ANPs are then engaged in the process of glycolysis and ethanol fermentation. The decrease in cytoplasmic pH leads to deactivation of *lactate dehydrogenase (LDH)* and activation of *pyruvate decarboxylase (PDC)* [7]. *PDC* is one out of 49 core hypoxia genes found in the hypoxia transcriptome dataset [8]. Therefore, *PDC* serves as the best indicator of plant response to hypoxia in terms of gene regulation.

Nevertheless, there are few resources found on the role of auxin in response to stress particularly towards flooding. Recently, it is reported that auxin-sensitive protein (Aux/IAA) mediates drought tolerance in *Arabidopsis* through the regulation of a secondary metabolite identified as glucosinolate [9]. In regards to this matter, auxin has shown to play vital regulatory roles towards morphological changes during adaptation induced by the stresses. Previously, researches have been focusing on the role of *Oryza sativa AUXIN BINDING PROTEIN57 (OsABP57)* in response to drought and salinity where *OsABP57* was found to function as an activator of plasma membrane  $H^+$ -ATPase [10] whereas the  $H^+$ -ATPase activity of the plasma membrane is responsible for proton extrusion from the cell. The extrusion of the proton is

important for intracellular pH homeostasis [11] since the electrochemical gradient of protons across the plasma membrane regulates membrane potential that results in a few physiological responses, for instances, phloem loading, stomatal opening, solute uptake by the roots and cell expansion [12]. Previous study showed that the overexpression of *OsABP57* in rice confers great tolerance to drought and salinity [13]. In addition, the yield remains unaffected due to the ability of the plant to recover after a long period of stress condition. This suggests a great improvement in the development of super rice with multiple stress tolerance attributes. In this study, the transgenic rice bearing overexpressed *OsABP57* gene driven by 35S promoter was analyzed in response to flooding and in comparison to MR219. MR219 rice variety was used in this study as a control rice for the genotype as the *OsABP57* overexpression construct was transformed into this variety previously. This variety was chosen mainly because it is a widely commercialized in the Malaysian rice agricultural system. Despite being a high producer, this variety suffers susceptibility to multiple biotic and abiotic factors including environmental stresses such as drought, salinity and flooding. Therefore, it is thought to be a great experimental candidate in the rice improvement strategy.

## **2. Materials and Methods**

### *2.1 Plant Materials and Stress Treatments*

The commercial rice *O. sativa* MR219 is widely planted in Malaysia yet susceptible to flooding. The MR219 seeds were collected from the Malaysian Agricultural Research and Development Institute (MARDI) while the *OsABP57* transgenic rice seed is a generous courtesy of Prof. Zamri Zainal from Universiti Kebangsaan Malaysia [13]. MR219 rice and *OsABP57* transgenic rice seeds were immersed overnight in water and left at 28 °C and monitored until germination. After 2 d, about 80% of the total seeds germinated. The seedlings were then monitored for two weeks

before being transferred to the container for a week. Later, the rice seedlings were moved into pots containing topsoil (0-20 cm depth) for approximately 26 d until they reached vegetative stage. The growth conditions were 30 °C under 16 h light and 8 h dark. Growth fertilizers were supplied as follows: 170:80:150 kg/ha N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O; the ratio is based on the total volume of soil used in the pot as recommended by the Integrated Agricultural Development Area, Ministry of Agricultural Malaysia. Then, the rice samples were used for hypoxia treatment where the samples were subjected to complete submergence in a 10 L container for one, two and three weeks. Six (6) biological replicates were made for each treatment for both varieties. Also, during this time, the rice is still at its vegetative growth.

### 2.2 Morpho-Physiological Analysis

Analyses of morpho-physiological were carried out to measure plant height and root architecture. Analysis of plant height was carried out from six biological replicates during one, two and three weeks of complete submergence by measuring the plant height from the rice stem above the soil level until the shoot tips. Root architecture analyses were carried out by observing the adventitious root development in both varieties during normal condition and two weeks of complete submergence.

### 2.3 Chlorophyll Content Analysis

Experimental setup was designed according to the randomized complete block design. Leaves from six biological replicates were selected randomly from three different parts of the plants: top, middle and bottom. Leaf chlorophyll content was measured using a chlorophyll measuring meter (SPAD-502, Konica Minolta/502, Japan) on three parts of the leaf for an average reading. The chlorophyll contents between rice varieties (MR219 and *OsABP57*) were compared for all three periods (Weeks 1, 2 and 3) for normal condition and treated samples and were analyzed

using analysis of variance (ANOVA) in SPSS V26 software.

### 2.4 RNA Extraction and Semi-quantitative Polymerase Chain Reaction (PCR)

Three MR219 and three *OsABP57* transgenic rice samples of control and treated were harvested and RNA extraction from leaves was performed according to TRIzol reagent (Life Technologies, Carlsbad, CA, USA) and purified using ethanol precipitation method [14]. Genomic DNA was lysed using TURBO DNase Kit (Ambion, Austin, TX, USA). One microgram (1 µg) RNA was used as the starting material for cDNA synthesis using the Superscript III Reverse transcriptase (Invitrogen, Paisley, UK) and the cDNAs were subjected to semi-quantitative PCR. Internal constitutive control, *OsU6* gene was used to normalize the expression result. Densitometry analysis was carried out to calculate the intensity of the expression band accurately. Gene expression is relative to the loading control gene from control plant (without treatment) and normalized to the internal constitutive gene (target gene × (loading gene (control)/internal constitutive gene)). To generate the fold change value from normalized value, all normalized samples were divided by the value of loading gene (control). PCR for *Oryza sativa pyruvate decarboxylase (OsPDC)* gene and *OsU6* gene were carried out as follows: initial denaturation (2 min, 95 °C), denaturation (50 s, 95 °C), annealing (1 min, 56 °C), elongation (50 s, 72 °C) and final elongation (5 min, 72 °C). PCR was carried out in 25 cycles. Primer used for both genes was as follows:

*OsU6* Forward: 5'-TAC AGA TAA GAT TAG CAT GGC CCC-3'

*OsU6* Reverse: 5'-GGA CCA TTT CTC GAT TTG TAC GTG-3'

*OsPDC* Forward: 5'-CTG TGA TTG CCG AGA CTG GT-3'

*OsPDC* Reverse: 5'-AGT CCA GCA TTT GCC CTC TC-3'

### 3. Results

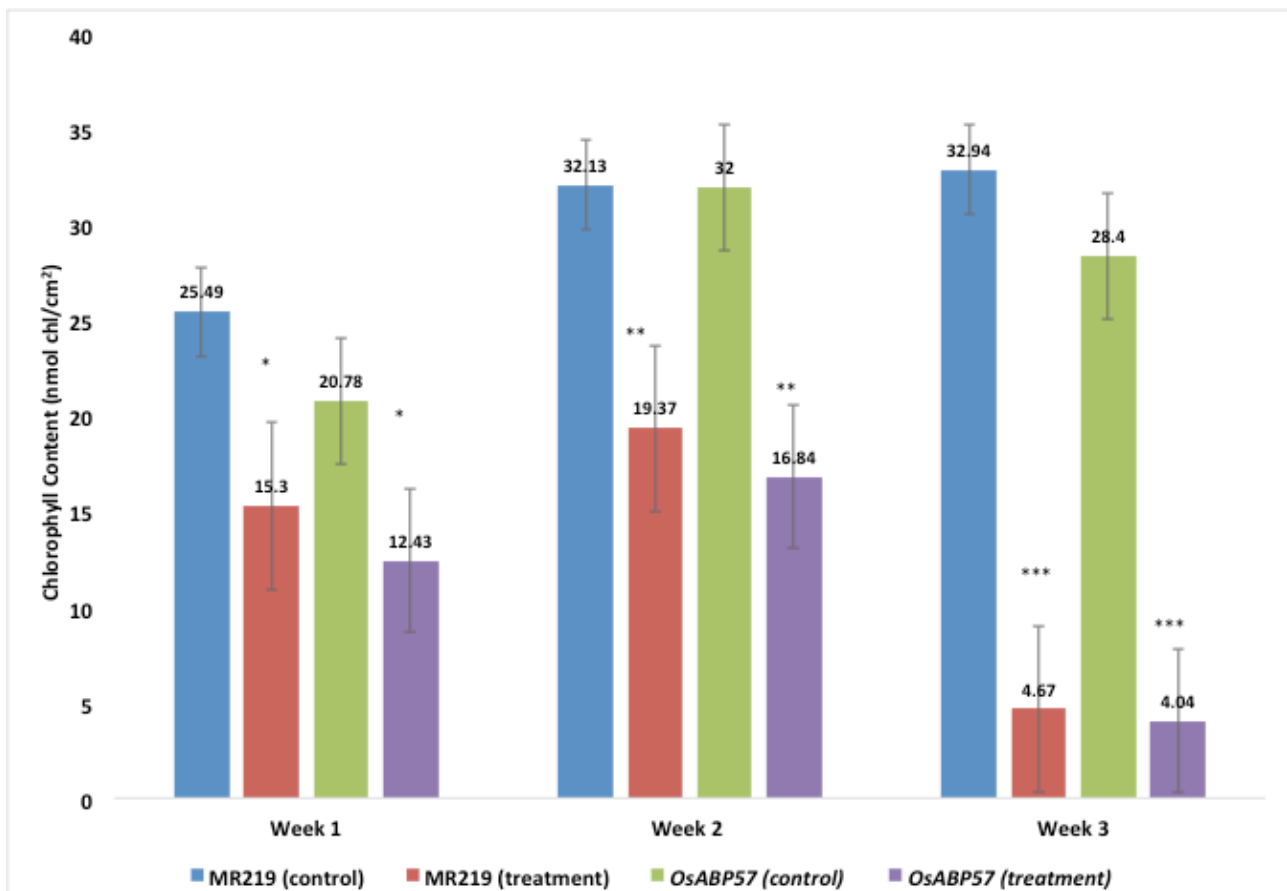
#### 3.1 Analysis of Relative Chlorophyll Content (nmol chl/cm<sup>2</sup>) during Flooding

To further analyze the effects of complete submergence and low oxygen condition for both rice varieties, chlorophyll content analysis was carried out. Control condition showed an increase of chlorophyll content in both rice varieties for Weeks 1-3 (Fig. 1). In treated samples, results showed that both MR219 and *OsABP57* have the same pattern of chlorophyll content under hypoxic condition from Week 1 to Week 3 of treatments (Fig. 1). Both varieties showed no significant difference in chlorophyll content during the first week of treatment (MR219 = 15.3 nmol chl/cm<sup>2</sup>, *OsABP57* = 12.43 nmol chl/cm<sup>2</sup>). During the second

week of treatment, the chlorophyll content for both rice varieties showed a slight increase (MR219 = 19.37 nmol chl/cm<sup>2</sup>, *OsABP57* = 16.84 nmol chl/cm<sup>2</sup>) before dramatical decrease in Week 3 of treatment (MR219 = 4.67 nmol chl/cm<sup>2</sup>, *OsABP57* = 4.04 nmol chl/cm<sup>2</sup>) (Fig. 1). However, after prolonged period of complete submergence of up to three weeks, both varieties showed decrease in chlorophyll content. Statistical analysis using ANOVA showed that both rice varieties did not show any significant difference for chlorophyll content for all three weeks with  $p > 0.05$ .

#### 3.2 Plant Height Analysis of *OsABP57* and MR219 under Low Oxygen State

Low oxygen condition caused by flooding also results in inhibition and growth retardation of the



**Fig. 1** Graph analysis of chlorophyll content (nmol chl/cm<sup>2</sup>) for both MR219 and *Oryza sativa* *AUXIN BINDING PROTEIN57* (*OsABP57*) under normal condition and complete submergence treatment (Weeks 1, 2 and 3).

Data were shown from six independent biological experiments, and bars indicate  $\pm$  SDs.

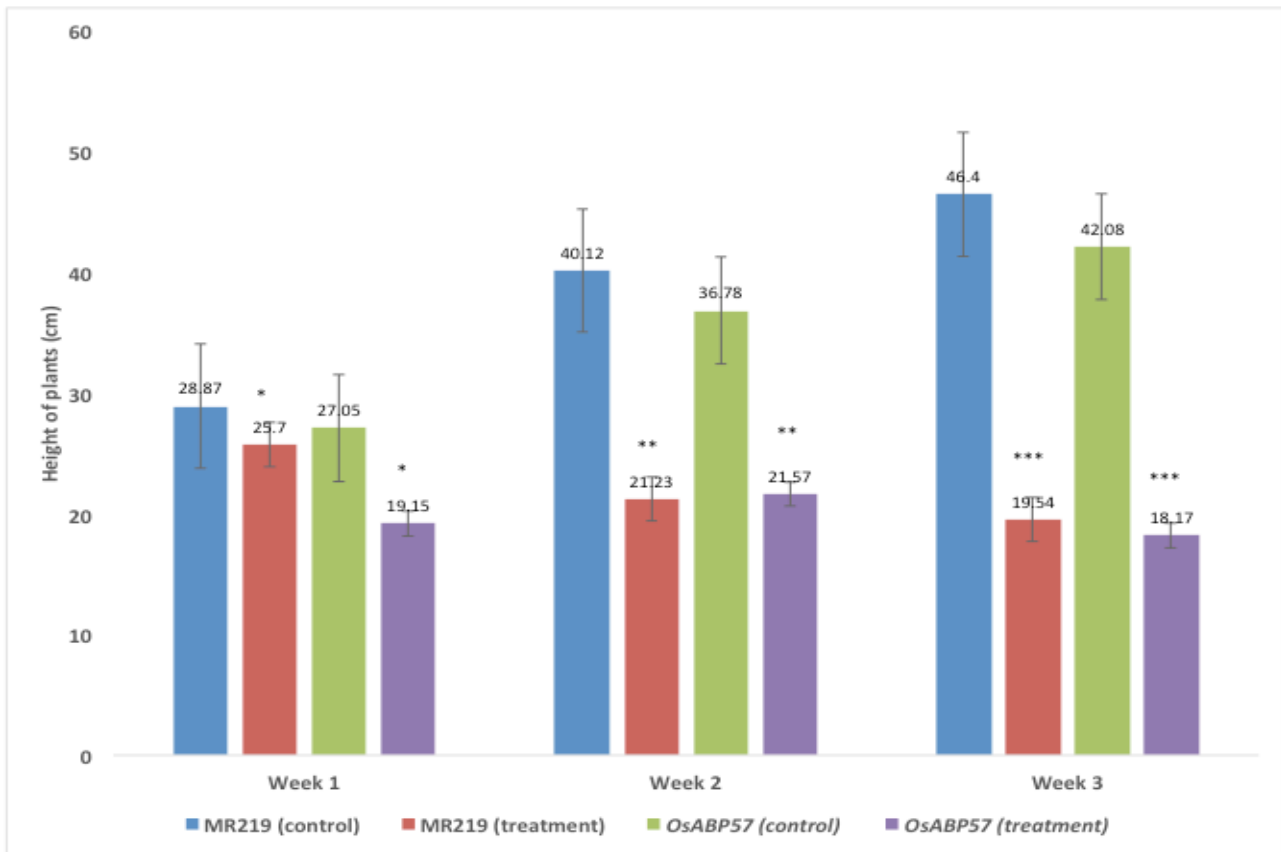
\*, \*\*, \*\*\* indicate  $p > 0.05$  between varieties ( $p = 0.542$ ).

plant. In this study, plant height was also measured to observe whether there are differences in both varieties in response to hypoxia. Results showed that, in control conditions, both varieties grew well whereby increased plant height was observed (Fig. 2). Submergence treatment from Week 1 till Week 3 showed a decrease in plant height was recorded for both varieties (Fig. 2). Decrease of plant height maybe due to the complete submergence treatment which resulted in deteriorate of plant growth where most of the plant parts were in the senescence phase which makes some of the leaves and stem detached from an intact plant. This situation influenced the plant height assessment which contributes to the decrease measurement of plant height. There was no significant difference in the plant height measurement for both varieties with  $p > 0.05$  which may indicate that both varieties have the same

response to submergence in terms of plant growth in response to flooding.

### 3.3 Root Architecture and Developmental Analysis

The growth of adventitious roots is part of the plant growth process and occurs naturally in almost all plants. In this study, the root growth architecture was also observed in order to analyze the growth and development of adventitious root in both varieties. Results indicated that there are differences in adventitious root growth between MR219 and *OsABP57*. The *OsABP57* showed more adventitious root growth as compared to MR219 even in normal condition without any treatment (Fig. 3a). However, after two weeks of complete submergence, there is no difference in adventitious root formation between *OsABP57* as compared to MR219 (Fig. 3b).

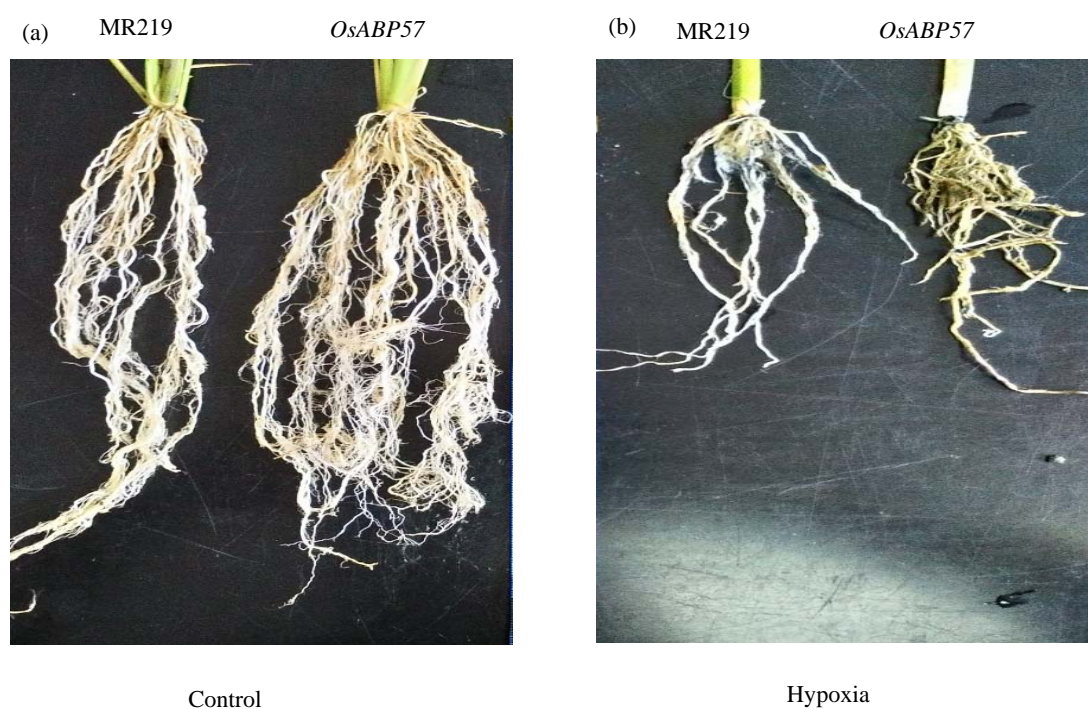


**Fig. 2** Graph analysis of plant height for both MR219 and *OsABP57* under normal condition and complete submergence treatment (Weeks 1, 2 and 3).

Data were shown from six independent biological experiments, and bars indicate  $\pm$  SDs.

\*, \*\*, \*\*\* indicate  $p > 0.05$  between varieties ( $p = 0.909$ ).

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**Fig. 3** Root architecture analysis of MR219 and *OsABP57* under (a) control, (b) hypoxia treatment.

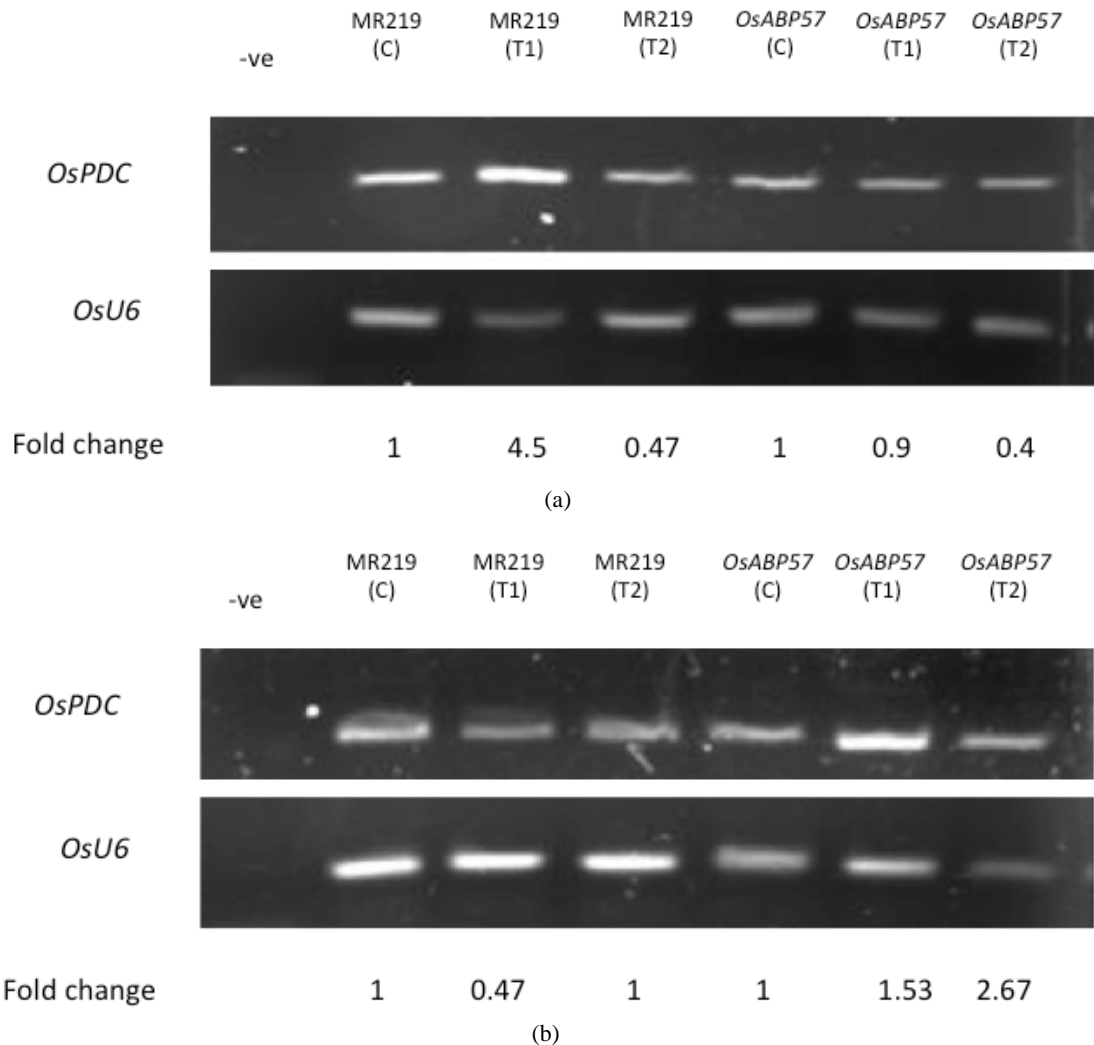
#### 3.4 Analysis of Hypoxia Core Gene in Both Varieties under Hypoxic Condition

In this study, the expression level of *OsPDC* gene in response to hypoxia in both varieties was compared. Densitometry analysis revealed that during Week 1 of complete submergence, the expression pattern of *OsPDC* in *OsABP57* transgenic treated plants was down-regulated as compared to *OsABP57* under normal condition (Fig. 4a). However, dissimilar pattern of *OsPDC* expression was observed in MR219 background where one of the biological replicates (replicate 1) showed a spike increase of *OsPDC* expression with 4.5 folds while the other biological replicates showed down-regulated expression by 0.47 folds. This descending expression pattern was the same as in the *OsABP57* treated sample (Fig. 4a). Interestingly, densitometry analysis carried out for Week 2 of complete submergence samples showed an increase of *OsPDC* gene expression in the *OsABP57* transgenic for both biological replicates with a fold change of 1.53 and 2.67, respectively (Fig. 4b). This,

however, unlike that of MR219, where one of the biological replicates showed reduced expression (0.47 folds) while other biological replicates showed no change in expression (one fold) as in the MR219 under normal condition. However, this expression is made through comparison in semi-quantitative PCR only, the precise expression measurement can be quantified through Real-Time PCR in absolute or relative expression.

#### 4. Discussion

Low oxygen state caused by flooding normally creates hypoxic or anoxic condition which can affect plant growth and development. There are several mechanisms of survival that have been studied such as efficient metabolic fermentation with limited oxygen supply as the plants are unable to undergo photosynthesis to synthesize food due to the lack of light perception [15]. Many studies have also focused on the roles of several hormones such as ethylene, however, quite limited information has been reported for auxin in response to flooding.



**Fig. 4** Expression pattern of *Oryza sativa* pyruvate decarboxylase (*OsPDC*) in both varieties during Week 1 (a) and Week 2 (b) under complete submergence compared to control condition.

“-ve” indicates PCR negative control; “MR219 (C)” indicates control condition; “MR219 (T1)” and “MR219 (T2)” indicate replicates 1 and 2. “*OsABP57* (C)” indicates control condition; “*OsABP57* (T1)” and “*OsABP57* (T2)” indicate replicates 1 and 2.

Densitometry analysis is determined by the qPCR. Gene expression is relative to the loading control gene from control plant (without treatment) and normalized to the internal constitutive gene. Fold change values were generated from normalized samples that were divided by the value of loading gene (control).

In this study, the effects of flooding on two different rice varieties of overexpressed *OsABP57* and MR219 local commercial rice was analyzed. This study aims to understand the roles of auxin in response to flooding by looking at the rice transgenic *OsABP57* performance under treatment. In addition, previous study has shown that transgenic *OsABP57* can tolerate and survive drought and saline conditions [13]. It is hypothesized that auxin may also impart

specific mechanisms during flooding. In this study, chlorophyll content between *OsABP57* and MR219 in the respective complete submergence treatments (Weeks 1, 2 and 3) showed no significant difference, indicating long period of complete submergence that causes chlorosis, senescence and reduction of chlorophyll content due to its decreased ability to absorb light. The increased concentration of ethylene in plant tissue has resulted in lower chlorophyll

content as compared to control rice [16, 17]. In this study, the plant height was also recorded to investigate water-escape attributes of *OsABP57* overexpressed rice. Unfortunately, results showed no significant difference in plant height between these two varieties inferring that none of these varieties have water-escape criteria in response to low oxygen state.

Another way to adapt to the flood situation is to create an adventitious root that has the aerenchyma structure to facilitate gaseous transport in the cell [18, 19]. Many plants respond to flooding or waterlogging by producing adventitious roots that develop into the soil or along the soil surface [20]. Unlike flooding, plants tend to grow longer primary root deep into the soil to scavenge for water sources [21] where all of the stored energy is transported into the root for growth and developmental purposes. However, the results in this experiment showed higher number of adventitious roots in overexpressed *OsABP57* line as compared to MR219 under normal condition but not in response to flooding. This infers that the alteration of *OsABP57* expression may have caused root architecture modification. Waterlogging condition caused by excessive water somehow has inhibited root growth and development in both varieties.

When plants are in a state of oxygen deprivation, several genes will be activated leading to glycolysis and ethanol fermentation [7]. The gene's expression is very dynamic and is regulated spatial-temporal as well as triggered by certain stimulus [22]. With the availability of hypoxia database, one of the hypoxia core genes in both varieties has been analyzed to observe the expression pattern. The qPCR results on *OsPDC* gene have shown an increase of expression during Week 2 of flooding treatment in *OsABP57* transgenic lines compared to MR219. Although there were no differences in morphophysiological analysis between these two rice varieties, the gene expression finding is interesting to look further in details to understand the alteration of *ABP57* gene in rice and its relationship to hypoxia core genes expression.

## 5. Conclusions

Abiotic stress affects plant growth and development either, physically or at the physiology and molecular level. Flooding which is one of the abiotic stresses causes oxygen deprivation to the plants. In this study, the effect of flooding on the overexpression transgenic of *OsABP57* in comparison to MR219 lowland commercial cultivar was reported. Data indicated that the *OsABP57* transgenics did not show any significant difference in terms of plant height and chlorophyll content compared to the MR219 local rice variety under flooding condition. This, however, is different from that of adventitious root results, where the alteration of *OsABP57* itself results in different root architecture even under normal growth. This relates to the main function of auxin in root development in terms of cell division and differentiation. Overall, it is concluded that the overexpression of *OsABP57* in rice has increased rice tolerance towards drought and salinity based on previous findings but not to flooding. Also, this indicates that the auxin roles particularly the *OsABP57* gene during plant response to water deficit might be different from that of flooding or low oxygen state.

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## References

- [1] Rahman, A. N. M. R., and Zhang, J. 2018. "Preferential Geographic Distribution Pattern of Abiotic Stress Tolerant Rice." *Rice* 11 (1): 10.
- [2] Cohen, S. P., and Leach, J. E. 2019. "Abiotic and Biotic Stresses Induce a Core Transcriptome Response in Rice." *Scientific Reports* 9: 6273.
- [3] Zhu, J. K. 2016. "Abiotic Stress Signaling and Responses in Plants." *Cell* 167 (2): 313-24.
- [4] Tamang, B. G., and Fukao, T. 2015. "Plant Adaptation to Multiple Stresses during Submergence and Following



- Desubmergence.” *International Journal of Molecular Sciences* 16 (12): 30164-80.
- [5] Fukao, T., Xu, K., Ronald, P. C., and Bailey-Serres, J. 2006. “A Variable Cluster of Ethylene Response Factor-Like Genes Regulates Metabolic and Developmental Acclimation Responses to Submergence in Rice.” *Plant Cell* 18 (8): 2021-34.
- [6] Hattori, Y., Nagai, K., Furukawa, S., Song, X. J., Kawano, R., Sakakibara, H., Wu, J., Matsumoto, T., Yoshimura, A., Kitano, H., Matsuoka, M., Mori, H., and Ashikari, M. 2009. “The Ethylene Response Factors SNORKEL1 and SNORKEL2 Allow Rice to Adapt to Deep Water.” *Nature* 460 (7258): 1026-30.
- [7] Mithran, M., Paparelli, E., Novi, G., Perata, P., and Loreti, E. 2014. “Analysis of the Role of the Pyruvate Decarboxylase Gene Family in *Arabidopsis thaliana* under Low-Oxygen Conditions.” *Plant Biology* 16 (1): 28-34.
- [8] Mustroph, A., Zanetti, M. E., Jang, C. J. H., Holtan, H. E., Repetti, P. P., Galbraith, D. W., Girke, T., and Bailey-Serres, J. 2009. “Profiling Translatomes of Discrete Cell Populations Resolves Altered Cellular Priorities during Hypoxia in *Arabidopsis*.” *Proceedings of the National Academy of Sciences* 106 (44): 18843-8.
- [9] Salehin, M., Li, B., Tang, M., Katz, E., Song, L., Ecker, J. R., Kliebenstein, D. J., and Estelle, M. 2019. “Auxin-Sensitive Aux/IAA Proteins Mediate Drought Tolerance in *Arabidopsis* by Regulating Glucosinolate Levels.” *Nature Communications* 10: 4021.
- [10] Kim, Y. S., Min, J. K., Kim, D., and Jung, J. 2001. “A Soluble Auxin-Binding Protein, ABP57: Purification with Anti-bovine Serum Albumin Antibody and Characterization of Its Mechanistic Role in the Auxin Effect on Plant Plasma Membrane H<sup>+</sup>-ATPase.” *The Journal of Biological Chemistry* 276 (14): 10730-6.
- [11] Palmgren, M. G. 2001. “Plant Plasma Membrane H<sup>+</sup>-ATPases: Powerhouses for Nutrient Uptake.” *Annual Review of Plant Physiology and Plant Molecular Biology* 52 (1): 817-45.
- [12] Takahashi, K., Hayashi, K. I., and Kinoshita, T. 2012. “Auxin Activates the Plasma Membrane H<sup>+</sup>-ATPase by Phosphorylation during Hypocotyl Elongation in *Arabidopsis*.” *Plant Physiol* 159 (2): 632-41.
- [13] Tan, L. W., Tan, C. S., Zuraida, A. R., Hossein, H. M., Goh, H. H., Ismanizan, I., and Zamri, Z. 2018. “Overexpression of *Auxin Binding Protein 57* from Rice (*Oryza sativa* L.) Increased Drought and Salt Tolerance in Transgenic *Arabidopsis thaliana*.” *IOP Conference Series: Earth and Environmental Science* 197 (1): 012038.
- [14] Weaver, D., Gopalakrishnan, K., and Joe, B. 2017. “Large-Scale Transcriptome Analysis.” In *Hypertension: Methods and Protocols*, edited by Touyz, R. M., and Schiffrin, E. L. Berlin: Springer Protocols, 1-26.
- [15] Voesenek, L. A. C. J., and Bailey-Serres, J. 2015. “Flood Adaptive Traits and Processes: An Overview.” *New Phytologist* 206 (1): 57-73.
- [16] Ceusters, J., and Van De Poel, B. 2018. “Ethylene Exerts Species-Specific and Age-Dependent Control of Photosynthesis.” *Plant Physiology* 176: 2601-12.
- [17] Ismail, A. M. 2018. “Submergence Tolerance in Rice: Resolving a Pervasive Quandary.” *New Phytologist* 218 (4): 1298-300.
- [18] Dawood, T., Rieu, I., Wolters-Arts, M., Derksen, E. B., Mariani, C., and Visser, E. J. W. 2014. “Rapid Flooding-Induced Adventitious Root Development from Preformed Primordia in *Solanum dulcamara*.” *AoB Plants* 6: 1-13.
- [19] Yamauchi, T., Colmer, T. D., Pedersen, O., and Nakazono, M. 2018. “Regulation of Root Traits for Internal Aeration and Tolerance to Soil Waterlogging-Flooding Stress.” *Plant Physiol* 176: 1118-30.
- [20] Visser, E. J. W., and Voesenek, L. A. C. J. 2004. “Acclimation to Soil Flooding-Sensing and Signal-Transduction.” *Plant and Soil* 274 (1-2): 197-214.
- [21] Guseman, J. M., Webb, K., Srinivasan, C., and Dardick, C. 2016. “DRO1 Influences Root System Architecture in *Arabidopsis* and *Prunus* Species.” *International Journal of Laboratory Hematology* 38 (1): 42-9.
- [22] Fukao, T., Barrera-Figueroa, B. E., Juntawong, P., and Peña-Castro, J. M. 2019. “Submergence and Waterlogging Stress in Plants: A Review Highlighting Research Opportunities and Understudied Aspects.” *Frontiers in Plant Science* 10: 340.