Effect of Submergence at the Tillering Stage on the Maximum Quantum Yield Efficiency and Leaf Gas Films of Thai Rice

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Abstract—The maximum quantum yield efficiency of PSII and leaf gas film production after 10 days of complete submergence at the tillering stage was compared among six Thai rice varieties. The treatments included two growth conditions (normal growing condition as control and complete submergence). The control group was treated with the conventional growing method in plastic pots, while the complete submergence was artificially simulated and transferred into plastic tubs at 15 days after transplanting with 1-meter depth of water. Compared with the control group, the submerged group showed significant increases in plant height. The flood tolerance variety, Chonlasit, presented a small increase, but there was a non-significant difference in plant elongation after submergence compared with susceptible varieties, PSL2, PTT, RD51, RD61, and KDML105. Chonlasit showed the greatest percentage of plant survival with 91.67%, while RD61 showed the lowest with 72.55%. Leaf gas films of all six varieties were observed after 6 hrs of submergence. Chonlasit and PSL2 presented much volume of leaf gas films in comparison with other varieties. There was little change in the maximum quantum yield efficiency of PSII (F_v/F_m) of the flood tolerance variety, Chonlasit. In contrast, the F_v/F_m value of susceptible varieties decreased significantly.

Index Terms—near-isogenic line, physical property, chemical property, bacterial blight

I. INTRODUCTION

Rice is the main staple and economic food crop in Thailand for national consumption and exportation. According to the FAO, global food production will need to increase by 70% by the year 2050 due to the rise in the world population [1]. Abiotic stresses, such as drought and flood are the major challenges which impact plants in many ecosystems worldwide, resulting in rice yield reduction [2], [3]. Submergence is a severe abiotic stress for rain-fed lowland rice. Rain-fed lowland rice often becomes submerged depending on the season. Different genotypes vary in submergence tolerance [4], [5]. Research has demonstrated that leaf gas film can promote submergence tolerance of rice [6], [7].

Leaves of submerged plants greatly restrict O_2 uptake from the surrounding environment, 10,000 times slower

in water than in the air [8]. Photosynthetic activity occurs during the day using dissolved CO_2 and produces O_2 by submerged leaves; whereas at night, the dissolved O_2 in water is the only source for photosynthesis [9], [10]. The term 'leaf gas film' has been used to describe the hydrophobic body surface trait that retains a thin layer of gas [7], [11]. Leaf gas film (LGF1) was identified as the responsive gene for leaf wax composition, epicuticular wax platelet abundance, surface hydrophobicity, and, therefore, leaf gas film retention in rice [12]. This trait is considered a mechanism to enhance tissue gas exchange with water [12]. In addition, leaf gas films have been demonstrated to improve underwater photosynthesis of rice, dark respiration, root pO2 and growth by greatly enhancing gas exchange of leaves under submerged conditions [7], [10]. Moreover, leaves with gas films might be able to keep stomata open when a plant is submerged, which would promote gas exchange [13].

Photosynthesis is the process that generates chemical energy from sunlight to support plant growth and development. Photosynthetic efficiency can be used as a key factor for determining plant resistance to various stresses. In recent years, chlorophyll fluorescence measurement has been wildly used to reveal photosynthetic efficiency without damaging plant tissue [14]-[17]. Generally, light energy captured by chlorophyll molecules is used in photochemical reactions to drive photosynthesis or, in the case of excess energy, it will dissipate as heat (non-photochemical quenching, NPQ) or emit chlorophyll fluorescence [18]. These three processes compete with each other; thus, by measuring the yield of chlorophyll fluorescence, the photosynthetic efficiency (photochemical reaction) and the degree of heat dissipation could also be estimated. Therefore, the current study aimed to investigate the ability of five Thai rice varieties to produce leaf gas film and compare the maximum photosynthetic efficiency among rice varieties under submergence conditions.

II. METERIAL AND METHODS

A. Plant Meterials and Growth Condition

Five rice accessions, including four Thai commercial accessions (RD61, PSL2, PTT1, KDML105 and RD51)

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and one flood tolerance accession (Chonlasit) were used in this study. Sterilized seeds were soaked in water at $37 \,^{\circ}$ C overnight to synchronize the germination. Germinated seedlings were transferred to a seedling tray with the soil water content maintained at 100%. The 21day-old seedlings were transferred into plastic pots 25.4 cm in diameter and 30 cm high containing 4 kg of clay soil with one plant per pot. The experiment was conducted in the greenhouse at Naresuan University, Thailand, from December 2018 to April 2019.

B. Characterization of Surface Gas Films, Flood Tolerance and Maximum Quantum Yield Efficiency

Flooding was simulated artificially in a plastic tub during the tillering stage (15 days after transplanting) with 1-meter depth of tap water. Three pots per accession were transferred into the submergence condition for 10 days. After flooding treatment, plant height and survival rate were recorded.

The two youngest fully expanded leaves of an individual plant were used to observe the formation of leaf gas film. Surface gas film can be observed by the naked eye as a silvery covering of the leaf. The observations were taken on three leaf segments of 50 mm length of the first leaf. The second leaf was used as a control by eliminating the leaf gas film formation by brushing with a dilution of Triton X (0.01% v/v of Triton X-100 in sterilized water). Measurements were taken at 0, 12 and 24 hours.

The youngest fully expanded leaves were measured for the maximum quantum yield efficiency before and after the flooding treatment. The maximum quantum yield efficiency was measured using PAR-FluoPen (Photon Systems Instruments, Czech Republic). The leaf sample was dark adapted for 30 min prior to measuring. The quantum vield efficiency of maximum PSII photochemistry F_v/F_m was calculated as $(F_m-F_0)/F_m$ [16], [18], where the minimum fluorescence (F_0) at open PSII centers was obtained by measuring light, while the maximum fluorescence (Fm) at closed PSII centers was determined after an application of a 0.8 s saturating light pulse (3,000 μ mol m⁻²s⁻¹) after being dark adapted for 30 min.

C. Statistical Analysis

Results were expressed as the mean of five replications \pm SE. Data were analyzed using one-way analysis of variance. Duncan's multiple range test at the 95% confidence level (p < 0.05) was used to compare the difference between genotypes. Statistical analysis was performed using the R software [19].

III. RESULTS

A. Plant Height, Elongation and %Survival under Submergence

Rice genotypes used in this study exhibited distinctively variable responses to the submergence for 10 days (Fig. 1a). Genotype KDML105 showed greater elongation with a highly significant difference in

comparison to before being submerged. The other genotypes: PSL2, PTT1, RD51, and RD61; however, exhibited medium elongation. Plant height did not increase much in the Chonlasit cultivar, resulting in a non-significant difference compared with before submergence. Survival percentage decreased in all the cultivars due to the imposition of 10 days of submergence treatment (Fig. 1b). Survival percentage of Chonlasit was 91.76% which was the highest among all cultivars. Survival percentage was significantly greater in Chonlasit in comparison to all cultivars. Among commercial cultivars, survival percentage was the least in RD61 (72.55%).



Figure 1. Plant height of 36 days-old rice plant of control and submergence condition for 10 days (a). Percentage of survival of six varieties after submergence for 10 days. The data represent average value ±SE from five replications.

B. Leaf Gas Films under Submergence

Six Thai commercial rice varieties were investigated for leaf gas film retention upon submergence (Fig. 2). The hydrophobic leaf blade surface presented immediately after submergence was seen as a silvery sheen on the leaf. Gas films on leaf blades of all six varieties could be observed during the first 6 hrs of submergence and lasted for 24 hrs. Chonlasit, RD51 and PSL2 genotypes presented greater volumes of gas films compared to other genotypes. Leaf gas films have been reported to be associated with leaf pubescence [12], [20]. Genotypes that produced much volume of gas films initially showed high values of F_v/F_m under submerged conditions. This result was similar to the study of Ref. [21]. They reported that leaves with gas films could potentially increase the carbohydrate status and increase underwater net photosynthesis, which contributes to submergence tolerance in rice. Leaf hydrophobicity and gas films on submerged leaves which related to the LGF1 gene have been confirmed to contribute to underwater photosynthesis, leading to flood tolerance in rice [12].



Figure 2. Comparison of the maximum quantum yield efficiency of PSII (F_v/F_m) between the control plants and submerged plants.

C. Changes in Chlorophyll Fluorescence under Submergence Conditions

The maximum quantum yield efficiency of PSII, as measured by chlorophyll fluorescence, was quantified for both before and after flooding treatment to determine how the submerged condition affected photosynthetic capacity in each variety. After 10 days of submergence, the overall performance of photosystem II (PSII) was comparatively lower compared to the control condition. As shown in Table I, the changes in the maximum quantum yield efficiency of PSII were greater in the control group compared to the submergence group. A varietal difference was observed in Fv/Fm under the control condition which suggested that the photosynthetic potential varies in nature by genotype and could be genetically regulated. The F_v/F_m values of all varieties declined at significantly different rates under the submergence condition except for Chonlasit, RD61 and KDML105 (Fig. 3). $F_{\nu}\!/F_m$ values of the three varieties: PSL2, PTT1, and RD51 decreased significantly under the submergence condition. Submergence at the tillering stage which resulted in a decrease in the maximum quantum yield efficiency of PSII can cause a reduction in yield and yield components of rice [22].



Figure 3. Leaves of six rice varies which present gas film formation at 24 hrs after complete submergence.

TABLE I.	THE MAXIMU	M QUANTUM	YIELD EFF	ICIENCY C	OF PSII (F _v /F	м)
OF CON	JTROL PLANTS	AND SUBMER	GED PLANT	'S OF SIX '	VARIETIES	

Variety/Treatment	Control	Submergence	
	Fv/Fm	Fv/Fm	
Chonlasit	0.73±0.02 ^a	0.68±0.04	
PSL2	0.73±0.01ª	0.58±0.11	
PTT1	0.73±0.04ª	0.59±0.08	
RD51	0.70±0.02 ^{ab}	0.64±0.07	
RD61	0.71±0.06 ^a	0.68±0.02	
KDML105	0.69±0.05 ^b	0.68±0.01	
Mean	0.71	0.65	
F-test			
Variety	*	ns	
CV%	4.86	9.77	

Means in the same column flowed by \pm SD

IV. DISCUSSION

To better understand the potential genetic resources for submergence tolerance in Thai commercial rice varieties, chlorophyll fluorescence parameters were measured together with the ability of the rice leaves to maintain gas films on leaf blades. Rice leaves retained a visible gas film layer upon submergence. The occurrence of hydrophobic leaves with gas film retention in different rice varieties is an important trait for rice to survive by during submergence continuing underwater photosynthesis [12]. There was a slight but nonsignificant decreased in the Fv/Fm value of Chonlasit, which has been reported to have flash flooding tolerance and non-photoperiod sensitivity [23]. This suggests that the tolerance mechanism in this variety might support the underwater photosynthesis which is linked to the high percentage of survival in comparison to other varieties. Unlike the high value of F_v/F_m after the submergence of RD61 and KDML105, this was because of the adaptation mechanism in these two varieties as seen in the high rate of plant elongation. The decrease of F_v/F_m in susceptible varieties, such as PSL2, PTT1, and RD51 suggests that using the chlorophyll fluorescence analysis to measure the maximum quantum yield efficiency of PSII is a promising method to determine plant tolerance in flood conditions.

V. CONCLUSION

Although submergence condition leads to plant elongation, the maximum quantum yield efficiency of PSII and leaf gas films were presented different responses to the period of submergence in rice at the tillering stage. Our results demonstrate that rice varieties responded differently in both control and submergence conditions. Tolerate and adapted varieties showed a small reduction of F_v/F_m and the greater amount of leaf gas film formation which help plants to cope with submergence. These results should accelerate our understanding of parameters involved in tolerance and adaptation of Thai rice varieties to excess water stress.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest and all ideas reflected in this manuscript have been agreed upon.

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