Oxidative Stress and its Relation to Radiation Use Efficiency in Rice Growing under Rainfed Condition in Northeast Thailand

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Abstract

In rainfed cultivation of rice, the scarcity of available water often inhibits plant growth. Under such conditions, the drought induces oxidative stress which damages to photosynthetic cells such as protein degradation, lipid peroxidation and enzyme inactivation. In this study, we evaluate oxidative damage for 10 rice genotypes under rainfed condition in relation to radiation use efficiency (RUE). Although the drought stress was relatively mild as shown in the slightly decreases of stomatal conductance and leaf water potential, RUE under rainfed condition was significantly decreased from that under flooded condition. The oxidative damages evaluated by chlorophyll fluorescence and lipid peroxidation were also increased under rainfed condition. The genotypic variation of RUE in both water conditions was negatively correlated to the value of midday Fv/Fm and it was thought to be affected by the canopy structure of each genotypes. Damage of leaf cells evaluated by membrane stability index under rainfed condition was not significantly different from that under flooded condition, but the larger photoinhibition and lipid peroxidation seems to cause severe cell damage when the water scarcity continues for long term.

Keywords: rice, photoinhibition, chlorophyll fluorescence, drought, oxidative stress

Introduction

Plant oxidative stress is induced by various abiotic stresses and damages to leaf cells (Mittler, 2002). Under drought and high light conditions, decreasing of energy use for Calvin cycle leads to over excitation of energy which causes generation of reactive oxygen species (ROS) and promote oxidative damage to photosynthetic cells (Murata *et al*., 2007). Oxidation of cell membrane increases water leakage and decreases leaf water potential. In addition, severe oxidative stress is thought to be a cause of metabolic limitation in Calvin cycle, inducing non-stomatal inhibition of photosynthesis (Lawlor, 2002). Drought is an increasingly important constraint of rice production: the scarcity of water resource for agriculture provides the opportunity for rice to have water deficit even for irrigated production as well as for rainfed production. However, the relation between oxidative stress and drought stress has not been examined in terms of crop response under field conditions with various environmental stresses.

To clarify the relationship between drought and oxidative stress under field condition, we firstly focused oxidative stress of rice in relation to plant water status. Effect of the oxidative stress on plant growth was evaluated in relation to RUE.

Materials and Methods

Field growth conditions

Ten rice genotypes from Surin1 backcross introgression lines with IR68586-FA-CA-143 (BC_3) were used. 3 weeks seedlings were transplanted with the plant density of 25 plants m^{-2} in rainfed and flooded field on 12th August 2010 at Ubon Rice Research Center, Northeast Thailand. Fertilizer was applied with 3-3-1.5 g m^{-2} of N-P_2O_5-K_2O for each field. The items below were measured from 2 to 9 weeks after transplanting.

Radiation use efficiency

Total shoot dry weight was measured at 2 and 9 weeks after transplanting, and the difference of dry weight was defined as total shoot growth. Radiation use efficiency (RUE) was calculated as RUE (g MJ^{-1}) = (total shoot growth)/(total intercepted solar radiation). To determine canopy light absorbance rate (kc), photosynthetic active radiation (PAR) at the top and bottom of the canopy in each plot was measured with a linear PAR intercept-meter (AccuPAR, Decagon, USA) under diffused solar radiation conditions once every week. The weekly accumulation of incident solar radiation was multiplied by the weekly value of kc, and summarized during the period as total intercepted solar radiation..

Chlorophyll fluorescence, gas conductance (gs) and leaf water potential (LWP)

Maximum quantum yield (Fv/Fm) and effective quantum yield (Φ_{PS2}) of photosystem Ⅱ was measured by chlorophyll fluorometer (OS-30p, Opti Science, USA) in early morning and midday of a clear sunny day every week. The topmost fully expanded leaves were used for the measurement. Simultaneously, gs and LWP of the topmost fully expanded leaves were also measured.

Membrane stability and lipid peroxidation

The topmost fully expanded leaves were sampled at 8 weeks after transplanting and used for the following analyses. The leaf membrane stability index (MSI) was determined according to Sairam and Saxena (2000). Leaf discs were soaked in distilled water at 40 °C for 60 min and its electrical conductivity recorded (C1). Subsequently the same samples were kept in boiling water for 10 min and its electrical conductivity also recorded (C2). MSI was calculated as MSI (%) = [1–(C1/C2)] × 100.

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation. Fresh leaf sample was homogenized in 20% trichloroacetic acid (TCA). The homogenate was centrifuged at 12000 G for 10 min. The 0.5 ml aliquot of the supernatant was added to 1.5 ml of 0.5% thiobarbituric acid in 20% TCA. The mixture was heated at 95°C for 30 min and then quickly cooled in ice. After centrifugation at 3000 G for 10 min, the absorbance of the supernatant was measured at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The MDA content was calculated using its extinction coefficient of 155mM^{-1} cm^{-1} and expressed as nmol MDA g^{-1} fresh weight.

Results and Discussion

Most genotypes decreased their total shoot growth under rainfed condition from that under flooded condition. The smaller shoot growth under rainfed condition was mainly caused by the lower RUE (Figure 1). The Surin1 which showed higher RUE under flooded condition also showed higher RUE under rainfed condition. Another parent line, IR68586-FA-CA-143 showed lower RUE under both water conditions (Table 1). However, except these two parent genotypes, the relationship of RUE between rainfed and flooded condition was not obvious. It implies that the effect of water limitation on RUE was different among genotypes. In the theory, RUE can be explained as the function of canopy photosynthesis (Sinclair and Horie, 1989, Reynolds *et al*., 2000). In terms of single leaf, photosynthetic rate is closely related with stomatal conductance, which is governed by LWP (Medrano *et al*., 2002). The slightly but significantly lower value of gs and LWP under rainfed condition suggested that mild drought stress was occurred (Table 1). The drought stress might decrease RUE.

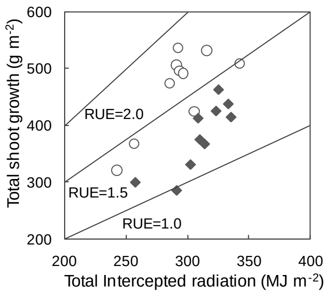


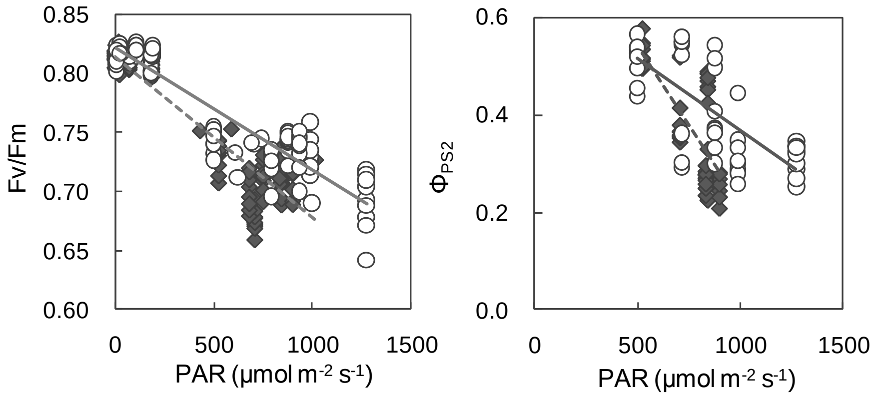
Figure 1. Genotypic variation in total shoot growth, total intercepted solar radiation and RUE under rainfed and flooded conditions. Open circle and closed diamond represents the genotype grown under flooded and rainfed condition, respectively. Data are average of three replicates.

Table 1. Radiation use efficiency, stomatal conductance and leaf water potential of 10 genotypes under flooded and rainfed conditions

	RUE (g MJ $^{-1}$)		g_s (mmol m^{-2})		LWP (Mpa)	
	Flooded	Rained	Flooded	Rained	Flooded	Rained
IR68586-FA-CA-143	1.42	0.97	218	184	-0.796	-0.836
Surin 1	1.83	1.34	214	224	-0.797	-0.864
IRUBN030055-5-87	1.38	1.43	239	244	-0.808	-0.906
IRUBN030055-5-112	1.73	1.17	261	192	-0.808	-0.856
IRUBN030055-5-190	1.68	1.25	286	230	-0.819	-0.853
IRUBN030056-10-42	1.49	1.32	280	220	-0.789	-0.850
IRUBN030056-10-107	1.65	1.32	229	189	-0.822	-0.847
IRUBN030062-1-9	1.68	1.20	249	222	-0.806	-0.864
IRUBN030063-9-4	1.33	1.14	311	229	-0.847	-0.847
IRUBN030070-9-32	1.66	1.09	229	194	-0.861	-0.861
Average	1.59	1.22	252	213	-0.803	-0.858
Genotype (G)	*		ns		ns	
Water condition (E)	***		***		***	
G x E	*		ns		ns	

Data are average of three replicates. * and ** represent statistically significant at 0.05, 0.01 level, and ns represents not significant.

The water limitation, even mild drought, decreases carbon fixation of rice and induces oxidative damage (Zhou *et al*., 2007). Measurement of chlorophyll fluorescence can easily evaluate photosynthetic capacity, and is widely applied for environmental stresses studies. The maximum quantum yield of photosystem Ⅱ (Fv/Fm) is an indicator of photoinhibition which relate to oxidative stress (Maxwell and Johnson, 2000). Generally, Fv/Fm decreases with increasing of exposed light intensity at midday. Decrease in Fv/Fm by high PAR was larger under rainfed condition than that under flooded condition (Figure 2). The same tendency was observed for the effective quantum yield (Φ_{PS2}) but the difference was not significant between rainfed and flooded conditions. It is known that the carbon fixation rate is closely related to the value of Φ_{PS2} under the environment of constant CO_2 concentration (Kato *et al*., 2003). Decrease of Φ_{PS2} induces over reduction in electron transport chain and increases oxidative damage. The lower value of Fv/Fm under rainfed condition is thought to be resulted by increased oxidative damage that derived from inhibition of carbon fixation. The genotypic variation in Fv/Fm and Φ_{PS2} was not significant, because of large residual variation in this study.



Open circle and closed diamond represents the genotype grown under flooded and rainfed condition, respectively. Data are average of four replicates.

Figure 2. Decrease of Fv/Fm and Φ_{PS2} with increasing of PAR in midday. Fv/Fm was measured in early morning and midday, and Φ_{PS2} was measured only in midday.

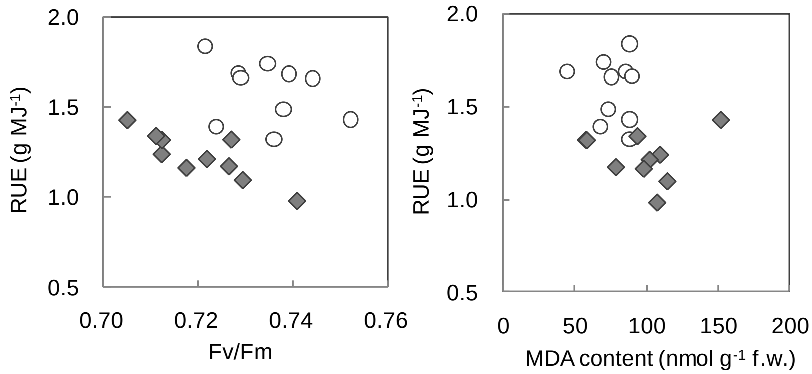
MDA content was higher under rainfed condition, indicating that damage of cell membrane by reactive oxygen species was larger under rainfed than that under flooded condition (Table 2). On the other hands, difference of MSI, another indicator of membrane damage, was not significant between the two water conditions. MDA is a product of lipid of peroxidation, while MSI indicates water leakage from cell induced by oxidative damage to cell membrane (Mittler, 2002). Accordingly, the result in this study may suggest that lipid peroxidation proceeded under rainfed condition but the damage was alleviated by antioxidation mechanisms.

Table 2 Malondialdehyde content and membrane stability index of 10 genotypes under flooded and rainfed conditions

	MDA (nmol g^{-1} f.w.)		MSI (%)	
	Flooded	Rainfed	Flooded	Rainfed
IR68586-FA-CA-143	88.4	107.3	81.7	76.1
Surin 1	88.3	93.5	81.5	76.2
IRUBN030055-5-87	67.7	151.9	79.7	78.6
IRUBN030055-5-112	70.2	78.4	77.8	75.7
IRUBN030055-5-190	85.5	109.3	75.7	77.1
IRUBN030056-10-42	73.1	57.2	79.6	76.0
IRUBN030056-10-107	75.3	58.0	78.5	79.0
IRUBN030062-1-9	44.2	102.0	82.3	80.7
IRUBN030063-9-4	88.1	97.8	83.1	78.1
IRUBN030070-9-32	89.6	114.5	76.5	80.0
Average	77.0	97.0	79.7	77.7
Genotype (G)	***		ns	
Water condition (E)	***		ns	
G x E	***		ns	

Data are average of three replicates. * and ** represent statistically significant at 0.05, 0.01 level, and ns represents not significant.

Drought stress decreases RUE and Fv/Fm, implying the RUE was positively correlated with Fv/Fm. However, the relationships for each water condition showed negative correlations (Figure 3). This suggests that the genotypes which showed larger canopy photosynthesis were suffered with larger photoinhibition. Because RUE is not determined only by single leaf photosynthesis but also canopy structure, further study is necessary to reveal the effect of photoinhibition on plant production.



Open circle and closed diamond represents the genotype grown under flooded and rainfed condition, respectively. Fv/Fm is the average of three measurement days from sixth to eighth weeks after transplanting.

Figure 3. Relationships of RUE to Fv/Fm and to MDA content.

In this study, oxidative stress in rice was evaluated under field environment of rainfed condition during wet season where drought stress was relatively mild. The drought stress decreased RUE and, increased photoinhibition and lipid peroxidation. The results suggest that the oxidative stress was associated with dry matter production. To elucidate the relation between drought and oxidative stress and to apply it for breeding program of drought tolerance, further studies are necessary.

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