Leaf Photosynthetic Activity and Antioxidant Defense Associated with Sub1 QTL in Rice Subjected to Submergence and Subsequent Re-aeration

Debabrata PANDA, Ramani Kumar SARKAR
(Division of Biochemistry, Plant Physiology and Environmental Sciences, Central Rice Research Institute, Cuttack-753 006, Orissa, India; * Present address: Rubber Research Institute of India Tura, Meghalaya-794 001, India)

Abstract: The influence of submergence on photosynthesis and antioxidant capacities in rice varieties Swarna and Swarna-Sub1 with or without Sub1 QTL were evaluated under control, simulated complete submergence and subsequent re-aeration. The leaf photosynthetic rate and stomatal conductance decreased in both varieties during the progression of submergence as compared to the control plants, but significant varietal differences were observed after 1 d of submergence. Submergence also altered the PSII activity, as reflected in a decrease in the values of $F_0$, $F_m$ and $F_v/F_m$ and degradation of chlorophyll, more in Swarna than in Swarna-Sub1. During early submergence period, the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR) against reactive oxygen species were increased in both varieties. However, with the progress of submergence period (after 7 d), the activities of SOD, catalase (CAT), APX, guaiacol peroxidase (GPX), GR and DHAR declined, more in Swarna than in Swarna-Sub1. During re-aeration, Swarna-Sub1 showed significant increase of above antioxidant enzymes but not in Swarna. Swarna-Sub1 improves photosynthetic activity, showing higher photosynthetic rate compared to Swarna under submergence and subsequent re-aeration because of less degradation of chlorophyll, higher stomatal conductance, and efficient PSII activity along with better antioxidant protection from oxidative damage.

Key words: antioxidant; chlorophyll fluorescence; photosynthesis; submergence tolerance

Submergence due to flash floods imposes a complex abiotic stress and major stress constraint to rice production, especially in rainfed lowland areas of the tropics (Sarkar et al, 2006; Bailey-Serres and Voeselek, 2008), and the extent of injury caused by complete submergence is largely dependent on floodwater conditions, particularly its temperature, turbidity and the extent of light penetration (Das et al, 2009). When rice plants are subjected to flash floods, they should adapt themselves to two drastic environmental changes: the changes from aerobic to hypoxic condition during complete submergence and the subsequent changes from hypoxic to aerobic condition when the flood water recedes. The visual damage caused by the submergence is generally not apparent immediately but develops soon after the water level recedes after complete submergence (Drew, 1997). At this point, plants are suddenly exposed to a completely different environment that is characterized by higher $O_2$ level and light intensity compared to the submerged condition. Light stress does not result from the high light intensity per se but from the absorbed light in excess of that used for the photosynthesis. It is generally assumed that submergence causes the closure of stomata and impairs gas exchange and $CO_2$ assimilation, further it induces photo bleaching of chlorophyll due to oxidative stress (Ella et al, 2003; Panda et al, 2008). Flooding/submergence and re-oxygenation can induce oxidative stress, causing an increased production of reactive oxygen species (ROS) (Ella et al, 2003; Fukao et al, 2011). The ROS acts as a cellular indicator of submergence stress and as secondary messenger involved in the stress response signal transduction pathway (Fukao and Bailey-Serres, 2004). Plants have active oxygen-scavenging systems consisting of several antioxidant enzymes. Among all the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR) and dehydroascorbate reductase (DHAR) play key roles in protecting plants from oxidative stress damage (Damanik et al, 2010).

Submergence tolerance in rice is physiologically complex but genetically simple (Xu et al, 2006). The Indian cultivar FR13A is the most widely studied and used source of submergence tolerance in rice breeding. A major QTL, designated Sub1, was identified on rice chromosome 9 and it controls most of the submergence tolerance of this genotype (Xu and Mackill, 1996). Sub1 was subsequently fine-mapped and cloned, and three genes encoding putative ethylene responsive factors (ERF), Sub1A, Sub1B and Sub1C, were...
identified, with Sub1A recognized as the primary determinant of submergence tolerance (Xu et al., 2006). Moreover, gene-based markers were designed for Sub1 and used for its successful introgression into popular high-yielding rice varieties (Neeraja et al., 2007; Septiningsih et al., 2009). Subsequent testing of introgression lines in the fields showed no apparent effects on agronomic performance, grain yield, or quality in the absence of submergence (Sarkar et al., 2006; Neeraja et al., 2007), but no report is available about its photosynthetic efficiency and antioxidant defense mechanism associated with Sub1 QTL during and after submergence. Recently, the Sub1 QTL has been introgressed into a highly submergence susceptible variety Swarna, and Swarna-Sub1, which has been the most popular Sub1 variety to date, has been well accepted by farmers (Reddy et al., 2010).

The present study is an effort to characterize photosynthetic efficiency and antioxidative defense in rice plants either possessing Sub1 QTL or not, i.e. Swarna-Sub1 and Swarna on the basis of measuring leaf gas exchange parameters, chlorophyll fluorescence and some selected antioxidant enzymes during control, different days after complete submergence and 1 d subsequent re-aeration. The study will be helpful to know whether the introgression of Sub1 QTL improves the photosynthesis and antioxidant properties in rice or not under submergence.

**MATERIALS AND METHODS**

**Rice materials and growth conditions**

The experiment was conducted with two indica rice (*Oryza sativa* L.) varieties: Swarna and Swarna-Sub1. Swarna-Sub1 is a popular rice variety developed through the marker-assisted backcrossing approach (Reddy et al., 2010). Seeds were sown directly in earthen pots containing 2 kg of farm soil and farmyard manure (3:1). Each pot was supplied with 80 mg urea, 192 mg single super phosphate (P2O5) and 70 mg murate of potash (K₂O). Plants were grown in a greenhouse subjected to natural solar radiation, with daily maximum photosynthetic photon flux density, air temperature and relative humidity being about 1660 µmol/(m²·s), 32.6 °C and 70%–75%, respectively. Fourteen-day-old seedlings were submerged in a concrete tank for 7 d filled with water to a height of 110 cm, so that at least 50 cm of water column remained above the top of the plants. This complete submergence imposed severe stress on the plants. The plants were studied in six treatments, i.e. 1, 3, 5, 7 d after complete submergence, complete submergence for 7 d followed by aeration for 1 d, and control growth condition (without submergence treatment). For re-aeration, the height of water column was brought down to 10 cm from the level of 110 cm. The experiments were carried out in three replications and were statistically analyzed.

The characteristics of the floodwater in terms of light transmission (%) were measured at 12:00 am (LI-COR, Lincoln, USA), and water temperature and oxygen concentration were determined at 06:00 am and 17:00 pm (Syland, Heppenheim, Germany), respectively. Light intensity at 60 cm water depth or at the vicinity of canopy level ranged from 215 to 319 µmol/(m²·s), whereas it was 1743 to 1812 µmol/(m²·s) above the water surface. The oxygen concentration at the same water depth was 2.5 to 3.1 mg/L at 06:00 am and 4.6 to 5.8 mg/L at 17:00 pm. The temperature did not vary much (about 4 °C), being 26.6 to 30.7 °C throughout the period of the experiment. **Measurement of photosynthetic rate, chlorophyll fluorescence and chlorophyll content**

Measurements of photosynthetic rate and stomatal conductance were made on the fully expanded leaves of five different plants within 30 min at the end of submergence treatment using an open system photosynthetic gas analyzer (PP Systems, USA) under normal ambient environmental conditions. The second and the third leaves from the top were selected and kept inside the chamber under natural irradiance until stable reading was recorded.

After measuring the photosynthetic rate, the same leaves were used for the measurement of chlorophyll fluorescence using a Plant Efficiency Analyzer, Handy PEA (Hansatech Instruments Ltd., Norfolk, UK). Leaves were maintained in darkness for 20 min before taking the data on chlorophyll fluorescence. The maximal intensity of the light source, providing an irradiance saturating pulse of 3 000 µmol/(m²·s) was used. Different chlorophyll fluorescence parameters like minimal fluorescence (Fₒ), maximal fluorescence (Fₘ), variable fluorescence (Fᵥ = Fₘ−Fₒ), maximum photochemical efficiency of PSII (Fᵥ/Fₘ) were calculated using the software supplied by the manufacture. After measuring the photosynthetic rate and chlorophyll fluorescence characteristics, the same leaves were used for the determination of chlorophyll content, which comprised both chlorophyll a and chlorophyll b. One hundred milligrams of finely chopped fresh leaves were placed in a capped measuring tube containing 25 mL of 80% acetone, and placed inside a
refrigerator (4 to 8 °C) for 28 h (Panda et al, 2008). The chlorophyll content was determined spectrophotometrically following Porra (2002).

Antioxidant enzyme activity

Leaf sample weighing 500 mg was homogenized in 10 mL of 50 mmol/L potassium phosphate buffer (pH 7.8) containing 1 mmol/L EDTA, 1 mmol/L ascorbate, 10% sorbitol and 0.1% triton X-100. The homogenate was centrifuged at 4 ºC at 15 000 × g for 20 min and the supernatant was used for enzyme analysis and native PAGE activity staining. All operations were performed at 0–4 ºC (Sarkar et al, 2001). Protein content was measured following Lowry et al (1951).

Superoxide dismutase (SOD, EC 1.15.1.1) was measured by the photochemical method described by Gianopolitis and Ries (1977) with modifications suggested by Choudhury and Choudhury (1985). One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of the rate of nitro blue tetrazolium chloride reduction at 560 nm. Catalase (CAT, EC 1.11.1.6) activity was measured in a reaction mixture containing 25 mmol/L phosphate buffer (pH 7.0), 10 mmol/L H₂O₂ and the enzyme extract. The decomposition of H₂O₂ was followed at 240 nm (Cakmak and Marschner, 1992). Ascorbate peroxidase (APX, EC 1.11.1.11) was assayed following Nakano and Asada (1981) by monitoring the rate of ascorbate oxidation at 290 nm [E = 2.8 mmol/(L·cm)]. The reaction mixture contained 50 mmol/L potassium phosphate buffer (pH 7.0), 0.1 mmol/L EDTA, 100 mmol/L H₂O₂ and 0.5 mmol/L ascorbic acid and the enzyme aliquot. The activity of guaiacol peroxidase (GPX, EC 1.11.1.7) was assayed following the method of Rao et al (1995). The reaction mixture contained 50 mmol/L phosphate buffer (pH 7.0), 0.1 mmol/L guaiacol, 0.1 mmol/L H₂O₂ and the enzyme aliquot. Enzyme activity was measured by the increase in absorbance at 470 nm caused by guaiacol oxidation [E = 26.6 mmol/(L·cm)]. Glutathione reductase (GR, EC 1.6.4.2) was assayed according to the method of Foyer and Halliwell (1976) by following the decrease in absorbance at 340 nm caused by NADPH oxidation [E = 6.2 mmol/(L·cm)]. Dehydroascorbate reductase (DHAR, EC 1.8.5.1) was assayed following Nakano and Asada (1981) by measuring the increase in absorbance at 265 nm.

Native PAGE activity staining of antioxidant enzymes

Native PAGE was performed on the crude extract using a stacking gel containing 4.5% acrylamide and a separating gel containing 7.5% acrylamide with a running buffer composed of 4 mmol/L Tris-HCl, pH 8.8 and 38 mmol/L glycine. In each treatment, 100 mg protein extract was loaded in the gel.

For SOD activity staining, the gel was incubated in 50 mmol/L Na-phosphate buffer, pH 7.5 (100 mL), containing 200 mg of NBT at 30 °C for 20 min in the dark. Then drain off the solution and incubate gel in another solution containing 50 mmol/L Na-phosphate buffer (pH 7.5), 0.4 mL of TEMED and 1 mg of riboflavin for about 15 min in the 40 V fluorescent tubes (two in number) at room temperature for development of acromatic bands (Beauchamp and Fridovich, 1971). For CAT activity staining, the gel was incubated in the solution containing 0.1% H₂O₂ for 5 min. Then the solution was drained off and the gel was placed in another solution containing 0.5% potassium ferricyanide and 0.5% ferric chloride for 10 min. The enzyme or zymogram was recorded as soon as the achromatic bands became evident according to Thorup et al (1961). For GPX activity staining, the gel was stained in 100 mL solution containing 0.5 mmol/L guaiacol and 2 mL of 30% H₂O₂ for about 5−10 min. The brownish bands appeared in the gel followed by Rao et al (1995). For APX activity staining, the gel was incubated in the solution with 100 mL of potassium phosphate buffer (50 mmol/L, pH 6.0), 4 mmol/L ascorbic acid and 0.1 mmol/L hydrogen peroxide for 15 min, then washed with distilled water and incubated in another solution with 100 mL of 0.125 mol/L HCl, 0.1% of potassium ferricyanide, 0.1% of ferric chloride for 10 min. APX was located as an achromatic bands on a coloured background (Tommasi et al, 2001).

Statistical analysis

Differences between various photosynthetic parameters and antioxidant enzymes were compared by ANOVA using IRRISTAT (International Rice Research Institute, Philippines) software’s least significant difference (LSD, P < 0.05), as it is a good test for determining whether means were significantly different. Correlation analysis was conducted following the standard procedure using IRRISTAT.

RESULTS

Survival under submergence

The extent of visible injury caused by flooding was
Chlorophyll content, photosynthetic rate, stomatal conductance and chlorophyll fluorescence measurements were used as an indicator of the sensitivity of a plant to submergence. In this experiment, two varieties gave distinctly different responses to submergence in terms of survival. Tolerant rice variety Swarna-Sub1 showed 90% survival after 7 d of submergence whereas it was less than 15% in Swarna (data not shown).

### Chlorophyll content, photosynthetic rate, stomatal conductance and chlorophyll fluorescence

Submergence resulted in significant reduction of chlorophyll content both in Swarna and Swarna-Sub1 rice varieties (Table 1). After 7 d of submergence, the percentage of reduction in chlorophyll content was greater in Swarna (76%) than in Swarna-Sub1 (56%) compared to the respective control plants. During subsequent re-aeration for 1 d, Swarna-Sub1 maintained significantly higher chlorophyll content than Swarna (Table 1). The main cause of variance proved to be the treatment with 91% of total variance, followed by the variety (8%) and the variety × treatment interaction for each parameter (Table 2).

The leaf photosynthetic rate decreased in both varieties during the progression of submergence as compared to control plants, but significant varietal differences were observed after 1 d of submergence. After 7 d of submergence, there was a substantial reduction in photosynthetic rate with maximum reduction in Swarna (96%) than Swarna-Sub1 (87%) in comparison to non-submerged control plants (Table 1). Recovery level after 1 d re-aeration varied among the varieties and Swarna-Sub1 maintained greater photosynthetic rate compared to Swarna. This parameter was greatly affected by treatment, which accounted for 86% of total variance (Table 2).

Stomatal conductance found to be significantly decreased in both varieties during the progression of submergence compared to control plants (Table 1). After 7 d of submergence, stomatal conductance decreased to 94% and 90% in Swarna and Swarna-Sub1 respectively compared to the control plants. Stomatal conductance found to be significantly higher

### Table 1. Changes in leaf chlorophyll content, photosynthetic rate, stomatal conductance, minimal (\(F_o\)) and maximal (\(F_m\)) fluorescence along with maximum photochemical efficiency of PSII (\(F_v/F_m\)) in Swarna and Swarna-Sub1 rice varieties under control, different days after complete submergence and 1 d re-aeration treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chlorophyll content (mg/g)</th>
<th>Photosynthetic rate ([\mu \text{mol/(m}^2\text{·s)]}</th>
<th>Stomatal conductance ([\mu \text{mol/(m}^2\text{·s)]}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Swarna</td>
<td>Swarna-Sub1</td>
<td>Swarna</td>
</tr>
<tr>
<td>C</td>
<td>2.88</td>
<td>2.38</td>
<td>33.8</td>
</tr>
<tr>
<td>1d</td>
<td>2.33</td>
<td>2.33</td>
<td>13.3</td>
</tr>
<tr>
<td>3d</td>
<td>2.25</td>
<td>2.00</td>
<td>9.1</td>
</tr>
<tr>
<td>5d</td>
<td>1.30</td>
<td>1.70</td>
<td>6.0</td>
</tr>
<tr>
<td>7d</td>
<td>0.71</td>
<td>1.05</td>
<td>1.4</td>
</tr>
<tr>
<td>1dA</td>
<td>0.52</td>
<td>1.02</td>
<td>1.5</td>
</tr>
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</table>

LSD (\(P < 0.05\)) 0.25 0.9 22

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(F_o) (relative)</th>
<th>(F_m) (relative)</th>
<th>(F_v/F_m) (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Swarna</td>
<td>Swarna-Sub1</td>
<td>Swarna</td>
</tr>
<tr>
<td>C</td>
<td>283</td>
<td>280</td>
<td>1.422</td>
</tr>
<tr>
<td>1d</td>
<td>285</td>
<td>287</td>
<td>1.360</td>
</tr>
<tr>
<td>3d</td>
<td>283</td>
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<td>246</td>
<td>0.596</td>
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<tr>
<td>7d</td>
<td>175</td>
<td>206</td>
<td>0.463</td>
</tr>
<tr>
<td>1dA</td>
<td>160</td>
<td>203</td>
<td>0.464</td>
</tr>
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LSD (\(P < 0.05\)) 23 75 0.025

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variety</th>
<th>Treatment</th>
<th>Variety + Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>0.637** (4)</td>
<td>18.193** (91)</td>
<td>1.187** (6)</td>
</tr>
<tr>
<td>PR</td>
<td>50.95** (12)</td>
<td>403.32** (86)</td>
<td>11.36** (3)</td>
</tr>
<tr>
<td>SC</td>
<td>25 711.1** (8)</td>
<td>339 435.0** (86)</td>
<td>3 546.8 (6)</td>
</tr>
<tr>
<td>(F_o)</td>
<td>1 965** (5)</td>
<td>66 358** (86)</td>
<td>4 478** (9)</td>
</tr>
<tr>
<td>(F_m)</td>
<td>176 680** (25)</td>
<td>461 949** (63)</td>
<td>117 340** (13)</td>
</tr>
<tr>
<td>(F_v/F_m)</td>
<td>0.0137** (12)</td>
<td>0.1196** (80)</td>
<td>0.0855** (8)</td>
</tr>
<tr>
<td>SOD</td>
<td>841.0** (7)</td>
<td>14 076.2** (90)</td>
<td>460.3** (3)</td>
</tr>
<tr>
<td>CAT</td>
<td>5.6169** (10)</td>
<td>53 566.2** (87)</td>
<td>1.7952** (3)</td>
</tr>
<tr>
<td>APX</td>
<td>0.4466** (11)</td>
<td>3.3168** (79)</td>
<td>0.4296** (10)</td>
</tr>
<tr>
<td>GPX</td>
<td>0.1225** (5)</td>
<td>2.2324** (91)</td>
<td>0.0857** (4)</td>
</tr>
<tr>
<td>GR</td>
<td>1.736** (6)</td>
<td>122 998** (84)</td>
<td>2.003** (8)</td>
</tr>
<tr>
<td>DHAR</td>
<td>0.5305** (9)</td>
<td>31.9947** (79)</td>
<td>0.6044** (12)</td>
</tr>
</tbody>
</table>

CC, Chlorophyll content; PR, Photosynthetic rate; SC, Stomatal conductance; \(F_o\), Minimal fluorescence; \(F_m\), Maximal fluorescence; \(F_v/F_m\), Maximum photochemical efficiency of PSII; SOD, Superoxide dismutase; CAT, Catalase; APX, Ascorbate peroxidase; GPX, Guaiacol peroxidase; GR, Glutathione reductase; DHAR, Dehydroascorbate reductase.

\(df\), Degrees of freedom; Total \(df\) is 35; **, Significant difference at the 0.01 level of over all ANOVA for variety, treatment and variety × treatment interaction for each parameter (\(P < 0.01\)).
in Swarna-Sub than in Swarna during 1 d re-aeration. The main cause of variance found to be the treatment (86%) (Table 2).

The leaf PSII activity was studied by measuring different chlorophyll fluorescence parameters in 20 min dark adapted leaf. In the non-submerged control plants, the values of different chlorophyll fluorescence parameters like $F_o$, $F_m$ and $F_v/F_m$ did not differ significantly between Swarna and Swarna-Sub1 (Table 1). This suggested that the responses of both tolerant and susceptible varieties were similar to non-stress conditions. Submergence would disorganise the photosynthetic apparatus as evident from the decline of chlorophyll fluorescence values in both Swarna and Swarna-Sub1, but Swarna-Sub1 maintained significantly higher values of $F_o$, $F_m$ and $F_v/F_m$ after 7 d of submergence and during re-aeration. For the different fluorescence parameters, e.g. $F_o$, $F_m$ and $F_v/F_m$, the main cause of variance proved to be the submergence treatment with 86%, 63% and 80%, respectively, of the total variance (Table 2).

**Levels of antioxidant enzyme activity**

The activities of all the antioxidant enzymes exhibited almost significant changes during prolonged submergence and subsequent re-aeration. Analysis of variance showed that the treatments describe the main cause of variance in all the antioxidant enzyme activities (Table 2). The SOD activity increased over the control up to 3 d submergence in Swarna and Swarna-Sub1 (Fig. 1-A). The SOD activity decreased more in Swarna (42%) than Swarna-Sub1 (32%) after 7 d of submergence over the non-submerged control plants. Subsequent re-aeration for 1 d, Swarna-Sub1 significantly increased the SOD activity but not in Swarna.

The CAT activity was not significantly changed up to 5 d after submergence in Swarna-Sub1 and significantly more decrease was observed in Swarna (67%) than Swarna-Sub1 (52%) after 7 d submergence. Later on, the CAT activity was not recovered during re-aeration (Fig. 1-B).

The APX activity found to be changed more significantly in Swarna-Sub1 than Swarna in control, submergence as well as re-aeration conditions (Fig. 1-C). The APX activity in Swarna-Sub1 showed an increasing tendency only after 1 d submergence then declined gradually when submergence condition was prolonged. It continued to decrease up to 7 d, showing the reduction by 65% and 48% in Swarna and Swarna-Sub1, respectively.

**Fig. 1.** Changes in antioxidant enzyme activities in rice leaves of Swarna and Swarna-Sub1 during non-submerged control (C), different days after complete submergence (dS) and subsequent re-aeration for 1 d (dA).

SOD, Superoxide dismutase; CAT, Catalase; APX, Ascorbate peroxidase; GPX, Guaiacol peroxidase; GR, Glutathione reductase; DHAR, Dehydroascorbate reductase. The measurements were carried out on fully expanded mature leaves. Data are the means of three replicates.
Sub1 respectively and significantly increased only in Swarna-Sub1 after 1 d re-aeration.

The GPX activity showed an increasing tendency up to 3 d of submergence from that of respective control plants in the both varieties, then declined gradually up to 7 d submergence and 1 d re-aeration (Fig. 1-D).

In the both varieties, the GR activity showed an increasing tendency up to 3 d submergence then declined gradually when submergence condition was prolonged (Fig. 1-E). It continued to decrease up to 7 d, showing the reduction by 73% and 63% in Swarna and Swarna-Sub1, respectively, and significantly increased only in Swarna-Sub1 after 1 d re-aeration.

The DHAR activity in the both varieties showed an increasing tendency only after 1 d submergence then declined gradually, and continued to decline up to 7 d, showing the reduction by 56% and 38% compared to the control plants in Swarna and Swarna-Sub1, respectively. The DHAR activity was significantly increased only in Swarna-Sub1 after 1 d re-aeration (Fig. 1-F).

Native PAGE activity of antioxidant enzymes

Isoenzyme banding patterns of SOD, CAT, APX and GPX were studied in control, different days after submergence and 1 d after re-aeration in leaf tissues of Swarna and Swarna-Sub1 (Fig. 2).

Two clear bands of SOD were observed in all the treatments in the both varieties. There was no distinct variation in band number though the band intensity was more prominent in Swarna-Sub1 compared to Swarna (Fig. 2-a). The APX banding pattern showed one clear band and two faint bands in the both varieties (Fig. 2-b). Three isoenzymes of CAT were noticed under the control and 1 d after submergence in the both varieties. During the progression of submergence, the band intensity decreased in the both varieties (Fig. 2-c). The GPX isoenzyme band pattern showed three bands in the both genotypes, but the band intensity gradually decreased during the progression of submergence and air adaptation in the both genotypes. Swarna-Sub1 exhibited higher band intensity compared to Swarna in all the treatments (Fig. 2-d).

DISCUSSION

Submergence or water logging imposes a complex abiotic stress on rice plants, and affects numerous physiological and metabolic processes (Ella et al., 2003; Sarkar et al., 2006; Bailey-Serres and Voesenek, 2008). In the present study, we hypothesized that introgression of Sub1 QTL may improve photosynthesis and oxidative defence in rice during and after submergence stress. To examine this, we studied Swarna with and without Sub1 QTL during different days of submergence followed by 1 d re-aeration along with the non-submerged control.

Like other abiotic stress, leaf photosynthetic rate is one of the earliest parameters responses to submergence and it decreased only after 1 d submergence in the both varieties along with the decrease of stomatal conductance (Table 1). Submergence also alters the PSII activity, as reflected in a decrease in the values of $F_o$, $F_m$ and $F_v/F_m$ and degradation of chlorophyll (Table 1). The rapid drop in CO$_2$ photosynthetic rate under submergence was probably due to the structural damage suffered by the photosynthetic apparatus as evident from the fall in the values of $F_o$, $F_m$ and $F_v/F_m$ (Panda et al., 2008). Damage of photosynthetic apparatus and impair of photosynthesis would then led to the decrease of light intensity and sub-optimal oxygen level in floodwater, especially during early morning as observed in this investigation (Mommer and Visser, 2005). The decline in the values of $F_m$ and $F_v/F_m$ reflects a reduction in the ability of PSII to reduce the primary acceptor $Q_A$ (Calatayud and Barreno, 2001). According to Gilmore et al. (1996), $F_o$ increases when the photochemical apparatus is damaged.
or, more specifically, when the number of functional chlorophyll does not connect to the reaction centres of PSII. In contrast, the decline in \( F_0 \) is an indication of a high-energy dissipation in the minor antenna (Pietrini et al., 2005). Several other factors like lipid peroxidation, stomatal closure and low internal CO\(_2\) concentration are resulting a concomitant decline in photosynthesis. Reduction in CO\(_2\) concentration and increase in the amount of ROS within the leaf due to ongoing light reaction, which leads to senescence and even death of the plants (Lin et al., 2004). Swarna-Sub1 showed higher photosynthetic rate compared to Swarna under submergence and subsequent re-aeration because of better protection of chlorophyll, high stomatal conductance and efficient PSII activity (Table 1).

To explain the difference observed between the two varieties and to understand how the protective mechanism might function in Swarna-Sub1 but not in Swarna, we measured the levels of the activities of some antioxidant enzymes. Submergence and re-oxygenation can induce oxidative stress, causing an increased production of ROS (Ella et al., 2003; Fukao et al., 2011). During early submergence period, the activities of SOD, APX, GR and DHAR were increased in the both varieties (Fig. 2). This result agreed with the reports on other crops (Ahmed et al., 2002). This early rise of enzyme activities was considered to be the response to active oxygen activities caused by submergence. Possibly, increased levels of active oxygen stimulate the cellular protective mechanism to mitigate damages, but with the progress of submergence period (after 7 d), the activities of antioxidant enzymes like SOD, CAT, APX, GPX, GR and DHAR decreased more significantly in Swarna than in Swarna-Sub1, indicating the low activity of rice leaves to decompose to H\(_2\)O\(_2\) and O\(_2^-\) as has been reported on other crops (Ahemed et al., 2002). Another possible cause of the reduction of these enzymes is the decrease of the production and/ or the activity of ROS. In our previous studies, the damaged light reaction system was evident as decrease in PSII activity (Panda et al., 2006, 2008). This may have led to a loss of chemical energy provided by light reaction system, which are thought to be used for the production of ROS under stress condition, in which chemical energy is not used for CO\(_2\) fixation. Thus, after 7 d of water logging, the production of ROS was retarded, resulting in the reduction of protecting enzymes.

Additionally, when submergence was removed, only in Swarna-Sub1 showed significant increase of SOD, APX, GR and DHAR activities, but not in Swarna (Figs. 1 and 2). During re-aeration, rice seedlings need protection from ROS (Sarkar et al., 2001). Potentially \(^1\)O\(_2\) are generated in PSII reaction centre and light-harvesting complex (LHC) and O\(_2^-\) is generated in PSI acceptor side (Niyogi, 1999). Ascorbic acid interacts with scavenging ROS in both PSII and PSI sides through xanthophyll cycle (Smirnoff, 1996) and ascorbate-glutathione cycle (Damanik et al., 2010). Though regeneration after submergence is more important for survival of rice seedlings (Panda et al., 2008), Swarna fails to encounter oxidative damage efficiently like Swarna-Sub1 during post submergence period.

CAT is considered as a key enzyme removing toxic hydrogen peroxide, and peroxidase have a complementary duty (Malecka et al., 2009). However, our results disagree with that of Malecka et al. (2009), because the activities of CAT and GPX were not significant in the rice plants under submergence and subsequent re-aeration compared to the control for the both varieties. SOD in combination with the effective ascorbate-glutathione cycle enzymes are found to be more important in antioxidative defense and participate in eliminating excessive ROS induced by submergence and subsequent re-aeration in rice.

Our experiment showed a positive significant correlation between antioxidant enzyme activities like SOD, CAT, APX, GPX, GR and DHAR with photosynthetic rate, chlorophyll content, stomatal conductance and different chlorophyll fluorescence parameters (Table 3). The results showed that the
maintenance of higher photosynthetic activity in Swarna-Sub1 during submergence and subsequent period of re-aeration might be due to the better antioxidative protection of photosynthetic apparatus than in Swarna.

In conclusion, Swarna-Sub1 improves photosynthetic activity, showing higher photosynthetic rate compared to Swarna under submergence and subsequent re-aeration because of less degradation of chlorophyll, higher stomatal conductance and efficient PSII activity along with better antioxidative protection from oxidative damage.

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