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ORIGINAL ARTICLE

Improvement of Photosynthesis by Sub1 QTL in Rice Under Submergence: Probed by Chlorophyll Fluorescence OJIP Transients

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Key words: Antioxidant; Chlorophyll fluorescence; Photosynthesis; Submergence

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Exposure to complete submergence by flash flooding is a major stress constraint to rice production, especially in rainfed lowland areas of the tropics (Sarkar et al., 2006; Bailey Serres and Voesenek, 2008). The adverse effect of submergence is a result of various inter related factors such as limited gas diffusion, reduced irradiance and decrease in membrane barrier function are just few factors that slow down photosynthesis during submergence (Sarkar et al., 2006; Das et al., 2009). In vivo chlorophyll fluorescence has been used frequently in the past as a convenient and non-intrusive method to determine the tolerance of different species to different environmental condition including submergence (Pietrini et al., 2005; Panda et al., 2006; Sarkar and

Panda, 2009). Fluorescence parameters derived by the theory of fluxes have been suggested to describe changes of absorbed, dissipative, trapping and electron transport fluxes (Force et al., 2003; Sarkar and Panda, 2009). The analysis of these parameters named JIP test is performed under typical fluorescence induction from the basal emission Fo (O) to a maximum emission Fm (P) through two intermediate steps i.e. J and I (Strasser et al., 1995).

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, and a major QTL, designated Sub1, was identified in chromosome 9 and that 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 field 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 associated with Sub 1 QTL during submergence.

The present study is an effort to characterize the photosynthetic efficiency in rice plants either possessing or not possessing Sub1 QTL *i.e* Swarna and Swarna Sub1 by measuring leaf gas exchange parameters and chlorophyll fluorescence OJIP transients during control and different days after complete submergence.

MATERIAL AND METHODS

Plant material and growth conditions:

The experiment was conducted with two *Indica* rice (*Oryza sativa* L.) cultivars: Swarna and Swarna Sub1. Swarna Sub1 is popular rice varieties were developed through the marker assisted backcrossing approach (Reddy et al., 2010). Seeds were sown directly in earthen pots containing two kg of farm soil and farmyard manure (3:1). Each pot was supplied with 80 mg urea, 192 mg single super phosphate (P_2O_5) and 70 mg murate of potash (K_2O). Fourteen days old seedlings were submerged in the concrete tank for 7 d filled with water to a height of 110 cm. The plants were studied in 5 treatments i.e. 1, 3, 5, 7days after complete submergence and control growth condition i.e. without submergence treatment. 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 h (LI-COR, Lincoln, USA), and water temperature and oxygen concentration were determined at 06:00 and 17:00 h (Syland, Heppenheim, Germany). Light intensity at 60 cm water depth or at the vicinity of canopy level ranged from 215 to 319 μ mol m^{-2} s^{-1} whereas it was 1743 to 1812 μ mol m^{-2} s^{-1} above the water surface. The oxygen concentration at the same water depth was 2.5 to 3.1 mg L^{-1} at 06:00 h and 4.6 to 5.8 mg L^{-1} at 17:00 h. The temperature did not vary much (≅ 4 ^{o}C), being 26.6 ^{o}C to 30.7 ^{o}C throughout the period of the experiment.

Measurement of photosynthetic rate:

Measurements of photosynthetic rate and stomatal conductance were made on the fully expanded leaves of five different plants within 30 minutes at the end of submergence treatment using an open system photosynthetic gas analyzer (PP Systems, USA) under normal ambient environmental conditions. The second and third leaf

from the top was selected and kept inside the chamber under natural irradiance until stable reading was recorded.

Measurement of chlorophyll fluorescence transients:

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). The chlorophyll fluorescence transients were induced by a red light of 3000μE m^{-2} s^{-1}, and recorded from 10 μs up to 1 s. All the measurements were done on fully dark-adapted attached leaves. From the first O-J-I-P transients, several bio-energetic parameters were derived according to the equations of JIP-test using BIOLYSER program (R. M. Rodriguez, Bioenergetic Laboratory, University of Geneva; available at

http://www.unige.ch/sciences/biologie/bioen).

Different Chlorophyll fluorescence parameters like minimal fluorescence (Fo), maximal fluorescence (Fm), variable fluorescence (Fv = Fm - Fo), Maximum photochemical efficiency of PS II (Fv/Fm) were calculated using the software supplied by the manufacture.

Measurement of chlorophyll content:

After measuring the photosynthetic rate and chlorophyll fluorescence characteristics, the same leaves were used for the measurement 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 ^{0}C) for 28 h (Panda et al, 2008). The chlorophyll was measured spectrophotometrically following Porra, (2002).

Statistical Analysis:

Differences between various photosynthetic and chlorophyll fluorescence parameters were compared by ANOVA using IRRISTAT (International Rice Research Institute, Philippines) software's least significant difference (LSD, *$p<0.05$), as this is a good test for determining whether means were significantly different.

RESULTS

Chlorophyll content, Photosynthetic rate and Stomatal conductance:

Submergence resulted in significant reduction of chlorophyll content both in Swarna and Swarna Sub1 cv. (Fig. 1A). After 7d of submergence the %reduction in chlorophyll content was greater in Swarna (76%) than Swarna Sub1 (56%) compared to the respective control plant.

The leaf photosynthetic rate decreased in both the varieties during the progression of submergence as compared to control plant but significant varietal differences was observed after 1d 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 (Fig. 1B).

Stomatal conductance found to be significantly decreased in both the cv during the progression of submergence as compared to control plant (Fig 1C). After 7d of submergence stomatal conductance significantly more in Swarna Sub 1 compared to Swarna.

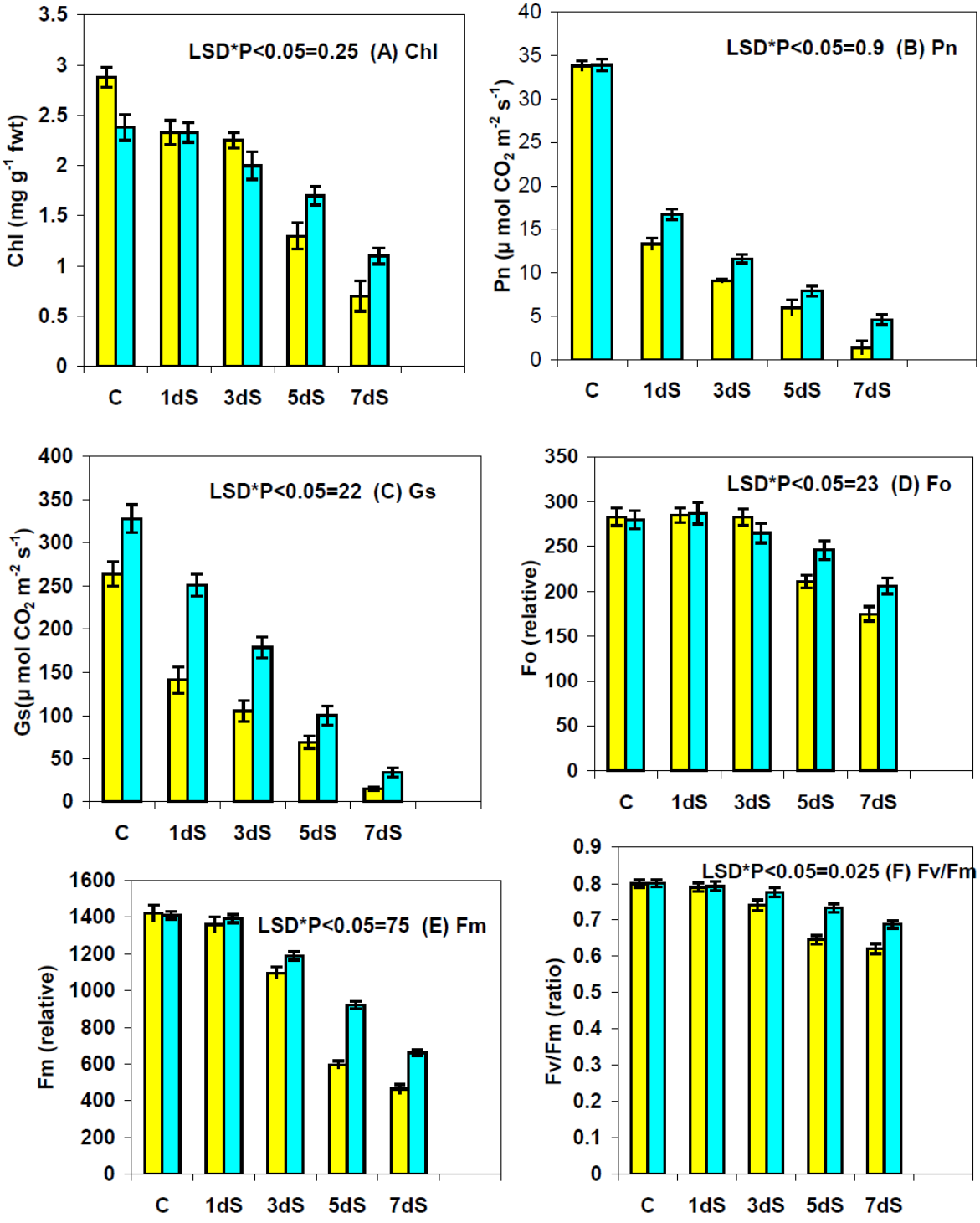
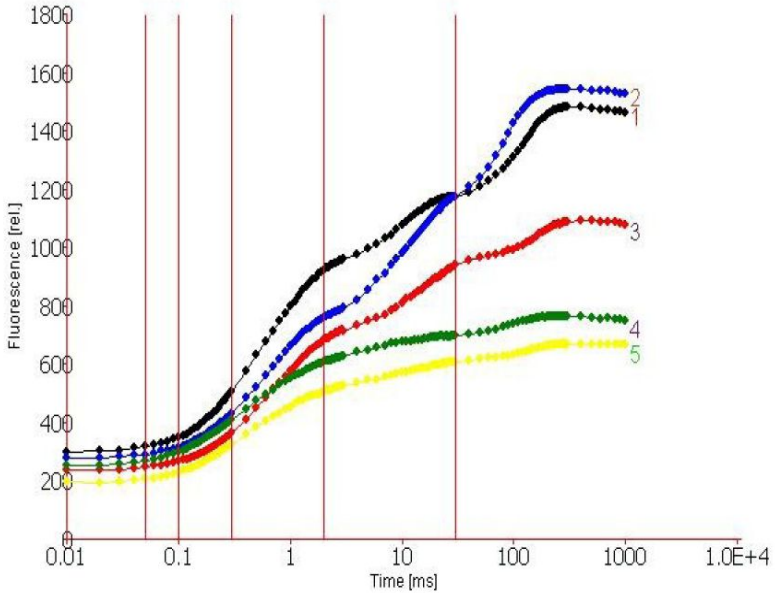


Fig. 1. Changes of leaf chlorophyll (Chl) content, photosynthetic rate (Pn), Stomatal conductance (Gs), Minimal (Fo) and Maximal (Fm) fluorescence along with maximum photochemical efficiency of PSII (Fv/Fm) in Swarna and Swarna Sub1 rice cultivars. Data are the mean of 3 replications and each replication has 5 readings. Vertical bar represent standard deviation. C, non submerged control; dS, days after complete submergence; LSD, least significant difference.

A. Swarna



B. Swarna Sub1

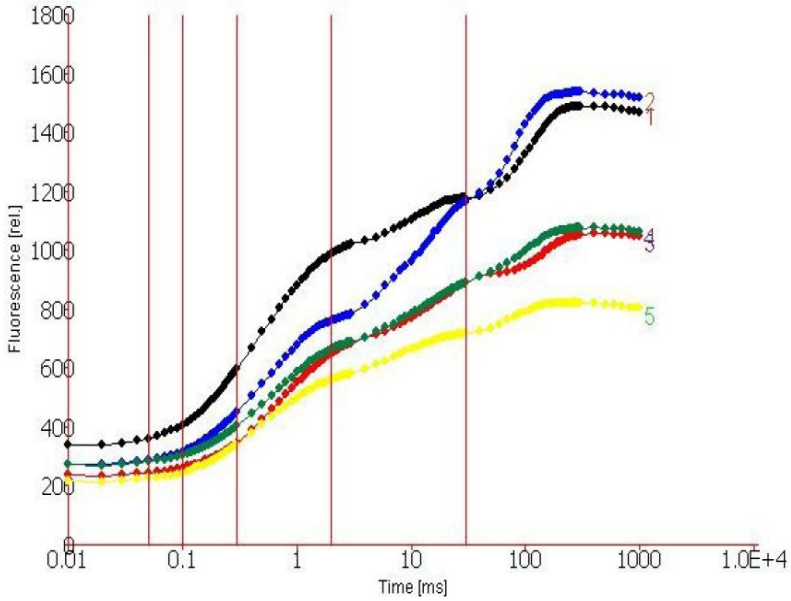


Fig. 2. Polyphasic Chlorophyll fluorescence transients of Swarna and Swarna Sub1 under non-submerged control (1) and after 1 (2), 3 (3), 5 (4) and 7 (5) d of complete submergence. Leaves were dark adapted for 20 min. The vertical lines represent the fluorescence intensity at a particular time spans. The first, second, third, fourth and fifth lines from left position demonstrate the fluorescence intensity at 50 µs, 100 µs, 300 µs, 2 ms, and 30 ms, respectively. The lines meet at the fluorescence curve at 50 µs, 2 ms and 30 ms are known as O-, J- and I-phase, respectively. The highest peak in the curve was designated as maximum fluorescence (P=Fm). A= Swarna, B= Swarna Sub 1.

Chlorophyll fluorescence OJIP transients:

The leaf PS II 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 Fo, Fm and Fv/Fm ratio did not differ significantly between Swarna and Swarna sub1 (Fig. 1D,E,F). This would suggest that responses of both the tolerant and susceptible cultivars were similar to non-stress conditions. Submergence would disorganise the photosynthetic apparatus as evident from the decline of chlorophyll fluorescence values in both the Swarna as well as Swana Sub1 but Swaran Sub1 maintained significantly higher values of Fo, Fm and Fv/Fm after 7d of submergence. Under complete submergence the shape of the OJIP transient also changed in rice leaves with decrease in maximal fluorescence (P=Fm) intensity, resulted lowering of variable fluorescence levels (Fig 2A,B). The decrease was more pronounced in Swarna compared to the Swarna Sub1 cultivar. The partial loosing of sigmoidal shape of O-J, J-I phase, and more suppression of P step after 5 and 7d of submergence was noticed in Swarna compared to Swarna Sub1 cv.

DISCUSSION

Submergence or water logging imposes a complex abiotic stress of rice plant, affects numerous physiological and metabolic processes (Ella et al., 2003; Sarkar et al., 2006; Bailey Serres and Voesenek, 2008). In the present study we examine the photosynthetic efficiency of cv Swarna with and without Sub1 QTL under submergence. Like other abiotic stresses, leaf photosynthetic rate is one of the earliest plant responses to submergence and it decreased only after 1 d submergence in both the cv. along with decrease of stomatal conductance (Fig. 1). Submergence also alters the PS II activity, as reflected in a decrease in the values of Fo, Fm and the Fv/Fm ratio and degradation of chlorophyll (Fig. 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 Fo, Fm and Fv/Fm ratio (Panda et al., 2008). Damage of photosynthetic apparatus and impair of photosynthesis would then be attributed to decrease of light intensity and sub-optimal oxygen level in floodwater, especially during early in the morning as observed in this investigation (Mommer and Visser, 2005). The decline in the values of maximal fluorescence (Fm) and Fv/Fm ratio reflects a reduction in the ability of PS II to reduce the primary acceptor QA (Calatayud and Barreno, 2001). According to Gilmore et al., (1996), Fo increases when the photochemical apparatus is damaged or, more specifically, when the number of functional chlorophylls not connected to the reaction centres of PS II. In contrast, the decline in Fo is an indication of a high-energy dissipation in the minor antenna (Pietrini et al., 2005). Swarna Sub1 showing more photosynthetic rate compared to Swarna under submergence because of better protection of chlorophyll, more stomatal conductance and efficient PS II activity (Fig 1).

The present investigation characterizes the main effect of submergence on the function of PS II in Swarna and Swarna Sub1 genotypes of rice as observed by the chlorophyll fluorescence induction kinetics (Fig. 2). All oxygenic photosynthetic organism investigated so far using this method shown the polyphasic rise with the basic step O-J-I-P and minor differences among the different phenotypes (Strasser et al., 2000). The present investigation is no way differs from earlier investigation (Fig. 1A, B). The shape of the O-J-I-P transient has been found to be very sensitive to stress caused by changes in different environmental conditions, e. g. light intensity, temperature,

drought, ozone elevation and chemical influences (Sayed, 2003; Govindachary et al., 2004; Sarkar and Panda, 2009). Under complete submergence the shape of the O-J-I-P transient also changed in rice leaves with decrease in maximal fluorescence (P=Fm) intensity, resulted lowering of variable fluorescence levels. The decrease was more pronounced in Swarna compared to Swarna Sub1 cv. The partial loosing of sigmoidal shape of O-J phase and the large suppression of P step during submergence in Swarna is attributed to some difference in the composition and organization of PS II antenna and damage of reaction center induced by submergence; they likely reflected the changes in PS II grouping (Panda et al., 2006). Complete suppression of P step especially after 5 and 7d of submergence indicated that the slower electron donation from PS II (Srivastava et al., 1997) together with the higher unbalance between a stable PS I activity and a damage of PS II (Stirbet et al., 1998) was more in Swarna compared to Swarna Sub1.

In conclusion, results showed that chlorophyll a fluorescence parameters provide a non-invasive method for investigation of structural and functional alteration of PS II. Due to submergence both donor and acceptor side of PS II is damaged and Swarna Sub1 improves photosynthetic activity showing more photosynthetic rate compared to Swarna under submergence because of less degradation of chlorophyll, higher stomatal conductance, and efficient PS II activity.

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