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Submergence effects on rice genotypes during seedling stage: Probing of submergence driven changes of photosystem 2 by chlorophyll *a* fluorescence induction O-J-I-P transients

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Abstract

The effects of submergence on chlorophyll (Chl) *a* fluorescence were compared in seven *Oryza sativa* (L.) cultivars, namely FR 13A, Khoda, Khadara, Kalaputia (tolerant), Sabita, and Hatipanjari (avoiding type), and IR 42 (susceptible). Seedlings were submerged for 4 d under complete darkness. Oxygen concentration of flood water decreased with the period of submergence with concomitant increase in concentration of carbon dioxide. Submergence caused diminution in the amount of total Chl. Genotypic differences were observed for Chl content and survival percentage. Quantification of the Chl *a* fluorescence transients (JIP-test) revealed large cultivar differences in the response of photosystem 2 (PS2) to submergence. The kinetics of Chl *a* fluorescence rise showed complex changes in the magnitudes and rise of O-J, J-I, and I-P phases caused by submergence. The selective suppression of the J-I phase of fluorescence especially after 2 d of submergence provided evidence for weakened electron donation from the oxygen evolving complex whereas under severe submergence stress (4 d) both O-J and J-I steps were suppressed greatly with highly suppressed P-step, which resulted in lowering of variable fluorescence. Grouping probability or energetic connectivity between PS2 obtained through JIP-test from the data after 2 d of submergence showed a direct relation with survival percentage, *i.e.* fluorescence measurements contained the information of the survival chance of a plant under submerged conditions. The information could be used in identifying the submergence tolerant cultivars when the damage is not very severe.

Additional key words: inundation; *Oryza sativa*; survival.

Introduction

Complete submergence of lowland rice crops occurs during flash flooding in wide areas of Southeast Asia resulting in an increased mortality of plants and low grain yield. A total of 22 million hectares of rice-growing area is adversely affected by flash flooding, half of which is in eastern India. Submerged plants experience low oxygen and low to almost zero irradiance under water relative to that in air which causes severe visible injuries and even death of the plants (Setter *et al.* 1995, Dennis *et al.* 2000, Sarkar *et al.* 2001).

The photosynthetic apparatus, and especially photosystem 2 (PS2), is very sensitive to different stresses. Stress and stress adaptation can therefore be monitored by following the behaviour of the photosynthetic apparatus (Strasser and Tsimilli-Michael 2001). A procedure for quantification of O-J-I-P fluorescence transients, known as the JIP-test, was developed by Strasser and Strasser (1995). The analysis of the fluorescence transient according to the JIP-test leads to the calculation of several phenomenological and biophysical expressions (Strasser *et al.* 1995, Sayed 2003). Chlorophyll (Chl) fluorescence and its association with submergence tolerance in rice are

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Abbreviations: area, the space above the fluorescence curve between F_0 and F_m; Chl, chlorophyll; DAS, days after submergence; DI_0/CS, dissipation of energy in a PS2 cross-section; ET_0/CS, electron transport in a PS2 cross-section; F_0, minimal fluorescence; F_m, maximal fluorescence; F_v (= $F_m - F_0$), variable fluorescence; F_v/F_m, maximum photochemical efficiency of PS2; $F_{50\mu s}$, $F_{300\mu s}$, and F_{2ms} fluorescence at 50 or 300 μs, and 2 ms, respectively; OEC, oxygen evolving complex; PG, grouping probability; PI_{ABS}, performance index on the basis of utilization of absorbed energy; PQ, plastoquinone; PS, photosystem; Q_A, primary electron acceptor of PS2; Q_B, secondary electron acceptor of PS2; RC/CS_0, number of reaction centres per excited cross-section; V_J $(F_{2ms} - F_0)/(F_m - F_0)$.
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yet to be worked out.

The present study characterizes the submergence sensitivity in rice plants on the basis of survival chance of the plant and of the changes in Chl *a* fluorescence transients, the polyphasic O-J-I-P-rise to prove the structural and functional alterations in PS2 in rice cultivars that differ in submergence tolerance. The main goal of the current investigation was to compare these responses and identify possible selection criteria for flooding tolerance in rice. At present, tolerant cultivars are identified by submerging them completely under water for a time period so that the susceptible cultivar exhibits the sign of mortality, *i.e.* softening of stem and yellowing of leaves where mortality of the plants is the sole criterion to differentiate between tolerant and susceptible cultivars. However, in this process sometimes valuable high yielding materials are lost.

Materials and methods

Plants and growth conditions: The experiment was conducted on seven *indica* rice cultivars [*Oryza sativa* (L.)], namely FR 13A, Khoda, Khadara, and Kalaputia (tolerant to submergence), Sabita and Hatipanjari (submergence-avoiding), and IR 42 (susceptible to submergence). All the cultivars were sown directly in earthen pots containing 2 kg of farm soil and farmyard manure in a 3 : 1 ratio. Each pot was supplied with 80 mg urea, 192 mg single super phosphate (P_2O_5), and 70 mg murate of potash (K_2O). Twenty one-day-old seedlings were completely submerged in concrete tanks at a depth of 110 cm of water. The tanks were covered with wooden planks so that no light could enter the tanks. Plants were submerged for 2 and 4 d (2 and 4 DAS). One more set kept outside under normal conditions served as control. Regeneration capacity was measured by counting the number of surviving plants 10 d after beginning of re-aeration.

Flood water characteristics: Water temperature and oxygen concentration were determined between 11:00 and 12:00 h (model *Simplair-F-5*, *Syland Scientific*, Heppenheim, Germany) at 60 cm water depth after 24, 48, and 72 h of submergence. Carbon dioxide concentration and pH of the flood water was measured using *pH/ISE* meter (model *250A*, *Orion*, Boston, USA) as described by Setter and Waters (1990).

Chl estimation: 100 mg of finely chopped fresh leaves were placed in a 25 cm^3 capped measuring tube containing 25 cm^3 of 80 % acetone, and kept inside a refrigerator (4 ^oC) for 28 h (Sarkar 1998). Chl amount was determined spectrophotometrically following Porra (2002).

Chl fluorescence was measured on the fully expanded leaves (second and third leaves from the top) after 1 h of de-submergence using a Plant Efficiency Analyzer, Handy *PEA* (*Hansatech Instruments*, Norfolk, UK) and recorded from 10 μs up to 1 s, with a data acquisition every 10 μs for the first 300 μs, then every 100 μs up to 3 ms, and after that 1 ms. The signal resolution was 12 bits (0–4 000). For each treatment, the Chl *a* fluorescence transients of 12 individual leaves were measured. Each replication had 4 measurements. All measurements were done on fully dark-adapted attached leaves. Maximal irradiance of 3 000 μmol(photon) m^{-2} s^{-1} was used. From the fast O-J-I-P transients, several bio-energetic parameters were derived according to the equations of the J-I-P test using the program *BIOLYSER* (R.M. Rodriguez, Bioenergetic Laboratory, University of Geneva; available at http://www.unige.ch/sciences/biologie/bioen). For the full explanation and derivation of different parameters see Strasser *et al.* (2000) and Force *et al.* (2003).

The overall grouping probability (PG) based on JIP-test was calculated as follows (Strasser and Stirbet 2001):

$$P_G = \frac{(W_{E,100\ \mu s} - W_{100\ \mu s})}{W_{100\ \mu s}(1 - W_{E,100\ \mu s}\ V_J)\ V_J}\ \frac{F_0}{F_m - F_0}$$

where

$$W_{E,100\ \mu s} = 1 - (1 - W_{300\ \mu s})^{1/5}$$

$$W_{100\ \mu s} = \frac{F_{100\ \mu s} - F_{50\ \mu s}}{F_{2\ ms} - F_{50\ \mu s}} \text{ and } W_{300\ \mu s} = \frac{F_{300\ \mu s} - F_{50\ \mu s}}{F_{2\ ms} - F_{50\ \mu s}}$$

Statistical analysis: Differences between various Chl fluorescence parameters of 7 rice cultivars 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

Flood water quality: There was not much variation in temperature, pH, and total inorganic carbon of flood water with submergence duration (Table 1), but appreciable variations in oxygen concentration were observed. As the submergence period progressed, the O_2 concentrations of flood water decreased sharply. After 4 DAS the oxygen concentration had decreased to less than one fourth of the original value. Carbon dioxide concentration increased approximately two fold.

Table 1. Changes of flood water characteristics due to submergence. Measurements between 11:00 and 12:00 h at 60 cm water depth. Means ± standard deviation.

Time after submergence [d]	pH	Temperature [°C]	Oxygen] [g m^{-3}]	Total inorganic C [mol m^{-3}]	CO_2 [mol m^{-3}]
Fresh water	7.20±0.04	29.9±0.0	6.5±0.1	0.120±0.005	0.0162±0.0013
2	6.85±0.05	30.1±0.2	2.3±0.3	0.123±0.004	0.0355±0.0032
4	6.88±0.04	30.0±0.0	1.5±0.2	0.125±0.010	0.0362±0.0039

Chl: Under control conditions, Chl content was significantly higher in susceptible cv. IR 42 followed by FR 13A (Fig. 1). Submergence decreased the content of Chl in all the cultivars. However, the rate of decrease was different in different cultivars. After 2 DAS the percent reduction of Chl content was greatest in IR 42 (36.9 %), followed by FR 13A (34.7 %), Khadara (33.8 %), Hatipanjari (22.8 %), Kalaputia (16.2 %), Khoda (15.4 %), and Sabita (12.4 %). However, after 4 DAS, the rate of decrease was low in tolerant cultivars; thereby these cultivars maintained higher Chl contents than the avoiding types (*e.g.* Sabita and Hatipanjari) and the susceptible cultivar IR 42.

Survival: After 2 DAS there was no mortality even in susceptible cultivars (data not given). However, after 4 DAS there was a clear cut difference in survival percentage. Survival percentage was nil in susceptible cultivar IR 42, whereas it was only 22 and 48 % in Hatipanjari and Sabita, respectively. In other four submergence tolerant cultivars the survival percentage was more than 80 %, among which higher survival was obtained in Kalaputia (90 %) followed by Khoda (89 %), FR 13A (83 %), and Khadara (83 %).

Analysis of Chl *a* fast fluorescence transients: To know the characteristics of the fast rise of the polyphasic Chl *a* fluorescence O-J-I-P transient under submergence, the data obtained from Kalaputia (tolerant) and IR 42 (susceptible) were used. The non-submerged control plants exhibited a typical polyphasic Chl *a* fluorescence O-J-I-P transients (Fig. 2*A*). The magnitude of fluorescence signal rose from the initial fluorescence level, F_0, to

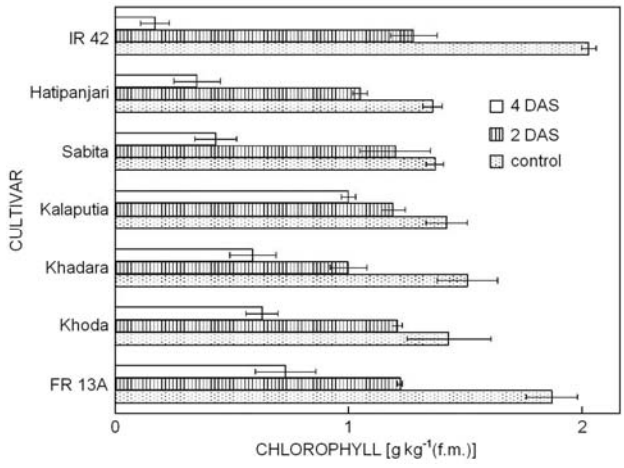

Fig 1. Contents of chlorophyll as affected by submergence. Means±standard deviation. Control = non-submergence; DAS = days of submergence.

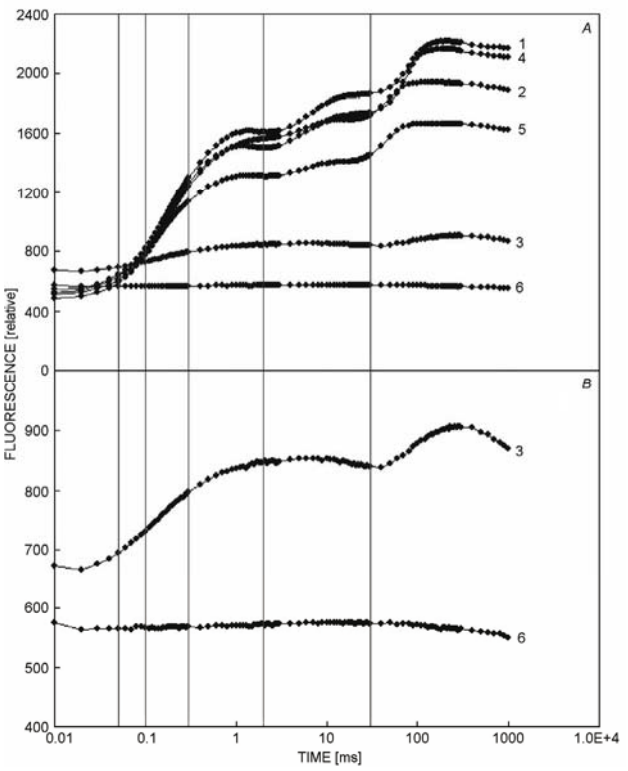

Fig 2. Polyphasic Chl *a* fluorescence transients of Kalaputia (tolerant) and IR 42 (susceptible) under control (non-submergence), and after 2 and 4 d of complete submergence (DAS) in rice leaves. Leaves were dark adapted for 20 min. *The vertical lines* represent the fluorescence intensity at a particular time spans. *The first, second, third, fourth, and fifth lines from left position* demonstrate the fluorescence intensities 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 F_m. *1*, *2*, *3* = Kalaputia; *4*, *5*, *6* = IR 42. *1*, *4* = control (non-submergence); *2*, *5* = after 2 DAS; *3*, *6* = after 4 DAS. (*A*) The scenario of fluorescence curve of Kalaputia and IR 42 with different treatments. (*B*) The fluorescence curve of severe submergence stress (4 DAS) with higher expansion to learn the characteristics of the curve in details.

the maximum level (F_m) with intermediate steps J and I. In Kalaputia the time taken to reach F_m was 213, 130, and 263 ms under control, 2 DAS, and 4 DAS, respectively whereas in IR 42 the values were 207, 203, and 14 ms, respectively. Thus under severe submergence stress (4 DAS), the time taken to reach the P-step was much shorter in a susceptible cultivar compared to the I-step, which generally took 30 ms under normal conditions, and a susceptible cultivar failed to show a typical polyphasic Chl *a* fluorescence O-J-I-P transients (Fig. 2*B*).

Changes of Chl *a* fluorescence parameters of different cultivars: F_m decreased under submergence in all the cultivars whereas no such trend was observed for F_0 (Table 2). Two days after submergence, the maximum decrease in F_m was observed in Khoda (26 %), followed by IR 42 (23 %) and Khadara (19 %). But after 4 DAS the maximum decrease in F_m was observed in Hatipanjari (85 %), followed by Khoda (76 %), IR 42 (73 %), and FR 13A (71 %). It showed that both tolerant and susceptible cultivars were affected with submergence. Under control conditions, the value of F_v/F_m was greatest in FR 13A (0.814), followed by Hatipanjari (0.796), Sabita (0.794), Kalaputia (0.788), Khoda (0.785), IR 42 (0.785), and Khadara (0.771). Submergence decreased the F_v/F_m ratio both under mild (2 DAS) and severe submergence stress (4 DAS). However, the decrease was different in different cultivars. The decrease in F_v/F_m value was within the 10 limit after 2 DAS whereas as much as 98 % decrease was noticed after 4 DAS in the susceptible cultivar IR 42, followed by Hatipanjari (97 %). Area, *i.e.* the space above the fluorescence curve between F_0 and F_m, decreased under submergence. After 2 DAS the magnitude of decrease in area was greatest in the submergence susceptible cultivar IR 42 (64 %) followed by the tolerant cultivars FR 13A (63 %). In other cultivars the rate of decrease varied between 49–61 %. After 4 DAS, the 100 % reduction in area was observed in IR 42 and Hatipanjari.

Table 2. Summary of the JIP-test formulae using data extracted from the fast fluorescence transient O-J-I-P. The units are relative. C = control, DAS = days after submergence, V = variety, T = treatment. In a column, means followed by a common letter are not significantly different at the 5 % level by Duncan's multiple range test.

Cultivar	F_0			F_m			F_v/F_m			Area		
	C	2 DAS	4 DAS	C	2 DAS	4 DAS	C	2 DAS	4 DAS	C	2 DAS	4 DAS
FR 13A	420^{bc}	504^{ab}	446^{c}	$2\,255^{b}$	2137^{a}	644^{bc}	0.814^{a}	0.765^{ab}	0.307^{c}	$32\,533^{a}$	$12\,203^{a}$	$8\,690^{b}$
Khoda	532^{a}	525^{a}	370^{d}	$2\,458^{a}$	1818^{bc}	599^{c}	0.785^{a}	0.712^{b}	0.387^{b}	$27\,000^{b}$	$10\,466^{a}$	$2\,517^{c}$
Khadara	484^{ab}	395^{c}	357^{d}	$2\,132^{bc}$	1720^{cd}	830^{ab}	0.771^{a}	0.774^{a}	0.561^{a}	$27\,200^{b}$	$11\,150^{a}$	$13\,639^{a}$
Kalaputia	470^{ab}	439^{bc}	666^{a}	$2\,219^{b}$	1941^{ab}	907^{a}	0.788^{a}	0.773^{a}	0.256^{d}	$29\,933^{a}$	$12\,000^{a}$	$6\,333^{b}$
Sabita	397^{c}	414^{c}	393^{cd}	$1\,760^{d}$	1566^{d}	844^{ab}	0.794^{a}	0.742^{ab}	0.416^{b}	$22\,866^{c}$	$10\,400^{a}$	$12\,423^{a}$
Hatipanjari	399^{c}	568^{a}	284^{e}	$1\,955^{cd}$	1995^{ab}	291^{d}	0.796^{a}	0.714^{b}	0.023^{e}	$20\,733^{c}$	$10\,678^{a}$	1^{d}
IR 42	463^{abc}	432^{c}	565^{b}	$2\,165^{b}$	1666^{cd}	576^{c}	0.785^{a}	0.741^{ab}	0.020^{e}	$31\,600^{a}$	$11\,466^{a}$	1^{d}
V×T means (LSD = 0.05)	66			198			0.050			2 358		

Cultivar	ET_0/CS			DI_0/CS			PI_{ABS}		
	C	2 DAS	4 DAS	C	2 DAS	4 DAS	C	2 DAS	4 DAS
FR 13A	151^{a}	94^{a}	64^{a}	78^{a}	119^{bc}	310^{c}	8.16^{a}	2.77^{ab}	0.42^{c}
Khoda	123^{b}	82^{a}	14^{c}	116^{a}	154^{ab}	228^{d}	3.33^{f}	1.61^{d}	0.13^{cd}
Khadara	134^{ab}	90^{a}	79^{a}	110^{a}	92^{c}	154^{e}	4.10^{e}	3.32^{a}	1.81^{a}
Kalaputia	128^{b}	85^{a}	42^{b}	97^{a}	100^{c}	495^{b}	4.92^{c}	3.03^{a}	0.14^{cd}
Sabita	102^{c}	86^{a}	76^{a}	75^{a}	112^{bc}	191^{de}	4.43^{d}	2.70^{b}	1.32^{b}
Hatipanjari	125^{b}	88^{a}	3^{c}	81^{a}	162^{a}	217^{d}	5.05^{bc}	1.51^{d}	0^{d}
IR 42	141^{ab}	91^{a}	3^{c}	100^{a}	112^{bc}	557^{a}	5.34^{b}	$2.41b^{c}$	0^{d}
V×T means (LSD = 0.05)	20			39			0.29		

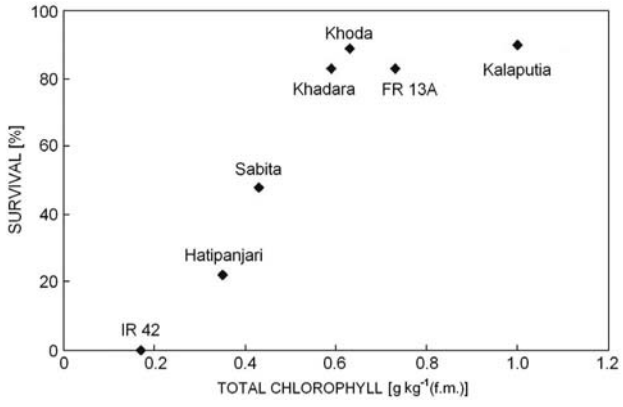

Fig. 3. Relationship between chlorophyll contents after 4 d of submergence and survival percentage.

In other cultivars the magnitude of decrease varied between 46 and 91 %. Like F_v/F_m, ET_0/CS and PI_{ABS} also decreased under submergence and after 4 DAS there was about 98–100 % reduction of these two parameters in IR 42 and Hatipanjari. The mortality percentage of these two cultivars after 4 DAS was also higher compared to the other cultivars (Figs. 3 and 4). Unlike F_v/F_m, the magnitude of dissipation per cross section (DI_0/CS) increased due to submergence.

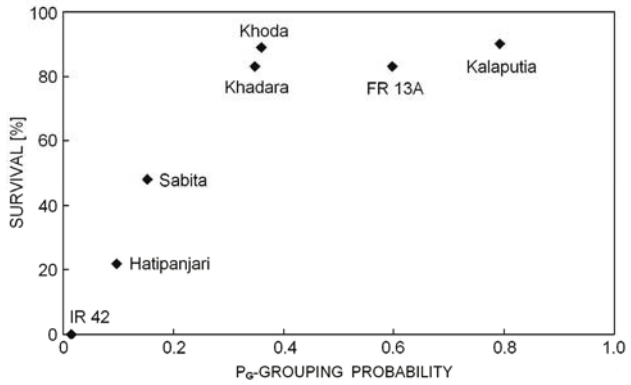

Fig 4. Relationship between grouping probability (PG) or energetic connectivity between photosystem 2 antennae and survival percentage.

Grouping probability (PG) and survival percentage: The JIP-test provides also the means to calculate the overall PG in living samples *in vivo* and *in situ* (Strasser *et al*. 2000). Grouping or energetic connectivity between PS2 antennae calculated from the data after 2 DAS showed a direct relation with survival percentage (Fig. 4). After 2 DAS there was no mortality even in susceptible cultivars, which appeared to be healthy but the internal damage was large enough to tell about the sensitivity of the plants towards submergence and if submergence were prolonged certain cultivars would expire in the early hours compared to the other cultivars.

Discussion

Flood water quality: Submergence tolerance depends upon genetic factors, but flood water quality also influences the survival. Under natural flooding, availability of radiant energy at canopy level is almost nil (Setter *et al*. 1995). As the plant did not receive any radiant energy, the senescence process was enhanced, affecting the photosynthetic apparatus. In addition to irradiance, another factor which affected the plant metabolism was the concentration of oxygen, which was sub-optimal (Table 1). Deleterious effects of hypoxia on plant survival and impairment of metabolic activities have been reviewed (Crawford and Braendle 1996, Drew 1997). Though the carbon dioxide concentration was high under submergence (Table 1), plant was unable to utilize the available CO_2 due to the deficiency in irradiance. Other parameters such as pH and temperature were optimal for plant growth; pH below 7.0 helped to survive (Ramakrishnayya *et al*. 1999).

Chl and survival: Reduction of Chl content due to submergence is common in rice (Sarkar *et al*. 1996, Das and Sarkar 2001). Maintenance of higher Chl content might be essential for survival or, in other words, degradation of Chl showed the loss of basic function in plants. Only after 4 DAS, Chl content showed a direct relation to survival percentage (Fig. 3). We suggest that maintenance of chloroplast integrity *vis-à-vis* Chl content could give a better option in predicting the survival due to submergence stress. However, the Chl based screening technique was not so sensitive that it could distinguish between tolerant and susceptible cultivars at mild stress, *i.e*. after 2 DAS (Fig. 1).

Chl *a* fluorescence: All oxygenic photosynthetic organisms investigated so far using this method have shown the polyphasic rise with the basic steps O-J-I-P and minor differences among different phenotypes (Strasser *et al*. 2000). The present investigation no way differs from earlier investigations (Fig. 2). The shape of the O-J-I-P transient is very sensitive to stress caused by changes in different environmental conditions, *e.g*. irradiance, temperature, drought, ozone elevation, and chemical influences (Zhang and Gao 1999, Calatayud and Barreno 2001, Strasser and Tsimilli-Michael 2001, Sayed 2003, van Heerden *et al*. 2003, Govindachary *et al*. 2004). Under complete submergence the shape of the O-J-I-P transient also changed in rice leaves with decrease in F_m, resulting in lowering of F_v (Fig. 2*A*). Under various stresses, such as heat (Lazár and Ilík 1997, Prakash *et al*. 2003) or drought (Guissé *et al*. 1995), an early fast step K was found at 200–300 µs leading to a polyphasic transient of the type O-K-J-I-P. Under strong heat stress, the K-step was predominant followed by a pronounced dip and later by a slight increase to a highly suppressed P-step. However, in the present investigation even with severe submergence stress no such step (*i.e*. K) was noticed, even though the P-step was highly suppressed (Fig. 2*B*). Appearance of the K-step is considered a deviation from the usually established balance of electron transport reactions responsible for the fluorescence rise (Srivastava *et al*. 1997). Unlike drought or heat stresses, submergence could not separate the step K in rice, which is usually hidden in the O-J rise (Strasser *et al*. 2000).

The rapid rise from O to J is photochemically controlled and the J-I rise is restricted by thermal reactions (Schreiber and Neubauer 1987). The donor side reactions of PS2 control the release of fluorescence quenching during the J-I phase. Any abiotic stress that perturbs the structure-function relations of OEC influences the rate of oxygen evolution which increases the quenching of fluo-

rescence rise at J- or I-steps. Therefore, the fluorescence rise at J- and I-steps envisages structural and functional integrity of OEC and is a useful indicator of water splitting activity (Govindachary *et al.* 2004). Under mild submergence stress (*e.g.* 2 DAS), the suppression of J-I step was comparatively more than O-J step whereas under severe submergence stress (4 DAS) both O-J and J-I steps were greatly suppressed with highly suppressed P-step (Fig. 2*A,B*). The rise of transient from O to J is due to the net photochemical reduction of Q_A to Q_A^- of PS2 (Prakash *et al.* 2003). Thus, under mild submergence stress the donor side of PS2 was more affected than the acceptor side whereas under severe submergence stress both the donor and acceptor sides of PS2 were severely affected by the inactivation of OEC with impairment of electron transport chain.

After 2 DAS the time to reach maximum fluorescence was lower than in the control. The maximal Chl *a* fluorescence yield F_m refers to complete reduction of PS2 acceptor Q_A, the quencher of fluorescence. This faster rise could be ascribed to a slowdown of electron transport beyond Q_A^- and a smaller pool size of electron acceptors between PS2 and PS1. However, after 4 DAS the time to reach maximum fluorescence especially in tolerant cultivars was higher than in the other treatments. Under severe submergence stress, both the donor and acceptor sides were damaged. If the donor sides produced fewer electrons, even with smaller pool size the electron acceptors between PS2 and PS1 would not be reduced quickly due to the shortage of sufficient electrons and thus after 4 DAS more time would be required to reach the maximum fluorescence. In susceptible cultivar the PS2 activity was completely lost under severe submergence stress, because the time taken to reach the P-step was much shorter (14 ms) than the time necessary to reach the I-step under normal conditions (30 ms).

The levels of F_0 either decreased or increased under submergence in different cultivars of rice (Table 2). An increase in F_0 fluorescence is one of the most direct signs of photoinhibition (Aro *et al.* 1993); it is due to the damage of the acceptor side of PS2 (Styring *et al.* 1990). However, under submergence F_0 in certain cultivars decreased and this was possible due to the disorganization at the antenna pigment level or decrease of the excitation trapping efficiency at the active centre of PS2 (Calatayud and Barreno 2001). Under submergence the Chl content decreased (Fig. 1); the low performance index (PI_{ABS}) based on absorption (Table 2) might be responsible for lowering the yield of F_0 in certain cultivars. It suggests that unlike other abiotic stresses, submergence could not affect uniformly and in dependence upon the cultivar the damage diagnosed either by higher or lower F_0. The decline of the values of F_m and F_v/F_m indicates the decreasing ability of PS2 to reduce the primary acceptor Q_A. Like other abiotic stresses, submergence also affected the photosynthetic apparatus and therefore, a decrease in the values of F_m and F_v/F_m was observed. The area above the fluorescence curve between F_0 and F_m represents the electron acceptor pool size of PS2 that includes Q_A, Q_B, and PQ (Strasser *et al.* 1995, Joliot and Joliot 2002) decreasing during submergence (Table 2). This reduction in pool size is in agreement with the overall deleterious effect of submergence on PS2. Electron transport in PS2 cross section (ET_0/CS) represents the re-oxidation of reduced Q_A *via* electron transport over a cross-section of active and inactive RCs (Force *et al.* 2003) that also decreased during submergence (Table 2). It shows that both the donor and acceptor sides of PS2 were damaged due to submergence but the damage was more pronounced in the susceptible cultivar (Table 2). In contrast, DI_0/CS increased due to the submergence effects. Hence maximum energy was lost in the form of dissipation due to the effects of submergence.

The JIP-test is the means to calculate the overall PG or energetic connectivity between PS2 antennae. Grouping probability accounts all possible ways of energetic communication of neighbouring PS2 core antennae. This can be explained by energy migration between the antennae of PS2 units (Strasser and Stirbet 2001). Among all the experimental parameters, PG calculated taking the samples after 2 DAS was highly sensitive to submergence (Fig. 4). The cultivars tolerant to submergence maintained the connectivity between the antennae of PS2 units while in susceptible cultivars the connectivity started to break down even when the submergence stress was not so severe (2 DAS) and therefore, a good association between grouping probability and survival percentage was observed (Fig. 4). Therefore, the fluorescence measurements contain the information of the survival chance of a sample under submerged conditions.

Our results show that the fast Chl *a* fluorescence transient measurements provide a non-invasive and rapid method for investigating stress effects on PS2. Since submergence induces structural disorganization, a general trend in the disorganization of the photosynthetic apparatus during submergence could be visualized. The PG obtained through the JIP-test measures the connectivity between PS2 antennae, highly sensitive to submergence stress. Loss of connectivity between the antennae of PS2 units determines the sensitivity of the plants to submergence and the greater is the loss of connectivity the greater is the probability of plant death. In susceptible cultivars, the loss of connectivity was faster compared to the content of Chl and Chl fluorescence parameters such as F_v/F_m, *etc*. Hence, the Chl *a* fluorescence parameters especially the PG, which represents the energetic connectivity between PS2 antennae, could determine the susceptibility/tolerance of the cultivars even under mild submergence stress.

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