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BRIEF COMMUNICATION

Response of senescing rice leaves to flooding stress

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Abstract

Flooding stress (FS) induced changes in pigment and protein contents and in photochemical efficiency of thylakoid membranes of chloroplasts were investigated during senescence of primary leaves of rice seedlings. Leaf senescence was accompanied by loss in 2,6-dichlorophenolindophenol (DCPIP) photoreduction, rate of oxygen evolution, quantum yield of photosystem 2 with an increase in MDA accumulation, and non-photochemical quenching (NPQ) of chlorophyll fluorescence. These changes were further aggravated when the leaves during this period experienced FS. The increase in NPQ value under stress may indicate photosynthetic adaptation to FS.

Additional key words: adaptation to stress; carotenoids; chlorophyll; non-photochemical quenching; photosystem 2; quantum yield; senescence.

Prolonged water-logging of rice plants is a major problem in rice growing countries. The work conducted so far to understand the mechanism of damage and adaptation of photosynthetic apparatus of green plants experiencing flooding stress (FS) is meager (Mauchamp and Methy 2004, Panda *et al.* 2006). The photosynthetic apparatus, especially its photosystem (PS) 2 is very sensitive to different abiotic stresses (Strasser and Tsimilli-Michael 2001, Biswal *et al.* 2003, 2006, Deo *et al.* 2006, Joshi *et al.* 2006). The degree of stress induced damage and stress adaptations can therefore be examined by monitoring the behaviour of the photosystem exposed to FS.

Most of the studies related to stress have been conducted in developing or mature leaves with scant regard to investigate the stress response in senescence phase. This is why we examined the photosynthetic response of leaves of a local cultivar of rice plant subjected to FS. The primary leaves of intact rice seedlings experiencing flooding stress were used for measurement of PS2 photochemistry during senescence to examine the stress induced damage of the photosystem and the possible adaptation mechanism the leaves develop to counter the stress effect.

FS was imposed by growing the rice seedlings on soil in submerged conditions as described by Panda *et al.* (2006). Rice (*Oryza sativa* L. cv. Swarna) seedlings were grown in 0.15×0.15 m pots with soil from the rice field around the campus of Sambalpur University for 7 d in continuous "white fluorescent light" (60 μmol m^{-2} s^{-1}) at 25±2 ºC in the laboratory. One set of 7-d-old seedlings was completely submerged in a thermocol tank of depth 0.6 m till 15th d whereas another set kept without submergence was taken as control. The photosynthetic response of plants to FS was studied during senescence (11–15 d) phase. In all cases the primary leaves of the rice seedlings were used for experiments. Pigments were extracted from leaves with chilled 80 % acetone. Estimation of chlorophyll (Chl) was performed spectrophotometrically as per Arnon (1949) and that of carotenoids as per Liaaen-Jensen and Jensen (1971). Total protein extracted from leaves was estimated following the method of Lowry *et al.* (1951). Chloroplasts from primary leaves of rice seedlings were isolated and 2,6-dichlorophenol indophenol (DCPIP) photoreduction in the isolated chloroplasts was measured spectrophotometrically as described by Biswal and

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Abbreviations: Chl – chlorophyll; DCPIP – 2,6-dichlorophenolindophenol; DPC – diphenylcarbazide; EDTA – ethylenediamine tetraacetate; F_0 – initial fluorescence; FS – flooding stress; F_v/F_m – photochemical efficiency of photosystem 2; OEC – oxygen evolving complex; Φ_{PS2} – quantum yield of photosystem 2; q_P – photochemical quenching coefficient; MDA – malondialdehyde; NPQ – non-photochemical quenching; PS – photosystem; RC – reaction centre.
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Mohanty (1976). Oxygen evolution was measured polarographically by an oxygen electrode *Leaf lab 2* (*Hansatech*, UK). Accumulation of malondialdehyde (MDA) was quantified as per Panda *et al*. (1987). The initial fluorescence (F_0), photochemical efficiency (F_v/F_m), quantum yield of PS2 (Φ_{PS2}), non-photochemical quenching (NPQ), and photochemical quenching coefficient (q_P) were determined by pulse amplitude modulated (PAM) chlorophyll fluorometer (*FMS 1*, *Hansatech*, UK) according to Schreiber *et al*. (1986).

The loss in Chl during senescence period was more pronounced when seedlings were subjected to FS (Table 1). The decline in pigment content could be attributed to the loss in irradiance and quality of transmitted radiation that the plants receive in submergence condition (Panda *et al.* 2006). The value of Chl *a/b* (Table 1) was taken as an index of the relative amount of reaction centres (RC) of the photosystems and light-harvesting complexes (LHCs). The decline in Chl *a/b* both in senescing leaves and leaves experiencing FS indicates relative stability of LHC. Relatively stable LHCs of thylakoids in submerged plants could be an adaptive strategy of the plants at low irradiance.

PS2 is very sensitive to water stress (Biswal *et al.* 2003) which damages the oxygen evolving complex (OEC) (Canaani *et al.* 1986) and PS2 RCs (He *et al.* 1995, Giardi *et al.* 1996). We found a significant decline in photochemical efficiency of FS-plants during senescence as indicated by the loss in oxygen evolution, DCPIP photoreduction, and the quantum yield of PS2 (Table 1). The DCPIP photoelectron transport with an exogenous electron donor, DPC, which feeds electrons directly to RC2 bypassing OEC, was lower in the senescing leaves experiencing stress than in their 15 d senescing counterparts (Table 1). The senescing leaves might experience stress due to submergence, which results in dismantling of core complex of RC2 structure.

Table 1. Effect of flooding stress on contents of chlorophyll [g kg^{-1}(FM)], carotenoids [g kg^{-1}(FM)], protein [g kg^{-1}(FM)], and MDA [mmol kg^{-1}(FM)], DCPIP photoreduction $H_2O \rightarrow$DCPIP [mmol kg^{-1}(Chl) s^{-1}], DPC→DCPIP [mmol kg^{-1}(Chl) s^{-1}] and O_2 evolution [μmol(O_2) m^{-2} s^{-1}]. Means of three independent measurements ± SD.

Parameter	7-d control	15-d control (senescing)	15-d flooding (senescing)
Chl	1.26±0.10	0.88±0.06	0.47±0.04
Chl *a/b*	3.35	2.34	1.67
Car	0.36±0.02	0.28±0.02	0.23±0.01
Protein	11.18±0.95	6.14±0.8	4.58±0.32
MDA	0.36±0.02	2.63±0.3	6.93±0.81
$H_2O \rightarrow$DCPIP	36.38±2.54	21.60±0.64	9.10±0.44
DPC→DCPIP	42.00±2.10	35.27±1.41	11.60±0.46
O_2 evolution	5.47±0.52	0.66±0.03	0.10±0.00

Studies with fluorescence probe confirm the damage of PS2 in thylakoids. Chl fluorescence is a very sensitive tool to study the stress induced damage of PS2 (Drew 1997). A significant increase in F_0 has been observed (Table 2) indicating an apparent sign of damage to the RC2 (Rintamäki *et al.* 1994, Nayak *et al.* 2003). However, more than one reason is ascribed to the rise in F_0 during various stress conditions. In our case, the increase in F_0 at day 15 is thought to arise from the inactivation of the RCs. Our proposition is supported by our data on FS-induced instability of Chl *a* and significant loss in DPC supported photoelectron transfer (Table 1). A significant decline in F_v/F_m in senescing leaves experiencing stress may be an estimate of the rate of linear electron transport in the leaf that was considerably reduced in submerged leaves due to low irradiance. A substantial enhancement of NPQ (Table 2) suggests the tendency of leaves to develop adaptation mechanism to counter the FS effect (Fernandez 2006).

Table 2. Fluorescence parameters indicating stress induced changes in photosystem 2 photochemistry of intact leaves of rice seedlings with or without flooding stress. Means of three independent measurements ± S.D.

Treatment	F_0	F_v/F_m	Φ_{PS2}	q_P	NPQ
7-d control	98.00±7.28	0.753±0.117	0.983±0.171	0.988±0.134	0.098±0.023
15-d control (senescing)	185.00±4.89	0.559±0.086	0.590±0.076	0.567±0.086	0.171±0.031
15-d flooding (senescing)	351.00±10.34	0.219±0.043	0.142±0.032	0.453±0.072	0.239±0.026

Although the intensity of transmitted radiation incident on submerged leaves is low, the stress causes a dramatic enhancement in MDA accumulation (Table 1). This needs an explanation. The data on the stress induced changes in the photosynthetic pigments and photochemical reactions provide a clue to explain it (Table 1). Relative stability of the pigments in the background of significant loss in F_v/F_m and oxygen evolution (Tables 1 and 2) could result in a photoinhibitory environment which is also evident by an increase in NPQ. The photoinhibitory condition even at low irradiance in submerged leaves could be an adaptive response and have a link with MDA accumulation. MDA accumulation is likely to further aggravate and dismantle the PS2 complex (Table 1). However, whether the loss in photochemical potential is a cause or consequence of MDA accumulation remains unclear.

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