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SHORT COMMUNICATION

BIOCHEMICAL IMPACT OF RE-OXYGENATION IN RICE SEEDLINGS AFTER SUBMERGENCE STRESS

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Rice cultivars treated with 2, 4 and 6d of submergence and post submergence exhibited increase in reactive oxygen species, hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$). The analysis of lipid peroxidation showed high increment in malondialdehyde (MDA) production only after 2d of treatment. Significant increase in peroxidase (POX) and superoxide dismutase (SOD) activities were enhanced under post submergence treatment after 4d, but reduced after 6d while catalase (CAT) activity declined after 4d of treatment. The contents of ascorbate (AsA) and glutathione (GSH) also showed increasing trends. The objective of this experiment was to test the hypothesis that submergence induces oxidative stress with exposure dependent intensity and variability and activates defence system in *Oryza sativa* L. Though water is essential for plants, but submergence-associated O_2 alteration causes the toxicity.

Key words: **Oxidative stress, *Oryza sativa*, re-aeration, submergence**

Oxygen deficiency obstructs respiration at the level of electron transport and decreases generation of ATP (Crawford and Brandle, 1996). Submergence stress is an important abiotic stress, which induces oxidative damage in rice plant, affecting antioxidant systems and causes significant crop losses (Ushimaru *et al*. 2001, Blokhina *et al.* 2003). The metabolism of sugar, protein and amino acids is profoundly altered during submergence (Ricard *et al*. 1994). Imposition of submergence may increase generation of reactive oxygen species (ROS) within the cell, particularly within the chloroplast of flood stressed plants, leading to lipid peroxidation, protein degradation, enzyme inactivation and affect nucleic acid and almost every component of cell leading to cell death (Rawyler *et al*., 2002 and Blokhina *et al.,* 2003). To overcome the effects of ROS, plant cells have well developed enzymic and non-enzymic antioxidant defense systems (Scandalios, 2002). In India, rice generally suffers from submergence during June to September. Though Ram *et al*., 2002 have suggested some adequate measures against submergence, there is dearth of information of oxidative stress management in relation to submergence acclimatization in various rice plants cultivated on this region. Our investigation aims in understanding the mechanism of submergence stress alters oxidative metabolism and antioxidant defense systems on submergence, its possible recovery on re-aeration in enhancing the recovery process in selected varieties of *O. sativa* L.

Dry graded uniform rice (*O.sativa* L.) seeds cv. Mahsuri, Basudeo, Tulashi and FR 13A were procured from Regional Agricultural Research Station (RARS), Karimganj, India. and Central Rice Research Institute (CRRI), Cuttack, India. The seeds were surface sterilized with 0.1% $HgCl_2$ for 5 – 10 mins and thoroughly

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washed with deionished water .The seeds were germinated in petri plates (15X90mm) containing Whattman no.1 filter paper moistened with deionised water and kept in B.O.D. incubator in dark at ± 25 °C. On the 3 d of germination, seeds were transferred to plastic glasses containing $^1/_2$Yoshida solution (Yoshida *et al*., 1976) and kept in a growth chamber at 30-33 °C (day/night) with a 12 h photoperiod and illumination under controlled environmental conditions at 52µmol m^{-2} s^{-1} (PAR). After every 6 h, seedlings were re-hydrated with fresh Yoshida solution. On the 5 d, rice seedlings were imposed to submergence for 2, 4, 6 days in plastic containers. Non-submerged seedlings were considered as control. The same method was used for recovery study after 6d of submergence. The roots and shoots after every 2, 4, 6 days for both submergence and recovery after submergence were sampled for various physiological and biochemical estimations.

Lipid peroxidation was measured as the amount of MDA determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). H_2O_2 was extracted in 5 % trichloroacetic acid from tissues using (0.2g) fresh leaves samples. The homogenate was used for the estimation of total peroxide content (Sagisaka, 1976). The estimation of O_2^- was done as suggested by Elstner and Heupal (1976) by monitoring the nitrate formation from hydroxylamine. Leaf tissues were homogenized with phosphate buffer pH 6.8 (0.1M) in prechilled mortar pestle. The extract was centrifuged at 4 °C for 15 min at 17 000 x g in a refrigerated cooling centrifuge. The supernatant was used for the assay of Catalase (CAT) [EC 1.11.1.6], Peroxidase (POX) [EC 1.11.1.7] and Superoxide dismutase (SOD) [EC 1.6.4.2]. CAT activity was assayed according to Chance and Maehly (1955). POX was extracted from tissue in 0 .2M sodium phosphate buffer (pH 6.6). POX was assayed using pyrogallol as substrate according to Kar and Mishra (1976). The assay of SOD was done as per the method of Gianopolitis and Ries (1977). Glutathione (GSH) was extracted and estimated as per the method of Griffith (1980). For the extraction and estimation of ascorbate (AsA), the method of Oser (1979) was followed. All experiments were done triplicates and repeated thrice and the data represent mean ± SE. The results were analyzed statistically with the Student's t-test at $P \leq 0.05$.

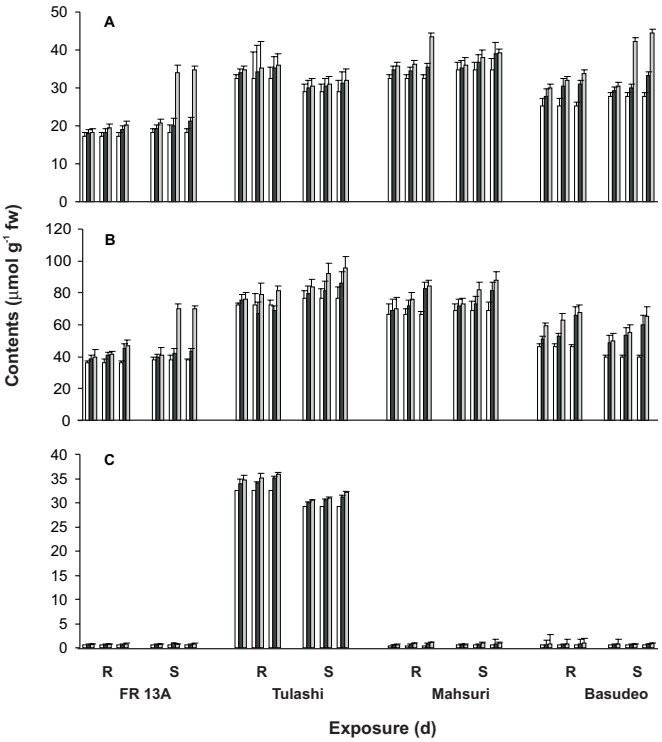

Fig. 1. Changes in H_2O_2 (A), O_2^- (B), and MDA (C) in roots and shoots of 4 genotypes of *Oryza sativa* L. under control (□), submergence (■) and post-submergence (▩). Data given are means of three different replicates ± standard errors. * represents statistically significant difference in contents in comparison with control at P < 0.05.

The time course of membrane lipid peroxidation in rice seedlings (determined as MDA content) is given in Figure 1C. The MDA content of roots increases in tolerant cultivar with maximum in Basudeo on 6 d during submergence. But it increases by 150.87% on same days of re-aeration. Whereas in susceptible cultivar, it shows maximum increase under submergence for 6 d but on post submergence, it gradually shows increase by 201.27% as compared to non-submerged seedlings. On the other hand, under submergence the MDA content of shoots increases with the increase in days of exposure. It shows maximum in Basudeo, the tolerant variety under submergence and it increases by 130.15% on 6 d of re-aeration. Whereas in susceptible cultivar, Mahsuri it shows maximum under submergence and increases more by 105.13% on 6 d of post submergence, as compared to non-submerged seedlings. The MDA contents was found to be accumulated more after exposure to air than in submergence which might be a

sign of oxidative stress inducing free radicals (Biemelt *et al*., 1998 and Blokhina *et al*., 2001).

The SOD activity of roots increases in tolerant cultivars with maximum in FR 13A on 6 d during submergence (Fig.2B). But it decreases by 56.25 % on same days of re-aeration. Whereas in susceptible cultivars, it shows maximum increase in Tulashi under submergence for 6 d but on re-aeration, it gradually shows more increase by 195.89 % as compared to non-submerged seedlings. On the other hand, under submergence the SOD activity of roots increases with the increase in days of exposure. The CAT activity increased during submergence, maximum to 153.62 % in FR 13A, the tolerant cultivars and simultaneously decreased by 104.43 % in 6 d on re-aeration (Fig.2A). But in susceptible cultivars, the CAT activity increases maximum in Tulashi (112.02 %) on 6 d under submergence and decreased by 71.09 % on 6 d in the same cultivars on re-aeration adaptation as compared to the non – submerged seedlings. The POX activity of roots and shoots increases in tolerant cultivars with maximum in FR 13A by 143.22 % on 6 d during submergence (Fig.2C). But it decreases by 89.43 % on the same days of recovery after submergence. Whereas in susceptible cultivars, it shows maximum increase to 112.10 % in Tulashi under submergence for 6 d but on re-aeration, it gradually shows more increase by 124.21 % as compared to non-submerged seedlings. On the other hand, under submergence the POX activity of roots and shoots increases with the increase in days of exposure. The activity of CAT, SOD and POX increases on re-admission to air with maximum in tolerant cultivars, suggesting a high H_2O_2 level regulated by a wide range of enzymes (Blokhina *et al.,* 2003 and Ella *et al*., 2003), under submergence the total peroxide (H_2O_2) content of roots increases with the increase in days of exposure. It shows maximum in FR 13A, the tolerant variety under submergence and it increases by 56.41 % on 6 d of re-aeration. Whereas in susceptible cultivars, it shows maximum in Tulashi under submergence and increases more by 105.13 % on 6 d of reaeration, as compared to non-submerged seedlings. The H_2O_2 content was found to be accumulated more after exposure to air than in submergence might be a sign of oxidative stress inducing free radicals (Biemelt *et al.*, 1998 and Blokhina *et al*., 2001). O_2^- on the other hand is incapable of crossing the biological membrane and together with H_2O_2 under stress forms highly reactive hydroxyl radicals (OH^-) via Haber – Weiss Reaction that might lead to severe oxidative load on cells. The Superoxide radical (O_2^{-}) content of shoots increases in tolerant cultivars with maximum in FR 13A on 6 d during submergence But it increases by 146.55 % on same days of subsequent re-aeration. The GSH content of roots increases in tolerant cultivars with maximum in FR 13A on 6 d during submergence. But it decreases by 99.55 % on same days of re-aeration (Fig.3B). On the other hand, under submergence the AsA content of root increases with the increase in days of exposure. It shows maximum in FR 13A, the tolerant variety under submergence and it decreases by 206.41 % on 6 d of re-aeration. Whereas in susceptible cultivars, it shows maximum in FR 13A under submergence and gradually decreases by 105.13 % on 6 d of re-aeration, as compared to non-submerged seedlings (Fig.3A).

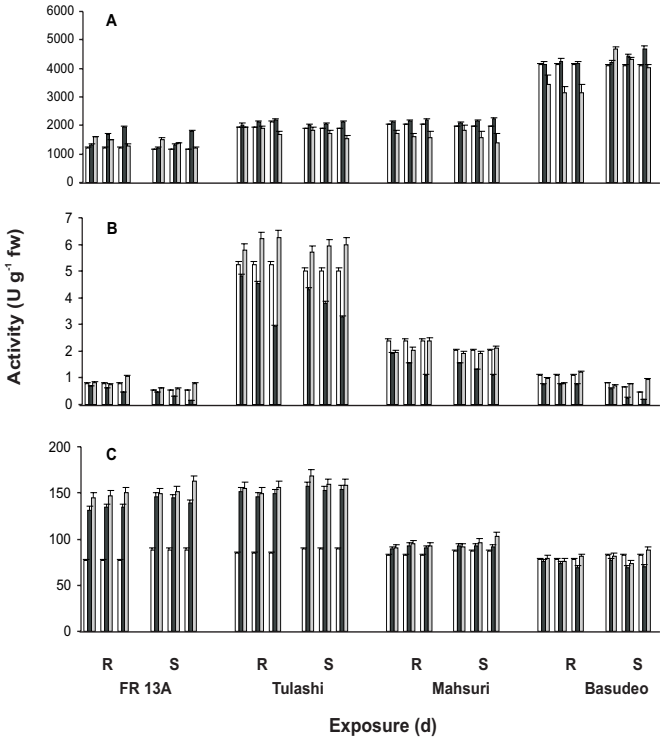

Fig. 2. Changes in activities of CAT (A), SOD (B) and POX (C) roots and shoots of 4 genotypes of *Oryza sativa* L. under control (□), submergence (■) and post-submergence (▩). Data given are means of three different replicates ± standard errors. * represents statistically significant difference in activity in comparison with control at P < 0.05

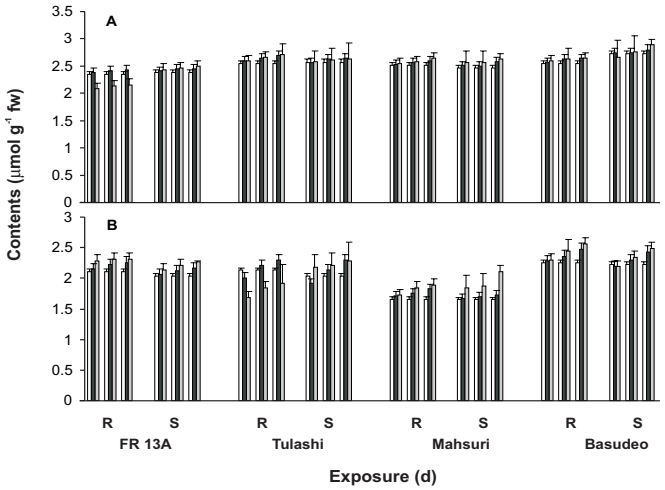

Fig. 3. Changes in AsA (A) and GSH (B) in roots and shoots of 4 genotypes of *Oryza sativa* L. under control (□), submergence (■) and post-submergence (▩). Data given are means of three different replicates ± standard errors. * represents statistically significant difference in contents in comparison with control at P < 0.05.

The present study revealed that submergence stress increased the membrane damage, as is evident from increased levels of lipid peroxidation. Damage may start after 4 days and the physiological chlorotic symptoms like yellowing occurs after 4-6 days of re-oxygenation/ post submergence in Tulasi, Mahsuri more than that of FR 13A and Basudeo. Summarizing the findings, it can be said that imposed submergence stress caused oxidative damage in rice cultivars, resulting in the decrease of its antioxidant potential with various physiological and biochemical alterations, and thus its possible recovery on re-aeration may helps in enhancing the recovery process in selected varieties of *O. sativa* L. Further, experiment in field condition would be appropriate.

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