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Efficiency of alcohol dehydrogenase activity as an indicator of submergence tolerance in rice

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ABSTRACT

The activity of Alcohol dehydrogenase (ADH), its isozyme profile and aldehyde level were studied in one-week-old submerged seedlings of four rice cultivars viz., FR13A, Gangasiuli (submergence tolerant); Mahsuri and Sarala (susceptible to submergence). ADH actvity increased under submergence and attained peak value after 48h of stress in all the varieties. Seedlings of the susceptible cultivar, Mahsuri exhibited maximum induction of ADH activity together with least rise in aldehyde content indicating that ADH activity alone may not be the sole determinant of submergence tolerance in all varieties of rice. Only one ADH isozyme was observed in the seedlings which was highly induced in all cultivars under submergence.

Key words: *rice, submergence tolerance, alcohol dehydrogenase, aldehyde, isozyme*

Rice is a semi aquatic plant adapted to survive submergence for limited period of time. Apart from anatomical adaptations such as formation of aerenchyma (Kawai and Uchimiya, 2000), rice plant undergoes complex metabolic changes like avoidance of self-poisoning and maintenance of adequate energy supply under submergence stress (Dhariwal *et al*. 1998). Oxygen deprivation results in cessation of TCA cycle and shift in the production of ATP from the mitochondrial electron transport linked oxidative phosphorylation to the substrate level phosphorylation of anaerobic glycolysis (Das and Uchimiya, 2002). Alcoholic fermentation is recognized as the principal catalytic pathway for recycling NAD to maintain glycolysis and substrate level phosphorylation in the absence of O_2 (Ricard *et al*., 1994). Alcohol dehydrogenase (ADH) is the main enzyme of alcoholic fermentation which catalyzes reduction of acetaldehyde to ethanol (Vartapetian and Jackson, 1997). Accumulation of acetaldehyde, on the other hand, is detrimental to plant cells (Crawford and Braendle 1996). ADH activity and the level of acetaldehyde in plants under submergence are, thus, thought to be indicators of plant survival under submergence. The present study was undertaken to find if the ADH activity can be correlated with degree of submergence tolerance in seedlings of some rice cultivars differing in degree of tolerance to submergence stress.

Seeds of four rice cultivars showing tolerant (FR13A), moderately tolerant (Gangasiuli) and susceptible (Mahsuri and Sarala) reaction to submergence were surface sterilized (10 min in 4% sodium hypochlorite solution) and were allowed to germinate in Petri plates lined with double layer of filter paper disks at 30 ± 1^{o}C in dark. Petri plates with one-week old seedlings were completely submerged in plastic trays (6 inch deep) containing water for 120 h. ADH activity was determined after every 24 h using only the leaf samples (Rumpho and Kennedy, 1981). The rate of the reaction was determined from the linear portion of the reaction curve. One Unit of enzyme activity was defined as the amount that brought about a change of 1.0 absorbance unit per min. Aldehyde estimation was carried out by the method of Sarkar (2001) with a calibration curve prepared by using formaldehyde (0.25µl ml^{-1}) as a standard. The results were expressed as µl aldehyde released per g dry weight of tissue. ADH isozymes were separated (Grover and Pental, 1992) by loading samples containing 60µg protein on 7.5% polyacrylamide gels using a vertical electrophoresis unit at a constant current of 20 mA.

Gels were stained for ADH activity following the method of Vallejos (1983). Soluble protein content was estimated by the method of Bradford (1976).

The basal level ADH activity was found to be higher in tolerant cultivars (0.70-1.125 units mg^{-1} protein) than the susceptible ones (0.42 to 0.52 units mg^{-1} protein). Submergence caused enhancement of activity by 5.24 to 29.86 fold in the seedlings of all four cultivars compared to the pre-submergence level (Fig. 1), with the peak values recorded at 48h of submergence. The susceptible cultivars, Mahsuri and Sarala showed higher induction (8.81 to 29.96 fold) than the tolerant cultivars which showed only 5.24 to 7.26 fold enhancement in activity. The specific activity of ADH in one of the susceptible cultivars, Mahsuri was 15.53 as compared to 5.90 and 5.01 units mg^{-1} protein observed in the tolerant cultivars FR13A and Gangasiuli, respectively. Decline in ADH activity was noted in all cultivars subsequent to 48h of submergence: the susceptible cultivars began to show symptoms of rotting particularly after 96 h.

These observations were supported by the intensity of the ADH activity bands, which was highly induced under submergence, especially in the susceptible cultivar Mahsuri. Only one ADH activity band was seen in control as well as submerged plants (Fig. 2). Submerged rice seedlings have been reported to have a very active alcoholic fermentation and enhanced ADH activity (Ellis and Setter 1999). Although the tolerant cultivars had higher pre-

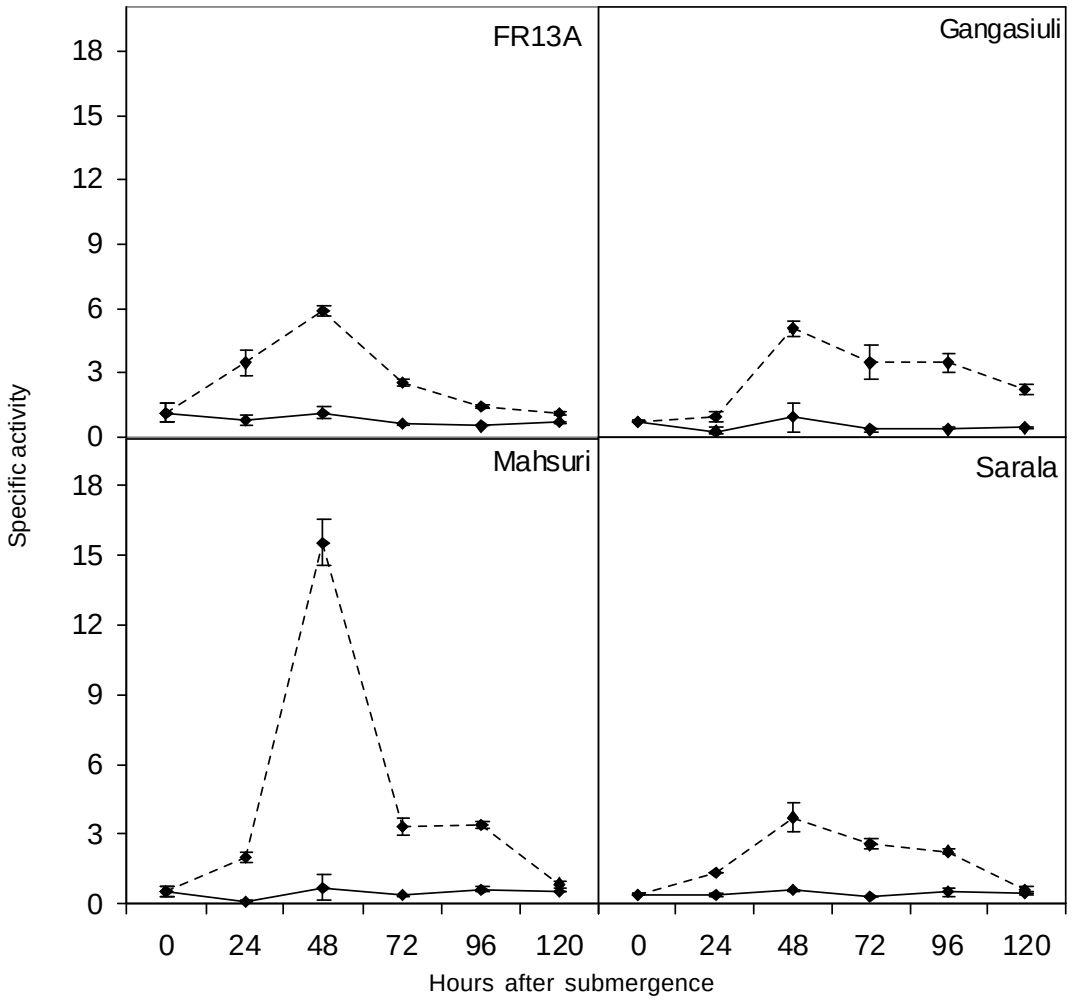

Fig 1. Alcohol dehydrogenase activity (unit mg^{-1} protein) in the seedlings of different rice cultivars under control (solid line) and submerged (dashed line) conditions. Data presented are mean ± SE, n=6.

submergence ADH activity, maximum rise in activity was observed in the susceptible cultivar Mahsuri. Findings of previous studies examining associations between ADH activity and flood tolerance have been contradictory with some studies reporting negative correlations, as in maize (Marshall *et al*., 1973) and others reporting a positive relationship as in sunflower (Torres,1981). Flood intolerant species typically found

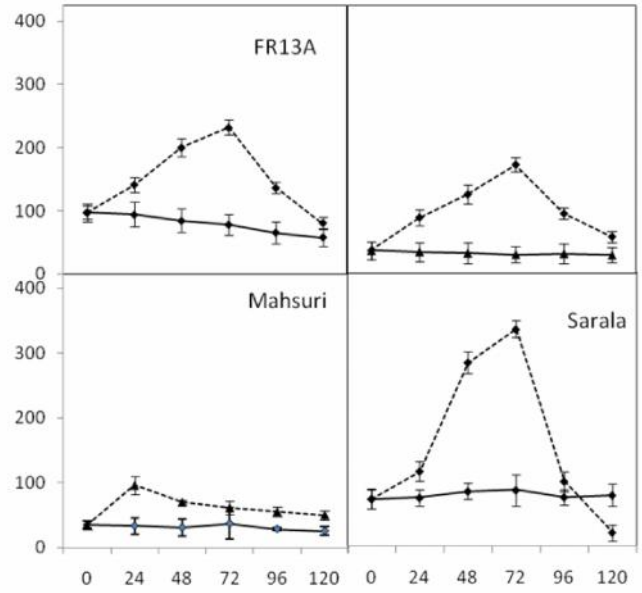

Fig. 2. Aldehyde released (µl g^{-1} dry wt.) by the seedlings of different rice cultivars under control (solid line) and submerged (dashed line) conditions. Data presented are mean ± SE, n=6.

in aquatic and terrestrial habitats tend to exhibit increased ADH activity in response to hypoxic soils, while flood-tolerant species typically have reduced ADH activity (McManmon and Crawford 1971).

Increased aldehyde activity under submergence in three of the cultivars with maximum accumulation at 72 h. The notable exception was Mahsuri, which showed a sudden increase upto 24h and then declined gradually (Fig. 2). Acetaldehyde has been detected in a number of plants including rice (Boamfa *et al*. 2003) and found to be produced in higher amounts under anaerobic conditions (Kim *et al*. 2007). Amount of acetaldehyde is an indication of the rate of alcoholic fermentation and also an index of survival under submergence stress (Sarkar, 2001).

Pattern of accumulation of aldehyde appeared to match closely with that of the ADH activity (Fig 1 and 2). The observed variation in the amounts of aldehyde released, like ADH activities, could be attributed to the cultivar differences. It seems possible that very high ADH activity of Mahsuri on the 2nd day i.e. 48h of submergence resulted in conversion of most of the acetaldehyde to ethanol. Higher accumulation of aldehyde in other three cultivars after 72h could also be the result of declining ADH activity after 72h (Fig 1 and 2). Moreover, aldehyde, being toxic might have made IR42 more vulnerable submergence stress.

Enzyme activity staining for ADH isozymes showed that its activity increased under anaerobiosis (Fig 3) reflecting activation of alcohol dehydrogenase genes. Activation of certain genes by environmental stimuli has previously been reported (Muench *et al*. 1993). Grover and Pental (1992) reported five isozymes of ADH in the germinating seeds of *Oryza sativa*. The

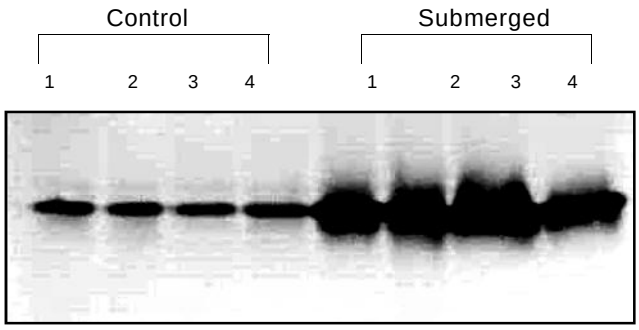

Fig. 3 Native-PAGE of alcohol dehydrogenase after 48h of submergence in one-week-old rice seedlings. (Where 1= FR13A, 2 = Gangasiuli, 3 = Mahsuri and 4 = Sarala). 60µg protein per lane was loaded.

pattern was same in both aerobically and anaerobically germinating seeds. As in present study, they too did not find any novel ADH isozyme under anaerobic conditions which they thought might be a common feature of the species belonging to the genus *Oryza*. Therefore, it appears that ADH activity alone may not determine the overall tolerance of all rice cultivars to submergence; other factors such as reserve carbohydrate status and the mode of their utilization (Das *et al*., 2000) may also play a role in survival of rice plants under submergence.

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