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Alcohol Dehydrogenase (ADh) Enzyme is a Potent Biochemical Marker for Submergence Tolerance in Rice (*Oryza sativa* L.) During Seedling Stage of Growth

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ABSTRACT

Aim: The incorporation of *sub* 1 gene in high yielding rice mega genotypes indicate that higher amount of Adh enzyme synthesis is controlled by the genetic factors. Adh enzyme will be the one of the vital factor for underwater respiration process of survival. Hence, rice genotypes with high amount of alcohol dehydrogenase enzyme synthesis during anoxic condition in compare with tolerant checks could be good promise for early introduction to the submergence prone area.

Methodology: Fresh and disease free seeds of fifty diverse rice genotypes including tolerant and susceptible checks were taken and germinated. 0.1 gm cut five days old rice seedlings of various submergence dose of day 1, 2, 3, 4 and 5 along with control were evaluated separately in three replications for Adh enzyme estimation by standard protocols with spectrophotometer.

Results: The peak time of Adh enzyme synthesis was during 72 hours to 96 hour of submergence period. Among paddy genotypes Mahananda along with Sabita and Purnendu performed well in compare with tolerant check paddy genotypes.

Conclusion: As it is controlled by genetic factors, so estimation of Adh enzyme activity under submerged stage could be the potent biochemical marker for the screening for paddy genotypes for seedling stage of crop growth during flash flooding condition.

Key Words: Alcohol dehydrogenase enzyme, Rice, Submergence, Biochemical marker

INTRODUCTION

Under flash flooding, few characters were identified as playing a key role in submergence tolerance in rice, the most critical are: maintenance of slow elongation of stem, maintenance of high carbohydrate concentration, retention of high chlorophyll percentages, optimum rates of alcoholic fermentation and energy conservation by maintaining low elongation growth rates during submergence (Jackson and Ram, 2003). Submergence tolerance is a metabolic adaptation in response to anaerobiosis that enables cell to maintain their integrity so that the plant survives hypoxia/anoxia without major damage (Sarkar *et al.*, 2006). Rapid increase of water due to various reasons is very much detrimental for crop establishment where direct sowing is practices (Kawano *et al.*, 2009). Tolerance to submergence stress during various stage of crop growth is also important aspect of submergence breeding (Ito *et al.*, 1999; Mohanty *et al.*, 2000). Tolerance to submergence stress during seedling stage of crop growth is very important for quick regeneration and initiation of new leaves after submergence (Setter *et al.*, 1997). Alcoholic fermentation is the alternative metabolic process that plants seems to get adapted under submerged or oxygen free atmosphere (Green Way and Setter, 1996). Under anaerobic conditions aerobic respiration shift to a less efficient anaerobic fermentation to provide energy for survival (Green Way and Setter, 1996). Alcohol dehydrogenase (Adh) involves in ethanol fermentation pathway that is responsible for the re-

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duction of toxic acetylaldehyde to ethanol, resulting in continuous regeneration of nicotinamide adenine dinucleotide (NAD) in the cytoplasm (Chung and Ferl, 1999). Hence, induction of Adh can enhance survival of plants under flooded conditions (Johnson *et al*., 1994). Changes in enzyme activity levels have been noted within a day under hypoxic conditions and may occur more quickly under anoxic conditions (Keeley and Franz, 1979). Variation of Adh activity was increased from aerial to submerged condition (Chan and Burton, 1992). Adh activity under submerged condition reaches to an optimum level which provide sufficient energy level for better survival. Estimation of Adh activity could be used as a tool to differentiate tolerant and susceptible level.

So an attempt was done to estimate the alcohol dehydrogenase enzyme activity in paddy genotypes which could provide an aid for bio chemical marker for submergence tolerance. Hence an attempt was made to study the alcohol dehydrogenase enzyme activity of fifty diverse paddy genotypes for tolerance under complete submergence condition during seedling stage of growth.

MATERIALS AND METHODS

Estimation of alcohol dehydrogenase enzyme: The Adh enzyme estimation was done by the modification of process of Tong, W.F. *et al*. 1989. The fresh and disease free seeds of fifty diverse rice genotypes were taken and germinated. Five days old rice seedlings was transferred to the test tube flooded with deionized water along with control. 0.1 gm cutted shoots of five days old rice seedlings of submergence dose of day 1, 2, 3, 4 and 5 along with control in separate set up with three replications were homogenated in a pre chilled mortar with 1.0 ml of 10 mM Tris-Hcl buffer solution (pH-7.6), containing 0.5mM zinc chloride, 0.5g polyvinylpyrolidone. The homogenate was centrifuged at 5600 x g for 20 mins, at -4^{0}C and the supernatant serve as a crude extract for assay of Adh activities. Adh activity was determined by modifying the method of Bonnichsen and Brink (1955). The assay mixture contained 0.1 M glycine- NaOH buffer (pH-9.0), 75mM ethanol and 0.26 mM NAD^+. After the addition of enzyme solution to the mixture the initial rate of NAD^+ reduction was measure at 340nm with spectrophotometer. One unit of Adh activity was defined as the amount which catalysed 1.0µ mole of NAD^+ per min. The calculation of Adh activity was based on manuals of Worthington and Worthington (2011), Units/mL = (A340/min x Cuvette volume x Enzyme dilution) / (6.22 x Sample volume).

This experiment was carried out under laboratory condition at the Dept. of Genetics and Plant Breeding, Calcutta University during 2015-2016.

RESULTS

Table 1 represents an account of estimated value of alcohol dehydrogenase enzyme units/gm/min of fifty diverse rice genotypes of five days aerial condition and five successive days of submergence during seedling stage of growth. The mean value ranges from 42.59 – 98.41 Adh units/gm/min. It was IR64 *sub*1 showed maximum activities of Adh enzyme. In compare with tolerant checks, rice genotypes like Mahananda, Sabita and Purnendu performed well. From chart 1, 2 and 3, it was revealed that gradually alcohol dehydrogenase synthesis took place during submergence period than the aerial condition. During seedling stage of rice genotypes, the enzyme synthesis reached its peak around 3-4 days of submergence. After that the synthesis of Adh enzyme falls rapidly during 5th day of submergence.

DISCUSSION

The interesting feature is that rice genotypes like Mahananda, Sabita and Purnendu showed Adh units above from the FR43B tolerant check genotypes and below the other tolerant check genotypes. But these rice genotypes performed appreciably well in compare with all tolerant check genotypes. Among three well performed rice genotypes Mahananda is best followed by Sabita and Purnendu. The exhaustion of reserve food materials of the nascent rice seedlings was unable to maintain the Adh activity so long period. It may be the age which determines the reserve food matters and Adh activity during extreme anaerobiosis stress condition. During early seedling stage of growth these genotypes may be considered to be a good promise for cultivation of paddy for submerged prone areas where direct sowing is practiced.

CONCLUSION

Flash flood and heavy rain during just grown rice seedling stage specially for direct seed sowing condition is very much fatal to the crop. As the rapid increase of water causes total inundation of the nascent rice seedlings, survival during seedling stage is very much crucial for regeneration after submergence. The well established *sub* 1 incorporated rice mega varieties like IR64 *sub* 1 and Swarna *sub* 1 showed commendable Adh enzyme activity in the experiment. In compare with the *sub* 1 introgressed rice varieties, genotype like Mahananda, Sabita and Purnendu performed at par during seedling stage of growth. So these rice genotypes could be a good promise for early selection to be introduced into the submerged prone area where flash flood may occur. So screening of Adh activity could a good tool for differentiating submergence tolerant and susceptible rice genotypes during seedling stage of growth.

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Table 1: Estimation of alcohol dehydrogenase enzyme activity in submergence condition in paddy genotypes during seedling stages of growth

Growth Condition / Name of the genotypes	5 Days Air	1 Day Submergence	2 Days Submergence	3 Days Submergence	4 Days Submergence	5 Days Submergence	Mean
	Adh units/ gm/min						
FR13A(TC)	18.81	50.64	102.73	156.27	165.12	66.23	93.30
Dudheswar	13.24	23.12	70.24	90.52	100.13	45.12	57.06
Mahananda	23.28	60.28	117.33	141.8	154.82	61.43	93.16
Lalat	14.23	19.33	69.24	92.13	100.53	34.23	54.95
Medi	13.52	21.23	56.52	78.14	88.23	42.12	49.96
Sonom	15.13	24.12	67.23	81.24	90.67	33.24	51.94
Raspanchali	11.12	26.23	65.31	80.52	99.89	30.12	52.20
Kataribhog	16.51	25.23	71.12	82.13	98.52	35.23	54.79
B-20	18.12	21.52	68.23	76.52	85.23	33.36	50.50

Table 1: ***(Continued)***

Growth Condition / Name of the genotypes	5 Days Air	1 Day Sub-mergence	2 Days Sub-mergence	3 Days Sub-mergence	4 Days Sub-mergence	5 Days Submer-gence	Mean
	Adh units/ gm/min						
Sita	19.23	23.34	72.12	82.32	87.23	42.12	54.39
Amulya	16.28	21.33	75.36	98.16	100.12	45.12	59.40
Vaidheli	21.23	35.16	98.23	110.52	115.23	49.34	71.62
SR26B	20.12	33.12	67.23	89.32	100.57	46.12	59.41
Swarna sub1(TC)	28.12	50.12	124.12	140.34	160.23	70.23	95.53
Lankagore	17.23	30.12	66.43	77.32	89.12	36.76	52.83
FR43B(TC)	26.34	45.12	97.23	124.12	145.34	54.23	82.06
Sabita	22.62	42.16	104.72	145.16	156.32	60.12	88.52
Barsatora	15.27	33.23	68.27	96.52	106.24	36.13	59.28
Ambika	17.52	42.27	73.16	99.16	105.16	41.23	63.08
IR64 sub1(TC)	27.16	56.23	99.54	153.61	178.82	75.12	98.41
Bhuri	23.32	34.16	68.27	89.23	102.24	38.23	59.24
Nagalmuda	24.13	37.27	98.16	112.13	138.24	43.23	75.53
Lakshmikajal	25.82	43.24	99.53	114.24	130.27	45.12	76.37
Khitish	19.12	33.23	89.12	100.12	125.12	42.23	68.16
Kalopahar	22.12	34.23	83.32	99.23	106.23	32.23	62.89
Malabati	21.13	37.27	82.13	89.16	116.27	35.25	63.54
Bakulpryia	20.21	32.27	85.32	99.57	105.21	30.12	62.12
Altanuti	18.21	30.23	75.32	89.52	100.21	29.23	57.12
Rajendraban	16.21	26.27	85.32	99.52	110.21	35.34	62.15
Dadswal	18.21	30.27	82.32	92.52	115.23	45.21	63.96
Morichswal	15.21	26.27	70.32	83.52	101.21	34.46	55.17

Table 1: ***(Continued)***

Growth Condition / Name of the genotypes	5 Days Air	1 Day Submergence	2 Days Submergence	3 Days Submergence	4 Days Submergence	5 Days Submergence	Mean
	Adh units/ gm/min						
Nonabakra	23.21	36.27	93.32	99.57	110.64	37.21	66.70
Pokkali	21.21	37.12	88.32	92.52	105.21	39.23	63.94
Ranjan	23.21	34.27	75.23	89.52	97.21	32.54	58.66
Bangalakshmi	14.21	26.27	65.32	79.52	80.21	30.23	49.29
Moulow	17.21	28.27	75.33	89.52	100.21	29.12	56.61
Palui	21.21	36.27	85.32	99.52	110.21	32.23	64.13
Akandi	18.12	34.32	72.13	89.24	108.52	34.67	59.50
Purnendu	21.53	52.34	98.24	132.21	145.24	61.26	85.14
CR-1280	19.21	45.12	67.23	78.23	98.12	30.12	56.34
Masuri	13.5	19.26	70.31	89.11	111.24	32.23	55.94
Niko	16.21	26.24	65.13	87.24	118.52	34.74	58.01
IR64(SC)	14.34	28.23	78.21	92.34	121.21	30.12	60.74
Swarna(SC)	13.02	20.26	72.35	91.16	117.2	37.23	58.54
N-Shankar	15.12	18.23	67.12	78.23	89.12	35.67	50.58
IR42(SC)	11.21	19.13	40.24	68.21	87.52	29.24	42.59
Jaladhi II	24.34	48.21	86.24	100.16	139.24	56.89	75.85
Khejurchori	13.24	23.43	56.12	78.12	89.12	33.33	48.89
Kanakchur	15.67	24.12	45.16	67.12	78.57	35.24	44.31
Lilabati	15.67	23.12	46.12	65.12	88.12	36.45	45.77
Mean	18.60	32.58	78.02	96.62	111.47	40.72	

TC- Tolerant check, SC-Susceptible check

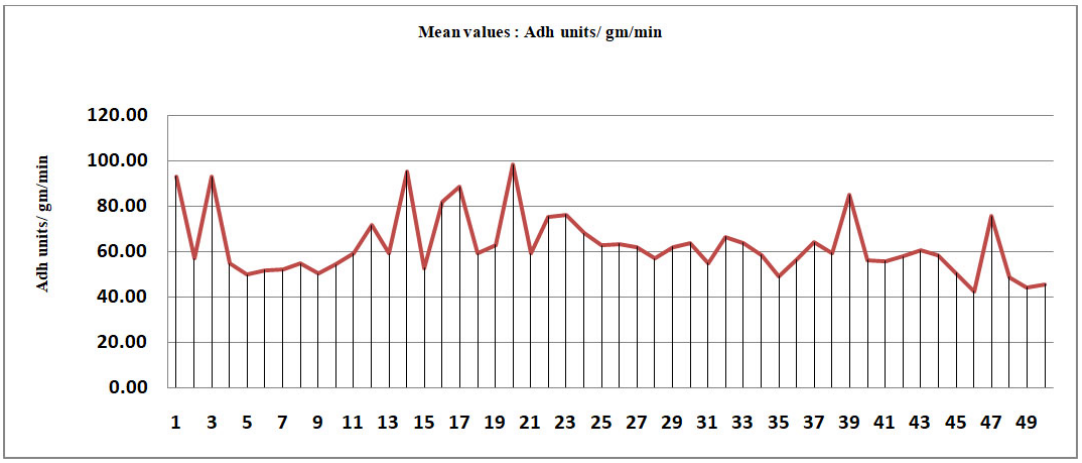

Chart 1: Mean values of Adh units of fifty diverse rice genotypes.

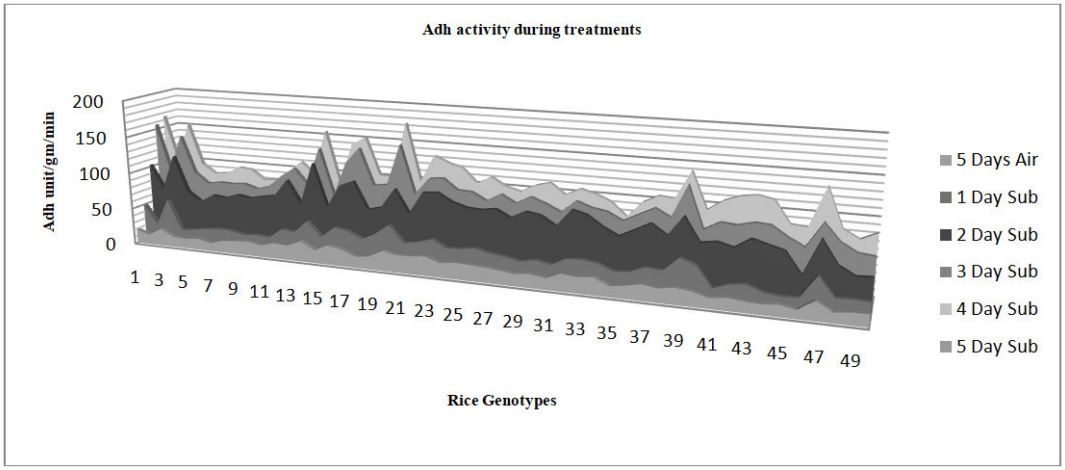

Chart 2: Adh activity during various treatments.

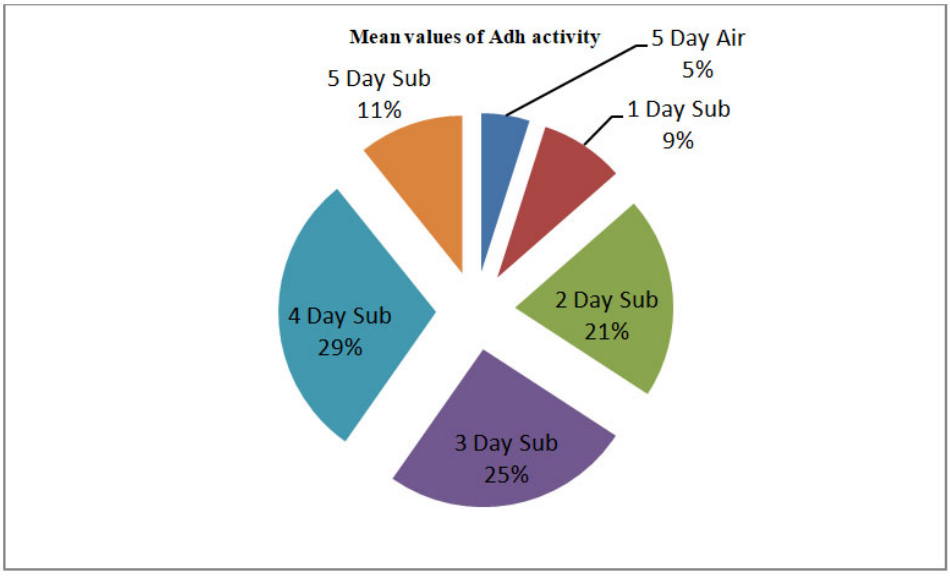

Chart 3: Pie chart showing difference in Adh activity during treatment effects.