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ROLE OF ACTIVE OXYGEN SCAVENGING SYSTEM (AOS) IN DETERMINING SUBMERGENCE TOLERANCE IN RICE

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ABSTRACT

The Rainfed lowland rice crop not only water deficits but also excess water to complete submergence. Rice is well adapted to aquatic environment. It has low survival if complete submergence persists for several days. Death of plant due to submergence depends on a number of factors. When plants are de-submerged after a period of submergence, a sudden exposure to air possibly produces free- radical of oxygen which may induce membrane lipid peroxidation, protein denaturation and changes in DNA leading to cell death. The present study was conducted to elucidate the role of active oxygen scavenging systems (AOS) in protecting from oxidative damage. In pot culture experiments, 14- and 21-days old plants of rice varieties differing in submergence tolerance were subjected to 4 and 7d complete submergence in outdoor pond under natural conditions. The activities of oxygen scavenging enzymes *viz.*, catalase, peroxidase and superoxide dismutase (SOD) were measured before submergence over a time course after de-submergence.

Key words : *Oryza sativa*, Submergence, Superoxide dismutase, Catalase, Peroxidase.

Introduction

Rice is extremely tolerant of standing water associated environmental stresses. Tolerance appears to be based on several morphological and biochemical features that are expressed to varying degrees depending upon the cultivar, environmental conditions and the stage of plant growth. Plants experience hypoxia to anoxic conditions under waterlogging and / or submergence leading to tissue damage and eventual death of plants when conditions are too harsh. Plants exposed to aperiod of anoxia survive, only to die on re-exposure to air (Monk *et al*., 1987a and Boamfa *et al*., 2002) suggesting oxidative damage during recovery phase. Anoxia is a major stress factor in flooded environments (Crawford, 1982; Ram *et al*., 2002), similar sequences of events could be expected to occur in plants which have undergone a period of submergence. Under stress condition, the production of reactive oxygen species occurs at a level much higher than could be removed by the oxygen scavenging systems. Plants, in general, are equipped with antioxidative defense system, which quickly remove the free radicals of oxygen preventing cellular damage. AOS are constitutive, they can show higher activity in response to re-exposure to oxygen after a period of anoxia induced by flooding or submergence.

Materials and Methods

Plant culture and imposition of submergence treatment

Seeds of four rice varieties namely FR 13A, Vaidehi (submergence intolerant) and Mahsuri IR 42 (submergence intolerant) were surface sterilized in 1% sodium hypochlorite solution for 2 minutes and thoroughly washed under running tap water and then placed for sprouting under dark at 30^0C. Sprouted seeds were direct seeded at 1 cm depth in 25 cm diameter earthen pots filled with 88 kg well pulverized farm soil fertilized with a recommended dose of NPK (60;40;40kg ha$^-$1). Ten replicates' pots with 5 plants in each pot were maintained per treatment under completely randomized design. Data were collected in triplicates and analyzed using standard procedures. Complete submergence treatment was

performed with 14- and 21-days old plants for 5 and 10 d durations under natural condition in an outdoor pond. All the end of submergence period, the pot was taken out of the ponds and ept in shade for 12 hours and then shifted to natural condition.

Measurements

Measurements on active oxygen scavenging systems *viz*., catalase, peroxidase and super oxide dismutase (SOD) were made just prior to submergence and within one hour after termination of submergence. For time SOD measurements, rice varieties FR 13A and Vaidehi (tolerant) and Mahsuri and IR42 (intolerant) were used. Plants survival was recorded 10d after removal of plants from submergence. Water quality for dissolved O2 and CO_2, temperature, pH and underwater irradiance in submergence pond was also monitored during submergence period in order to define the submergence environments.

Superoxide dismutase activity in leaves was assayed following the method described by Asada *et al*. (1974) based on the inhibition of photo chemical reduction of dye nitro blue tetrazolium (NBT) by the enzyme SOD, Peroxidase and catalase activities in rice leaves were assayed calorimetrically following the methods described by Sinha (1972) and Mc Curne Gulstan (1959), respectively.

Results

Flood water quality is known to influence survival by changing morpho-physiological manifestations of plants (Ram *et al*., 2002, Setter *et al*., 1989).

Superoxide dismutase (EC. 1.15.1.1)

Superoxide dismutase the key enzyme for dismutation of superoxide free radicals was also measured in rice leaves before submergence and 5 and 10d of submergenc. The enhancement in SOD enzyme activity on submergence as compared to non-submerged control was around 12.0 to 12.8 folds, in tolerant varieties and 7.0 to 9.5 folds approximately in intolerant varieties after 5d of submergence. With further increase in submergence duration the enzyme activity went up by 14.6 folds in Vaidehi and 15 folds in FR13A whereas, Mahsuri and IR42 showed 11.7- and 9.0-folds higher activity.

Catalase and peroxidase (EC.1.11.1.6 and EC.1.11.1.7)

In general, catalase activity increased by roughly 2-

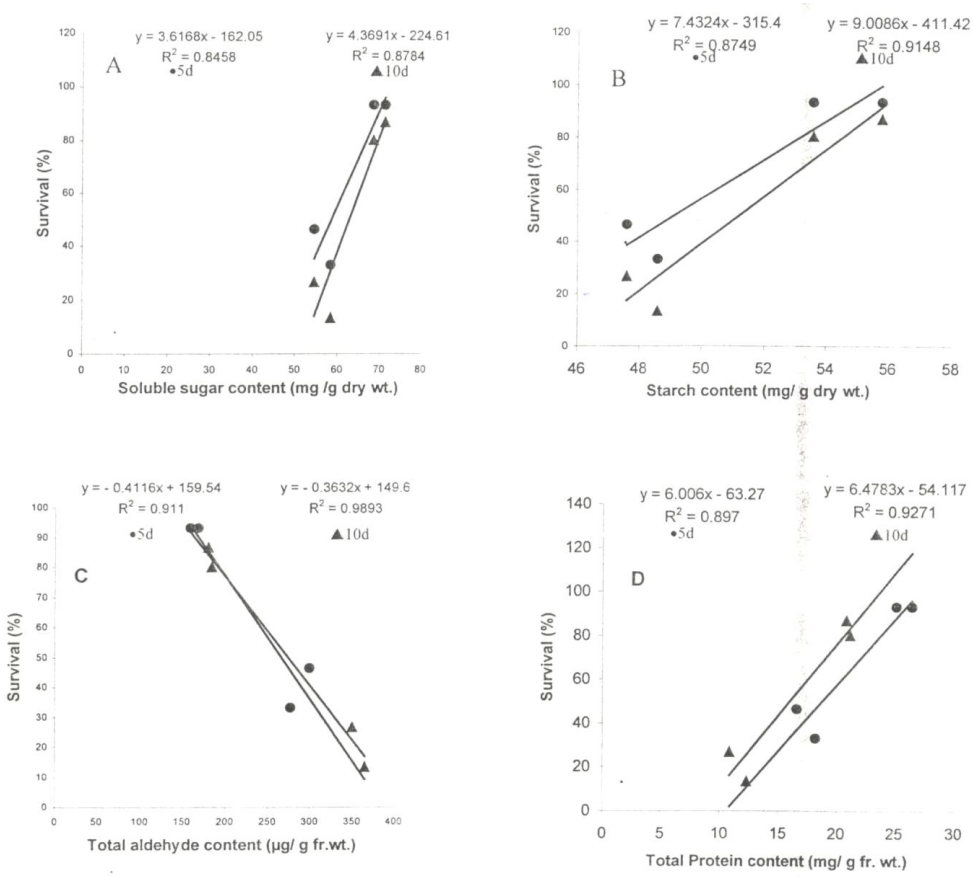

Fig. 1 : Correlation between survival and soluble sugars (A) and starch (B) in shoots prior to submergence and survival and total aldehyde (C) and total protein (D) in leaves just after de-submergence of lowland rice varieties. 30d old plants were completely submerged for 5 and 10 d in outdoor pond under natural conditions.

Table 1 : Environmental characterization of flood water in submergence tank during 5 and 10 d submergence durations (measurements were made at plant canopy level and averaged for the entire submergence durations).

Submergence duration (days)	Time of measurement	Flood water parameters				
		CO_2 (mol m^{-3})	O_2 (mol m^{-3})	Temp. (0^0C)	pH	Irradiance* (PAR) (µ mol m^{-2} S^{-1})
0d	0600h	0.41±0.08	0.09± 0.02	28.6± 0.05	7.9 ± 0.03	107.5±2.1
	1600h	0.15±0.01	0.16 ± 0.02	32.6± 0.09	8.2± 0.05	(405±3.6)
5d	0600h	1.76±0.04	0.11±0.02	32.0± 0.05	8.0 ± 0.02	103.5±1.2
	1600h	0.99±0.04	0.15± 0.01	34.3 ±0.04	8.2 ± 0.02	(395±4.5)
10d	0600h	1.37 ± 0.10	0.10 ± 0.01	29.6 ±0.07	8.1 ±0.03	99.6±0.8
	1600h	1.33 ± 0.07	0.11 ±0.01	32.3 ±0.05	8.2±0.02	(415±3.5)

* Irradiance was measured at 1100h, figures in parentheses are corresponding irradiance in air.

Table 2 : Effect of submergence durations on superoxide dismutase, catalase and peroxidase activities in leaves of lowland rice varieties.

Variety	Submergence duration		
	0d	5d	10d
***SOD (units g^{-1} fr.wt.)**			
FR 13A	416	5367	6305
Vaidehi	437	5286	6413
Mahsuri	395	3787	4644
IR 42	427	3032	3908
CD AT 5 %	**59.3**	**168.4**	**161.5**
****Catalase activity (units g^{-1} fr. wt. min^{-1}) x 10^2**			
FR 13A	1.52	6.20	8.08
Vaidehi	1.80	6.48	8.84
Mahsuri	1.32	3.88	6.56
IR 42	1.60	3.64	6.80
CD AT 5 %	**0.80**	**1.30**	**1.50**
****Peroxidase activity (units g^{-1} fr. wt. min^{-1}) x 10^2**			
FR 13A	1.50	6.9	9.4
Vaidehi	1.50	7.0	9.6
Mahsuri	1.30	4.0	5.8
IR 42	1.35	3.6	6.0
CD AT 5 %	**0.10**	**0.16**	**0.11**

* One unit of enzyme activity is defined as the amount of enzyme catalyzing 50% inhibition in the reduction of dye NBT under specified assay conditions.

**1 Unit of enzyme activity is defined as the amount of enzyme that catalyzes increase in absorbance of 0.1 per minute in reaction mixture under specified assay.

3 folds in intolerant varieties and 3-4 folds in tolerant varieties over their respective non-submerged control with 5 days submergence period. Like catalase, peroxidase activity also did not vary significantly in tolerant and intolerant varieties when measured prior to submergence (Table 2). However, tolerant varieties (FR13A and Vaidehi) showed significantly greater enhancement in enzyme activity on submergence than Mahsuri and IR42(intolerant). The enhancement in enzyme activity on submergence was around 2.6-3.0 folds in intolerant varieties and approximately 4.6 folds in tolerant varieties after short period (5d) of submergence. The maximum being 6.4 folds in FR13A with 10 days of submergence.

Discussion

SOD activity submergence of rice varieties for 5 and 10d durations increased SOD activity survival folds showing greater increase in tolerant varieties through the initial level of enzyme was almost similar irrespective of the submergence tolerance level of the varieties (Table 2). Other two enzyme of antioxidative defence system namely catalase peroxidase also increased during post submergence phase. Enhancement of SOD, catalase and peroxidase activities during post-submergence/ post anoxic phase has been reported in a number of plants indicating the occurrence of oxidative stress (Monk *et al*., 1987 a & b; Yu and Rangel, 1999; Ushimaru *et al*., 1992, 1997, 1999).

Yu and Rangel (1999) reported over production of FeSOD and MnSOD in transgenic tobacco induced by waterlogging. However, there was no difference in waterlogging induced growth reduction between transgenic lines over-expressing FeSOD and MnSOD. Transgenic lines in tobacco and lupins over producing enzyme activity suffer less growth reduction than the non-transgenic parental lines (Yu and Rengal, 1999).

Higher catalase peroxidase activity observed especially intolerant rice varieties could possibly reduce the damage by scavenging hydrogen peroxide generated during oxidative stress. These antioxidative defence system effectively scavenge free radicals of oxygen

produced during post submergence/ post anoxic phase productivity plant membrane from lipid peroxidative and protein denaturation. Role of superoxide dismutase, catalase and peroxidase in combating oxidative stress in plants has been well reviewed (Raychaudhuri, 2000; Jackson and Ram, 2002; Ram *et al*., 2002). Direct experimental evidence implicating active oxygen species in post submergence injury is still at large. However, submergence of rice plants has been reported to increase the generations of free radicals as detected in leaves by electron paramagnetic resonance (Thongbai and Goodman, 2000). Supplying plants with ascorbate 24 h before de-submerging scavenge these free radicals improved survival rates especially in a submergence sensitive cultivar (Thongbai and Goodman, 2000). Damage of submerged rice plants by oxidative stress has been demonstrated by measuring ethane evolution, a product of lipid peroxidation, which was produced in a greater amount by intolerant rather than tolerant rice lines (Santosa *et al*., 2001).

The experimental evidences on oxidative damage even during submergence has not been explored, though it is possible that anoxic core can develop in the enterior of the tissues during submergence which are oxygenated during day time from photosynthetically derived O_2 inducing oxidative damage. Santosa *et al*. (2001) demonstrated ethane production by submerged rice plants.

Conclusion

In conclusion, short term submergence depressed plant survival possibly through oxidative damage during the recovery phase. Rice varieties capable of inducing ant oxidative defense systems during anoxic submerged phase have advantage of surviving better than those with ill equipped defense system. Manipulation of SOD and other defense systems lie catalase, peroxidase, glutathione reductase and antioxidants like a- tocopherol, ascorbate and glutathione may enrich the defense system of rice plants to combat the ill effects of submergence and anoxia.

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