Indian Journal of Agricultural Sciences **79** (9): 687–93, September 2009

Physiological marker traits associated with submergence tolerance of lowland rice (*Oryza Sativa*)

A K SINGH[1], SANJAY SINGH[2], ANURADHA SINGH[3], S K PRADHAN[4], J P TYAGI[5] and ALOK SINGH[6]

Narendra Deva University of Agriculture and Technology, Faizabad, Uttar Pradesh 224 229

Received: 27 January 2009; Accepted: 5 June 2009

ABSTRACT

A field experiment was conducted during 2005–06 to elucidate the possible pre- and post-emergence traits associated with injury and submergence tolerance of lowland rice (*Oryza sativa* L.). Ten replicate pots with 5 plants in each pot were maintained per treatment under completely randomized design, respectively. The total protein content though was not much variable among varieties before submergence but decreased appreciably during submergence showing much lower values in intolerant varieties. A strong positive correlation between survival and protein content of leaves just after de-submergence was observed (R^2= 0.90 for 5d) and (R^2=0.93 for 10d) of complete submergence indicating a direct relationship of protein content with survival. Intolerant rice varieties accumulated 21 to 23 folds aldehyde on submergence against only 10 folds in tolerant ones showing a negative correlation (R^2=0.9) between survival and aldehyde accumulation which might prejudice the submergence tolerance of rice. Activities of superoxide dismutase, catalase and peroxidase enhanced on submergence in all the varieties though the induction was more in tolerant varieties. 'FR13A' and 'Vaidehi' had 15 folds increase in superoxide dismutase activity against only 9 to 11 folds in 'Mahsuri' and 'IR42' respectively with 10 days complete submergence. These traits could possibly be looked into as markers for structuring submergence tolerant lowland rice.

Key words: Aldehyde, Catalase, Cycocel, MBTH (3-methyl-2 Benzo-thiozolinone hydrazone),*Oryza sativa*, Porosity, Peroxidase, Paclobutrazol, Submergence, SOD (superoxide dismutase)

Lowland rice (*Oryza sativa* L.) experiences partial to complete submergence during monsoon rains and this occurs more than once during the growing seasons. Although rice is known to grow well under flooded conditions. Complete submergence may devastate the crop, especially during early stage of crop growth. Flooding and submergence is known to create hypoxia or anoxia in plants altering their normal functioning. Plants tolerant to hypoxia/anoxia develop inducible aerenchyma in addition to the constitutive aerenchyma to facilitate oxygen transport through plants and poor aeration of soils increases root porosity (Gibberd *et al.* 1999). Low O_2 concentrations in water of flooded rice fields may induce anoxic conditions which produce toxic metabolites causing injury to plants (Setter *et al.* 1997).

Anoxia induced aldehyde production during submergence could be particularly important, since accumulation of aldehyde could be damaging to plant tissues. Under anoxia, alcoholic fermentation tends to increase, resulting in increased synthesis of acetaldehyde and ethanol (Boamfa *et al.* 2005). Any hindrance in the conversion of acetaldehyde to ethanol may eventually lead to higher acetaldehyde in tissue which might trigger cell death. Carbohydrate which is the substrate for alcoholic fermentation has long been recognized as an important factor in submergence tolerance. Treatments which alter the carbohydrate status of the plants prior to submergence greatly affect the submergence tolerance (Ram *et al.* 2002, Ismail *et al.* 2009). Post-submergence/postanoxic generation of free radicals of oxygen which triggers membrane lipid peroxidation, protein denaturation and DNA mutation may induce injury leading to cell death. However, physiological and biochemical traits studied so far did not clearly underpin the precise timing of

[1]Assistant Professor (e mail: ashok_singh@india.com); [2]Senior Scientist (e mail: anjay_singh777@yahoo.com), Genetics Division, IARI, NewDelhi 110 012, [3]Ph D Scholar (email: ashok_singh @india.com), Department of Crop Physiology, NDUniversity of Agriculture and Technology, Kumarganj, Faizabad 224 229, [4]Senior Scientist (e mail: pradhansk @yahoo.com), Central Rice Research Institute, Cuttack, Orissa, [5]Technical Officer (e mail: sanjay_singh777@yahoo.com), Genetics Division, IARI, New Delhi, 110 012, [6]Ph D Scholar (e mail: singhalok@redfmail.com), Department of Crop Physiology.

cell death especially in lowland rice. Therefore, the present investigation was carried out to elucidate the possible pre and post submergence traits associated with injury and submergence tolerance of lowland rice.

MATERIALS AND METHODS

The experiment was evaluated in a randomized block design with 3 replications at the University during rainy *(kharif)* season of 2005–06. Seeds of 4 rice varieties, namely 'FR 13A', 'Vaidehi' (submergence tolerant) and 'Mahsuri', 'IR 42' (submergence intolerant) were surface sterilized in 1% sodium hypochlorite solution for 2 min. and thoroughly washed under running tap water and then placed for sprouting under dark at 30°C. Sprouted seed were direct seeded at 1 cm depth in 25 cm diameter earthen pots filled with 8 kg well pulverized farm soils fertilized with recommended doses of NPK (90: 60: 40 kg/ha). Ten replicate pots with 5 plants in each pot were maintained per treatment under completely randomised design. Complete submergence treatment was performed with 30-days- old plants for 5 and 10days durations under natural condition in outdoor pond with water depth of 1 m to ensure that plants remained completely under water during submergence treatment. At the end of submergence period, the pots were taken out of the ponds and kept in shade for 12 hr and then shifted to natural conditions to quantify submergence environment. The floodwater quality for O_2, CO_2, temperature, *p*H and underwater irradiance was monitored during submergence period.

All measurement were made in triplicate and using standard procedures. Measurement on plant height, shoot dry weight and root porosity were made just prior to submergence and within an hour after termination of submergence. Plant height was measured from the base of the plants at the soil surface to the tip of longest leaf used to express elongation during submergence in different varieties. Sample for dry weight were collected and quickly fresh frozen in liquid nitrogen, brought to laboratory and were oven-dried at 70±1°C until constant weight. Root porosity is based on buoyancy of the tissue by measuring fresh weight in air and water.

For protein analysis, fresh leaf sample were collected and quickly fresh frozen in liquid and analyzed protein content in laboratory by using the modified method of (Lowry *et al.* 1951).Total aldehyde content in second and third fully expanded leaves from the top was measured prior to submergence and just after removal from submergence using the standard procedure described by (Sawicki *et al.* 1961) and standardized for rice samples. 1 g fresh sample was extracted with 5 ml, 1 M aqueous chilled perchloric acid (PCA) and the extract was neutralized to *p*H 6.5 by 1m KOH (the volume of KOH was recorded for calculation). 05 ml of neutralized extract was reacted with 0.5 ml of 0.4% aqueous 3-methyl-2 benzo-thiozolinone hydrazone (MBTH) in a screw cap vials at 98°C exactly for 3 min, cooled quickly to room temperature under running cold tap water. To each tube, 2.5ml of 0.2% aqueous ferric chloride solution was added and 5 min later, 6.5 ml acetone was mixed in each tube with vortexing. Absorbance was measured at 635 mm against reagent black prepared with neutralized 1m PCA treated as per samples. The amount of aldehyde was calculated from standard methods.

Enzyme associated with post submergence oxidative damage, namely catalase (EC. 1.11.1.6), Peroxidase (EC.1.11.1.7) and superoxidase dismutase (EC.1.15.1.1) were extracted in phosphate buffer and assayed as per standard procedures described by (Sinha 1972, Mc Curne and Galstan 1959, Asada *et al.* 1974) respectively. Recovery checks were performed to validate the methods for rice samples.

RESULTS AND DISCUSSION

Floodwater parameters

Floodwater quality is known to influence plant survival, hence, was monitored regularly during submergence period. On the day of submergence, floodwater CO_2 concentrations were lower (0.4 mol/m^3) which increased by 4 folds (1.76 mol/m^3) when measured in the morning at the end of 5 and 10 d of submergence. The concentration was higher in morning 0600 hr and lower in evening 1600 hr, possibly due to utilization of CO_2 in photosynthesis. In contrast, O_2 concentrations were higher in the afternoon (0.16 mol/m^3) and lower in the morning (0.10 mol/m^3) again indicating the contribution from underwater photosynthesis. Floodwater temperature ranged between 29 and 34°C, whereas *p*H varied between 7.9 and 8.2. Underwater irradiance measured at the top of the canopy of submerged plants was (105 µmol/m^2/S^1) about 1/4 of the irradiance in air above the water surface. Lower irradiance under water may decrease photosynthesis by the submerged plants (Ram *et al.* 1999).

Growth, root porosity and survival

Plant height measured prior to submergence and just after the termination of submergence showed an increased underwater elongation. The elongation in tolerant varieties ('FR 13A' and 'Vaidehi') was about 7 and 12%. while, in intolerant varieties ('Mahsuri' and 'IR 42') was from 13 to 21% for 5 and 10 d submergence respectively. Survival recorded 10 days after termination of submergence, showed 93% survival in both the tolerant varieties whereas intolerant varieties 'Mahsuri' and 'IR 42' had 47 and 35% survival with 5d complete submergence. When submergence period increased to 10days, survival further decreased to 87% for 'FR 13A' and only 13% for 'IR 42'. It is clear from the above observations that rice varieties having greater elongation on submergence had lower survival.

Root porosity, an indication of internal gas space, is known to facilitate gas transport and are particularly important during complete submergence of plants. In the present experiments,

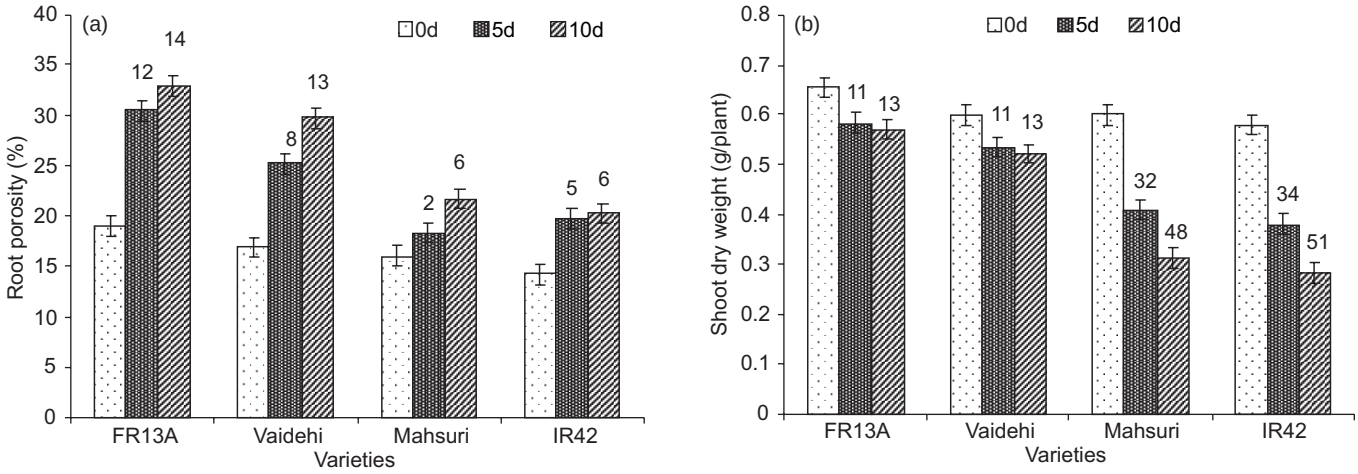

Fig 1 Effect of submergence durations on root porosity (a) and shoot dry weight (b) in lowland rice. Figures at the top of bars are increase in porosity (a) and per cent reduction in dry weight (b) over control (0d) on submergence

root porosity of different rice varieties showed only a small variation before submergence but a sharp increase occurred on submergence (Fig 1). The increase in root porosity over non-submerged controls was higher in tolerant varieties ranging between 12 and 14% in 'FR 13 A' and 8 to 13% in 'Vaidehi' for 5 and 10 d submergence. Intolerant varieties on the other hand, showed only 2 to 6% increase in porosity on submergence.

Ram *et al.* (2002) reported that rice plants elongate on complete submergence in an attempt to escape O_2 deprivation. We observed variable shoot elongation, which was distinctly higher in intolerant varieties. Rapid elongation seems to deplete plant reserves leaving only little amount to support recovery growth after submergence period is over (Mallik *et al.* 2002). This is clearly reflected in lower shoot dry weight in intolerant varieties of rice in comparison to the tolerant ones (Fig 1). Since, underwater irradiance during submergence was only 1/4 of the normal radiation, production of new photosynthates (dry matter) is greatly reduced. Hence, varieties having higher dry matter prior to submergence and also lower depletion during submergence will have advantage in survival of submergence. Higher survival was observed in less elongating 'FR 13A' and 'Vaidehi' whereas more elongating 'Mahsuri' and 'IR 42' had lower survival. A clear-cut negative correlation between elongation by submerged plants and various symptoms of injury or rates of survival was demonstrated with large number of genetically linked rice genotypes. Overall, the balance of evidence points to greater underwater leaf extension being associated with poorer rates of survival (Setter *et al.*1996). The likely explanation is that extra growth exhaust limited substrates supplies or redirects them away from maintenance process needed to prolong survival.

The most common and important adaptation to survive a long-term flooding is the development of a gas space continuum primarily in the cortical tissue, which may stretch from the stomata to the root tips(Jackson and Armstrong 1999, Mc Donald *et al.* 2001). This changes the root porosity and oxygen transport to the otherwise anoxic roots during submergence. High root porosity observed in 'FR 13 A' and 'Vaidehi' during submergence (Fig 1), therefore, might be advantageous for survival through enhanced O_2 transport (Singh *et al.* 2002, Singh *et al.* 2001). In our studies, submergence-intolerant rice varieties deplete dry matter faster than the tolerant ones which probably is utilized in enhanced underwater elongation in intolerant varieties prejudicing survival. Lower dry matter observed in rice plants completely submerged for 4 to 12 days could either be from reduced dry matter production or loss in dry weight due to utilization in respiration or both. Similarly, shoot mass of wheat plants waterlogged for 28 days decreased by 2 to 3 folds than the well drained plants and even after the recovery period of 25 days shoot biomass did not recover fully in waterlogged plants (Mallik *et al.* 2002).

Shoot dry weight before submergence was almost similar in all the 4 rice varieties but decreased during 5 and 10 days submergence periods at a variable rates. The decrease in shoot dry weight of both the tolerant varieties was 11 and 13% with 5 and 10d submergence respectively while corresponding decrease in 'Mahsuri' was 32 and 48% and 34 and 51% in IR42, respectively (Fig 1). It indicates a rapid depletion of shoot dry weight in intolerant varieties during submergence possibly due to rapid utilization in anaerobic respiration.

Total aldehyde content

Accumulation of aldehyde on submergence is an indicator of oxygen stress-induced alcoholic fermentation in plants. Total aldehyde before submergence was very nominal but increased during submergence in all the rice varieties (Fig 2). The magnitude of aldehyde accumulation was to the tune of only 10 folds in tolerant varieties whereas, it was

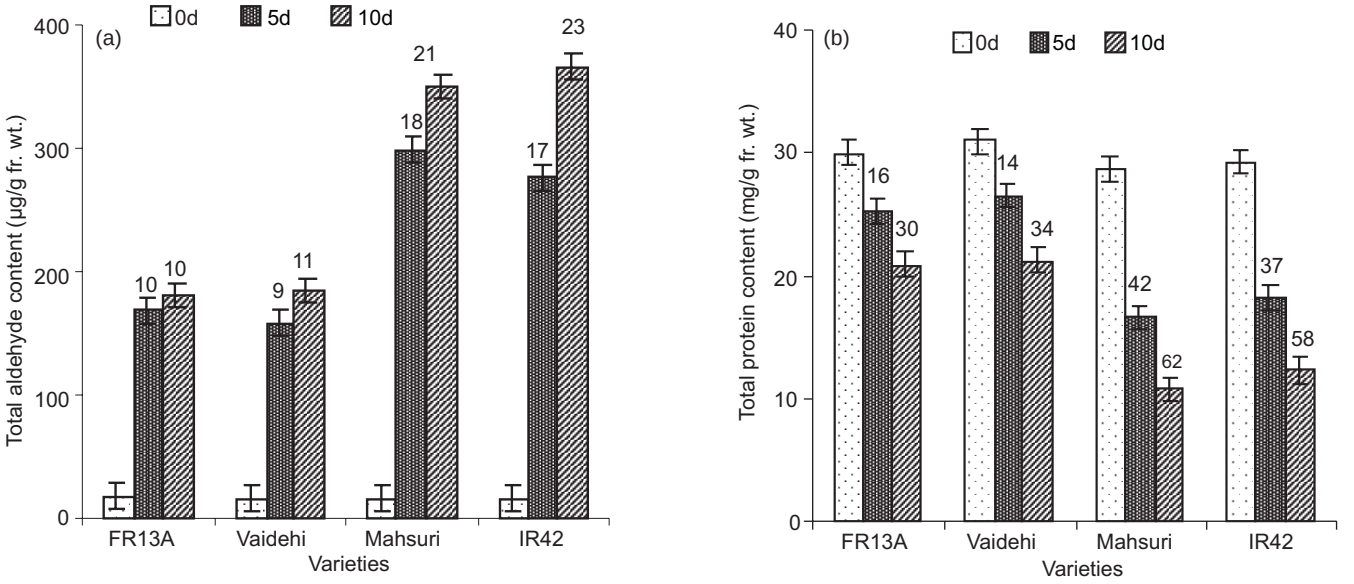

Fig 2 Effect of submergence durations on total aldehyde (a) and total protein (b) contents in leaves of lowland rice varieties. Figures at the top of (a) are per cent reduction and in (b) are times accumulation over non-submerged control (0d)

about 20 to 23 folds in intolerant varieties. This clear-cut difference between tolerant and intolerant varieties indicates about the status of alcoholic fermentation in different plants. Higher aldehyde accumulation was negatively correlated with lower plant survival with R^2 values of 0.91 and 0.99 with 5 and 10 days submergence respectively (Fig 3) showing damaging effects of aldehyde. Recent study indicated that alcoholic fermentation in excised rice coleoptiles which was 7.5 µmol/g fresh weight/hr during the first 12 hr was down regulated after an initial period of acclimation to anoxia producing less anaerobic proteins and with lower consumption of sugars (Colmer *et al.* 2001). Differential accumulation of aldehyde was observed in a number of rice varieties and was correlated with submergence tolerance of rice (Boamfa *et al.* 2005). However, this emission does not account for the sizeable amounts of acetaldehyde and ethanol which leaked out into the surrounding submergence water. Another difficulty in interpreting the survival data as influenced by total aldehyde content is that, we do not know how much of the measured aldehyde is acetaldehyde and what is its actual concentration in the growing rice shoot tips which are directly related to survival growth after submergence is over. But the observed differential behaviour of total aldehyde accumulation in previously known tolerant and intolerant rice varieties, could be useful trait for screening lowland rice for submergence tolerance.

Total protein content

Leaf protein content in all the 4 rice varieties was almost similar before submergence. On submergence, however, protein content decreased gradually showing maximum reduction with 10 days submergence (Fig 2). 'FR 13A' and 'Vaidehi' had only 30 and 34% reduction in protein content, whereas, 'Mahsuri' and 'IR 42' showed 62 and 58% decrease respectively with 10days of submergence. The drastic reduction in protein content of leaves of intolerant varieties shows a more damaging effects on protein machinery including enzymes rendering them susceptible of submergence. Higher protein content probably imparts better survival on submergence which is also evident from a strong positive correlation (R^2 values of 0.90 for 5days and 0.93 for 10days submergence) between survival and protein content of leaves at the end of submergence period (Fig 3). Total protein content (soluble plus insoluble) of rice varieties though did not differ significantly prior to submergence but declined gradually with increasing submergence durations. Intolerant varieties had almost 58-62 % reduction in total protein content after 10 days submergence, indicating that submergence has more damaging effects on these varieties, possibly through accelerated protein hydrolysis and/or reduced protein synthesis. Anaerobiosis induces the repression of most aerobic protein synthesis and induction of a number of anaerobic proteins (ANP's) in different plants (Raymond 1985). Thus, it looks obvious that the observed decline in protein content on submergence could mainly be due to the degradation of aerobic proteins (soluble and insoluble). Positive correlation between survival and total protein content (at the end of submergence) with R^2 values of 0.90 and 0.93 for 5 and 10days of submergence respectively (Fig 3) revealed that tolerant varieties somehow were able to stabilize protein structures, in addition to the synthesis of new anaerobic proteins under adverse submergence environments.

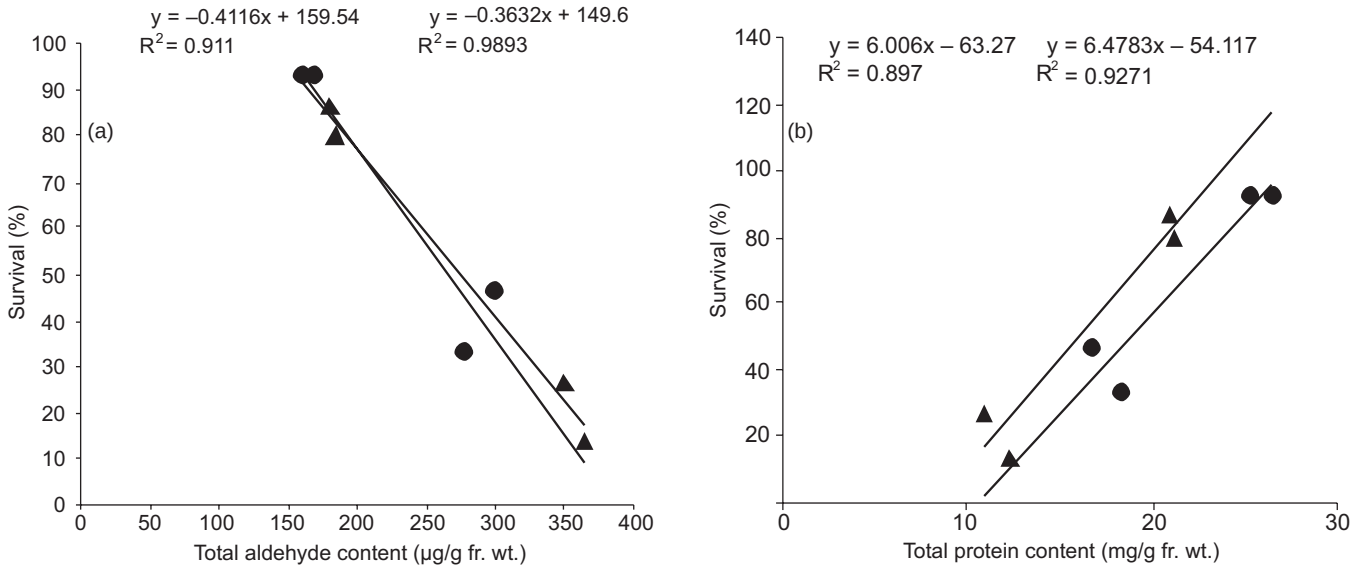

Fig 3 Correlation between survival and total aldehyde (a) and total protein (b) in leaves of lowland rice varieties during 5 and 10d of complete submergence just after de-submergence

Superoxide dismutase

The activity of superoxide dismutase, the key enzyme for dismutation of superoxide free radicals, increased on submergence as compared to non-submerged control (Table 1). The increase was about 13 folds in tolerant varieties only 7.0 to 9.5 folds in intolerant varieties after 5days of submergence. With further increase in submergence duration to 10days, the enzyme activity went up by 15 folds in 'FR13A' and 'Vaidehi' whereas, 'Mahsuri' and 'IR42' showed 11.7 and 9.0 folds higher activity over non submerged controls.

In general, catalase and peroxidase activities did not vary significantly in tolerant and intolerant varieties when measured prior to submergence (Table 1). However, on submergence catalase activity.increased roughly by 2-3 folds in intolerant varieties and 3-4 folds in tolerant varieties over their respective non-submerged control with 5 days submergence period. Peroxidase activity also showed significantly greater enhancement in tolerant varieties ('FR13A' and 'Vaidehi') than the intolerant ('Mahsuri,' and 'IR42') on submergence. The enhancement in enzyme activity on submergence was around 3 folds in intolerant varieties and approximately 5 folds in tolerant varieties after short period (5days) of submergence. The maximum enhancement being about 6 folds in tolerant and only 4 folds in intolerant varieties with 10 days of submergence (Table 1). Submergence of rice varieties for 5 and 10days durations increased SOD activity several folds showing greater increase in tolerant varieties though the initial level of enzyme was almost similar, irrespective of the submergence tolerance level of the varieties (Table 1). Other 2 enzymes of anti-oxidative defense system namely catalase and 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 (Roy Chaudhary 2000).

Higher catalase and peroxidase activity observed, especially in tolerant rice varieties might minimize the damage by scavenging hydrogen peroxide generated during oxidative stress. These anti-oxidative defense system effectively scavenge free radicals of oxygen produced during post submergence/post anoxic phase protecting plant membrane from lipid peroxidation and protein denaturation. Rio *et al.* (2006) has been well reviewed the role of superoxide dismutase, catalase and peroxidase in combating oxidative stress in plants. 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 generation of free radicals as detected in leaves by electron paramagnetic resonance (Thongbai and Goodman 2000). Damage of submerged rice plants by oxidative stress has been demonstrated by emission of ethane, a product of lipid peroxidation, which was produced in a greater amounts by intolerant rather than tolerant rice lines (Yu and Rengel, 1999). The experimental evidences on oxidative damage during submergence has not been explored, though it is possible that anoxic core can develop in the interior of the tissues during submergence which are oxygenated during day time from photo synthetically derived O_2 inducing oxidative damage.

The basic mechanisms associated with submergence injury and tolerance of lowland rice is now becoming clearer. The damage seems to be associated with impeded gas exchange which reduces photosynthesis, accelerated shoot elongation during submergence and limitation to substrate availability for anaerobic metabolism and recovery growth

Table 1 Effect of submergence durations on superoxide dismutase, catalase and peroxidase activities in leaves of lowland rice varieties

Variety	Submergence duration		
	0d	5d	10d
	**SOD (/g fresh weight)*		
'FR 13A'	416	5 367	6 305
'Vaidehi'	437	5 286	6 413
'Mahsuri'	395	3 787	4 644
'IR 42'	427	3 032	3 908
CD *(P*=0.05)	20.9	218.4	265.8
	***Catalase activity (/g fresh weight/min)×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 *(P*=0.05)	0.07	0.25	0.37
	***Peroxidase activity (/g fresh weight/min)×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 *(P*=0.05)	0.21	0.26	0.38

* 1 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/min. in reaction mixture under specified assay conditions.

during post submergence/post anoxic phases. Additional injury may occur during post submergence oxidative stress through generation of free radicals of oxygen causing lipid peroxidation and protein denaturation. Tolerance to submergence and post submergence oxidative stress could partly be explained on the basis of higher activities of active oxygen scavenging systems and higher content of respiratory substrates (sugars and starch) and reduced under water elongation conserving metabolic energy for maintenance processes rather than spending them in undesirable rapid elongation. However some more exploratory research is needed to underpin the precise timing and nature of cell death and related biochemical mechanisms imparting tolerance to submergence of lowland rice

REFERENCES

Asada K, Takahasi M, Nagate M. 1974.Assay and inhibitors of spinach super oxide dismutase. *Agricultural Biochemistry* **38**: 471–3.

Boamfa E L, Veres A H, Ram P C, Jackson M B, Reuss J and Harren PJM. 2005. Kienetics of ethanol and acetaldehyde suggest a role for acetaldehyde production in tolerance of rice seedling to microaerobic condition. *Annals of Botany* **90**: 727–6.

Colmer T D, Huang S, Greenway H. 2001. Evidence for down-regulation of ethanolic fermentation and K + effluxes in the coleoptile of rice seedlings during prolonged anoxia. *Journal of Experimental Biology* **52**: 1507–17.

Gibberd M R, Colmer T D, Cocks P S. 1999. Root porosity and oxygen movement in waterlogging tolerant *Trifolium tomentosum* and intolerant *Trifolium glomeratum*. *Plant Cell and Environment* **22**: 1161–68.

Ismail A M, Ella E S, Vergara G V and Mackill D J. 2009. Machenism associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza sativa*). *Annals of Botany* **103**: 197–9.

Jackson M.and Armstrong W. 1999. Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biology* **1**: 274 –7.

Lowry O H, Rosebrough N J, Fare A and Land Randall R J. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**: 265–5.

Mallik A I, Colmer T D, Lambers H, Setter T and Schortemeyer M. 2002. Short term waterlogging has long term effect on the growth and physiology of wheat. *New physiologist* **153**: 225–6.

Mc Donald M P, Galwey N W, Ellneskog-Staam P, Colmer T D. 2001. Evaluation of *Lophopyrum elongatum* as a source of genetic diversity to increase the waterlogging tolerance of hexaploid wheat (*Triticum aestivum*). *New Phytologist* **151**: 369–80.

M. Curne D C and Galstan.W 1995. Inverse effect of gibberellins on peroxidase activity during growth in dwarf strain of peas and corn. *Plant Physiology* **65**: 245–8.

Ram P C, Singh A K, Singh B B, Singh V, Singh H P, Setter T L, Singh V P and Singh R K. 1999. Environmental characterization of flood water in eastern India: Relevance to submergence tolerance of lowland rice. *Experimental Agricultural* **35**: 141–2.

Ram P C, Singh B B, Singh A K, Ram Parashu, Singh P N, Singh H P, Boamfa I E, Harren F J M, Edi Santosa., Jackson M B, Setter T L, Reuss J, Wade L J, Singh V P and Singh R K. 2002. Submergence tolerance in rainfed lowland rice. Physiological basis and prospects for cultivar improvement through marker-aided breeding. *Field Crop Research* **76**: 131–2.

Raychaudhuri S S. 2000. The role of superoxide dismutase in combating oxidative stress in higher plants. *The Botanical Review* **66**: 89–98.

Raymond P, Al-ani A and Pradet A. 1985. ATP production by respiration and fermentation and energy charge during aerobiosis and anaerobiosis in twelve fatty and starchy germinating seeds, *Plant Physiology* **79**: 879–4.

Rio L A, Sandale L M, Corpas F J. 2006. Reactive oxygen spicies in peroxisome, production of scavenging and role in cell signaling. *Plant Physiology* **141**: 330–5.

Sawicki E, Hauser T R, Stanley T W and Elbert W. 1961. The 3-methyl-2-benzothizolinone hydrazones test sensitive, new method for the detection, rapid estimation and determination of aliphatic aldehydes. *Annals of Chemistry* **33**: 93–96.

Setter T L, Ellis M, Laureles E V, Ella E S, Senadhira D, Mishra S B and Datta S. 1997. Physiology and Genetics of Submergence Tolerance in rice. *Annals of Botany* **79**: 67–77.