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BIOCHEMICAL TRAITS ASSOCIATED WITH SUBMERGENCE TOLERANCE OF RAINFED LOWLAND RICE

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Abstract

Rainfed lowland rice possesses sufficient genetic variability in tolerance to submergence. A large variability was observed with respect to the level of soluble sugar and starch, total protein content and aldehyde accumulation in response to submergence. Tolerant varieties (FR13A, Vaidehi) had higher soluble sugar and starch before submergence than intolerant varieties (Mahsuri, IR42) which decreased on submergence at variable degrees among the varieties. Total protein content though was not much variable among varieties before submergence but decreased appreciably during submergence especially in intolerant varieties. Strong negative correlation (R^2=0.9) between survival and reduction in protein content was observed indicate more adverse effect during submergence. Adverse effect of submergence was also apparent through accumulation of about 21-23 folds total aldehyde in intolerant varieties against only 10 folds in tolerant ones. Correlation studies indicated strong negative correlation (R^2=0.9) between survival and aldehyde accumulation. Beneficial effects of soluble sugars and starch on survival was evident from positive correlation with R^2 values of 0.85 and 0.88 (for soluble sugar) and 0. 87and 0.91 (for starch) with 5 and 10d submergence respectively. Activities of superoxide dismutase, catalase and peroxidase were enhanced by submergence treatment. Genotypic variability were also observed for differential regulation of antioxidant defense system in tolerant and intolerant rice varieties catalase and peroxidase activities during post submergence phase did not show significant differences among varieties. SOD activity however, had around 15 folds increased in FR13A and Vaidehi against only 9-11 fold in Mahsuri and IR42 for a 10d submergence period. These traits could be used as markers for structuring submergence tolerant lowland rice.

Key words: *Oryza sativa,* submergence, porosity,soluble sugars, starch, aldehyde, total protein, SOD, catalase, peroxidase.

Introduction

Flooding or submergence is known to create hypoxia or anoxia in the plant system due to 10^4 times slower diffusion of gases in water (Armstrong, 1979). The movement of oxygen from shoot to root occurs by diffusion possibly through aerenchyma which is greatly impeded due to submergence altering the physiology of rice plants. Plant tolerant to hypoxia/anoxia develop inducible aerenchyma in addition to the constitutive aerenchyma to facilitate oxygen transport through plants. Root porosity increase due to poor aeration of soils (Gibberd *et al*., 1999). The normal oxidative pathway of carbohydrate metabolism is shifted to anaerobic respiration. Low O_2 concentrations in the water of flooded rice fields induces hypoxia or anoxia in root system which produces toxic metabolites causing injury to plants. The biochemical mechanisms underlying the adaptation of plants to anoxia are still not completely understood but high ethanolic fermentation has been stated to be of importance for plant tolerance to anaerobiosis (Bertani *et al*., 1980, AlAni *et al*., 1985, Raymond *et al*., 1985) which facilitates regeneration of NAD and production of ATP, though only 2 ATP molecules are generated through fermentation, 18 times lower than that of aerobic respiration.

Anoxia induced aldehyde production during submergence could be particularly important, since accumulation of aldehyde could be damaging to plant tissue. Under anoxia, alcoholic fermentation tends to increase, resulting in increased synthesis of acetaldehyde and ethanol (Davies, 1980). 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 at the time of submergence greatly affect the submergence tolerance (Ram *et al*., 2002, Setter *et al*., 1989, 1996) under anoxia and just after the anoxic treatment the plants usually look quite healthy, subsequently, after re-exposure to air they become soft, start to disintegrate and die very quickly. The main reason of this injury is probably the generation of free radicals of oxygen which triggers lipid peroxidation resulting in membrane damage (Hendry and Brocklebank, 1985). However, physiological and biochemical traits associated with submergence tolerance are not clearly underpinned as yet especially in lowland rice. Therefore, the present investigation was carried out to elucidate the possible traits, associated with submergence tolerance of lowland rice varieties.

Materials and Methods

Plant Culture and Imposition of Submergence Treatment

Seeds of four rice varieties namely FR 13A, Vaidehi (submergence tolerant) and Mahsuri and 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°C. Sprouted seed were direct seeded at 1cm depth in 25cm diameter earthen pots filled with 8kg well pulverized farm soils fertilized with recommended doses of NPK (120:60:60 kg ha^{-1}). Ten replicate pots with 5 plants in each pot were maintained per treatment under completely randomised design. Complete submergence treatment was performed with 30 day old plants for 5 and 10d durations under natural condition in outdoor pond water depth of 1 meter to insure that plant remained completes under water during submergence treatment. At the end of submergence period, the pots were taken out the ponds and kept in shade for 12 hours and then shifted to national conditions To quantify submergence environment, the flood water quality for O_2, CO_2, temperature, pH and under water irradiance was monitored during submergence period.

Measurements: Growth, Root Porosity and Sruvival

All measurement were made in triplicate and allies using standard procedure. Measurement on plant height, shoot dry weight and root porosity were made just prior to submergence and with in an hour after termination of submergence. Plant height was measured from base of soil surface to the tip of longest leaf use to express elongation during submergence in different varieties. Samples for dry weight were collected were quickly frozen in liquid nitrogen and brought to laboratory were they were oven dried at 70 ± 1°C until constant weight. Root porosity was measured as per method described in Raskin (1983). This method is based on measurement of the buoyancy of the tissue by measuring fresh weight in air and water. Emergence of new leaf on the plants which has gone under submergence before as indication of survival and was recorded 10 days after termination of submergence treatment.

Bio Chemical Measurement

Soluble sugar and starch in shoots were measured in previously oven dried samples following the methods of Yemm and Willis (1954) and Mc-Cready *et al*., (1950) respectively. Fresh leaf samples (200 mg) were homogenized with 80% ethanol and centrifuged at 4,000g in a laboratory centrifuge for 10 minutes. Supernatant was discarded and residue washed twice with 80% ethanol to remove chlorophyll. The residue left after removal of chlorophyll was resuspended in 5 ml 1N NaOH and kept at 30°C over night. On centrifugation 4,000g, the supernatant was collected and use for protein analysis using the modified method 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.

Enzyme Activity

Enzyme related to 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 as per standard procedure of Sinha (1972), Mc-Curne and Galston, (1959) and Asada *et al*., (1974) respectively. Recovery checks were done to validate the method for rice samples.

Results

Flood Water Parameters

Flood water quality is known to influence plant survival hence was monitored regularly during submergence period. The CO_2 and O_2 concentrations in flood water had inverse relationship when measured in the morning 0600 h. and afternoon 1600h. (Table 1.). On the day of submergence, flood water CO_2 concentration was lower which increased by 4-6 folds when measured at the end of 5 and 10 d of submergence. The concentration was higher in morning 0600h. and lower in evening 1600h. possibly due to utilization of CO_2 in photosynthesis. In contrast, O_2 concentration was higher in the afternoon and lower in the morning again indicating the photosynthetic production of O_2. Flood water temperature ranged between 29-34°C where as, pH range was 7.9-8.2.

Table 1: Environmental characterization of flood water in submergence tank during 5 and 10d 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^{O}C)	pH	Irradiance* (PAR) (μ mol m^{-2} S^{-1})
0d	0600h	0.41$\pm$0.08	0.09$\pm$0.02	28.6$\pm$0.05	7.9$\pm$0.03	107.5$\pm$2.1
	1600h	0.15$\pm$0.01	0.16$\pm$0.02	32.6$\pm$0.09	8.2$\pm$0.05	(405$\pm$3.6)
5d	0600h	1.76$\pm$0.04	0.11$\pm$0.02	32.0$\pm$0.05	8.0$\pm$0.02	103.5$\pm$1.2
	1600h	0.99$\pm$0.04	0.15$\pm$0.01	34.3$\pm$0.04	8.2$\pm$0.02	(395$\pm$4.5)
10d	0600h	1.37$\pm$0.10	0.10$\pm$0.01	29.6$\pm$0.07	8.1$\pm$0.03	99.6$\pm$0.8
	1600h	1.33$\pm$0.07	0.11$\pm$0.01	32.3$\pm$0.05	8.2$\pm$0.02	(415$\pm$3.5)

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

Under water irradiance measured at the top of canopy of submerged plant was about ¼th of irradiance in air above the water surface. Lower irradiance under water may decrease submerged plant photosynthesis.

Growth, Porosity and Survival

Plant height measured prior to submergence and just after termination of submergence showed an increased elongation while under water. The elongation in tolerant varieties, (FR 13A and Vaidehi) was about 7 and 12 percent for 5 and 10 d submergence while, in intolerant varieties (Mahsuri and IR 42) had from 13-21 percent elongation respectively. (Actual data not presented) Survival recorded 10 days after end of submergence revealed 93 percent survival in both the tolerant varieties whereas intolerant varieties Mahsuri and IR 42 had 47 and 35 percent but survival with 5d complete submergence. When submergence period increase to 10d survival was still lower and ranged between 87 percent for FR 13A and only 13 percent for IR 42. It is clear from the above observation that rice varieties having greater elongation on submergence had lower survival. Root porosity as an indication of internal gas spaces which are known to facilitates gas transport and are particularly important during complete submergence of plants. In the present experiment porosity of rice roots showed only a small variation before submergence whereas, a sharp increase occurs on submergence (Fig. 1A). The increase in root porosity was higher in tolerant varieties ranging between 12-14 percent in FR 13A and 8-13 percent in Vaidahi for 5 and 10 d submergence. Intolerant varieties on other hand showed 2-6 percent increase in porosity on submergence.

Shoot Dry Weight

Shoot dry weight before submergence was almost similar in all the four rice varieties but decreased during 5 and 10 d submergence periods at a variable rates. The decrease in shoot dry weight of both the tolerant varieties was 11 and 13 percent with 5 and 10d submergence while corresponding decrease in Mahsuri was 32 and 48 percent and in IR42 34 and 51 percent respectively (Fig. 1B). These indicates Ba rapid depletion of shoot dry weight intolerant varieties.

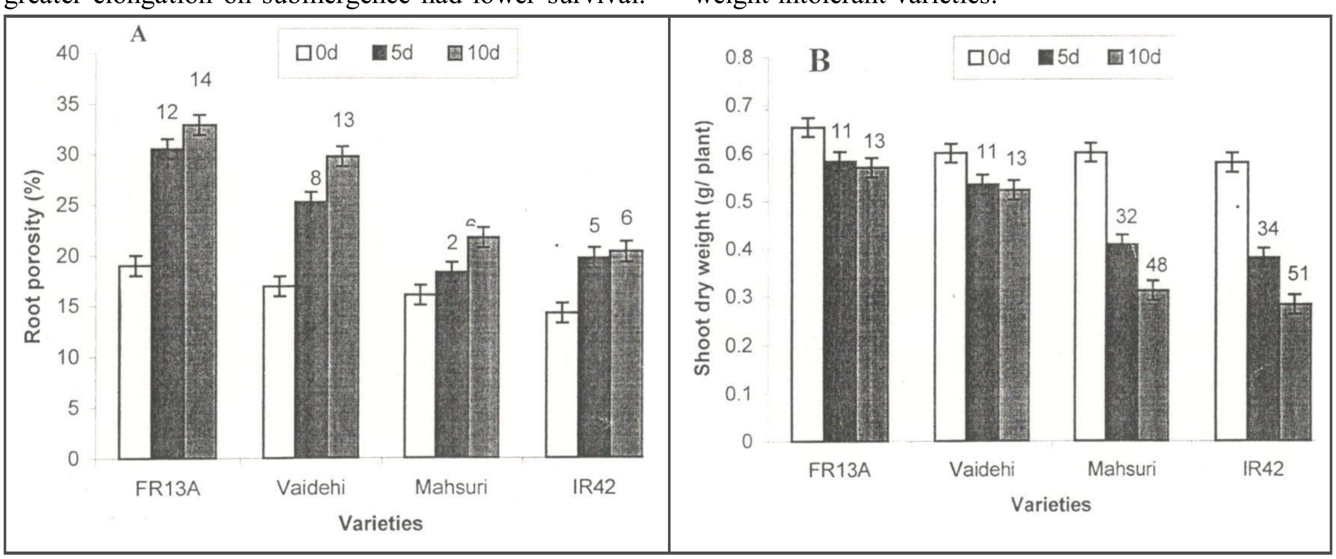

Fig. 1: Effect of submergence durations on root porosity (A) and shoot dry weight (B) in lowland rice. Figures at the top of bars are increase in porosity (A) and percent reduction in dry weight (B) over non-submerged control (0d). 30d old plants were completely submerged for 5 and 10d durations in outdoor ponds under natural conditions.

Biochemical

Total Soluble Sugars and Starch Content

Total soluble sugar and starch content in shoots of four rice varieties measured just before submergence and end of 5 days of submergence revealed a reduction a irrespective of the varieties. The difference between tolerant and intolerant varieties were not very distinct (Fig. 2A and 2B). However, when submergence duration was increased 10 days a drastic reduction in soluble sugar and starch was observed in intolerant varieties. The corresponding decrease in tolerant varieties was just below 20 percent. This huge reduction in carbohydrate level of intolerant rice varieties evidently depletes them of respiratory substrates which other wise, would have been essential for their survival on desubmergence (Fig. 2A and 2B). A strong positive correlation between soluble sugar and survival was observed with R^2 value of 0.85 (5d submergence) and 0.88 (10d submergence) fig. 3A. Correlation between starch and survival was still strong with R^2 value 0.87 and 0.91 for 5 and 10d submergence durations. (Fig. 3B).

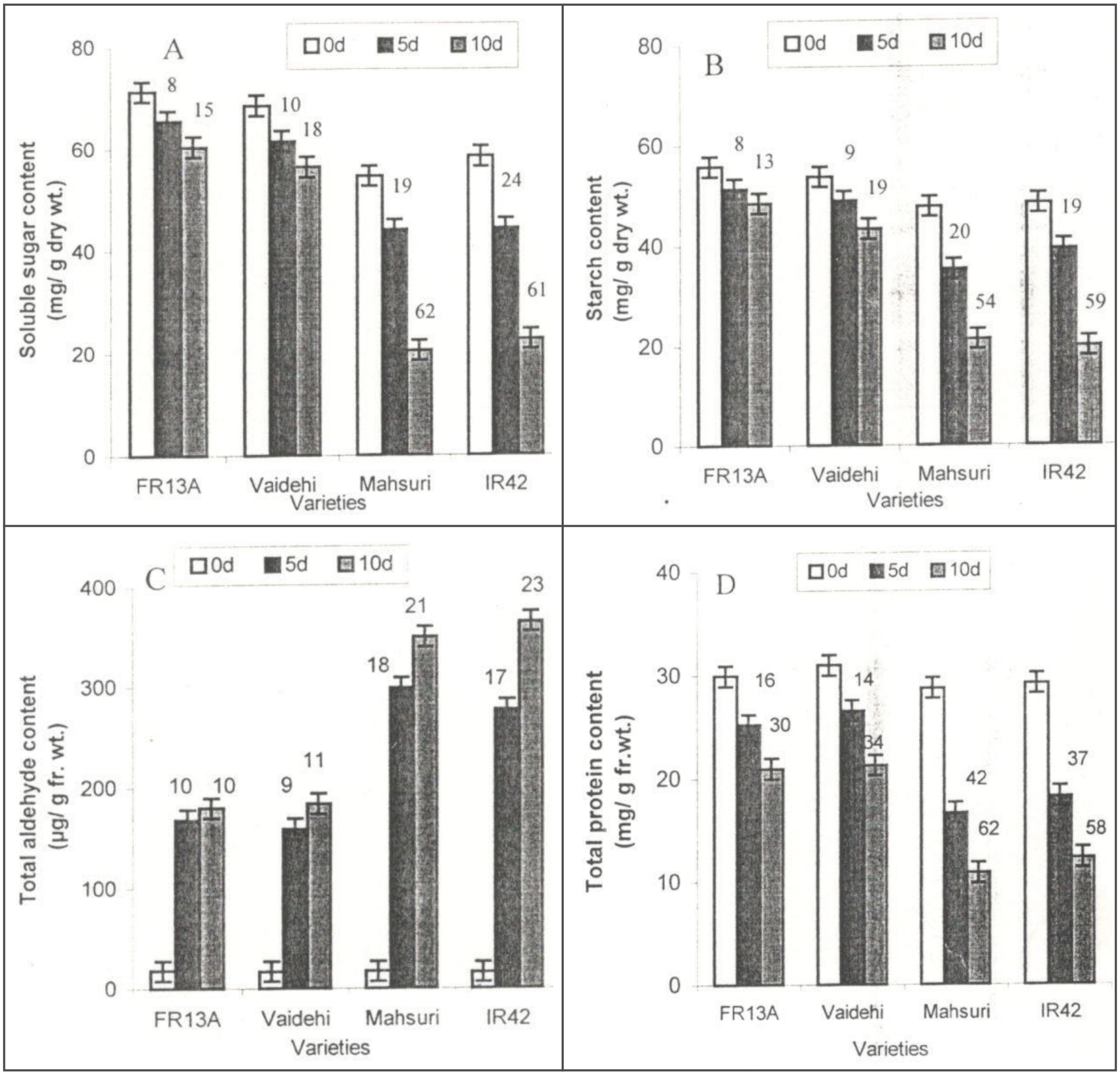

Fig. 2: Effect of submergence durations on soluble sugar (A), starch (B) in shoots and total aldehyde (C) and total protein (D) contents in leaves of lowland rice varieties. Figures at the top of bars A, B and D are percent reduction and in C are times accumulaion over non-submerged control (0d). 30d old plants were completely submerged for 5 and 10d in outdoor ponds under natural conditions.

Total Aldehyde Content

Total aldehyde content which is an indicative of oxygen stress during submergence was very nominal before submergence but increased during submergence in all the rice varieties studied (Fig. 2C). The magnitude of aldehyde accumulation was the tune of 10 folds in tolerant varieties whereas, it was about 20-23 folds in intolerant varieties. This clear cut difference between tolerant and intolerant varieties indirectly indicates about the variability in alcoholic fermentation ability of plants. Higher aldehyde accumulation was negatively correlated with lower plant survival with R^2 value of 0.91 and 0.99 with 5 and 10 d submergence respectively (Fig. 3C).

Total Protein Content

Leaf protein content in all four rice varieties was almost similar before submergence. On submergence, protein content decreased gradually showing maximum reduction with 10d submergence (Fig. 2D). FR 13A and Vaidehi had only 30 and 34 percent in protein content whereas, Mahsuri and IR 42 showed 62-52 percent decreased with respectively with 10d of submergence. The drastic reduction and protein content in leaves of intolerant varieties shows a destruction of protein machinery including enzymes rendering them susceptible of submergence. Higher protein content also impart better survival on submergence which is evident by a strong positive correlation with R^2 value of 0.90 (5d) and 0.93 (10d) submergence (Fig. 3D).

Antioxidant Enzymes

• Superoxide Dismutase (E.C. 1.15.1.1):

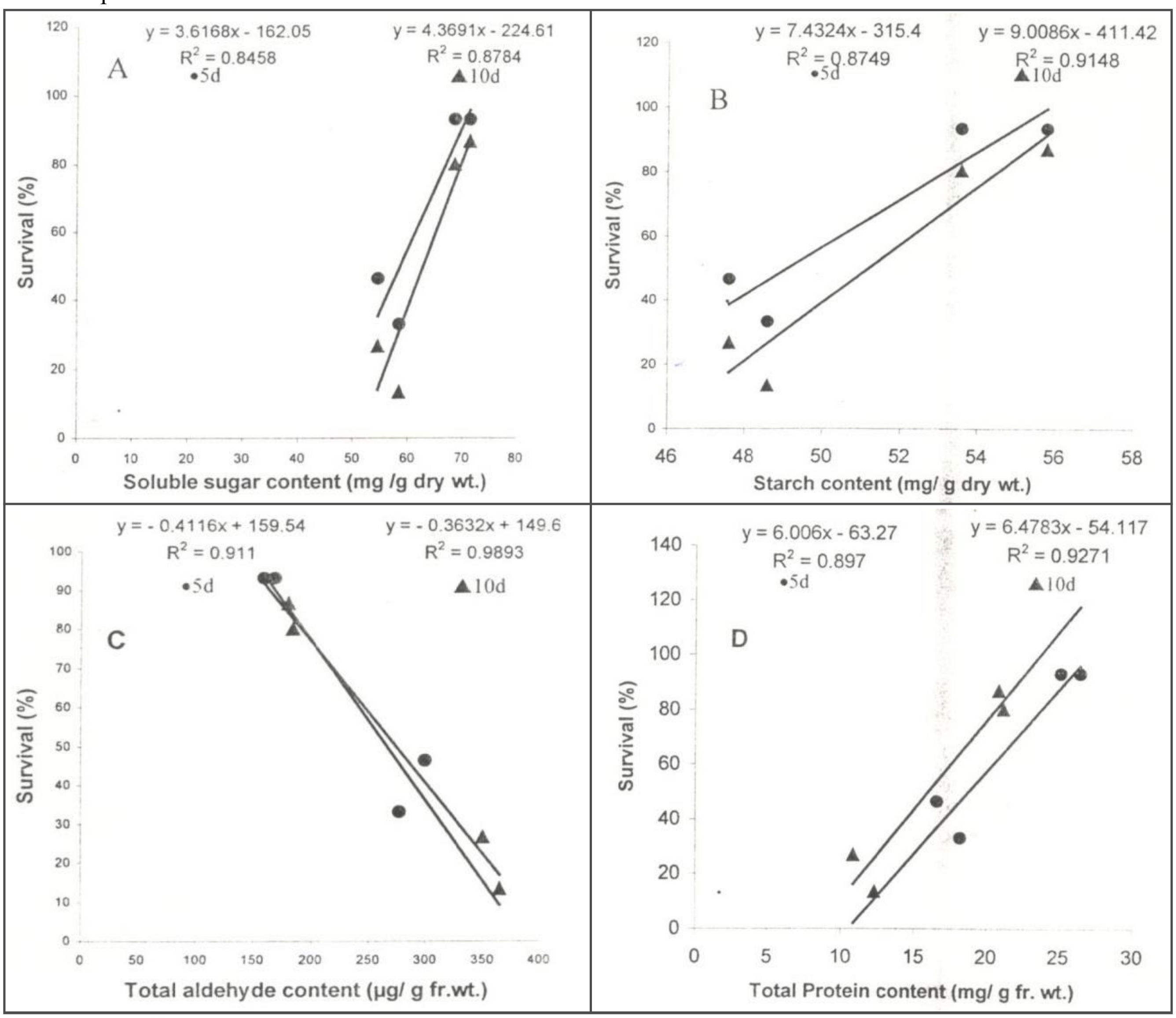

Fig. 3: Correlation between survival and soluble sugars (A) and starch (B) in shoots prior to submergence and survival and total aldehyde (C) and total protein (D) in leaves just after de-submergence of lowland rice varieties. 30d old plants were completely submerged for 5 and 10d in outdoor ponds under natural conditions.

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}) × 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}) × 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. ** One 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 conditions.

Superoxide dismutase the key enzyme for dismutation of superoxide free radicals was also measured in rice leaves before submergence and 5 and 10d of submergence. 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 (E.C.1.11.1.6 and E.C.1.11.1.7):

In general, catalase activity increased by roughly 2-3 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

Growth, Porosity and Survival

Rice plants elongate on complete submergence in an attempt to escape O_2 deprivation (Armstrong, 1979, Drew *et al*., 1979). In the present study, we observed variable shoot elongation, which was distinctly higher in intolerant varieties. Rapid elongation seems to deplete plant reserves leaving only meager amount to support recovery growth after submergence period is over. This is clearly reflected in lower shoot dry weight in intolerant varieties of rice in comparison to the tolerant ones (Fig. 1B). Since, under water irradiance during submergence was only ¼ th of the normal radiation production of new photosynthates (dry matter) is greatly reduced. Hence, varieties having higher dry matter prior the submergence and also lower depletion during submergence will have advantage in survival of submergence. Higher survival we observed in less elongating FR 13A and Vaidehi whereas more elongating in Mahsuri and IR 42 had lower survival. Higher underwater shoot elongation could be beneficial for deep-water rice but is of no great significance to rainfed lowland rice, which lodge and eventually die once the floodwater recedes. Shoot elongation in low land rice on submergence possibly depletes the plants with their reserves leading to eventual death later on. (Setter and Laureles, 1996, Ram *et al*., 2002, Catling, 1992).

The beneficial effects of reduced under water elongation in lowland rice was proved experimentally by Setter and Laureles, (1996) and Ram *et al*., (2002) where, induction of elongation by foliar application of GA_3 changed the tolerance level of FR 13A to relatively intolerant. In addition, intolerant variety IR42 behaved like a tolerant variety showing higher survival when treated with growth retardant paclobutrazol and cycocel.

The under priming theory in that the metabolic cost of underwater elongation shortened under water survival times by competing with cell maintenance processes for limited energy and hydrocarbon skeletons (Greenway and Setter, 1996, Sarkar *et al*., 1996, Setter *et al*., 1997). Several factors may be sensed by submerged shoot that initiate faster extension growth. Prominent among these is ethylene, submergence increases endogenous ethylene to active level by simple entrapment of basal ethylene production (Voesenek *et al*., 1993b) resulting from the water covering that impedes radial diffusive loss. Ethylene levels also increase because of extra biosynthesis induced by partial O_2 shortage through promotion of ACC synthesis. Ethylene acts in concert with other hormones notably gibberellins (Raskin and Kende, 1984) hormone upon which ethylene promoted elongation depends. Ethylene can enhance, the elongation promoting properties of gibberellins (Voesenek *et al*., 1996). A submergence induced increase in GA (Kende *et al*., 1988) and an associated decline in hormone abscisic acid (Van der Straeten *et al*., 2001) may also play a role in promoting underwater extension. Ethylene action can be enhanced by a decreased in it growth inhibitor abscisic acid (Hoffman-Benning and Kende, 1992) by the action of CO_2 and low levels of oxygen (*eg*. 1-5 k Pa) in *Rumex palustris*, (Blom *et al*., 1994) and buoyant tension generated by high porosity of most tissue of aquatic plants (Musgrave and Walters, 1974). Toojinda *et al*., (2002) working with large number of genetically linked rice genotypes demonstrated a clear cut negative correlation between elongation by submerged plants and various symptoms of injury or rates of survival. Overall, the balance of evidence points to greater underwater leaf extension being associated with poorer rates of survival. 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 tip (Jackson and Armstrong, 1999). This changes the root porosity and oxygen transport to the otherwise anoxic roots during submergence. High root porosity facilitates diffusion of O_2 originating either from the atmosphere or from photosynthesis to the roots allowing aerobic metabolism. High root porosity observed in FR 13 A and Vaidehi, therefore, might be advantageous during submergence (Fig. 1A). O_2 transported to root may oxygenate rhizoshphere through radial oxygen loss facilitating detoxificiation of chemically reduced iron, manganese, hydrogen sulphides (Gambrell *et al*., 1991) and may also support ammonia to less toxic nitrate (Blom *et al*., 1994). Aerenchyma also promotes the counter flow of volatile compounds accumulated in the anaerobic soil and plant tissue. Thsese includes ethanol (Crawford and Finegan, 1989, Vertapetian and Jackson, 1997) acetaldehyde, methane, CO_2 and ethylene.

Submergence/waterlogging adversely affects shoot dry weight by reducing number of tillers and production of new leaves. The rate of production of new growth slows down due to less availability of irradiance under water and difficulty in CO_2 diffusions inside the leaf. Since there is very little addition of new dry matter during submergence and the existing dry matter is continuously being utilized during anaerobic respiration, the rate of utilization eventually regulates the dry matter content of different varieties. In our studies, submergence intolerant rice varieties deplete dry matter fasters than the tolerant ones which probably is utilized in enhanced under water elongation in intolerant varieties prejudicing survival. Setter *et al*., (1989) observed lower dry matter in rice plants completely submerged for 4-12 days resulting either from reduced dry matter production or loss in dry weight due to utilization in respiration or both. Shoot mass of wheat plants waterlogged for 28 days decreased by 2-3 folds than well drained plants. Even after the recovery period of 25 days shoot biomass did not recover fully in waterlogged plants (Mallik *et al*., 2002).

Biochemical

Total Soluble Sugars and Starch

Soluble sugars and starch contents were initially higher in FR13A and Vaidehi (tolerant) as compared to Mahsuri and IR42 (intolerant) which decreased gradually during submergence of 5 and 10d durations (Fig. 2A and 2B). The decrease was comparatively higher in intolerant varieties. This greater reduction in soluble sugars and starch on submergence could possibly be due to (i) higher level of utilization of carbohydrate in anaerobic respiration and also to increased shoot elongation, (ii) very low level of underwater photosynthesis in intolerant rice varieties due either to low irradiance or low CO_2 during the day (Palada and Vergara, 1972, Ram *et al*., 1999). Higher level of soluble sugars and starch observed in tolerant varieties even after 10d of submergence indicates sustained underwater photosynthesis and controlled level of reserved substrate utilization during submergence. However, there is no experimental evidence that tolerant types undergo more vigorous underwater photosynthesis than intolerant types (Mazaredo and Vergara, 1982). Since the assimilation of carbohydrate slows down, the submerged plants have to rely on reserves (starch) for their respiratory substrate leading to decline in starch content on submergence (Fig. 2B). Induction of starch degradation by elevated levels of α-amylase activity is reported in cereals grains (Perata *et al*., 1998), floating and deepwater rices (Yamaguchi and Sato, 1963, Raskin and Kende, 1984). The amount of carbohydrate is often positively correlated with level of submergence (Chaturvedi *et al*., 1995, Mallik *et al*., 1995, Singh *et al*., 2001, Ram *et al*., 2002). We observed a strong positive correlation between survival and soluble sugar (R^2= 0.85 and 0.88) and survival and starch (R^2= 0.87 and 0.91) for 5 and 10d submergence respectively. Convincing evidence for the involvement of carbohydrate in submergence tolerance for rice came from experiments in which 14d old plants of submergence tolerant variety IR 42 were submerged at 0600h and 1800h in the dark. Plants submerged in the morning (0600 hr) before onset of the dawn showed poor survival (0-25%) compared with those submerged in the evening (100% survival) (Ram *et al*., 2002). As expected, carbohydrate (soluble sugars and starch) concentrations of shoots were in plants submerged in the evening as compared with those submerged in the morning. Low carbohydrate reserves though prejudice survival of submergence, it is less certain that differences in tolerance between cultivars are linked to differences in carbohydrates status. However, high carbohydrate prior to submergence has been advocated as marker trait for submergence tolerance (Singh *et al*., 2001, Ram *et al*., 2002). Ella and Setter, (1999) also reported the use of specific gravity of seeds as selection in criteria for high carbohydrate, which putatively helps seedlings establishment under anoxia in waterlogged fields. However, field screening of a double haploid population derived from a cross between FR 13A (tolerant) and CT-6241 (intolerant) rice varieties did not support the proposal that large seeds are necessarily associated with better submergence tolerance (Ram *et al*., unpublished data).

Aldehyde Content

Submergence induced accumulation of aldehyde in leaves of all the rice varieties tested indicates fermentive activity which might influence submergence tolerance of rice. Greater accumulation with increase in submergence durations indicates that either the efficiency of enzyme ADH to convert acetaldehyde into ethanol is reduced or conversion of pyruvate to acetaldehyde catalysed by PDC is accelerated or both.

Aldehyde accumulation through was observed in all the four varieties but is was almost two folds greater in intolerant varieties than that of tolerant ones (Fig. 2C). A strong negative correlation (R^2= 0.91 and 0.99 for 5 and 10d submergence) was observed between survival and aldehyde content of leaf (Fig. 3C). Differential accumulation of aldehyde in a number of rice varieties has also been reported by Mallik *et al*., (1995) and Singh *et al*., (2001) and was correlated with submergence tolerance of rice. More recent, on line measurement of acetaldehyde production using line-tunable Co-laser Photo Acoustic, revealed there was no substantial difference in acetaldehyde and ethanol emission between submergence tolerant FR 13A and the less submergence tolerant CT-6241 under submergence treatment (Boamfa *et al*., 2002). However, this emission does not account for the acetaldehyde and ethanol which leaked out into the surrounding submergence water. Another difficulty in interpreting the survival data as influenced by total aldehyde content in 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 low land rice for submergence tolerance.

Total Protein Content

Total protein content (soluble and 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 percent reduction in total protein content after 10d submergence indicating that submergence has a more damaging effect in 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). These it looks obvious that the observed decline in protein content submergence treatment could mainly be due to the degradation of aerobic proteins (soluble and insoluble). Positive correlation between survival and total protein content with R^2 values of 0.90 and 0.93 for 5 and 10d of submergence (Fig. 3D) revealed that tolerant varieties somehow were able to stabilize protein structures, in addition to the synthesis of new anaerobic proteins under adverse submergence environments. Anaerobiosis induced initial decline in protein synthesis due to destabilization of polyzones was also reported by Bailey-Serres and Freeling (1990).

Antioxidative Defence System

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 antioxidataive 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*., 1987a and 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 specially intolerant rice varieties could possibly reduced 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 24h before desubmerging 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 amounts 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 interior 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

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, a reduced photosynthesis, accelerated shoot elongation during submergence and limitation to substrate availability for anaerobic metabolism. 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 substrate (sugar and starch) and reduced under water elongation conserving metabolic energy for maintenance process rather than spending them in undesirable rapid elongation. However, some more exploratory research is needed to under pin the precise timing and nature of cell death and related biochemical mechanisms imparting tolerance to submergence of lowland rice.

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