Journal of Phytology 2009, 1(6): 425–432
© Journal of Phytology, 2009
ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

GROWTH, CHLOROPHYLL AND ELECTRIC CONDUCTIVITY RESPONSES OF RICE CULTIVARS TO DIFFERENT LEVELS OF SUBMERGENCE AND POST-SUBMERGENCE STRESS

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SUMMARY

Submergence stress is one of the most adverse factors on plant growth and productivity. The present investigation evaluated submergence tolerance and susceptibility of rice (*Oryza sativa* L.) cultivars with root and shoot respond differently. The submergence tolerant and intolerant cultivars respond with better ability to synthesized reserve carbohydrates inducing the enzymes with hydrolyzing reserve polysaccharides. The increased percentage of electric conductivity indicated that the rice cultivars have better emergence ability on air adaptation. The MDA content was found to accumulate more after exposure to air than in submergence, which might be a sign of oxidative stress inducing free radicals. The present study revealed that submergence stress increased the membrane damage, as is evident from increased value of electrical conductivity and lipid peroxidation. The root and shoot physiology, as described above, presumably contributes to the ability of rice genotypes to grow in diverse environments that differ in submergence.

Keywords: Ion leakage, Lipid peroxidation, Physiology, Submergence, *Oryza sativa*.

R.K. Upadhyay et al. Growth, Chlorophyll and Electric Conductivity Responses of Rice Cultivars to Different Levels of Submergence and Post-Submergence Stress. J Phytol 1 (2009) 425-432.
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1. Introduction

Submergence or the excess of water in natural locations is an important environmental factor limiting plant productivity, which is often called flooding. This hinders metabolic processes of plant, which ultimately retards growth and yield (Hossain and Loborte, 1996). Submerged plants experience low oxygen and low light intensity under water relative to that in air. Oxygen deficiency obstructs respiration at the level of electron transport and decreases generation of ATP (Crawford and Brandle, 1996). Decrease in growth ability has been attributed to a shortage of oxygen due to submergence or waterlogging inhibiting respiration and ATP formation (Magneschi and Perata, 2009). When ATP formation is reduced, the oxidation-reduction state between cell membranes becomes unbalanced and membrane permeability is increased. Hence, the solute leakage and electrical conductivity are increased, deteriorating cell membrane (Kawano et al 2009). Submergence stress is an important abiotic stress, which induces oxidative damage in rice plant, affecting antioxidant systems and causes significant crop losses (Ushimaru et al 2001; Blokhina et al 2003; Jackson et al 2009). The metabolism of sugar, protein and amino acids is profoundly altered during submergence (Ricard et al 1994). Imposition of submergence may increase generation of reactive oxygen species (ROS) within the cell, particularly within the chloroplast of flood stressed plants, leading to lipid peroxidation, protein degradation, enzyme inactivation and affect nucleic acid and almost every component of cell leading to cell death (Rawyler et al 2002; Blokhina et al 2003; Sakagami et al 2009). To overcome the effects of ROS, plant cells have well developed enzymic and non-enzymic antioxidant defense systems (Scandalios, 2002).

Rice, though a semi - aquatic plant adapted to survive submergence for a certain period of time. A total of 22 million hectares of rice-growing area is adversely affected by submergence, half of which is in eastern India (Roy, 1993). In India, generally rice suffers from submergence during June to September. Though Singh et al 2001 and Ram et al 2002 have suggested some adequate measures against submergence; there is dearth of information of oxidative stress management in relation to submergence acclimatization in various rice plants cultivated on this region.

In this investigation we have compared the susceptibility and tolerance of rice cultivars for understanding the mechanism of submergence stress induced oxidative damage on submergence, its possible recovery on re-aeration and the possible role of antioxidants in enhancing the recovery process in selected varieties of *O. sativa* L. The ability of varieties to recover and resume rapid growth following submergence imposition and subsequent reaeration is important for crop yield. Submergence tolerance in rice plant can be accessed through some physiological, biochemical parameters under submergence stress and these parameters can be used as selection criteria for breeding flood tolerant rice.

2. Materials and Methods

Plant materials, growth conditions and stress imposition

Dry graded uniform rice (*O. sativa* L.) seeds of two different cultivars, submergence-tolerant namely, Basudeo and intolerant, Mahsuri were procured from Regional Agricultural Research Station (RARS), Karimganj, India. The seeds were surface sterilized with 0.1% $HgCl_2$ for 5 – 10 mins and thoroughly washed with deionished water. The seeds were germinated in petri plates containing Whattman no.1 filter paper moistened with deionised water and kept in B.O.D. incubator in dark at ± 25°C. On the 3 d of germination, seeds were transferred to plastic glasses containing $^1/_2$Yoshida solution (Yoshida et al 1976) and kept in a growth chamber at 30-33°C (day/night) with a 12 h photoperiod and illumination at 52µmol $m^{-2} s^{-1}$. After every 6 h., seedlings were rehydrated with fresh Yoshida solution. On the 5 d, rice seedlings were imposed to submergence for 2, 4, 6 days in plastic containers. Non-submerged seedlings were considered as control. The same method was used for recovery study after 6d of submergence. The roots and shoots after every 2, 4, 6 days for both submergence and recovery after submergence were sampled for various physiological and biochemical estimations.

Growth parameters

Root and shoot length of same age plants were measured by detaching the shoot from the roots using centimeters (cm) scale. Samples from uniform root and shoot were washed with distilled water and blotted in tissue paper and weighed with the help electronic balance (Systronics, *Gujarat,* India). The measured samples were kept in oven at 80°C for 72h and weighed. Both fresh mass and dry matter were expressed as biomass produced in g/root or shoot.

Determination of Electrical conductivity, Chlorophyll and Carotenoid contents

Electrical conductivity was determined as per the method of Dionisio-Sese and Todita (1998) and calculated using the formula-

$EC = EC_1 / EC_2 \times 100$, where EC1 is the initial conductivity of the sample before autoclaving and EC_2 is the final conductivity of the sample measured after autoclaving the sample. Leaves were extracted in cold 80% acetone and chlorophyll and carotenoid content was estimated as per the methods of Arnon (1949).

Determination of Proline contents

Proline concentration in plant tissues was determined following the method of Bates et al. (1973). Tissue (0.5g) was homogenized with 5 ml of sulfosalicylic acid (3%) using mortar and pestle and filtered through Whatman No.1 filter paper. The volume of filtrate was made upto 10 ml with sulfosalicylate acid 2 ml of filtrate was incubated with 2 ml glacial acetic acid and 2 ml ninhydrin reagent and boiled in a water bath at 100°C for 30min. After cooling the reaction mixture, 6 ml of toluene were added and after cyclomixing it, absorbance was read at 570 nm.

Determination of lipid peroxidation

Lipid peroxidation was measured as the amount of MDA determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). The leaf tissues (0.2g) were homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 x *g* for 20min. To 1 ml of the resulting supernatent, 1 ml of TCA (20%) containing 10.5% (w/v) of TBA and 10µl (4% in ethanol) BHT (Butylated hydroxytolune) were added. The mixture was heated at 95 °C for 30min in a water bath and then cooled in ice. The contents were centrifuged at 10 000 x *g* for 15 min and the absorbance was measured at 532 nm and corrected for 600 nm. The concentrations of MDA were calculated using extinction coefficient of 155m $M^{-1} cm^{-1}$.

Statistical analyses

All experiments were done triplicates and repeated thrice and the data represent mean ± SE. The results were analyzed statistically with the Student's t- test at $P \leq 0.05$.

3. Results and Discussion

Our investigation highlights the rapid and deliberating effects of exposing rice plants to submergence stress. It has been observed that before submergence the total fresh mass of roots was highest in Mahsuri but it decline in Basudeo. However, the fresh biomass of roots increased maximum in Mahsuri and minimum in Basudeo with increase in days of submergence as compared to non-submerged seedlings. Shoots are less susceptible to submergence than roots. The fresh mass accumulation in roots and shoots seems to increase more in tolerant cultivars, Basudeo on air adaptation suggesting their better ability to metabolized reserve carbohydrates in contrasts of which can induce the enzymes that hydrolyzed reserve polysaccharides (Emel' yanov et al 2003; Ismail et al 2009; Kang et al 2009). On post submergence, root fresh mass increased in Mahsuri with lowest in Basudeo as compared to non-submerged seedlings (Table 1). As shown in table 1, the total fresh mass of shoot was highest in Basudeo as compared to Mahsuri. On submergence, the shoot fresh mass accumulation increased maximum in Basudeo with lowest in Mahsuri with the increase in days of exposure as compared to non-submerged seedlings. However on post submergence, maximum increase was observed in Basudeo, followed by Mahsuri as compared to non-submerged seedlings.

In non-submerged genotype, the total dry mass of roots was highest in Mahsuri as compared to Basudeo. Under submergence the dry weight biomass of roots increased maximum in Mahsuri with the increase in days of submergence as compared to non-submerged seedlings. However on post submergence, root dry mass showed greater accumulation in Mahsuri followed by Basudeo with respect to non-submerged seedlings (Table 2).

Table 1. Root and Shoot fresh mass of 2 cultivars of rice subjected to submergence stress followed by recovery after submergence

Cultivar	Root fresh mass (g)							Shoot fresh mass (g)						
		Submergence (d)			Recovery (d)				Submergence (d)			Recovery (d)		
	Control	2	4	6	2	4	6	Control	2	4	6	2	4	6
Mahsuri	0. 140± .012	0.120± .014*	0.109± .03	0.132± .008*	0.118± .009	0.121± .009	0.118± .012	0.144± .009	0.131± .008*	0.118± .009	0.401± .012*	0.129± .003	0. 139± .14	0.131± .013*
Basudeo	0.163± .013	0.142± .014*	0.140± .019*	0.152± .012	0.162± .018*	0.160± .014*	0.150± .012*	0.170± .012	0.150± .014*	0.148± .013*	0.180± .014	0.162± .013	0.176± .018*	0.162± .011*

Values given are means of three replicates ± standard errors. * indicates stastistically significant differences at P < 0.05 level .

Table 2. Root and Shoot dry mass of 2 cultivars of rice subjected to submergence stress followed by recovery after submergence

Cultivars	Root dry mass (g)							Shoot dry mass (g)						
		Submergence (d)			Recovery (d)				Submergence (d)			Recovery (d)		
	Control	2	4	6	2	4	6	Control	2	4	6	2	4	6
Mahsuri	0.040± .010	0.98± .002	0.94± .004*	0.100± .009	0.99± .018	0.91± .014	0.99± .020*	0.067± .009	0.045± .002	0.032± .009*	0.072± .010	0.044± .012	0.99± .014	0.90± .015*
Basudeo	0.015± .012	0.012± .004*	0.020± .012	0.034± .033*	0.022± .004*	0.010± .002	0.023± .014*	0.012± .009	0.012± .004*	0.022± .010*	0.024± .031*	0.020± .002	0.010± .009*	0.019± .012

Values given are means of three replicates ± standard errors . * indicates stastistically significant differences at P < 0.05 level .

Table 3. Root and Shoot Length of 2 cultivars of rice subjected to submergence stress followed by recovery after submergence

Cultivars	Root Length (cm)							Shoot Length (cm)						
		Submergence (d)			Recovery (d)				Submergence (d)			Recovery (d)		
	Control	2	4	6	2	4	6	Control	2	4	6	2	4	6
Mahsuri	2.43± .012	2.58± .014	2.43± .012*	2.45± .012	2.48± .015*	2.50± .012	2.60± .011*	3.36± .008	3.40± .009	3.40± .009	3.46± .010*	3.43± .009	3.36± .011*	3.40± .009*
Basudeo	6.66± .008	6.71± .015*	6.73± .006	7.33± .008*	6.80± .005	7.40± .005	6.66± .033*	8.80± .005	8.86± .003*	8.66± .043	9.10± .015	9.13± .013*	9.03± .023*	9.36± .023*

Values given are means of three replicates ± standard errors. * indicates stastistically significant differences at P < 0.05 level .

Table 4. Chlorophyll and Carotenoid contents of 2 cultivars of rice subjected to submergence stress followed by recovery after submergence.

Cultivars	Chlorophyll content (μmol g^{-1} fr.wt)							Carotenoid content (μmol g^{-1} fr.wt)						
		Submergence (d)			Recovery (d)				Submergence (d)			Recovery (d)		
	Control	2	4	6	2	4	6	Control	2	4	6	2	4	6
Mahsuri	449.91±3.49	364.81±2.35*	339.04±5.06	320.08±4.42*	374.32±5.28	362.08±4.09*	401.20±4.42	12.62±4.01	12.33±3.18*	12.18±4.19	11.97±4.28*	11.96±3.24	12.12±4.30	12.34±6.39*
Basudeo	4813.88±11.33	4612.19±12.66	3225.63±11.6*	3157.89±13.57*	4089.24±14.67	4612.18±12.66*	4961.01±12.05	18.11±4.30	17.73±3.34*	17.10±4.03	16.97±5.04	17.12±3.12	18.30±5.30*	18.24±7.01

Values given are means of three replicates ± standard errors. * indicates stastistically significant differences at P < 0.05 level .

Table 5. Root and Shoot Solute Leakage of 2 cultivars of rice subjected to submergence stress followed by recovery after submergence

Cultivars	Root Solute Leakage (%)							Shoot Solute Leakage (%)						
		Submergence (d)			Recovery (d)				Submergence (d)			Recovery (d)		
	Control	2	4	6	2	4	6	Control	2	4	6	2	4	6
Mahsuri	66.39±1.25	66.82±1.55	73.34±3.35*	73.12±3.92	66.87±8.27*	62.12±9.81	61.81±9.05*	66.39±1.25	33.03±1.50	40.29±1.09*	57.5±2.52	68.14±2.04	60.55±2.57*	78.64±3.45*
Basudeo	42.37±1.35	90.76±1.04*	33.08±1.33	33.05±1.07*	47.49±1.16	37.93±1.33*	38.87±1.02	58.63±1.38	57.68±1.29*	47.75±1.03*	40.89±1.08	62.78±2.02*	51.61±2.01	50.97±1.02

Values given are means of three replicates ± standard errors . * indicates stastistically significant differences at P < 0.05 level .

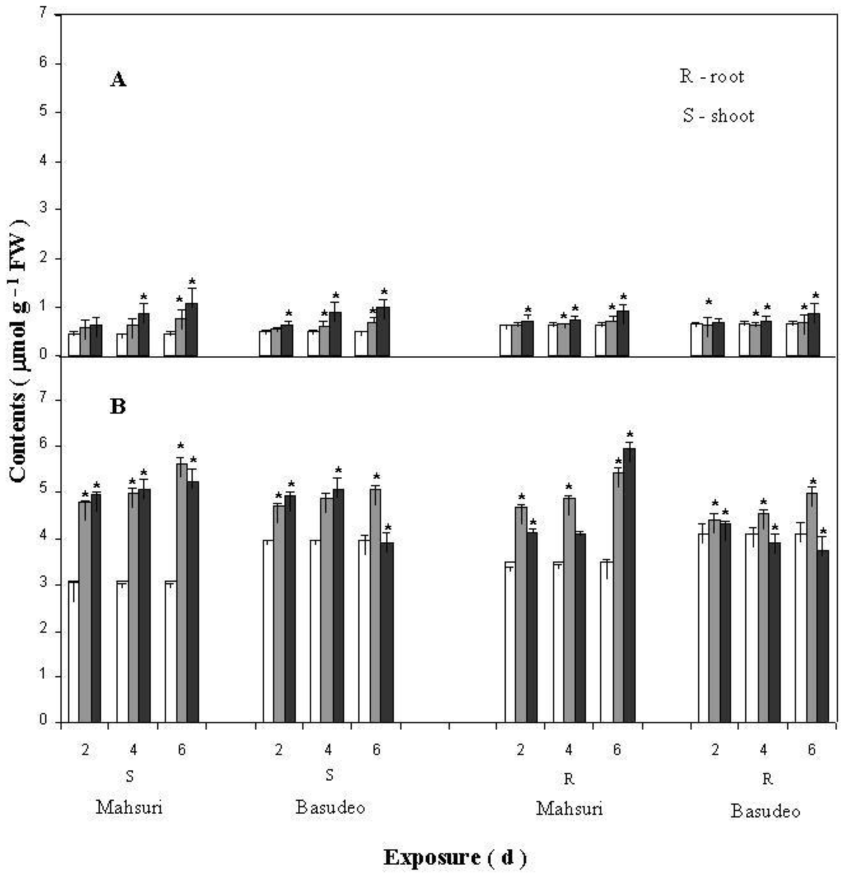

Figure 1. Changes in Malondialdehyde (A) and Proline (B) in roots and shoots of two cultivars of *Oryza sativa* L. (cv. Mahsuri and Basudeo) under non submerged control (□), submergence (▩) and recovery after submergence (■). Data given are means of three different replicates ± standard errors. * represents statistically significant difference in contents in comparison with control at $P < 0.05$.

It was further observed that before submergence, the total dry mass of shoot was highest in Basudeo followed by Mahsuri. On submergence, the shoot dry mass accumulation increased maximum in Basudeo with minimum in Mahsuri with the increase in days of exposure as compared to non-submerged seedlings. However on post submergence, maximum increase was observed in Basudeo followed with a minimum increase in Mahsuri as compared to non-submerged seedlings (Table 2). The dry matter accumulation was higher in tolerant cultivars, mostly in Basudeo on post submergence rather than on submergence. This may be because of the entry of water into intercellular space that is ordinarily gas – filled (Jackson and Ram, 2003; Mommer et al 2005; Ismail et al 2009). Maintenance of more dry matter content during submergence and subsequent re-aeration might contribute to the higher regeneration capacity as exhibited by Basudeo followed by Mahsuri in our case. This suggests that tolerant cultivars were able to maintain greater cell integrity than the susceptible cultivars such as Mahsuri during the same period of submergence and subsequent post submergence increases root and shoot length to greater height in tolerant cultivars like, Basudeo and Mahsuri which might be due to their better elongation ability as induced by ethylene (Jackson and Ram, 2003).

Before submergence the root length was highest in Basudeo with lowest in Mahsuri. But after submergence, the highest root length was recorded in Basudeo with lowest in Mahsuri. Maximum root length was recorded in Basudeo followed with a minimum increase in Mahsuri. Whereas, the shoot length was highest in Basudeo followed by Mahsuri. On submergence the maximum increase was observed in Basudeo followed by a minimum increase in Mahsuri. On re-aeration/post submergence, the survival of shoots increases. Maximum shoot length was observed in Basudeo with a minimum increase in Mahsuri (Table 3).

As a specific measure of the submergence stress – induced oxidative damage, the time course of membrane lipid peroxidation in rice seedlings (determined as MDA content) is given in Fig.1A. The Malondialdehyde (MDA) content of roots increases in tolerant cultivar with maximum in Basudeo on 6 d during submergence. But it increases by 150.87% on same days of re-aeration. Whereas in susceptible cultivar, it shows maximum increase under submergence for 6 d but on post submergence, it gradually shows increase by 201.27% as compared to non-submerged seedlings. On the other hand, under submergence the Malondialdehyde (MDA) content of shoots increases with the increase in days of exposure. It shows maximum in Basudeo, the tolerant variety under submergence and it increases by 130.15% on 6 d of re-aeration. Whereas in susceptible cultivar, Mahsuri it shows maximum under submergence and increases more by 105.13% on 6 d of post submergence, as compared to non-submerged seedlings. The MDA contents was found to be accumulated more after exposure to air than in submergence which might be a sign of oxidative stress inducing free radicals (Blokhina et al 2001; Kumutha et al 2009).

Submergence increased pigment degradation and electrical conductivity in plant. Such response varies among the susceptible and tolerant cultivars. In the present study, the percentage of electrical conductivity in roots of non-submerged seedlings was highest in Basudeo with lowest in Mahsuri. Under submergence, maximum increase in percentage of electrical conductivity was observed in Basudeo on the 2 d, followed by Mahsuri. After transfer of the seedlings to air, the maximum increase was recorded in Mahsuri on the 2 d followed by 6 d with a minimum increase in Basudeo on the 4 d of air adaptation as compared to roots of non-submerged seedlings. Whereas, in shoots of non-submerged seedlings, the percentage of electrical conductivity was maximum in Basudeo followed by Mahsuri. Under submergence, the percentage was maximum to 131.99% in Mahsuri on the 6d, as compared to non-submerged seedlings. After exposure to air, Basudeo showed 130% on the 6 d followed by122% on the 4 d with lowest in Mahsuri on the 6 d of re-aeration as compared to shoots of non-submergence seedlings (Table 5). On the other hand, the percentage of electrical conductivity was higher in tolerant cultivar on post submergence implicating increase in the emergence ability during post submergence (Hsu et al 2000; Jackson and Ram, 2003). However, the chlorophyll and carotenoid content increases in tolerant cultivar, Basudeo followed as compared to susceptible cultivars, Mahsuri suggesting that the tolerant cultivars re-attain their synthetic activities to the emergence of the leaves above the submergence level, whereas susceptible cultivars, still being under water (Ushimaru et al 1994; Drew 1997; Dias filho et al 2000).

On the other hand, the non-submerged seedlings showed maximum to 439.02% chlorophyll content in Basudeo with minimum to 180.21% in Mahsuri. Whereas, the non-submerged seedlings showed maximum carotenoid content in Basudeo followed by

Mahsuri. Under submergence, the chlorophyll content was maximum to 98.84% in Basudeo on 2 d with minimum to 64.08% in Mahsuri on 6 d of exposure. Whereas carotenoid contents were maximum in Basudeo by 92.98% on the 2 d of submergence with minimum by 84.15% in Mahsuri on the 6 d of submergence as compared to non-submerged seedlings (Table 4). But, after exposure to air chlorophyll content rises to 103.70% in Basudeo on 6 d followed by 4 d with minimum induction to 84.29% in Mahsuri on the 4 d of exposure to air as compared to non-submerged seedlings. Whereas, the carotenoid content showed maximum to 99.88% in Basudeo on the 4 d followed by 6 d with the minimum by 85.28% in Mahsuri on the 2 d of re-aeration, as compared to non-submerged seedlings (Table 4).

Other plant responses such as proline accumulation also followed an increasing pattern according to submergence and recovery after submergence. Proline could have a protecting role of stabilizing membrane and protein. The plant on post submergence after 6 d of exposure showed a peak in proline accumulation (Fig.1B). The proline (PR) content of roots increases in tolerant cultivar with maximum in intolerant, Basudeo on 6 d during submergence. But it decreases by 90.82% on same days of post submergence. Whereas in tolerant cultivar, it shows maximum increase in Basudeo under submergence for 6 d but on post submergence, it gradually shows more increase by 161.67% as compared to non-flooded seedlings. On the other hand, under submergence the proline (PR) content of shoots increases with the increase in days of exposure. It shows maximum in Basudeo, the tolerant variety under submergence but decreases by 89.10% on 6 d of re-aeration. Whereas in susceptible cultivars, it shows maximum in Mahsuri under submergence and gradually increases by 23.13% on 6 d of air adaptation, as compared to non submerged control. However, on air adaptation the tolerant cultivar showed decrease in proline content suggesting the use of excess proline in air adaptation as compared to susceptible cultivar (Alia and Saradhi, 1993; Garcia et al. 1997). The present study revealed that submergence stress increased the membrane damage, as is evident from increased value of electrical conductivity and lipid peroxidation. In non-submerged rice seedlings, conductivity decreased with increasing days of exposure in submerged plants increases indicating the ion leakage due to membrane damage caused by oxygen depletion due to submergence. Damage may start after 4 days and the physiological chlorotic symptoms like yellowing occurs after 4-6 days of re-aeration/post submergence in Mahsuri more than that of Basudeo. Based on our results the electrical conductivity of the seedlings could help in evaluating the growth rate ability of rice seedlings. Further, the growth, shoot length of the seedlings was significantly inhibited by submergence stress.

Acknowledgment

The Rice seeds received from Regional Rice Research Station (Assam Agricultural University), Karimganj, Assam is gratefully acknowledged.

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