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Physio-chemical Response of Rice (*Oryza sativa* L.) Genotypes to Complete Submergence during the Vegetative Stage under Coastal Agro-climatic Zone of Odisha

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ABSTRACT: Globally, submergence is regarded as one of the most dangerous abiotic stresses that affect the world's rice (*Oryza sativa* L.) production system. A pot experiment was conducted to assess the effects of complete submergence that happens at the vegetative growth stage on three rice genotypes. The rice genotypes cv. Swarna, cv. Swarna-Sub1 and cv. Binadhan-11, significantly showing different characteristics, were undertaken with four levels of complete submergence treatments (no submergence i.e. control, 4 and 8 days of submergence and re-aeration for a day after 8 days of submergence) in factorial completely randomized design and that were replicated thrice. Seedlings at 30 DAS were subjected to submergence in a poly-pit to study the performance of rice genotypes under varying levels of complete submergence stress. Among the three test-genotypes of rice, cv. Swarna-Sub1 showed the highest survival rate due to the least shoot elongation, highest tiller number and leaf area under 4 and 8 days of complete submergence in comparison to the control. It accumulated more non-enzymatic anti-oxidants like carotenoid, proline over controlled condition. Higher carbohydrate, chlorophyll, and protein content were associated with submergence tolerant genotypes in comparison to the susceptible one (cv. Swarna). The antioxidant system of the plant to scavenge the ROS was almost at par in all three genotypes before submergence but increased significantly in tolerant ones under complete submergence. Elevated enzymatic antioxidant levels manifested the ability of cv. Swarna-Sub1 to overcome the oxidative stress through up-regulation of SOD, catalase, glutathione peroxidase activity under 8 days of complete submergence and subsequent re-aeration. The performance of cv. Swarna-Sub1 followed by cv. Binadhan-11 and cv. Swarna were in diminishing mode under all four submergence treatments.

Keywords: Complete submergence, Rice (*Oryza sativa* L.), Binadhan-11, Vegetative Stage, Osmoregulants, Anti-oxidants.

INTRODUCTION

Rice (*Oryza sativa* L.), a semi-aquatic annual grass being used as staple food for about half of the human race (Hawksworth and Bridge 1985 and David 1989), is the most important cereal crop in the developing world. It is considered as grain of life across Asia where half of the world's poorest people live. Its ability to grow under wet environment is unique; hence, it is cultivated in tropical and sub-tropical belts with high rainfall. The burgeoning population in the developing countries like India has compelled the farmers to produce more rice per unit area and time so as to feed the teeming millions. But, the yield of this crop is leveling out. So how to increase the current annual global rice production of 435 million (M) tons (t) to 704.5 M t so as to feed the additional 269 M global population by 2050 is the greatest challenge before us.

In India, rice occupies about 42.9 M ha of land with an annual production of about 135.54 M t (2023) and it continues to hold the key to sustain food production by contributing to 20 to 25 per cent of agriculture GDP and assures food security for more than half of the total population. The *Kharif* (wet season) rice in India predominantly occupies 41.1 M ha (2023) with larger share by West Bengal, Uttar Pradesh, Odisha, Punjab and Andhra Pradesh states in the descending order producing 96.4 M t of rice. Rice production is extremely affected by a wide range of biotic and abiotic stresses but in Eastern India, submergence of arable fields is considered as the third most principal cause of damage which disrupts higher rice productivity. Crop loss in India due to excess moisture is very often observed in *Kharif* crops, mostly in the coastal tracts. Some rice cultivars that are economically important but intolerant to submergence show difficulties in survival, delay in growth and poor grain yield.

Among the rice growing states, the state Odisha occupies a prime position in terms of production as well as consumption of rice. It is the major food crop (75%) of the state grown in about 4.3 M ha of land area with a total annual production of 5.3 M t, which accounts for nearly 7.3 per cent of the national rice production. However, the productivity per hectare is deplorably low (1.4 t ha^{-1}) as compared to the National and global averages which is 1.7 t ha^{-1} and 2.3 t ha^{-1}, respectively. Uncertainty of rainfall and water logging are major

factors affecting rice production in Eastern India as well as in Odisha (Sarkar *et al.*, 2006).

Flooding from the monsoon, flash floods, and tidal waves cause submergence. When a significant amount of the soil's pore spaces are filled with water and restricts oxygen diffusion and gaseous exchange among soil, plants, and atmosphere, this leads to submergence stress. This reduces the growth and functionality of the roots, thus has a detrimental effect on the survival and growth of the plants (Sultana *et al*., 2019). Rice is generally affected by two forms of flooding: flash flooding and stagnant flooding. Flash flooding occurs when water levels rise quickly and submerge the crop for one to two weeks; whereas, stagnant flooding occurs when water levels fall below 100 cm and stay there for several weeks. The rice plant's semi-aquatic characteristics allows it to grow for longer period in water logged and/or submerged conditions. This is due to quicker elongation of the submerged shoot parts by the development of aerenchyma, which permits adequate internal oxygen transfer to submerged plant parts from the elongated shoot. Surviving in low oxygen environments is made possible by the production of energy and the detoxification of fermented products by coleoptile elongation (Kumar *et al*., 2021).

During the lifespan of rice plants, submergence usually happens during the vegetative stage while drought occurs during the reproductive stage. Tolerant rice plants demonstrate suppression of shoot elongation, hold onto chlorophyll in leaf tissues, and remain viable during submergence. When plants remain submerged under water for longer than a few days gradually loses oxygen due to the effects on respiration and photosynthesis, and this causes the plants to wither and eventually die. Submergence may affect every aspect of a plant's growth and development, including its morphology, physiology, biochemistry, and anatomy. Plant damage is caused by a variety of factors, including low gas exchange rates, intense turbid water shading, high flow rates that cause mechanical damage, and flooded water's ability to carry solutes. When totally submerged, rice and other aquatic plants have a tendency to extend their shoots in an attempt to emerge on the water's surface, often at the price of reserve substrates needed for energy production. In Eastern India's flash flood rice-growing regions, where submergence lasts only one to two weeks, excessive shoot elongation eventually causes lodging and plant death when the flood water recedes (Kumar *et al*., 2020).

Due to the changing climate, the number of rainy days has reduced but the intensity of rainfall has increased. Heavy rainfall and poor drainage lead to accumulation of water for few days to weeks. Hypoxic or anoxic conditions arise in the plant system due to water logging or submerged condition as diffusion of gases in submerged soil is reduced by 10^4 folds as compared to air. Respiration of the plant roots and soil micro-organisms leads to exhaustion of soil O_2 concentration. This condition causes poor aerobic root metabolism which further terminates the energy- dependent processes like ion uptake, root growth etc. (Jackson *et al.*, 1987). Slow gas diffusion also results in the accumulation of gases produced by roots which includes CO_2, methane, ethylene etc. The primary adaptive response of plants to submergence stress is ethylene-mediated which includes shoot elongation, aerenchyma development and adventitious root formation. Shoot elongation enables a totally submerged plant to reach the water surface. But, such strategy is only beneficial in case of shallow but prolonged floods, where the plant comes above the water surface before the stress becomes lethal. Aerenchyma tissues enhance the oxygen diffusion from shoot to the submerged root (Armstrong *et al.*, 1994), thus reducing the hazardous effects of anaerobiosis on submerged plants. Under continuous submergence, ethylene also facilitates carbohydrates depletion, chlorophyll degradation and ethanolic fermentation as a result of which the plant perishes. When conditions are too harsh, the plants die due to restricted photosynthesis and respiration mediated energy crisis. Another adaptive response is low oxygen quiescence strategy which is adopted by the species when fast shoot extension is insufficient so also the rapid growth by the plant is futile to re-surface the plant.

When floodwater recedes, re-oxygenation follows submergence which results in overproduction of reactive oxygen species (ROS) causing oxidative stress (Fukao *et al.*, 2011). These ROS includes super oxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen. These are unavoidable by-products of cell metabolism and common components of biochemical changes in chloroplast, mitochondria and peroxisomes. Injury to membrane integrity is the major symptom during anoxia, where the elevated levels of these oxygen free radicals cause damage to the plants, measured as changes in lipid content and composition and activation of lipid peroxidation. In order to withstand during submergence condition, rice plants have evolved a variety of survival mechanisms (including metabolic adaption and/or escape) under hypoxia (low O_2) or anoxia (zero O_2) (Ma *et al*., 2020; Loreti and Striker 2020). Rice plants have active oxygen-scavenging systems consisting of several antioxidant enzymes and some low level of non-enzyme antioxidants, which counteract the free radicals and decelerate the progress of many injuries associated with oxidative stress and ROS accumulation. Among all the enzymatic antioxidants, catalase, ascorbate peroxidase, superoxide dismutase, guaiacol peroxidase, glutathione reductase etc. play the key roles in ROS detoxification in plant cells under stress.

There is a huge pool of rice genotypes in Eastern India that are cultivated under rainfed condition in various agro-climatic conditions. Screening the rice genotypes under submergence stress conditions by observing morphological traits along with analyzing the physiological and biochemical traits helps to determine which genotypes are naturally adapted to survive flooding conditions (Maity *et al*., 2023). Rice genotypes with less chlorosis, reduced leaf elongation, high amount of reserved carbohydrates, and prompt re-adaption to aerial environment in post- submergence period can tolerate the flash flood conditions. Rice

genotypes behave differentially under complete submergence for 4 to 8 days during vegetative stage. However, many studies have already been done on the well accepted submergence tolerant rice genotype cv. Swarna-Sub1 but no such work could be traced for the newly released submergence tolerant rice variety cv. Binadhan-11. It needs to be evaluated for studying the genetic potential and physiological significance in contrast to the popular rice genotypes; susceptible and tolerant ones under complete submergence so as to achieve the targeted yield. Keeping all these things in view, the present investigation was undertaken to study the effect of complete submergence during vegetative stage on morpho-physiological, biochemical and enzymatic ability of the rice genotypes during the submergence period and after desubmergence or re-aeration.

MATERIALS AND METHODS

A. Experimental design and layout of the experiment

The experiment was conducted during *Kharif*, 2018 as pot culture in Agronomy main research field of Odisha University of Agriculture and Technology (OUAT), Bhubaneswar, Odisha, India under Agro-climatic zone of 'East Coast Plain' at 64 km air distance from the Bay of Bengal at East. The experimental site in particular was located at 21°15′N latitude, 85°48′E longitude and 26.9 m above the mean sea level. Its climate falls in the 'moist hot' group (Lenka, 1976). The experimental site had loamy sand soil. Its pH was 5.48 and its electrical conductivity was 0.03 dsm^{-1}. The soil possessed 0.47% organic carbon, 100 kg ha^{-1} total N and 50 kg ha^{-1} each of available P_2O_5 and available K_2O.

The pot culture experiment was carried out in a factorial completely randomized design with 12 treatment combinations which were replicated thrice. The treatments consisted of three rice genotypes i.e. cv. Swarna-Sub1, cv. Swarna and cv. Binadhan-11 and four complete submergence conditions (S_0, S_1, S_2 and S_3) i.e. no submergence (control), complete submergence for 4 days, complete submergence for 8 days and re-aeration for 1 day after 8 days of complete submergence, respectively. The seeds were sterilized with Bavistin @ 2.0 g lt^{-1} of water for two hours. Then those seeds were washed with distilled water thoroughly followed by surface soaking with blotting papers. Then the seeds were soaked in distilled water for the period of 24 hours and allowed to sprout by placing them on moist filter paper. Those sprouted seeds were sown directly in poly-pots measuring 21 cm depth and 17 cm diameter filled in with 3 kg of farm soil and FYM at 3:1 allowing 10 seedlings per pot to grow. After germination, the seedlings were thinned properly and five vigorous plants were kept in each pot. Required amount of fertilizers N, P_2O_5 and K_2O were supplied @ 80:40:40 kg ha^{-1}. A trench was dug to a height of 70cm and filled-in with water where all the poly-pots with 30 days old seedlings were completely submerged for varying durations as per treatments. Standing water level of at least 10 cm was maintained above the top of the plant. Thus the plants were exposed to complete submergence stress for a period of 4 and 8 days. Next day after 8 days (in S_3), pots were removed from the trench and allowed to grow under normoxia condition. Subsequently, the recovery was studied after 24 hours of re-aeration. Special attention for timely watering and drainage of excess rainfall was given.

B. Measurement of morphological parameters

Observations on different morpho-physiological and biochemical parameters of rice genotypes under each treatment were recorded before and after submergence as well as during the period of re-aeration. Plant height (cm), leaf area (cm^2) and days taken to 50% flowering were recorded.

C. Measurement of leaf photosynthetic pigments

Finely chopped 1 g fresh leaf sample was put in 80% v/v acetone solution and kept in dark for 24 hours. O.D value was then taken at 645, 663 and 480 nm. The chlorophyll and carotenoid content were calculated using the formula suggested by Arnon (1949) and expressed as mg g^{-1} fresh weight of leaves. Chlorophyll Stability Index (CSI) was calculated as per the formula given by Kar *et al*. (2005) i.e. [total chl. content (stress) / total chl. content (non-stress)] x100.

D. Measurement of membrane stability index (MSI)

MSI was determined by following the method given by Sairam and Srivastava (2002). 0.1 g fresh leaf tissues were taken and made into small pieces followed by placing those in test tubes containing 20ml de-ionised water in two sets. One set of the leaf samples were placed in hot water bath maintained in 40ºC for 30 minutes and their conductivity was measured. The second set was placed in hot water bath maintained at 100ºC for 10 minutes. Samples were cooled to room temperature and conductivity was measured. Then MSI was calculated and expressed in percentage.

E. Measurement of proline, total carbohydrate and protein content

Proline content of leaf samples was determined by following the procedure given by Bates *et al*. (1973). 100 mg fresh leaf sample was ground in mortar and pestle with 10 ml of 3% sulpho-salicylic acid and the homogenate was centrifuged at 3000 rpm for 10 minutes. The extract was filtered and 2 ml of filtrate was transferred into a clean test tube to which 2 ml each of glacial acetic acid, orthophosphoric acid and acid ninhydrin were added. Then test tubes were incubated in boiling water bath for 1 hour at 100°C, followed by cooling to cease the reaction. Samples and standards were poured into separating funnel to which 4 ml of toluene was added and shaken vigorously. Toluene layer was discarded and OD value was taken at 520nm. A standard curve of data was used to calculate the amount of proline present in leaf sample and expressed as mg $gram^{-1}$ fresh weight of leaves.

Protein estimation was done as per the method given by Lowry *et al.* (1951). Fresh leaf sample of 0.1 g were ground in mortar and pestle using 1 ml of 10% TCA and was then centrifuged at 5,000 rpm for 10 minutes. After discarding the supernatant, the residue was collected and into that, 1 ml of 1N NaOH was added followed by thoroughly mixing and centrifuging at

10,000 rpm for 10 minutes at 4°C. From that, 0.1 ml of supernatant was taken and to that 0.1 ml of 1N NaOH, 0.9 ml water and 5 ml reagent C added. After 10 minutes, Folin-ciocalteau reagent was added and was kept in dark for 30 minutes. Absorbance reading was taken at 660nm. A standard curve of protein was prepared and from that sample protein content was calculated.

Carbohydrate content of leaf samples was estimated by following the procedure of Yoshida *et al*. (1976). 0.1 g powdered dry sample was taken in a test tube along with 5 ml of 2.5 N HCl and kept in boiling water bath at 100ºC for 3 hours followed by cooling to room temperature. Then solid Na_2CO_3 was added to stop effervescence and volume was made up to 100 ml with distilled water in a volumetric flask. 1ml of supernatant was taken in another test tube and volume was made up to 3ml with distilled water followed by addition of 4ml anthrone. It was heated in boiling water bath for 8 minutes and then cooled rapidly. The absorbance reading was taken at 630 nm and expressed as mg g^{-1} dry weight.

F. Measurement of ROS scavenging enzymes

For the estimation of the activity of superoxide dismutase (SOD) enzyme, about 1 g of fresh leaf sample was taken and macerated in a clean, dry mortar and pestle keeping in a nice bath using 50 M potassium phosphate buffer (pH 7.8). Then the sample was centrifuged at 10,000 rpm for 10 minutes at 4°C in cooling centrifuge. The supernatant was collected in a test tube and a 3ml of reaction mixture was prepared containing 50 mM p-buffer, 50 µM Methionine, 2 µM Riboflavin, 0.1 mM EDTA,75 µM NBT, 0.1 ml riboflavin and 0.9 ml water. A blank was set without the enzyme and NBT and another reference was prepared by taking NBT without enzyme along with other chemicals. Thus 2 sets of test tubes were prepared, one for dark and another for light. Dark set of test tubes were kept under completely dark condition where a slight set of test tubes were exposed to 400W bulb for 15 minutes. OD at 560 nm was taken. Enzyme activity was expressed as unit g^{-1} fresh weight of leaves.

Catalase (CAT) activity was estimated by macerating 0.05 g of fresh leaf sample in 1 ml of potassium phosphate buffer and homogenate was centrifuged at 12,000 rpm for 20 minutes in cooling centrifuge at 4°C. Then the supernatant was collected and diluted to 10 ml with distil water. 3 ml of assay was prepared in a cuvette by adding 0.01 ml of enzyme, 2.99 ml of $H_2O_2.PO_4$ (0.036% w/w) and a blank containing only 2.99 ml $H_2O_2.PO_4$ simultaneously. Absorbance reading was taken at 240 nm against blank.

For the estimation of the activity of glutathione peroxidase (GPX) enzyme, 1 g leaf sample was macerated with 0.4 M sodium phosphate buffer (pH 7) using a pre-chilled mortar and pestle. Homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C. Supernatant was collected and used for enzyme estimation. 0.1 ml enzyme extract was pipetted out in a test tube and other reagents such as 0.4 ml buffer, 0.1 ml sodium azide, 0.2 ml glutathione and 0.1 ml hydrogen peroxide was added. Then the volume was made up to 1 ml with 0.1 ml of distil water. The tubes were incubated at 37°C for 5-10 minutes and after that 10% TCA was added to stop the reaction. To determine the residual glutathione content, the assay was again centrifuged. Again 3 ml of reaction mixture was prepared by adding 1.9 ml buffer, 1.0 ml DTNB and 0.1ml supernatant. Then O.D. of the enzyme mixture was taken at 412 nm against blank containing 2 ml of buffer and 1 ml DTNB reagent. The activity of enzyme was expressed as µg of glutathione consumed per minute per gram fresh weight.

G. Statistical analysis

The difference between various parameters was analyzed and compared statistically by ANOVA technique in a factorial completely randomized design laid down by Panse and Sukhatme (1985), Fisher (1925), and Gomez and Gomez (1984). Standard error of means i.e. S.Em (±) were used in all cases. The significance of variance was tested by 'Error mean square" method of Fisher Snedecor's F-test at the probability level of 0.05 for appropriate degrees of freedom.

RESULTS AND DISCUSSION

A. Effect of complete submergence on morphological parameters

From the experiment, it was observed that plant height increased significantly in all the genotypes under submergence as compared to control but at different rate (Table 1). However, the increase in shoot elongation was found to be the maximum in cv. Swarna with a tune of 38.50% followed by cv. Binadhan-11 with 14.26% which was at par with cv. Swarna-Sub1 with 13.9% at 4 days.

Table 1: Effect of complete submergence at vegetative stage on plant height (cm) and leaf area (cm^2) of rice genotypes.

Genotype	Plant height (cm)					Leaf area (cm^2)				
	S_0	S_1	S_2	S_3	Mean	S_0	S_1	S_2	S_3	Mean
Swarna	37.13	51.43	57.60	59.33	51.37	16.00	17.33	18.58	18.60	17.69
Swarna-Sub1	39.51	45.00	50.59	51.07	46.54	16.98	18.79	20.57	20.61	19.24
Binadhan-11	41.28	47.17	53.00	54.00	48.86	19.78	21.75	23.6	23.8	21.65
Mean	39.31	47.87	53.73	54.80	48.92	17.60	19.21	20.63	20.66	19.53
	G	**S**	**G*S**			**G**	**S**	**G*S**		
S Em (±)	0.763	0.881	1.526			0.231	0.267	0.463		
C.D.at 5%	2.26	2.57	4.45			0.68	0.78	1.35		

(*C: Control; G: Genotype; S:Stress; RA: 24 hr re-aeration)

The similar trend was found among the cultivars at 8 days of submergence and also during 24 hours of re-aeration after 8 days of complete submergence with the values being 55.12% and 59.78% in cv. Swarna followed by 28.39% and 30.81% in cv. Binadhan-11 and 28.04% and 29.25% in cv. Swarna-Sub1 over control.

It is evident that under submerged condition susceptible variety showed faster elongation which resulted in lodging and death of the plant when water level receded. Cv. Binadhan-11 showed more elongation as compared to cv. Swarna-Sub1 during the period of submergence as well as re-aeration. So the present findings reported that cv. Swarna-Sub1 followed by cv. Binadhan-11 showed better performance and survival proficiency than cv. Swarna with respect to shoot elongation. These findings are in agreement with Singh *et al*. (2001) and Sultana *et al*. (2019) who reported better survival with lower shoot elongation during submergence. Sarkar and Bhattacharjee (2011) observed that plant height did not increase much in sub1 introgressed cultivars, resulted significantly lower elongation compared to other genotypes. The ethylene-response factor transcription encodes the locus Sub1A which provides the ability for growth under submergence conditions. Bailey-serres and Voesenek (2008) documented that shoot emergence seems to represent a higher cost of energy and might compromise eventual recovery when the water recedes. The enhancement of the height of the plant was chiefly attributed to accumulation of ethylene in the plant cells under submerged condition. It was concluded that rice plants that exhibit only limited elongation during submergence often show tolerance to complete submergence. In this study, Swarna-Sub1 showed quiescence strategy where ethylene mediated suppression of stem elongation is the key factor.

Similarly, plant leaf area significantly increased in all the genotypes in response to submergence stress as compared to control (Table 1). However, the percentage increase in leaf area was found to be the maximum in cv. Swarna-Sub1 followed by cv. Binadhan-11 and cv. Swarna. In Swarna-Sub1, the percentage increase in leaf area was 10.7%, 21.1% and 21.4% at 4 days, 8 days and 8 days complete submergence with 24 hours re-aeration respectively. This corroborates with the findings of Sarkar *et al*. (2003). They found that the genotypes which showed higher stabilization of yield under submergence, maintained higher leaf area and leaf dry weight.

Ethylene stimulates cell elongation in association with auxin, gibberellins, or both. Faster elongation underwater is highly reliant on the prior break down of growth-inhibiting hormone abscisic acid. Other than ethylene, rice leaves may also react to other signals. That signal could be attributed to either partial oxygen scarcity or accumulating carbon dioxide, as rice coleoptiles elongate more quickly underwater in response to and their synergistic effects as well as ethylene (Kumar *et al*., 2020).

Number of days required to 50 % flowering in all the genotypes under non- stressed and stressed conditions was observed and presented in Table 2. Data revealed that delay in 50% flowering in response to complete submergence increased with the increase in duration of submergence. The delay was found to be higher in cv. Swarna (5-8 days) followed by cv. Swarna-Sub1 (4-7days) and cv. Binadhan-11 (3-6 days).

B. Effect of complete submergence on photosynthetic pigment content of the leaves

Complete submergence resulted in significant reduction of chlorophyll content of all the genotypes is presented in Table 3. After 4 days and 8 days of submergence, the percentage reduction in total chlorophyll content was the maximum in cv. Swarna with 27.88% and 44.87% followed by cv. Binadhan-11 with 9.27% and 21.4% and Swarna-Sub1 with 4.14% and 15.75% respectively.

Table 2: Effect of complete submergence at vegetative stage on days to 50% flowering of rice genotypes.

Genotypes	Days to 50% flowering				
	S_0	S_1	S_2	S_3	Mean
Swarna	103.00	108.00	111.00	108.00	17.69
Swarna-Sub1	101.00	105.00	108.00	105.00	19.24
Binadhan-11	94.00	97.00	100.00	108.00	21.65
Mean	17.60	19.21	20.63	20.66	19.52
	G	**S**	**G*S**		
SEm(±)	0.231	0.267	0.463		
C.D.at 5%	0.68	0.78	1.35		

(*C: Control; G:Genotype; S:Stress; RA: 24 hr re-aeration)

Table 3: Effect of complete submergence at vegetative stage on total chlorophyll content and carotenoid content of rice genotypes.

Genotypes	Total chlorophyll content (mg g^{-1} FW)					Carotenoid content (mg g^{-1} FW)				
	S_0	S_1	S_2	S_3	Mean	S_0	S_1	S_2	S_3	Mean
Swarna	3.12	2.25	1.72	1.74	2.21	0.15	0.10	0.09	0.13	0.12
Swarna-Sub1	3.62	3.47	3.05	3.17	3.32	0.38	0.35	0.30	0.34	0.34
Binadhan-11	3.45	3.13	2.71	2.78	3.01	0.25	0.22	0.18	0.21	0.22
Mean	3.40	2.95	2.49	2.56	2.85	0.26	0.22	0.19	0.23	0.23
	G	**S**	**G*S**			**G**	**S**	**G*S**		
SEm (±)	0.019	0.022	0.039			0.003	0.004	0.009		
C.D.(p=0.05)	0.06	0.07	0.11			0.01	0.01	0.02		

(*C: Control; G: Genotype; S: Stress; RA: 24 hr re-aeration)

After 24 hours of re-aeration, the total chlorophyll content was found to be slightly increased in all the genotypes compared to during submergence period and Swarna-Sub1 was found to perform better than others indicating its high regeneration capacity. However, the concentration of chlorophyll remained significantly lower compared to the control even in the tolerant cultivar during re-aeration. Similar trend as the leaf chlorophyll concentration was found in case of carotenoid content of leaves (Table 3). After 4 days of complete submergence, Swarna recorded the highest reduction rate of 31.82% followed by cv. Swarna-Sub1 (7.89%) and cv. Binadhan-11 (6.76%) over control. After 8 days of complete submergence, cv. Swarna-Sub1 was found to maintain its high carotenoid content with 0.3 mg g^{-1} FW of leaves followed by cv. Binadhan-11 (0.18 mg g^{-1} FW) and cv. Swarna (0.09 mg g^{-1} FW). After the period of re-aeration also, Swarna-Sub1 was found to have highest carotenoid content (0.34 mg g^{-1} FW) followed by cv. Binadhan-11 (0.21 mg g^{-1} FW) and Swarna (0.13 mg g^{-1} FW). Jackson *et al.* (1987) reported that complete submergence has direct effect on chlorophyll degradation. Ethylene synthesis was increased during submergence which triggered the gene expression and chlorophyllase enzyme activity, responsible for quicker chlorophyll breakdown in treated plants. Chlorophyll degradation was less in submergence tolerant cultivars due to reduction in ethylene production (Das *et al*., 2005; Sarkar *et al*., 2006). In this study, concentrations of these pigments were found to be increased after water level receded, which was reflected after 24 hours of re-aeration. Susceptible genotype cv. Swarna showed highest chlorophyll and carotenoid degradation as compared to cv. Swarna-Sub1. This investigation indicated that new leaf formation was faster at recovery period in tolerant genotype that facilitated regaining their synthetic activities which was found to be absent in susceptible cultivar. Data reflected in the Table 4 indicated that CSI (%) was highly affected by the submergence as it reduced with the increase in duration of submergence. There was significant variation observed among the genotypes. Cv. Swarna-Sub1 showed best performance with 94.76% and 81.49% and the worst by cv. Swarna with 72.12% and 55.13% after 4 days and 8 days of complete submergence respectively. After one day re-aeration also, cv. Swarna-Sub1 was found to have the higher regeneration capacity than others with the highest CSI value (87.5%). This corroborates with the findings of Dwibedi *et al.* (2017) who reported that physiological traits like chlorophyll stability, sugar content were higher intolerant genotypes as compared to susceptible ones during submergence.

Table 4: Effect of complete submergence at vegetative stage on chlorophyll stability index and membrane stability index of rice genotypes.

Genotypes	CSI (%)					MSI (%)				
	S_0	S_1	S_2	S_3	Mean	S_0	S_1	S_2	S_3	Mean
Swarna	100.00	72.12	55.13	55.78	70.76	71.65	56.42	51.80	55.50	58.84
Swarna-Sub1	100.00	94.76	81.49	87.5	90.90	74.55	67.25	62.70	70.18	68.67
Binadhan-11	100.00	90.70	78.50	80.50	87.40	81.15	72.33	68.09	73.67	73.81
Mean	100.00	85.86	71.70	74.60	83.02	75.78	65.33	60.86	66.45	67.11
	G	**S**	**G*S**			**G**	**S**	**G*S**		
SEm (±)	0.78	0.90	1.55			0.942	1.087	1.883		
C.D.(p=0.05)	2.27	2.62	4.53			2.75	3.17	5.50		

(*C: Control; G: Genotype; S: Stress; RA: 24 hr re-aeration)

C. Effect of complete submergence on membrane stability index (MSI)

The data on MSI depicted in the Table 4 indicated its drastic reduction under complete submergence irrespective of the genotypes. Before the induction of submergence, the highest value was recorded in cv. Binadhan-11 (81.15%) followed by cv. Swarna-Sub1 (74.55%) and cv. Swarna (71.65%). However, after 4 days and 8 days of submergence, highest value was recorded in cv. Swarna-Sub1 with the values 67.25% and 62.7% followed by cv. Binadhan-11 with 72.33% and 68.09% and cv. Swarna with 56.42% and 51.8%, respectively. Similar trend was found even during the re-aeration period. For the present study it was highly significant for genotypes, stress levels and stress x genotype interactions. Submergence-tolerant plant can maintain membrane stability under submerged conditions, but up to a certain extent, consistent with the results of Lei *et al*. (2012). MSI is important for cellular function, and only cells with stable membrane systems can maintain their normal physiological functions. Therefore, membrane stability is usually used for measuring the extent of injury in plants under stress. The coexistence of submergence and anaerobic stresses affects the biochemical and physiological processes of plants including cell membrane function. The increased permeability and leakage of ions out of the cell has been used as a measure of MSI.

D. Effect of complete submergence on proline accumulation

Proline accumulation differs significantly among all the genotypes under treated and untreated conditions. A survey of data presented in Table 5 revealed that amount of proline was quite similar in all the genotypes under controlled condition. But when plants were submerged for a period of 4 days, huge percentage of increase was noticed in cultivars, the highest being found in cv. Swarna (118.83 µg g^{-1} FW) and least in Swarna-Sub1 (86.75 µg g^{-1} FW). But it gradually decreased with the increase in the submergence period. Proline content again decreased in all the genotypes in response to the re-aeration compared to the stress period. The maximum decrease was found in cv. Swarna-Sub1 (from 70.84 to 62.50 µg g^{-1} FW) followed by cv. Binadhan-11 (from 68.9 to 61.7 µg g^{-1} FW).

Alia and Sarathi (1993) reported that suppression of mitochondrial electron transport was the primary reason

for stress- induced proline accumulation in plants. Under submergence, normal growth of mitochondria is affected, resulting in accumulation of proline (Shibasaka and Tsuji 1988). Proline is referred as a supportive index for assuming osmotic deficits out of submergence. There is a broad consensus that accumulation of amino acids such as proline serves as osmoprotectants that compensate for osmotic potential that can increase because of rapid consumption of soluble carbohydrates under the stress (Magneschi and Perata 2009). In addition to being an osmoticum, proline may also act as a sink of energy, a nitrogen storage compound, a scavenger for hydroxyl-radicals and a compatible solute that protects enzymes and cellular structures (Smirnoff and Cumbes 1989). This study revealed that, proline was submergence-inducible and gradually declined over 24 hours of re-aeration. This was more rapid in cv. Swarna-Sub1 and cv. Binadhan-11 than cv. Swarna. The rate at which proline disappeared after air adaptation suggested that excess proline might be used in adaptation when plants were transferred from hypoxia to normoxia (Sarkar *et al*., 2011). It is noteworthy that the abundance of proline during submergence and recovery is not positively correlated with submergence tolerance. It can be expected that the amount of proline accumulated in the Sub1 introgressed genotypes is sufficient to provide the beneficial effects on adaptations to submergence and re-oxygenation. It appears that these biochemical adjustments to the stress via amino acid accumulation are regulated in a SUB 1A-independent manner.

Table 5: Effect of complete submergence at vegetative stage on proline and total soluble protein content of rice genotypes.

Genotypes	Proline content (µg g^{-1} FW of leaves)					Total soluble protein (mg g^{-1} FW of leaves)				
	S_0	S_1	S_2	S_3	Mean	S_0	S_1	S_2	S_3	Mean
Swarna	68.47	118.83	91.06	87.40	91.44	18.54	9.59	4.83	5.30	9.57
Swarna-Sub1	70.84	86.75	75.44	62.50	73.89	20.00	15.55	10.49	15.72	15.44
Binadhan-11	68.90	84.92	74.54	61.7	72.33	19.10	14.67	9.67	14.67	14.53
Mean	69.40	96.84	80.35	70.36	79.24	19.21	13.27	8.33	11.90	13.18
	G	**S**	**G*S**			**G**	**S**	**G*S**		
SEm(±)	0.452	0.522	0.904			0.106	0.122	0.212		
C.D.(p=0.05)	1.32	1.52	2.64			0.31	0.36	0.62		

(*C: Control; G: Genotype; S: Stress; RA: 24 hr re-aeration)

E. Effect of complete submergence on total soluble protein status of leaves

The data on total soluble protein synthesis was estimated at different stages of submergence for all the genotypes and presented in Table 5. It was observed that protein degradation significantly increased with the increase in the duration of submergence irrespective of genotypes. After 4 days of submergence, the depletion in the protein content was least in Swarna-Sub1 (22.25%) and highest in cv. Swarna (48.27%) compared to the control. Similar trend was found for the genotypes under 8 days of complete submergence where the percentage degradation was lowest in Swarna-Sub1 (47.55%) followed by cv. Binadhan-11 (49.39%) and Swarna (73.95%). As water level receded, after 24 hours of re-aeration protein content was found to be increased in all the genotypes compared to the stress period but at a different rate indicating their regeneration ability and adaptation to submergence. Swarna-Sub1 recorded the highest increase followed by cv. Binadhan-11 and cv. Swarna. This is in accordance with the findings of Shingaki-Wells *et al*. (2011).

F. Effect of complete submergence on total carbohydrate content

Complete submergence resulted in a remarkable depletion of the total carbohydrate content in the leaves of all of the cultivars in comparison to control (Table 6). The level of depletion of carbohydrate content was lower in tolerant cultivar compared to the susceptible cultivar, which further widened as the days to submergence progressed as evident in the percentage change in carbohydrate contents between control and submerged plants. The rate of reduction was highest in case of cv. Swarna followed by cv. Binadhan-11 and Swarna-Sub1 both after 4 days and 8 days of complete submergence. After 8 days of submergence and subsequent re-aeration for one day, the amount of dry matter in the susceptible cultivar cv. Swarna was only 36.8 mg g^{-1} dry weight of the leaves, whereas the accumulation in the tolerant cultivar Swarna-Sub1 was 55.7 mg g^{-1} dry weight and that of cv. Binadhan-11 was 53.1 mg g^{-1} dry weight. These results were in conformity with the earlier findings of Sarkar *et al.* (2006); Bailey-serres *et al.* (2008).

High carbohydrate status during submergence is related to the submergence tolerance of rice crops (Yamada, 1959). In this study, carbohydrate content was estimated before and after submergence, where under control condition it was recorded relatively similar in all the cultivars. But irrespective of cultivars, there was decrease in carbohydrate content with the increase in duration of submergence. This might be due to rapid consumption, particularly in susceptible cultivar, to maintain elongation growth during submergence, synthesis of cell wall and cell elongation. This might be also due to the depletion of photosynthetic rate under submerged condition attributed to reduction in leaf area, chlorophyll fluorescence, low stomatal conductance and inter-cellular CO_2 concentration. Moreover, submergence also limits the carboxylation by lowering the intercellular CO_2 concentration and suppressing the RuBisCO activity, vis-à-vis enhancing the oxygenation process. Carbohydrate status after submergence is the key factor that determines the ability of plant to withstand submergence. After receding water level, regeneration of new leaves and rapid recovery for better

survival requires high energy reserves. So presence of sufficient carbohydrates after submergence is crucial for speedy recovery which was noticed in Swarna-Sub1 during re-aeration. Srivastava *et al*. (2007) reported that greater mortality in the susceptible genotype Swarna was due to starvation and lower energy supply for maintenance and repair processes of membrane integrity during submergence and recovery.

Table 6: Effect of complete submergence at vegetative stage on total carbohydrate content and SOD activity of rice genotypes.

Genotypes	Total carbohydrate content (mgg^{-1} DW of leaves)					SOD activity (unit min^{-1} g^{-1} FW of leaves)				
	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean
Swarna	66.83	43.00	36.00	36.80	45.66	1.61	2.49	1.12	1.29	1.63
Swarna-Sub1	68.8	56	53.70	55.7	58.55	1.76	3.01	1.50	1.68	1.99
Binadhan-11	66.3	53.5	51.2	53.10	56.03	1.81	2.95	1.43	1.54	1.93
Mean	67.31	50.83	46.97	48.53	53.41	1.73	2.82	1.35	1.50	1.85
	G	S	G*S			G	S	G*S		
SEm (±)	0.443	0.511	0.886			0.02	0.02	0.04		
C.D.(p=0.05)	1.29	1.49	2.59			0.05	0.06	0.11		

(*C: Control; G: Genotype; S: Stress; RA: 24 hr re-aeration)

G. Effect of complete submergence on the activity of ROS scavenging enzymes

SOD activity of all genotypes was estimated at different stages of submergence stress and presented in Table 6. This study revealed that SOD activity increased up to 4 days of complete submergence in all the genotypes over control but beyond that the activity started to decrease significantly in all varieties. On subsequent air adaptation for 24 hours, the activity again increased; however, the activity was below the level of non-submerged controlled plants especially in cv. Swarna. During the period of re-aeration, highest increase in SOD activity was found in Swarna-Sub1 (1.68 unit g^{-1} FW) followed by cv. Binadhan 11 (1.54 unit g^{-1} FW) and cv. Swarna (1.29 unit g^{-1} FW). This result agreed with the reports on the other crops (Panda and Sarkar, 2012).

The data analyzed for CAT activity of leaves was presented in Table 7. CAT activity increased in all the genotypes up to 4 days of complete submergence but beyond that the activity decreased. At 4 days of submergence, increase in the CAT activity compared to the respective control was highest in Swarna-Sub1 than other genotypes. The rate of reduction in the CAT activity was minimum in Swarna-Sub1 (5.09%) followed by cv. Binadhan 11 (8.68%) and cv. Swarna (47.34%) at 8 days of submergence. CAT activity again increased after 24 hours of cessation of submergence treatment compared with the 8 days of submergence treatment, the maximum increase being found in Swarna-Sub1 (2.05 unit min^{-1} g^{-1} FW). However, it always remained significantly lower during submergence and re-emergence period than in the control plants. This corroborates with the results of Kumar *et al*. (2020).

GPX activity (unit min^{-1} g^{-1} FW) was assessed during the experiment and depicted in the Table 7. Before submergence the enzyme activity was almost similar in all the genotypes. Its activity showed an increasing tendency up to 4 days of complete submergence from that of respective control plants. The magnitude of increase was highest in Swarna-Sub1 (30.86%) and lowest in cv. Swarna (18.55%). GPX activity then declined gradually when submergence condition was prolonged. It continued to decrease up to 8 days, showing the reduction by 12.29% in Swarna-Sub1, 13.14% in cv. Binadhan-11 and 41.74 % in cv. Swarna over the control plants. After 24 hours of re-aeration, GPX activity again increased but non-significantly in cv. Swarna.

Table 7: Effect of complete submergence at vegetative stage on catalase and GPX activity of rice genotypes.

Genotypes	Catalase activity (unit min^{-1} g^{-1} FW of leaves)					GPX activity (unit min^{-1} g^{-1} FW of leaves)				
	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean
Swarna	2.07	2.46	1.03	1.09	1.66	3.45	4.09	2.01	2.25	2.52
Swarna-Sub1	2.16	3.09	1.56	2.05	2.22	3.5	4.58	3.07	2.4	3.60
Binadhan-11	2.19	2.99	1.45	1.86	2.12	3.4	4.46	2.95	3.06	3.47
Mean	2.14	2.85	1.35	1.67	2.00	3.45	4.38	2.68	2.90	3.35
	G	S	G*S			G	S	G*S		
SEm (±)	0.02	0.03	0.05			0.019	0.022	0.04		
C.D.(p=0.05)	0.07	0.08	0.14			0.06	0.06	0.11		

(*C: Control; G: Genotype; S:Stress; RA:24 hr re-aeration)

Submergence and re-aeration can induce oxidative stress, causing an increased production of ROS as reported in many studies (Ella *et al.,* 2003 and Fukao *et al.*, 2011). ROS act as a cellular indicator of submergence stress and as secondary messenger involved in the stress response signal transduction pathway (Fukao *et al.,* 2004). These are very harmful for cellular components. High level of some antioxidant enzymes such as SOD, CAT, and GPX etc. are important in order to survive oxidative stress after the plants are subjected to different levels of submergence stress. SOD acts on the superoxide anions, converting it to another reactive and toxic intermediate, H_2O_2 (Mates, 2002). CAT is considered as a key enzyme removing H_2O_2, and peroxidase has a complementary duty (Malecka *et al*., 2009).

This early rise of enzymes was considered to be the response to active oxygen activities caused by

submergence. Possibly, increased levels of active oxygen stimulate the cellular protective mechanism to mitigate damages. However, with the progress of submergence period beyond 4 days, there was significant decrease in the activities of antioxidant enzymes irrespective of the genotypes. This might be due to the damaged light reaction system and reduced PS II activity under prolonged submergence (Panda *et al.*, 2006 and 2008). This may have led to a loss of chemical energy provided by light reaction system, which are thought to be used for the production of ROS under stressful condition, in which chemical energy is not used for the CO_2 fixation. Thus, beyond 4 days of submergence, production of ROS was retarded resulting in the reduction of protecting enzymes.

Again during re-aeration, enzyme activity was found to be increased to scavenge the ROS and this was significant in cv. Swarna-Sub1 and cv. Binadhan-11. These findings agree with Panda *et al*. (2008). During re-aeration, rice seedlings need protection from ROS (Blokhina *et al*., 2001). Though regeneration after submergence is more important for survival of rice seedlings (Panda *et al.*, 2008), cv. Swarna failed to encounter oxidative damage efficiently as like cv. Swarna-Sub1 and cv. Binadhan-11 during post submergence recovery period. In the present study decrease in antioxidant enzymes activity during submergence and their slow recovery after submergence in the susceptible cultivar caused more oxidative damage to the susceptible cultivar. The results showed that tolerant cultivars somehow maintained greater quantities of antioxidant enzymes levels during submergence and subsequent period of re-aeration might help it to encounter the oxidative damage efficiently.

CONCLUSIONS

In the present investigation, it was found that submergence has a very harmful effect on growth, development and productivity of rice. The results revealed that Swarna-Sub1 maintained greater quantities of chlorophyll, carotenoid, protein content under submerged condition. Leaf and internodal elongation are the processes that occur in all rice species during development of foliage but, maintenance of growth under water and tolerance to complete submergence are traits indispensable for survival. This is accompanied with higher activity of SOD, CAT, GPX which facilitated scavenging mechanism against production of ROS that might be responsible for the tolerance to complete submergence. These antioxidant enzymes level during submergence and subsequent re-aeration might help it to encounter the oxidative damage efficiently. Hence, on the basis of all the observations recorded during the course of investigation, it was concluded that performance of cv. Binadhan-11 is at par with Swarna-Sub1, an already known submergence tolerant cultivar; hence it is likely to be tolerant to complete submergence but for a limited duration.

FUTURE SCOPE

As submergence is a very common but very harmful stress not only in the coastal belt of Odisha but also in the whole world, development of new submergence tolerant varieties are must. However, for confirmation of the results, this warrants further investigation.

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