Physiological and biochemical attributes of rice (*Oryza sativa* L.) genotypes under complete submergence during the vegetative stage

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By

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CERTIFICATE-I

This is to certify that the thesis entitled **"Physiological and biochemical attributes of rice (*Oryza sativa* L.) genotypes under complete submergence during the vegetative stage"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Plant Physiology)** to the **Odisha University of Agriculture and Technology, Bhubaneswar** is a faithful record of bonafide and original research work carried out by **Pragyani Dash, Adm. No. 07PP/17** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged.

29/6/19

(Mrs. Prabhasmita Shatpathy)
CHAIRMAN
ADVISORY COMMITTEE

CERTIFICATE-II

This is to certify that the thesis entitled **"Physiological and biochemical attributes of rice (*Oryza sativa* L.) genotypes under complete submergence during the vegetative stage"** submitted by **Pragyani Dash, Adm. No. 07PP/17** to the **Odisha University of Agriculture and Technology, Bhubaneswar** in partial fulfilment of the requirements for the degree of **Master of Science in Agriculture (Plant Physiology)** has been approved/~~disapproved~~ by the students' advisory committee and the external examiner.

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Bhubaneswar **(Pragyani Dash)**

Date : **Adm. No. 07PP/17**

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ABBREVIATIONS

%	:	Percentage
@	:	At the rate
^{0}C	:	Degree Celsius
BSH	:	Bright sunshine hour
CD	:	Critical difference
Cm	:	Centimetre
Cv	:	Coefficient of variation
et al.	:	Co-workers
FCRD	:	Factorial completely randomized design
Fig	:	Figure
FW	:	Fresh weight
G	:	Genotype
Max	:	Maximum
Min	:	Minimum
Mg	:	milligram
g	:	gram
RH	:	Relative humidity
S.E.m	:	Standard error of mean
µg	:	Microgram
S	:	Stress
RA	:	Reaeration
c.v.	:	Cultivar
DAS	:	Days after sowing

ABSTRACT

The present pot culture experiment was conducted in the Agronomy Main Research Field of the Odisha University of Agriculture and Technology (OUAT), Bhubaneswar during *kharif* 2018 to study "Physiological and biochemical attributes of rice (*Oryza sativa* L.) genotypes under complete submergence during the vegetative stage". Three rice genotypes (cv. Swarna, cv. Swarna sub 1 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 reaeration 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 sub 1 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 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 sub 1 to overcome oxidative stress through up-regulation of SOD, catalase, glutathione peroxidase activity under 8 days of complete submergence and subsequent reaeration. With regard to yield attributes, panicles per plant and grains per panicle were significantly the highest in cv. Swarna sub 1 and cv. Swarna recorded the least. The performance of cv. Swarna sub 1 followed by cv. Binadhan 11 and cv. Swarna were in diminishing mode under all four submergence treatments.

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 and 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 110.9 M t (2017) 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 38.8 M ha (2017) 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).

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. 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. Among all the antioxidant enzymes, catalase, ascorbate peroxidase, superoxide dismutase, guaiacol peroxidase, glutathione reductase etc. play the key roles in ROS detoxification in plant cells under stress.

Genotypes of rice 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 genotypes Swarna *Sub* 1 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, a pot culture experiment has been designed to study the **"Physiological and biochemical attributes of rice (*Oryza sativa* L.) genotypes under complete submergence during the vegetative stage"** with the following objectives.

Objectives

1) Effect of complete submergence during vegetative stage on morpho-physiological traits in rice genotypes.
2) Biochemical and enzymatic analysis during the submergence period and after desubmergence or reaeration.
3) Effect of complete submergence during vegetative stage on yield and yield attributes in rice.

REVIEW OF LITERATURE

Rice production is adversely affected by numerous abiotic stresses such as submergence, water logging, drought, cyclone and salinity, which normally prevail throughout the year and mostly during *kharif* season. Although rice plant is well adapted to aquatic environment, but it is unable to survive under complete submergence for several weeks. Submergence stress is more obviously detrimental when rice plants experience some sort of oxidative exposure. The greater prevalence of submergence stress in farming systems that severely depresses crop yields. This is more prevalent for rice seedlings being completely inundated under water and thereafter a sudden recession of water level. In areas where rain fed lowland rice is subjected to flash floods, elongation results in lodging and death of plants after the water recedes. Hence, plants adapted to these areas must have submergence tolerance. In majority of cases, the successful strategy is probably a combination of morphological, anatomical and metabolic adaptations. Submergence tolerance is a metabolic adaptation in response to anaerobiosis that enables cells to maintain their integrity such that the plant survives hypoxia without major damage. The relevant literature pertaining to these aspects have been reviewed under following heads. In this present study, three tolerant and susceptible rice genotypes were evaluated for underwater shoot elongation, carbohydrate status, constitutive and stress inducible antioxidative defence system in order to ascertain if they are related to submergence tolerance. The aim of the present study is to find out physiological and biochemical basis of survival of rice genotypes to complete submergence during their vegetative stage.

2.1 Morpho-physiological character

2.1.1 Plant height

Palada and Vergara (1972), Singh and Bhattacharjee (1987) revealed a positive correlation between seedling survival rate during the submergence and their initial and final height, length and elongation rate of leaf sheath of rice. However, Sarkar (1997) opined that plant height of a cultivar before submergence has no significance with the survival percentage during submerged condition.

Plant height did not increase much in sub1 introgressed cultivars that resulted significantly lower elongation compared to other genotypes (Sarkar and Bhattacharjee, 2011). Re-orientation of petioles in a more upright position and faster leaf or stem elongation enable the shoot to regain contact with the water surface and the open atmosphere (Jackson, 1985; Voesenek *et al*., 2004; Fukao and Biley Serres, 2008). Deepwater flooding-adapted rice can maintain aerobic metabolism during submergence through development of its canopy above the water surface due to elongation of its long leaves (Sakagami *et al*., 2009 and Kawano *et al*., 2002). However, a negative correlation between survival percentage and rapid elongation of stem under submerged condition of rice has been reported by Singh *et al*. (2011). Shoots of the intolerant genotypes usually senesced and degraded with time when submerged and surviving plants recovered more slowly by slower shoot elongation and leaf and tiller formation (Mackill *et al.*, 2014). Submergence increases the endogenous levels of GAI (bioactive form) and GA20 (inactive form; an immediate precursor of GAI) in the intercalary meristem and elongation zone of internode sections in deep water rice (Hoffmann-Benning, 1992).

2.1.2 Leaf area

Mansab *et al.* (2003) opined that for maximum crop growth enough leaves must be present in the canopy to intercept most of the incident NAR. Therefore, growth is often expressed on leaf-area basis.

Leaf area of rice increases under submerged condition (Yajie Zhang *et al.*, 2015) but susceptible rice genotypes showed higher reduction in root oxidase activity, leaf area and leaf dry weight under stagnant flooding compared to control.

2.2 Biochemical characters

2.2.1 Chlorophyll content

According to Jackson *et al.* (1987), quicker loss of chlorophyll in leaves of submerged plants is caused by ethylene, which triggers gene expression and chlorophyllase enzyme activity which is involved in chlorophyll breakdown. Sarkar *et al.* (2001) reported that complete submergence stress accelerates degradation of chlorophyll content in susceptible cultivars compared to the tolerant one which can

also be used as an indicator to measure degree of tolerance to submergence. However, Panda *et al.* (2008) reported significant reduction of chlorophyll content in both susceptible and tolerant cultivars under submergence but tolerant cultivars maintained higher level chlorophyll during submergence and the subsequent period re-emergence. The efficiency of light captured to drive photosynthesis is directly correlated to the photosynthetic capacity of a plant which is determined by several factors, including photosynthetic pigment composition and chlorophyll concentration in the leaf (Netondo *et al.,* 2004). According to Sahu (2007), the major physiological traits in rice involve in maintenance of high carbohydrate concentration, minimum elongation growth, optimum rates alcoholic fermentation of regulation of antioxidant system and low synthesis of ethylene during submergence on recession of water.

Singh *et al.* (2014) the sensitive and tolerant genotypes show similarly high leaf chlorophyll concentrations before submergence but, when submerged, the tolerant genotypes maintained more chlorophyll than the intolerant genotypes. Chlorophyll content decreases proportionately with increase in submergence period (Das *et al.,* 2009). Singh *et al.* (2014) reported that the sensitive genotypes exhibited 10–20 % greater reduction in leaf chlorophyll concentration than did the tolerant checks and the Sub1 introgression lines. Lesser degradation of total chlorophyll helps in survival of the plants as well as in speedy recovery after submergence. Concentration of chlorophyll during re-emergence is significantly lower compared to the non-submerged control plants even in tolerant cultivars (Sarkar *et al.,* 2007).

2.2.2 Carotenoid content

According to Rai *et al.* (2004), yellowing of leaves (chlorosis) was a symptom of submergence of seedling due to the increase in carotenoid content.

2.2.3 Proline content

Suppressions of mitochondrial electron transport is the primary reason for stress induced proline accumulation in susceptible genotypes (Alia and Sarathi *et al.,*1993). Proline has also been referred to as a supportive index for assuming osmotic deficits out of submergence as studied earlier (Mostajeran and Rahimi-Echi, 2009). Puckridge *et al*. (1995) during the study observed that accumulation of proline was the maximum in susceptible cultivar under submerged condition. So, high

accumulation is considered to be an indication of submergence damage which occurs due to the hydrolysis of the protein.

2.2.4 Chlorophyll stability index

Sarkar *et al.* (2006) during his study on physiological basis on rice under submergence, concluded that in complete submergence stress, more degradation of chlorophyll content in susceptible cultivar compared to tolerant on which can be used as indication of submergence tolerance.

2.2.5 Membrane stability index:

Sakae Agarie *et al*. (1995) indicates that silica involved in the stress tolerance, assumbaly with increasing membrane permeability to water stress condition, during his study, electrolyte was increased in silica deficit leaf to the same levels as water-stressed leaf. Upadhyay *et al.* (2009) revealed that submergence stress increased the membrane damage, as is evident from increased value of electrical conductivity and lipid peroxidation.

2.2.6 Protein content

Goswami *et al. (2017)* reported that during submergence stress condition protein degradation is high in susceptible cultivars.

2.2.6 Carbohydrates content

Cultivars that maintained more than 6% of their initial NSC at the time of re-aeration were found to be capable of developing new leaves, rather quickly. High level of NSC before submergence is associated with enhanced survival under flooded condition, possibly by supplying the required energy for maintenance metabolism through anaerobic respiration (Das *et al.,* 2005) which enables cell to maintain their integrity so that the plant survives hypoxia/ anoxia without major damage (Sarkar *et al.,* 2006).

Tolerant genotypes were identified and key traits such as high levels of NSC (starch and soluble sugars) and limited underwater elongation were found to be associated with tolerance (Das *et al.,* 2005). According to Sarkar *et al.* (2006) and

Panda *et al.* (2008), cultivars are needed that can withstand repeated flooding and have better regeneration capacity as well as faster growth after flooding to produce sufficient biomass in a shorter period when submerged. The submergence tolerant landraces used in breeding, such as FRI3A, are more tolerant than their Sub l derivatives and inherently accumulate more NSC before submergence, whereas Sub l varieties maintain NSC concentrations similar to their original parents, suggesting their reliance on concurrent photosynthesis during submergence (Singh *et al.*, 2014).

Reports suggested that in water logged condition rice plant could utilize sugar, mostly reducing type by hydrolysing the sucrose and other storage carbohydrate on demand of respiratory substrate for better survival (Sujata *et al.,* 2008). Under inundated condition of submergence plant use its storage carbohydrate as a source of respiratory substrate for better survival (Kawano *et al.,* 2009). Maintenance of high levels of stored carbohydrates in the seedlings prior to submergence coupled with minimum shoot elongation is a desirable trait for submergence tolerance. The cultivar that maintained higher carbohydrate content at the time of re-emergence were found to develop new leaves very quickly (Sarkar *et al.*, 2006)

The major morphological and physiological submergence tolerant traits are slow leaf elongation, less chlorosis, high carbohydrate reserve storage during submergence and prompt re-adaptation to the aerial environment after de-submergence. Given the above traits, carbohydrate metabolism during submergence seems to be an important factor in flash-flood tolerance, and this 'quiescence strategy' is characterized by slow expansion growth that is presumed to conserve energy and carbohydrates (Singh *et al.,* 2001). Ram *et al.* (2002) also observed that the amount of NSC contained within the dry seed or in the shoots of 10-day-old seedlings prior to submergence was not necessarily higher in submergence-tolerant types; yet these genotypes tend to lose less carbohydrate when under water and recover faster after submergence (Mazaredo and Vergara, 1982; Das *et al.*, 2005).

The amount of stored carbohydrates in plant organs is positively correlated with the level of submergence-tolerance in rice under submerged conditions (Ella *et al.,* 2006). Takeshi *et al.* (2008) concluded that carbohydrate reserves spared during submergence can facilitate re-initiation of meristems and leaf development upon de-submergence. The increase in carbohydrate consumption for cell division, cell

elongation and maintenance of elongated leaves are adverse factors in the escape strategy (Setter and Laureles, 1996; Voesenek *et al.,* 2006). The consumption of carbohydrate during submergence and lack of photosynthetic carbon gain are expected to cause serious inhibition of recovery after desubmergence (Kawano *et al.,* 2002).

Therefore, it is sufficiently reasonable to conclude that high carbohydrate status after submergence, which is the consequence of its level before submergence and extent of turnover and consumption during submergence, is the key factor that determines the ability of plants to withstand submergence stress.

2.2.7 Antioxidative enzymes

Superoxide dismutase (SOD) merely acts on the superoxide anion, converting it to another reactive intermediate (H_2O_2), and catalase (CAT) acts on H_2O_2, converting it to water and oxygen (Mates *et al.,* 2000). Flood tolerant genotypes under submergence can thrive by effective energy maintenance through lower leaf expansion, minimum elongation, less chlorosis, high DMP, high carbohydrate reserve, and a strong antioxidant enzyme system (Sarkar *et al.,* 2001).

Colmer and Jackson (2008) revealed that oxidative damage of chlorophyll, DNA, protein lipids, nucleic acids and other macromolecules occurs by oxygen radicals which can severely disrupt normal metabolism due to their cytotoxicity. Plants have evolved an antioxidant defence system comprising enzymes such as SOD, CAT, and peroxidase to minimize and eliminate oxidative damage by removing and scavenging reactive oxygen species (ROS) at different cellular locations (Mittler, 2002). Under limited oxygen availability, photosynthesis and respiration are restricted, leading to an energy crisis, resulting formation of toxic products from anaerobic respiration and the accumulation of reactive oxygen species (ROS) in plant cells (Licausi and Perata, 2009; Pucciariello *et al.,* 2013; Yang and Hong, 2015). When the submerged plants are promptly exposed to an environment with high light and oxygen tension, the formation of ROS occurs such as superoxide anion, hydroxyl radical and hydrogen peroxide that if not mitigated, can cause severe damage to cellular organization leading to plant death (Sarkar *et al.,* 2006). Cultivars that are more efficient in detoxifying the ROS upon exposure to air are more capable of

retaining their chlorophyll, maintain growth of older leaves, regenerate new leaves relatively fast and hence sustain plant growth (Sarkar *et al.,* 2006).

Damage from the action of free radicals during submergence is also less in genotypes containing Sub 1 and this may contribute to a stronger recovery after submergence (Santosa *et al*., 2007).

Anandana and Arunachalamb (2012) reported that the activities of all antioxidant enzymes were significantly greater in tolerant/avoidance genotypes than in the susceptible genotypes under both submergence and de-submergence. Seedlings of tolerant genotypes had higher survival, and the roots were more viable with greater capacity to elongate (Manjri *et al.*, 2018). Moreover, they had higher peroxidase activity which could be utilized as the selection criteria for evaluating submergence tolerance in rice and lesser increases in both electrolyte leakage and malondialdehyde production during submergence.

Singh *et al.* (2001) reported that submergence for 4-7 days increased SOD, CAT, peroxidase activity, and just after 24 hours of submergence, the SOD activity in tolerant lines was twice that of susceptible rice varieties. SOD in combination with the effective ascorbate glutathione cycle enzymes are more important in antioxidative defence and participate in eliminating excessive ROS induced by submergence and subsequent re-aeration in rice. ROS generated during and following submergence are usually lower and the concentration of antioxidants (ascorbic acid, glutathione, phenolics and antioxidant enzymes) are higher in tolerant landraces, contributing to their chlorophyll retention (Ella *et al*., 2003).

Ethylene accumulates in plant tissue during submergence because of both enhanced synthesis and entrapment when its diffusive escape is inhibited by water and subsequently prompts underwater leaf senescence. This effect is suppressed in tolerant cultivars such as FR 13A (Jackson and Ram, 2003; Fukao *et al.,* 2006).

2.3 Yield and yield attributes

Reddy *et al.* (2008) revealed that the attributes like HI, biological yield, tillers per plant flag leaf width and spikelet per panicle exhibited high positive direct effect on grain yield per plant. The survival and productivity of rice increased with the age

of seedling at the time of submergence (Haque, 1974; Hasanuzzaman, 1974; Pande *et al.,*1979). By increasing the duration of submergence, the tiller number of all the tested varieties decreased after de-submergence but the decrease was comparatively less in flood tolerant variety FR 13A than other varieties (Reddy *et al.* 1985). Tillering could be adversely affected as a result of the rapid increase in plant height during submergence (Reddy and Mittra, 1985). Sarkar and Bhattacharjee (2012) also observed cultivars with SUB1 (Swarna-Sub1, IR64-Sub1 and Samba Mahsuri-Sub1) maintained greater biomass at the end of submergence, while resuming faster growth during recovery than their respective recurrent parents.

According to Ashura (1998), a positive correlation was found between yield and plant height, tillers per plant, grains per panicle, panicles/plant, 1000 seed weight and panicle length under low land condition in rice. Tolerant genotypes produced significantly more tillers per unit area than the sensitive ones after submergence. Shoots of the intolerant genotypes usually senesced and degraded with time when submerged and surviving plants recovered more slowly as shown by slower shoot elongation, and leaf and tiller formation. Lodging was also higher in sensitive genotypes due to weaker culms (Mackill *et al.*, 2014).

According to Panda *et al.* (2008) prompt resumption of growth following submergence is a desirable trait as it supports production of new photosynthesizing shoot biomass and earlier tillers, both essential for higher yields. Grain yield is influenced by high direct positive effects of productive tillers, and grains per panicle, filled grains per panicle, panicles per plant and grains per panicle, plant height (Hairmansis *et al.,* 2010 and Sadeghi, 2011). Ram *et al.* (2006) observed yield improvement in introgression lines in rice was due to increase in number of ear bearing tillers and number grains per panicle.

According to Fisher (2007) high photosynthetic rate and slow leaf senescence are the crucial physiological aspects responsible for higher grain yield. Submergence delays 50% flowering in rice genotypes, which is inevitable because majority of existing leaves or shoots die during submergence and the new growth occurs during the recovery period (Srivastava *et al.,* 2007). Faster recovery of tolerant genotypes was also associated with a shorter delay in flowering and maturity (Singh *et al*., 2009).

MATERIALS AND METHODS

The 'Materials and Methods' include detailed description of the various experimental techniques and materials employed during the course of investigation to study the **"Physiological and biochemical attributes of rice (*Oryza sativa* L.) genotypes under complete submergence during the vegetative stage".** The details of materials used, methods and procedures followed during the course of this investigation have been presented here under briefly in a systematic way.

3.1 Experimental site

The present trial was conducted in the 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 21^0 15' N latitude, 85^0 48' E longitude and 26.9 m above the mean sea level during *kharif,* 2018.

3.2 Experimental soil characteristics

A true representative soil sample was drawn by quartering method from the composite soil collected from the Agronomy Main Research (AMR) field and then it was air dried under shade for four days. After grinding by means of a soil sample grinder, it was put to mechanical analysis. Some soils after grinding were passed through 2.0 mm sieve and ultimately used for chemical analysis. The details of the methods of analyses are in table 3.1 and the physico-chemical properties of the experimental soil are delineated under table 3.2.

Table 3.1 Methods of soil analyses

Sl. No.	Particulars	Method(s) employed
01	Mechanical analysis	Boyuoucos Hydrometer Method (Piper, 1950)
02	Soil pH	Glass electrode (Jackson, 1973)
03	Organic carbon	Volumetric method (Walkley and Black, 1947) method as described by Muhr *et al.,* 1965.
04	Available nitrogen (N)	Alkaline permanganate method (Subbiah and Asiha, 1956).
05	Available phosphorous (P_2O_5)	Brays' method No.1 (Bray and Kurtz, 1945) as described by Muhr *et al.*, 1965.
06	Available potassium (K_2O)	Flame photometer method as described by Muhr *et al.*, 1965.

Table 3.2 Physico-chemical properties of the experimental soil

pH	Textural class	Organic Carbon (%)	EC (ds m^{-1})	Total N (kg ha^{-1})	Available P_2O_5 (kg ha^{-1})	Available K_2O (kg ha^{-1})
5.48	Loamy sand	0.47	0.03	100	50	50

3.3 Climatic condition

The location of the experiment is characterized by warm and moist climate with a hot and humid summer and mild winter. Broadly, the climate falls in the 'moist hot' group (Lenka, 1976). The experiment was carried out during *kharif* (August-December, 2018). The monthly weather parameters during the petri dish culture and pot culture of rice viz. maximum and minimum temperature, relative humidity (RH), rainfall, and bright sunshine hours were recorded at the meteorological observatory nearby the experimental site which are delineated under Table 3.3.

Meteorological parameters during crop growth (*kharif*, 2018)

Table 3.3 Weekly mean of weather parameters during pot culture recorded at Agronomy Main Research Field, OUAT, Bhubaneswar

Std. Week No.	Temperature (°C)		Relative Humidity (%)		Bright sunshine hours	Total Rainfall (mm)	Wind velocity (km h^{-1})
	Max.	Min.	Max.	Min.			
30th	32.1	26.5	94	71	2.3	34.4	4.0
31st	32.5	25.5	91	71	2.8	35.6	3.5
32nd	32.4	25.7	93	78	4.2	216.7	2.8
33rd	32.6	26.1	93	76	4.3	149.2	3.7
34th	32.5	26.1	94	80	3.3	31.3	1.8
35th	31.3	25.5	94	79	3.5	59.5	0.3
36th	30.8	24.7	94	77	3.6	28.7	0.7
37th	34.1	25.9	91	68	6.6	12.9	0.4
38th	31.7	24.8	94	78	3.3	155.9	0.9
39th	33.8	25.4	95	70	6.5	39.2	0.2
40th	35.1	25.5	94	54	4.5	0.0	0.1
41st	30.7	23.3	96	72	3.4	230.4	1.0
42nd	33.4	22.5	94	53	7.5	0.0	0.1
43rd	35.9	20.6	92	58	8.7	3.6	0.2
44th	30.0	21.8	94	61	6.7	2.8	0.9

3.4 Experimental details

The experiment was conducted to study the physiological and biochemical attributes of rice genotypes under complete submergence during the vegetative stage.

For this purpose, pot culture experiment was conducted in the AMR during *kharif*, 2018. *i. e*. from 14-08-2018 to 09-12-2018.

3.4.1 Treatments

The treatments consisted of three rice genotypes (G_1, G_2 and G_3) i.e. Swarna, Swarna Sub 1 and Binadhan 11 and four excess water situations (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 reaeration for 1 day after 8 days of complete submergence, respectively. The details of the treatments are as in Table 3.4.

3.4.2 Varietal characteristics

3.4.2.1 Swarna (MTU 7029, IET 5656)

It is one of the oldest but popular ruling variety in Eastern India suitable for medium land, released from Rice Research Station, Maruteru, APAU, Andhra Pradesh in 1980 and notified in 1980 by Central Variety Release Committee. It is of medium duration (140-145 days), 95-100 cm tall variety with good tillering habit, medium bold grains, brown husk, low glycemic index (GI) and yielding 5.0 t ha^{-1}. It is resistant to bacterial leaf blight (BLB) but tolerant to many diseases including sheath rot. It is also highly susceptible to submergence stress.

3.4.2.2 Swarna Sub-1(CR 2539-1; IET 2026)

It is developed from ruling Swarna (MTU 7029) variety of rice by introducing the Sub-1 gene by marker–assisted backcrossing, which enables it to tolerate complete submergence to considerable period. It was released in 2009. It has medium slender grains with 140-145 days duration, 95-100 cm height and suitable for flood prone shallow low land producing grain yield of 5.2 t ha^{-1}. It is tolerant to complete submergence between 15-17 days and resistant to sheath rot.

3.4.2.3 Binadhan 11

It has been released by Bangladesh Institute of Nuclear Agriculture in 2013 as a submergence tolerant early maturing rice variety for aman season. It is of 130-135 days (under 20-25 days submergence) and 115-120 days for non submerged condition. Medium long grain with yield potential yield of 4.5 t ha^{-1} and but can produce more under non submerged condition.

3.5 Design and layout

The pot culture experiment was carried out in a factorial completely randomized design with 12 treatment combinations (3 x 4), replicated thrice (R_1, R_2 and R_3). Healthy seeds of three different rice genotypes were collected from the National Rice Research Institute, Cuttack, Odisha for this experiment. 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 55cm in AMR was 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 subsequently the recovery was studied after 24 hours of re-aeration.

Special attention for timely watering and drainage of excess rainfall was given. Measured quantity of (300 ml) of tap water was applied to each pot regularly up to physiological maturity. The plan of the layout of experiment is shown in Fig. 3.1.

Table 3.4 Details of treatment and symbols used

Treatments	**Symbols used**
Rice genotypes	
Swarna Sub-1	G_1
Swarna	G_2
Binadhan-11	G_3
Submergence	
No submergence (control)	S_0
Submergence for 4 days	S_1
Submergence for 8 days	S_2
Submergence for 8 days followed by re-aeration for 1 day	S_3

	G1				G2				G3			
	S0	**S1**	**S2**	**S3**	**S0**	**S1**	**S2**	**S3**	**S0**	**S1**	**S2**	**S3**
R1	**R1G1S0**	**R1G1S1**	**R1G1S2**	**R1G1S3**	**R1G2S0**	**R1G2S1**	**R1G2S2**	**R1G2S3**	**R1G3S0**	**R1G3S1**	**R1G3S2**	**R1G1S3**
R2	**R2G1S0**	**R2G1S1**	**R2G1S2**	**R2G1S3**	**R2G2S0**	**R2G2S1**	**R2G2S2**	**R2G2S3**	**R2G3S0**	**R2G3S1**	**R2G1S2**	**R2G3S3**
R3	**R3G1S0**	**R3G1S1**	**R3G1S2**	**R3G1S3**	**R3G2S0**	**R3G2S1**	**R3G2S2**	**R3G2S3**	**R3G3S0**	**R3G3S1**	**R3G3S2**	**R3G3S3**

Fig. 3.1 Layout plan of the pot culture experiment

3.5.4 Calendar of operation

The experiment was conducted during *kharif*, 2018 and calendar of operation is showed below in the Table 3.5.

Table 3.5 Calendar of operations

Sl. No.	Operations	Date
1	Pre-soaking seed germination	14/08/18
2	Sowing	18/08/18
3	Thinning	22/08/18
4	Weeding	29/08/18
5	1st Observation (before submergence i.e. S_0)	17/09/18
6	Date of complete submergence	17/09/18
7	2nd Observation (after 4 days of complete submergence i.e. S_1)	21/19/18
8	3rd Observation (after 8 days of complete submergence i.e. S_2)	25/09/18
9	4th Observation (after 8 days of complete submergence and 24 hours of reaeration i.e. S_3)	26/09/18
10	Date of 50% flowering in Swarna	30/11/18
11	Date of 50% flowering in Swarna sub 1	28/11/18
12	Date of 50% flowering in Bina 11	21/11/18
13	Harvesting	9/12/18

The details of the methods of observations are as below.

3.6 Pre-harvest observations

3.6.1 Plant height (cm)

The height of five plants from each pot was recorded replication wise from the ground level of plant to the upper most leaf tip. The data were measured in centimetre before and after submergence as well as during the period of re-aeration.

3.6.2 Leaf area ($cm^{2)}$

Second leaf was selected in each pot for the estimation of leaf area. The leaf samples were collected in a beaker containing a small amount of water to prevent leaf rolling before taking the measurement. For each leaf sample, the length and maximum width were measured (in centimetre) and the area was computed by multiplying each other. Then each leaf area (in cm^2) was multiplied with an adjustment factor of 0.75 as suggested by IRRI (1972).

(Usually the adjustment factor is determined by plotting the leaf on a graph paper and the actual leaf area is found out. Actual leaf area can also be determined by leaf area meter. Then the adjustment factor is found out by the formula, Adjustment factor = Actual leaf area/ (length x width). But as per IRRI (1972), the value 0.75 can be used for all stages of growth in rice except the seedling stage and harvesting stage where the value of 0.67 is used.)

3.7 Biochemical and physiological parameters

3.7.1 Estimation of total chlorophyll content (Arnon, 1949)

Total chlorophyll content of the leaves was determined by using the method stated by Arnon (1949). The second leaf from the top was sampled for the purpose. The leaf samples were immediately kept in moist polythene bags to keep them turgid. 100 mg of fresh leaves were taken from the middle portion of the leaves and were cut into small pieces. The leaf discs were then put in 80% v/v acetone solution and kept in dark for 24 hours. Then they were filtered by Whatman No. 1 filter paper and the filtrate was used to record the absorbance (OD) at 645 nm and 663 nm. The respective chlorophyll content was calculated using the following formulae and expressed as mg g^{-1} FW leaf.

$$\text{Chlorophyll-a} = (12.7 \times OD_{663} - 2.69 \times OD_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll-b} = (22.9 \times OD_{645} - 4.68 \times OD_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total Chlorophyll} = (20.2 \times OD_{645} + 8.02 \times OD_{663}) \times \frac{V}{1000 \times W}$$

Where, OD_{645} = Absorbance (OD value) at 645 nm

OD_{663} = Absorbance (OD value) at 663 nm

V = Volume of 80% acetone (in ml)

W = Fresh weight of leaf (in gram)

3.7.3 Carotenoid content (Litchenthaler and Wellburn, 1983)

Carotenoid content in the leaves were determined by using the following method. The 3rd leaf from the top was sampled for the purpose. The leaf samples were immediately capped in moist polythene bags to keep them fresh. 100 mg of fresh leaf was taken from the middle portion of the leaf and were cut into small pieces. The leaf discs were then put in 80% v/v acetone solution and kept in dark for 24 hours. Then they were filtered by Whatman No. 1 filter paper and the filtrate was used to record the absorbance (OD) at 480 nm. The respective chlorophyll content was calculated by using the following formula and expressed as mg/g FW of leaf.

$$\text{Carotenoid} = [(OD_{480} + (0.114 \times OD_{663}) - (0.638 \times OD_{645})] \times \frac{V}{1000 \times W}$$

3.7.4 Chlorophyll Stability Index (%)

Chlorophyll Stability Index (CSI) was calculated by taking leaf samples of control untreated and those imposed with drought stress as per and the formula given below (Kar *et al*., 2005).

$$\text{CSI (\%)} = \frac{\text{Total chl. content (stress)}}{\text{Total chl. content (non - stress)}} \times 100$$

3.7.5 Membrane stability Index (%)

Membrane stability index was determined by estimating the electrolyte leakage from the fresh leaf tissue into distilled water 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 40 ºC for 30 minutes. After that their conductivity was measured using electrical conductivity meter. 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.

MSI= {1-(conductivity AT 40ºC / conductivity at 100 ºC)}×100

3.7.5 Proline estimation: (Bates, L.S., R.P. Waldron and I.D. Teare, 1973)

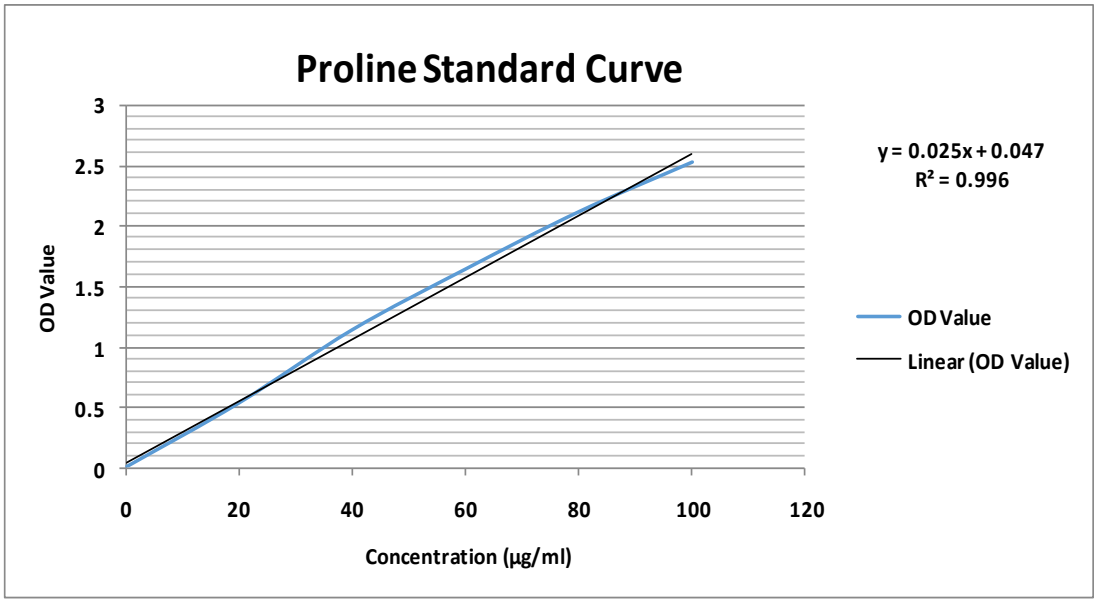

Fig. 3.2 Proline standard curve

Proline content of leaf samples was determined by following procedure given by Bates *et al*. (1973). Fresh leaf sample of 100 mg was ground in mortar and pestle with 10 ml of 3% sulphosalicylic acid and the homogenate was centrifuged at 3000 rpm for 10 minutes. The extract was filtered through Whatman No. 2 filter paper and 2 ml of filtrate was transferred into a clean test tube to which 2 ml of each glacial acetic acid, orthophosphoric acid and acid ninhydrin were added. Then test tubes were incubated in boiling water bath for 1 hour at 100 ^{0}C, followed by cooling to cease the reaction. Samples and standards were poured into separating funnel to which 4 ml of toluene added and shaken vigorously. Toluene layer was discarded and OD was read

at 520 nm. A standard curve of data was used to calculate the amount of proline present in leaf sample and expressed as mg per gram FW.

Calculation- mg of proline/g tissue =**(X/2) × (10/100)×1000**

3.7.6 Total carbohydrate of leaf

Carbohydrate content of leaf samples was estimated by following procedure (Yosidha *et al*., 2005). Powdered dry sample of 0.1 g 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 then volume was made up to 100 ml with distilled water in a volumetric flask. 1ml of supernatant taken in a test tube and with distil. Water volume made up to 3ml followed by addition of 4ml anthrone. It was heated in boiling water bath for 8 minutes and cooled rapidly. The absorbance reading was taken in spectrophometer at 630 nm.

Amount of carbohydrate present in 100 mg of sample

= (mg of sugar from graph/ml of aliquot sample) × (Total volume of extract in ml of sample in mg) x 100

3.7.7 Estimation of Protein Content by Lowry's Method: (Lowry, OH, NJ Rosbrough, AL Farr and RJ Randall, 1951)

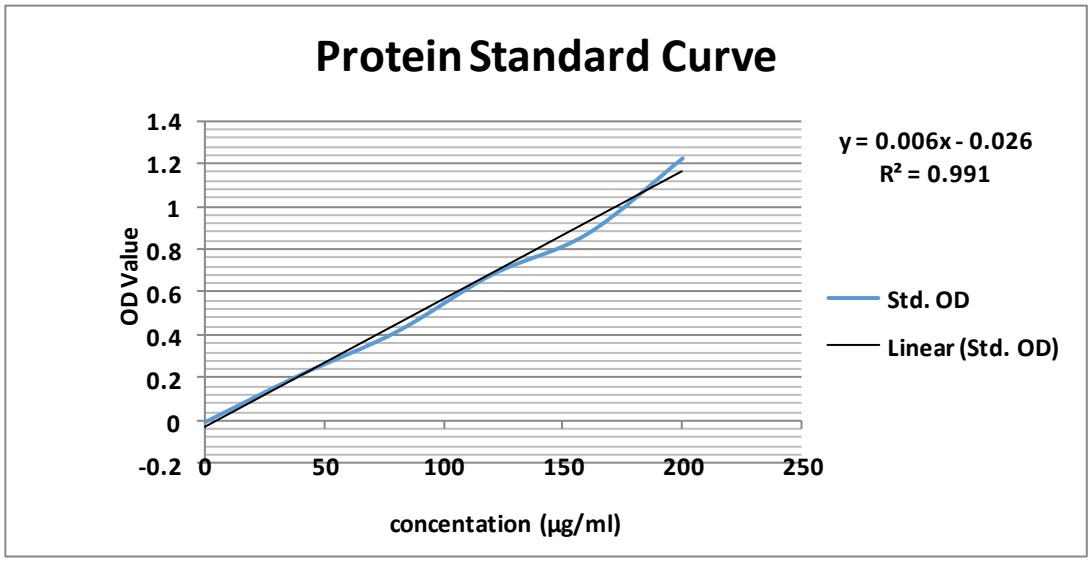

Fig 3.3 Protein Standard Curve

Protein content was measured by following the Lowry's method. Fresh leaf sample of 1 g was macerated in 10 ml TCA (10%) solution using mortar and pestle. After transferring into a centrifuge tube it was taken for centrifugation at 5,000 rpm for 10 minutes. The supernatant was thrown out and residue was collected to which 10 ml of 1N NaOH was added followed by mixing thoroughly by using a glass rod. Again centrifuged at 10,000 rpm for 10 minutes at 4 ^{0}C. Then 0.2 ml of sample extract was pipette out into a test tube and the volume was made up to 2 ml with water. In each test tube including blank 10 ml of reagent C was added and allowed to stand for 10 minutes. 1 ml of reagent D was added to it, mixed well and incubated at room temperature and kept in dark for 30 minutes. At last blue colour was noticed. Finally, absorbance was taken at 660 nm. A standard protein curve was prepared and from that sample protein content was calculated as mg g^{-1} sample.

3.7.8 Superoxide dismutase activity: (Madamanchi, N. R., Donahue, J. L., Cramer, C. L., Alscher, R.G. and Pedersen, K., 1994)

This assay was based on the capacity of the extracts to inhibit the photochemical reduction of Nitroblue tetrazolium (NBT) in the presence of the riboflavin-light-NBT system (Beauchamp and Fridovich, 1971). About 1 g of fresh leaf sample was weighed (cut into pieces) and macerated in a clean, dry mortar and pestle keeping in an ice bath using 50 M potassium phosphate buffer (pH 7.8). Sample was centrifuged at 10,000 rpm for 10 minutes at 4 ^{0}C in cooling centrifuge. 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 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 another for light. Dark set of test tubes were kept under completely dark condition whereas light set of test tubes were exposed to 400 W bulb for 15 minutes. Then at 560 nm absorbance was taken immediately using a spectrophotometer. It was calculated as 50 % inhibition of the reaction between riboflavin and NBT in the presence of methionine was taken as 1 unit of SOD activity. Enzyme activity was expressed as unit/g FW.

$$\textbf{SOD (U/g FW)} = \frac{(\text{Control OD} - \text{Treatment OD})}{\text{Control OD}} \times 100$$

50% inhibiton = 1 unit of enzyme activity

3.7.9 Catalase activity: Dhindsa *et al.* (1981) (CAT; EC 1.11.1.6)

0.05 g of fresh leaf sample was macerated in 1 ml of potassium phosphate buffer using mortar and pestle and homogenate was centrifuged at 12,000 rpm for 20 minutes in cooling centrifuge at 4 ^{0}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.

Calculation

Catalase (U/min/g FW) =

$$\frac{(\text{Change in absorbance/min}) \times \text{Total volume (ml)} \times \text{Dilution factor}}{\text{Extinction coefficient} \times \text{Volume of sample taken (ml)}}$$

Specific activity (EAU/mg protein) = $\frac{\text{Enzyme activity}}{\text{Protein content (mg/g FW)}}$

3.7.10 Glutathione peroxidase activity: (Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B, Hafeman, D. G. and Hoekstra, W. G., 1973)

1 g leaf sample was macerated with 0.4 M sodium phosphate buffer (pH 7.0) using a pre-chilled mortar and pestle. Homogenate was centrifuged at 10,000 rpm for 15 minutes at 4 ^{0}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, 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 ^{0}C for 5-10 minutes and 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 0.1 ml supernatant. Thus the enzyme mixture was allowed to take absorbance 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 FW.

Calculation

Peroxidase (U/min/g FW) =

$$\frac{(\text{Change in absorbance/min}) \times \text{Total volume (ml)} \times \text{Dilution factor}}{\text{Extinction coefficient} \times \text{Volume of sample taken (ml)}}$$

Specific activity (EAU/mg protein) = $\frac{\text{Enzyme activity}}{\text{Protein content (mg/g FW)}}$

3.8 Yield parameters

3.8.1 No. of tillers/plant

The total number of tillers of the 5 tagged plants was counted and their average was taken to express the no. of tillers per plant.

3.8.2 No. of panicles per plant

The total number of panicles of five plants from each genotype was counted and their average was recorded at the time of maturity.

3.8.3 No. of grains per panicle

Average number of filled grains per panicle of each genotype was recorded.

3.8.5 Panicle weight

At maturity, mean panicle weight was measured by taking average of weight of ten random panicles.

3.8.6 Rachis weight

Average rachis weight was recorded from each 10 random rachis

3.8.7 Straw weight

After harvest mean straw weight was measured by taking average weight od 3 random straws.

3.8.8 Test weight

Thousand bold grains were selected and their weight was taken.

3.8.9 Spikelet Sterility percentage

Number of filled grains and sterile grains were counted in each ten random panicles and the mean of their ratio was expressed in percentage

3.8.11 Harvest Index (HI)

HI is calculated by taking the ratio between economic yield and the biological yield, as the formula given by Nichiporovic, 1960.

Harvest Index (HI) = (Economic yield/Biological yield) × 100

3.8.12 Yield per plant

Average grain yield was recorded after harvest from each variety and expressed in grams per plant.

3.9 Statistical analysis

The data related to various morpho-physiological, biochemical and yield parameters collected in this experiment was analysed statistically by applying analysis of variance technique (AVNOVA) technique in a factorial completely randomized design (CRD) laid down by Panse and Sukhatme (1978), Cochran and Cox (1977), 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.

Fig. 3.4 Swarna sub 1 after submergence stress

Fig. 3.5 Swarna after submergence stress

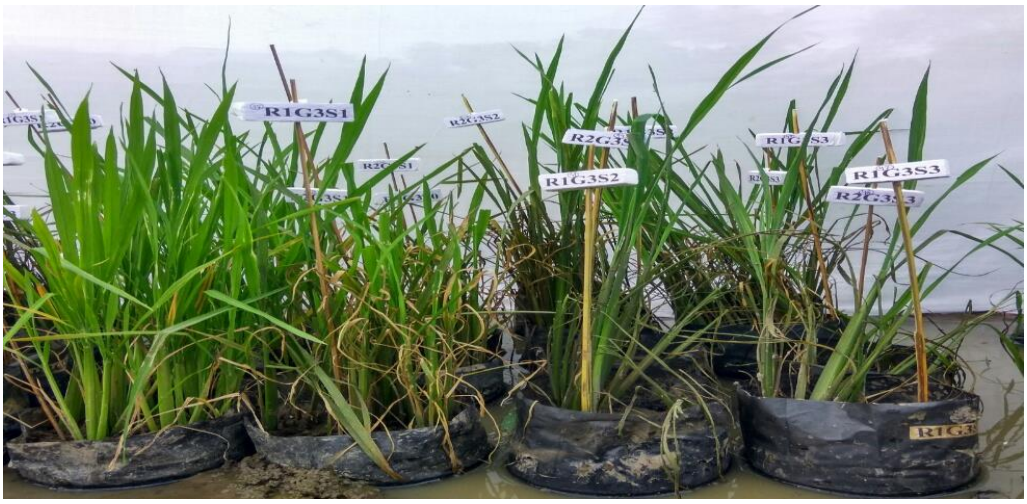

Fig. 3.6 Binadhan 11 after submergence stress

RESULTS

The present experiment was performed during *kharif* (August to December), 2018 under completely randomized design (factorial) to study the **"Physiological and biochemical attributes of rice (*Oryza sativa* L.) genotypes under complete submergence during the vegetative stage"** in the Agronomy Main Research Field of Odisha University of Agriculture and Technology (OUAT), Bhubaneswar. The changes in biochemical parameters of rice genotypes such as leaf chlorophyll, carotenoid, proline, carbohydrate content and activity of oxygen scavenging system were examined during the research to find out the physiological and biochemical efficiency of three different rice genotypes in response to complete submergence and subsequent re-aeration. The results recorded through the statistical analysis are presented below in tables and figures.

4.1 Effect of complete submergence on morphological characters

4.1.1 Plant height (cm)

Plant height measured at different duration of complete submergence induced during the vegetative stage of rice genotypes were presented in Table 4.1. In general, it was observed that plant height increased significantly in all the genotypes under submergence as compared to control but at different rate. However, the increase in shoot elongation was found to be the maximum in Swarna with a tune of 38.50% followed by Binadhan 11 with 14.26% which was at par with Swarna sub 1 with 13.90% at 4 days. 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% & 59.78% in Swarna followed by 28.39% & 30.81% in Binadhan-11 and 28.04% & 29.25% in Swarna sub 1 over control.

Table 4.1 Effect of complete submergence on plant height (cm)

Genotype	Days to complete submergence				
	0 day (C)	**4 day**	**8 day**	**8 day + RA**	**Mean**
Swarna	37.13	51.43 (38.50)	57.60 (55.12)	59.33 (59.78)	51.37
Swarna sub 1	39.51	45.00 (13.90)	50.59 (28.04)	51.07 (29.25)	46.54
Binadhan 11	41.28	47.17 (14.26)	53.00 (28.39)	54.00 (30.81)	48.86
Mean	39.31	47.87	53.73	54.80	48.92
			G	**S**	**G*S**
SEm(±)			0.763	0.881	1.526
CD at 5%			2.26	2.57	4.45
CV%			5.40		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

Table 4.2 Effect of complete submergence on leaf area (cm^2)

Genotype	Days to complete submergence				
	0 day (C)	**4 day**	**8 day**	**8 day + RA**	**Mean**
Swarna	16.00	17.33 (8.3%)	18.58 (15.9%)	18.60 (16.0%)	17.69
Swarna sub 1	16.98	18.79 (10.7%)	20.57 (21.1%)	20.61 (21.4%)	19.24
Binadhan 11	19.78	21.75 (10.0%)	23.6 (19.35%)	23.8 (20.3%)	21.65
Mean	17.60	19.21	20.63	20.66	19.53
			G	**S**	**G*S**
SEm(±)			0.231	0.267	0.463
CD at 5%			0.68	0.78	1.35
CV%			4.10		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.1.2 Leaf area (cm^2)

Plant leaf area was recorded at different days of complete submergence and presented in Table 4.2. It was revealed that the leaf area significantly increased in all the genotypes in response to submergence stress as compared to control. However, the percentage increase in leaf area was found to be the maximum in cv. Swarna sub 1 followed by cv. Binadhan-11 and cv. Swarna. In Swarna sub 1, 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 reaeration respectively.

4.1.3 Days to 50% flowering

Number of days required to 50 % flowering in all the genotypes under non-stressed and stressed conditions was observed and presented in Table 4.3. 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 Swarna (5-8 days) followed by Swarna sub 1 (4-7days) and Binadhan 11 (3-6 days).

Table 4.3 Effect of complete submergence on days for 50% flowering

Genotype	**Days to complete submergence**				
	0 day (C)	**4 day**	**8 day**	**8 day + RA**	**Mean**
Swarna	103.00	108.00	111.00	108.00	17.69
Swarna sub 1	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
CD at 5%			0.68	0.78	1.35
CV%			4.10		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.2 Effect of complete submergence on physiological parameters

4.2.1 Chlorophyll content

Complete submergence resulted in significant reduction of chlorophyll content of all the genotypes is presented in Table 4.4. After 4 days and 8 days of submergence, the percentage reduction in total chlorophyll content was the maximum in Swarna with 27.88% & 44.87% followed by Binadhan 11 with 9.27% & 21.4% and Swarna sub 1 with 4.14% & 15.75% respectively. After 24 hours of reaeration, the total chlorophyll content was found to be slightly increased in all the genotypes compared to during submergence period and Swarna sub 1 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-emergence.

Table 4.4 Effect of complete submergence on total chlorophyll content (mg/g FW)

Genotype	**Days to complete submergence**				
	0 day (C)	**4 day**	**8 day**	**8 day + RA**	**Mean**
Swarna	3.12	2.25 (-27.88)	1.72 (-44.87)	1.74 (-44.23)	2.21
Swarna sub 1	3.62	3.47 (-4.14)	3.05 (-15.75)	3.17 (-12.4)	3.32
Binadhan 11	3.45	3.13 (-9.27)	2.71 (-21.4)	2.78 (-19.42)	3.01
Mean	3.40	2.95	2.49	2.56	2.85
			G	**S**	**G*S**
SEm(±)			0.019	0.022	0.039
CD at 5%			0.06	0.07	0.11
CV%			2.34		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

Under control condition, chlorophyll-a content of the leaves was highest in Swarna sub 1 (2.73 mg g^{-1} FW) followed by Binadhan 11 (2.42 mg g^{-1} FW) and

Swarna (2.23 mg g^{-1} FW) (Table 4.5). At 4 days and 8 days of complete submergence, its percentage reduction was the maximum in Swarna (36.62% and 50.07% respectively) as compared to other genotypes. During reaeration, Swarna sub 1 outperformed others with minimal reduction of 12.80% followed by Binadhan 11 (14.00%).

Similarly, Chlorophyll-b content was found to be decreased as compared to control under submerged conditions with increase in its duration (Table 4.6). After 4 days of complete submergence, the least percentage reduction was recorded in Swarna sub 1 (4.21%) followed by Swarna (5.65%) and Binadhan 11 (5.81%). But after 8 days of submergence, Swarna was having the highest reduction value of 32.66%. After 24 hours reaeration period, Swarna sub 1 was having the highest chlorophyll b molecule (0.79 mg/g FW) than the others.

Table 4.5 Effect of complete submergence on chlorophyll a content of leaves (mg/g FW)

Genotype	**Days to complete submergence**				
	0 day (C)	**4 day**	**8 day**	**8 day + RA**	**Mean**
Swarna	2.23	1.41 (-36.62)	1.11 (-50.07)	1.13 (-49.18)	1.47
Swarna sub 1	2.73	2.62 (-4.27)	2.33 (-14.63)	2.38 (-12.80)	2.52
Binadhan 11	2.42	2.32 (-4.13)	2.03 (-16.00)	2.08 (-14.00)	2.21
Mean	2.46	2.11	1.82	1.86	2.06
			G	**S**	**G*S**
SEm(±)			0.021	0.024	0.041
CD at 5%			0.06	0.07	0.12
CV%			3.54		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

Table 4.6 Effect of complete submergence on chlorophyll b content (mg/g FW)

Genotype	Days to complete submergence				
	0 day (C)	**4 day**	**8 day**	**8 day + RA**	**Mean**
Swarna	0.83	0.78 (-5.65)	0.56 (-32.66)	0.55 (-33.06)	0.68
Swarna sub 1	0.87	0.83 (-4.21)	0.71 (-18.39)	0.79 (-9.19)	0.78
Binadhan 11	0.86	0.81 (-5.81)	0.68 (-20.9)	0.77 (-10.4)	0.78
Mean	0.85	0.80	0.65	0.70	0.75
			G	**S**	**G*S**
SEm(±)			0.010	0.012	0.021
CD at 5%			0.03	0.03	0.06
CV%			4.74		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.2.2 Carotenoid content

Similar trend as the leaf chlorophyll conc. was found in case of carotenoid content of leaves (Table 4.7). After 4 days of complete submergence, Swarna recorded the highest reduction rate of 31.82% followed by Swarna sub 1 (7.89%) and Binadhan 11 (6.76%) over control. After 8 days of complete submergence, Swarna sub 1 was found to maintain its high carotenoid content with 0.30 mg/g FW of leaves followed by Binadhan 11 (0.18 mg/g FW) and Swarna (0.09 mg/g FW). After the period of reaeration also, Swarna sub 1 was found to have highest carotenoid content (0.34 mg/g FW) followed by Binadhan 11 (0.21 mg/g FW) and Swarna (0.13 mg/g FW).

4.2.3 Chlorophyll stability index (CSI)

Data reflected in the Table 4.8 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. Swarna sub 1 showed best performance with 94.76% & 81.49% and the worst by Swarna with 72.12% & 55.13% after 4 days and 8 days of complete submergence respectively. After one-day reaeration also, Swarna sub 1 was found to have the higher regeneration capacity than others with the highest CSI value (87.5%).

Table 4.7 Effect of complete submergence on carotenoid content of leaves (mg/g FW)

Genotype	Days to complete submergence				
	0 day (C)	**4 day**	**8 day**	**8 day + RA**	**Mean**
Swarna	0.15	0.10 (-31.82)	0.09 (-36.36)	0.13 (-11.36)	0.12
Swarna sub 1	0.38	0.35 (-7.89)	0.30 (-21.05)	0.34 (-9.65)	0.34
Binadhan 11	0.25	0.22 (-6.76)	0.18 (-25.68)	0.21 (-13.51)	0.22
Mean	0.26	0.22	0.19	0.23	0.23
			G	**S**	**G*S**
SEm(±)			0.003	0.004	0.009
CD at 5%			0.01	0.01	0.02
CV%			3.37		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

Table 4.8 Effect of complete submergence on chlorophyll stability index (%)

Genotype	Days to complete submergence				
	0 day (C)	**4 day**	**8 day**	**8 day + RA**	**Mean**
Swarna	100.00	72.12 (-27.9)	55.13 (-44.9)	55.78 (-44.2)	70.76
Swarna sub 1	100.00	94.76 (-5.2)	81.49 (-18.5)	87.5 (-12.5)	90.9
Binadhan 11	100.00	90.7 (-9.3)	78.5 (-21.5)	80.5 (-19.5)	87.4
Mean	100.00	85.86	71.7	74.6	83.02
			G	**S**	**G*S**
SEm(±)			0.78	0.90	1.55
CD at 5%			2.27	2.62	4.53
CV%			3.23		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

Table 4.9 Effect of complete submergence on membrane stability index (MSI)

Genotype	Days to complete submergence				
	0 day (C)	**4 day**	**8 day**	**8 day + RA**	**Mean**
Swarna	71.65	56.42 (-21.26)	51.80 (-27.70)	55.50 (-22.54)	58.84
Swarna sub 1	74.55	67.25 (-9.79)	62.70 (-15.90)	70.18 (-5.87)	68.67
Binadhan 11	81.15	72.33 (-10.86)	68.09 (-16.90)	73.67 (-9.22)	73.81
Mean	75.78	65.33	60.86	66.45	67.11
			G	**S**	**G*S**
SEm(±)			0.942	1.087	1.883
CD at 5%			2.75	3.17	5.50
CV%			4.86		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.2.4 Membrane stability index (MSI)

The data on membrane stability index depicted in the Table 4.9 indicated its drastic reduction under complete submergence irrespective of the genotypes. Before the induction of submergence, the highest value was recorded in Binadhan 11 (81.15%) followed by Swarna sub 1 (74.55%) and Swarna (71.65%). However, after 4 days and 8 days of submergence, highest value was recorded in Swarna sub 1 with the values 67.25% and 62.7% followed by Binadhan 11 with 72.33% and 68.09% and Swarna with 56.42% and 51.8% respectively. Similar trend was found even during the reaeration period. For the present study it was highly significant for genotypes, stress levels and stress x genotype interactions.

4.3 Effect of complete submergence on biochemical parameters

4.3.1 Protein content

The data on protein synthesis was estimated at different stages of submergence for all the genotypes and presented in Table 4.10. It was observed that protein degradation significantly increased with the increase in the duration of submergence

was irrespective of genotypes. After 4 days of submergence, the depletion in the protein content was least in Swarna sub 1 (22.25%) and highest in 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 sub 1 (47.55%) followed by Binadhan 11 (49.39%) and Swarna (73.95%). As water level receded, after 24 hours of reaeration 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 sub 1 recorded the highest increase followed by Binadhan 11 and Swarna.

4.3.2 Proline content

Proline accumulation differs significantly among all the genotypes under treated and untreated conditions. A survey of data presented in Table 4.11 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 Swarna (118.83 µg g^{-1} FW) and least in Swarna sub 1 (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 reaeration compared to the stress period. The maximum decrease was found in Swarna sub 1 (from 70.84 to 62.50 µg g^{-1} FW) followed by Binadhan 11 (from 68.9 to 61.17 µg g^{-1} FW).

Table 4.10 Effect of complete submergence on protein content

Genotype	**Days to complete submergence**				
	0 day (C)	**4 day**	**8 day**	**8 day + RA**	**Mean**
Swarna	18.54	9.59 (-48.27)	4.83 (-73.95)	5.30 (-71.41)	9.57
Swarna sub 1	20.00	15.55 (-22.25)	10.49 (-47.55)	15.72 (-21.40)	15.44
Binadhan 11	19.10	14.67 (-23.21)	9.67 (-49.39)	14.67 (-23.21)	14.53
Mean	19.21	13.27	8.33	11.90	13.18
			G	**S**	**G*S**
SEm(±)			0.106	0.122	0.212
CD at 5%			0.31	0.36	0.62
CV%			2.79		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

Table 4.11 Effect of complete submergence on proline content (µg/g FW of leaves)

Genotype	Days to complete submergence				
	0 day (C)	**4 day**	**8 day**	**8 day + RA**	**Mean**
Swarna	68.47	118.83 (73.56)	91.06 (32.99)	87.40 (27.65)	91.44
Swarna sub 1	70.84	86.75 (22.46)	75.44 (6.49)	62.50 (-11.78)	73.89
Binadhan 11	68.90	84.92 (23.25)	74.54 (8.19)	61.7 (-11.22)	72.33
Mean	69.40	96.84	80.35	70.36	79.24
			G	**S**	**G*S**
SEm(±)			0.452	0.522	0.904
CD at 5%			1.32	1.52	2.64
CV%			1.87		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.3.3 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 (Fig. 4.1). 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 Swarna followed by Binadhan 11 and Swarna sub 1 both after 4 days and 8 days of complete submergence. After 8 days of submergence and subsequent reaeration for one day, the amount of dry matter in the susceptible cultivar Swarna was only 36.8 mg g^{-1} dry weight of the control value, whereas the accumulation in the tolerant cultivar Swarna sub 1 was 55.7 mg g^{-1} dry weight and that of Binadhan 11 was 53.1 mg g^{-1} dry weight compared to their respective control plants.

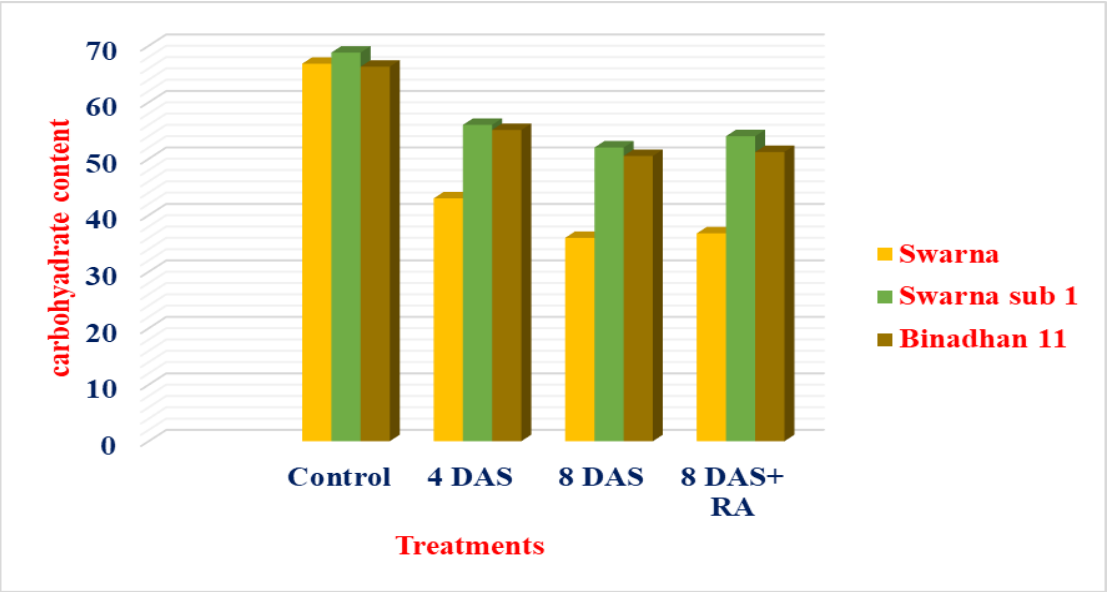

Fig. 4.1 Effect of complete submergence on total carbohydrate content of rice genotypes

4.4 Effect of complete submergence on the activity of enzymes

4.4.1 Superoxide dismutase (SOD)

SOD activity of all genotypes was estimated at different stages of submergence stress and presented in Fig. 4.2. 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 Swarna. During the period of reaeration highest increase in SOD activity was found in Swarna sub 1 (1.68 unit g^{-1} FW) followed by Binadhan 11 (1.54 unit g^{-1} FW) and Swarna (1.29 unit g^{-1}).

4.4.2 Catalase

The data analysed for catalase activity (unit min^{-1} g^{-1} FW) of leaves was presented in Fig. 4.3. Catalase 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 catalase activity compared to the respective control was highest in Swarna sub 1 than other genotypes. The rate of reduction in the catalase activity was minimum in Swarna sub 1 (5.09%) followed by Binadhan 11 (8.68%) and Swarna

(47.34%) at 8 days of submergence. Catalase 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 sub 1 (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.

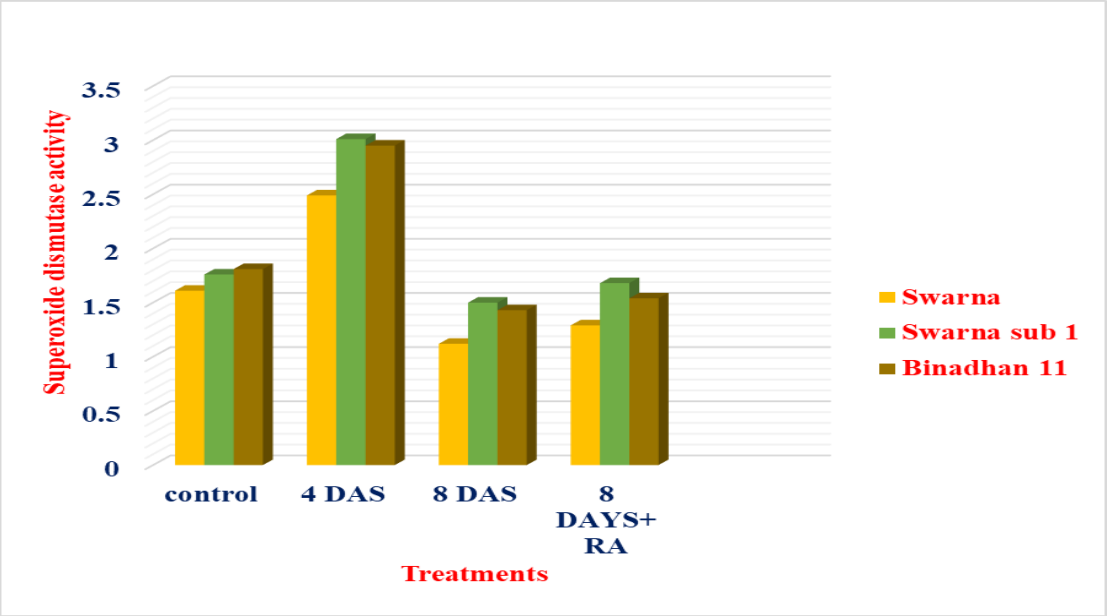

Fig. 4.2 Effect of complete submergence on superoxide dismutase (unit min^{-1} g^{-1} FW) activity in rice genotypes

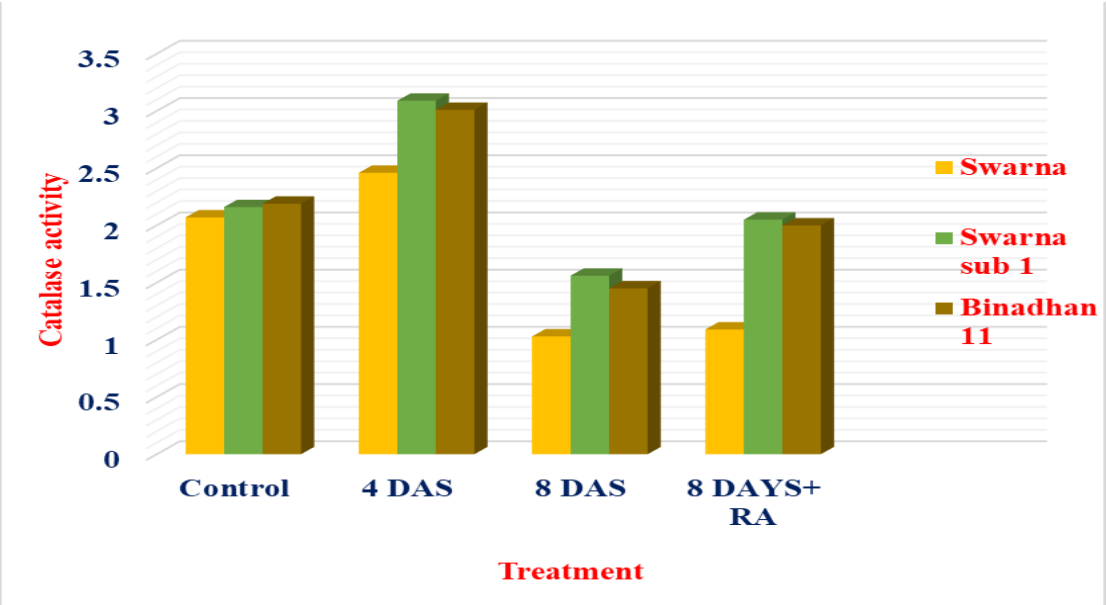

Fig. 4.3 Effect of complete submergence on catalase activity (unit min^{-1} g^{-1} FW) in rice genotypes

4.4.3 Glutathione peroxidase (GPX) activity

GPX activity (unit min^{-1} g^{-1} FW) was assessed during the experiment and depicted in the Fig. 4.4. 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 sub 1 (30.86%) and lowest in 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 sub 1, 13.14% in Binadhan 11 and 41.74 % in Swarna over the control plants. After 24 hours of reaeration, GPX activity again increased but non-significantly in Swarna.

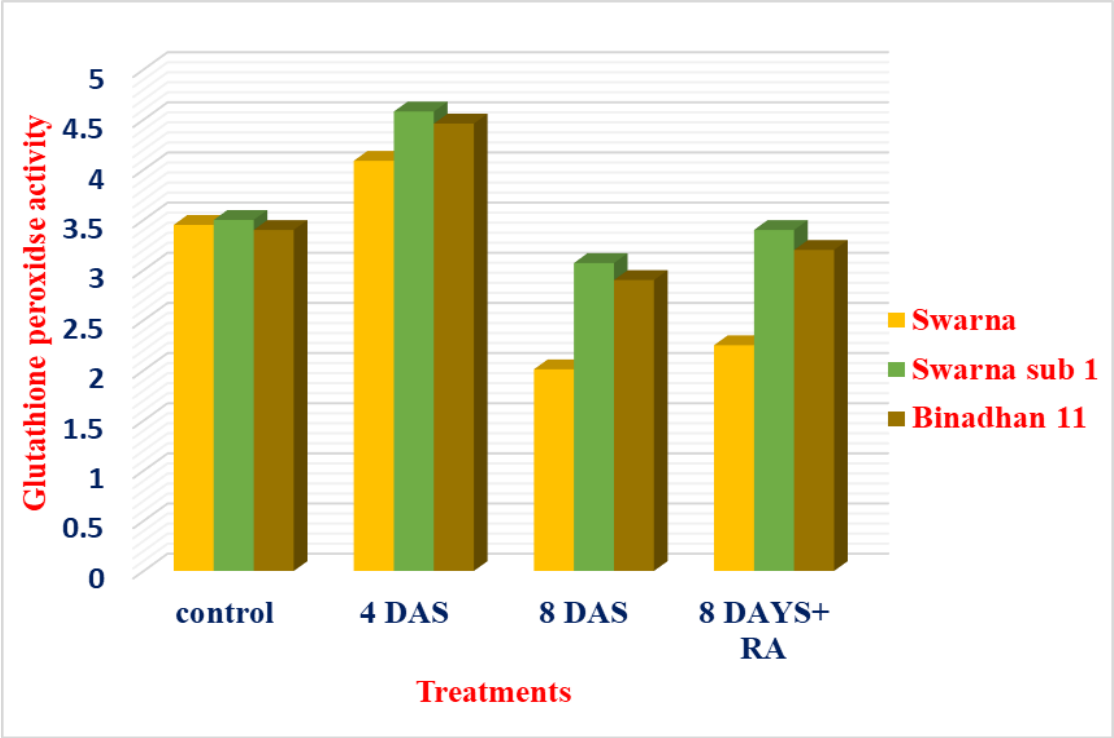

Fig. 4.4 Effect of complete submergence on GPX (unit min^{-1} g^{-1} FW) activity of rice genotypes.

4.5 Effect of complete submergence on yield and yield attributing characters

Observations were taken on the yield and its contributing characters during the harvesting stage of all the genotypes and treatments. However, the variation between the genotypes with 8 days of complete submergence and reaeration treatments was found to be non-significant, hence not presented in the tables.

4.5.1 Number of tillers per plant

A close perusal of the data on tiller numbers evidently revealed that number of tillers per plant reached maximum in all the genotypes under control condition but gradually decreased with the increase in days to submergence (Fig. 4.5). Swarna sub 1 showed comparatively better performance than Binadhan 11 and Swarna. The rate of decrease in the tiller number was highest in the susceptible cultivar Swarna (21%) followed by Binadhan 11 (13%) and Swarna sub 1 (10%) after 8 days of submergence as compared to their respective control plants.

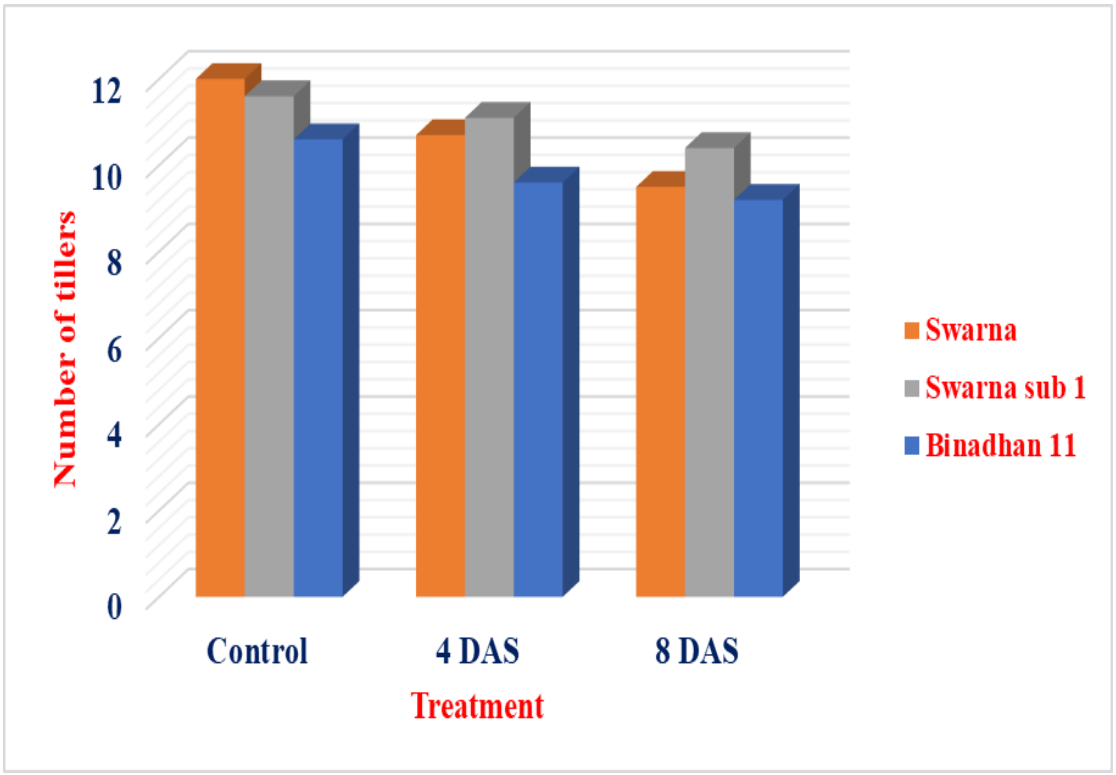

Fig. 4.5 Effect of complete submergence on number of tillers in rice genotypes

4.5.2 Straw weight per plant

As similar to the tiller number, a drastic reduction in the straw weight per plant was also noticed under complete submergence. Table 4.12 revealed that among the genotypes with 4 days of submergence treatment, Swarna showed the maximum reduction in the straw weight (19.80%) followed by Binadhan 11 (8.33 %) and Swarna sub 1 (7.14%) over the plants under control. This gap widened further in plants under 8 days of submergence with a similar trend among genotypes as in case of the 4 days of submergence treatment.

4.12 Effect of complete submergence on straw weight per plant

Genotype	**Days to complete submergence**			
	0 day (C)	**4 day**	**8 day**	**Mean**
Swarna	21.20	17.00 (-19.80)	16.30 (-23.10)	18.17
Swarna sub 1	21.00	19.50 (-7.14)	17.92 (-14.67)	19.47
Binadhan 11	24.00	22.00 (-8.33)	21.20 (-11.67)	22.40
Mean	22.07	19.50	18.47	20.01
		G	**S**	**G*S**
SEm(±)		0.437	0.437	0.757
CD at 5%		1.30	1.30	2.25
CV%		6.55		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.5.3 Number of panicles per plant

The data on number of panicles per plant under treated and untreated conditions for all the three genotypes were obtained and presented in Table 4.13. It was revealed that the number of panicles reduced with the severity of the treatment in all the genotypes. The decrease in numbers was the maximum in Swarna with 25.00% & 38.00% followed by Binadhan 11 with 11.5% & 26.4% and Swarna Sub 1 with 10.83% & 25.00% under 4 days and 8 days of complete submergence respectively.

4.13 Effect of complete submergence on number of panicles per plant

Genotype	Days to complete submergence			
	0 day (C)	4 day	8 day	Mean
Swarna	10.00	7.50 (-25.00)	6.20 (-38.00)	7.90
Swarna sub 1	12.00	10.70 (-10.83)	9.00 (-25.00)	10.57
Binadhan 11	13.00	11.50 (-11.5)	9.57 (-26.4)	11.36
Mean	11.67	9.90	8.26	9.94
		G	**S**	**G*S**
SEm(±)		0.209	0.209	0.363
CD at 5%		0.62	0.62	1.08
CV%		6.32		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.5.4 Panicle weight per plant

Table 4.14 revealed that panicle weight reduced under the influence of submergence. There was significant difference in the reduction in the panicle weight among the genotypes in response to the submergence. Exposure to 4 days of submergence resulted decrease in the panicle weight over control which was found to be highest in Swarna (24.8%) followed by Binadhan 11 (17.1%) and Swarna sub 1 (11.2%). Similar trend was noticed in the plants with 8 days of submergence.

4.14 Effect of complete submergence on panicle weight per plant

Genotype	Days to complete submergence			
	0 day (C)	4 day	8 day	Mean
Swarna	17.85	13.43 (-24.8)	7.65 (-57.1)	12.98
Swarna sub 1	17.33	15.39 (-11.2)	12.25 (-29.3)	14.99
Binadhan 11	18.52	15.35 (-17.1)	12.04 (-35.0)	15.30
Mean	17.90	14.72	10.65	14.42
		G	**S**	**G*S**
SEm(±)		0.350	0.350	0.606
CD at 5%		1.04	1.04	1.80
CV%		7.27		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.5.5 No. of grains per panicle

Table 4.15 revealed that the number of grains per panicle declined in all the genotypes after submergence treatment as compared to their respective control. Among the genotypes with 4 days and 8 days of complete submergence treatment, Swarna showed the maximum reduction with 31.54% & 57.69% followed by Binadhan 11 with 22.75% & 35.15% and minimum being noticed in Swarna sub 1 with 14.67% & 27.80% respectively over the respective control plants.

4.15 Effect of complete submergence on number of grains per panicle

Genotype	**Days to complete submergence**			
	0 day (C)	**4 day**	**8 day**	**Mean**
Swarna	130.00	89.00 (-31.54)	55.00 (-57.69)	91.33
Swarna sub 1	150.00	128.00 (-14.67)	108.30 (-27.80)	128.77
Binadhan 11	141.10	109.00 (-22.75)	91.50 (-35.15)	113.87
Mean	140.37	108.67	84.93	83.49
		G	**S**	**G*S**
SEm(±)		2.561	2.561	4.435
CD at 5%		7.61	7.61	13.18
CV%		6.90		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.5.6 Grain yield per plant

Significant reduction in grain yield was noticed in plants with submergence treatment (Table 4.16). Among the genotypes with 4 days and 8 days of complete submergence, Swarna sub 1 showed the lowest reduction in the yield with 10.2% & 29.5% followed by Binadhan 11 with 18.6% & 37.1% and Swarna with 25.6% & 6.75% respectively over their respective control.

4.16 Effect of complete submergence on grain yield per plant

Genotype	Days to complete submergence			
	0 day (C)	4 day	8 day	Mean
Swarna	16.80	12.50 (-25.6)	6.75 (-59.8)	12.02
Swarna sub 1	16.25	14.60 (-10.2)	11.45 (-29.5)	14.10
Binadhan 11	17.50	14.25 (-18.6)	11.00 (-37.1)	14.25
Mean	16.85	13.78	9.73	13.46
		G	**S**	**G*S**
SEm(±)		0.313	0.313	0.542
CD at 5%		0.93	0.93	1.61
CV%		6.97		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.5.7 Spikelet sterility percentage

Spikelet sterility percentage recorded during the harvesting stage of the treated and untreated genotypes was presented in table 4.17. It was evident from the data that the sterility percentage increased in all the genotypes with increase in their duration of submergence but the magnitude of increase was different for all cultivars. Among the genotypes with 4 days of submergence treatment during their vegetative stage, the percentage of increase was the lowest in Swarna sub 1 (39.3%) followed by Binadhan 11 (44.2%) and Swarna (67.8%). The same trend was found among the genotypes with 8 days of submergence, but with increased gap between the treatments.

4.17 Effect of complete submergence on spikelet sterility percentage

Genotype	Days to complete submergence			
	0 day (C)	4 day	8 day	Mean
Swarna	5.9	9.90 (67.8)	13.10 (122.0)	9.63
Swarna sub 1	6.10	8.5 (39.3)	11.10 (82.0)	8.57
Binadhan 11	4.60	6.63 (44.2)	8.70 (89.1)	6.64
Mean	5.53	8.34	10.97	8.28
		G	**S**	**G*S**
SEm(±)		0.154	0.154	0.267
CD at 5%		0.46	0.46	0.79
CV%		5.58		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.5.8 Test weight (1,000 grain weight)

Data on 1,000 grain weight was observed and depicted under the Table 4.18. It was found that the test weight decreased in all the genotypes with submergence treatment as compared to the control. There was significant difference among the genotypes and treatments. With 4 days and 8 days of complete submergence, the rate of reduction in test weight was found to be the minimum in cv. Binadhan 11 (1.7 % and 4.7% respectively) followed by cv. Swarna sub 1 (3.8 % and 6.5% respectively). The maximum reduction was observed in Swarna with 11.3% and 13.0% with 4 and 8 days of complete submergence respectively.

4.18 Effect of complete submergence on test weight

Genotype	**Days to complete submergence**			
	0 day (C)	**4 day**	**8 day**	**Mean**
Swarna	23.00	20.40 (-11.3)	20.00 (-13.0)	21.13
Swarna sub 1	23.50	22.60 (-3.8)	21.97 (-6.5)	22.69
Binadhan 11	26.13	25.70 (-1.7)	24.90 (-4.7)	25.58
Mean	24.21	22.90	22.29	23.13
		G	**S**	**G*S**
SEm(±)		0.397	0.397	0.688
CD at 5%		1.18	1.18	2.04
CV%		5.15		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.5.9 Rachis weight per plant

From the data presented in the Table 4.19**,** the decreasing tendency of the rachis weight in response to the increase in the duration of submergence was revealed. Among the genotypes with 8 days of complete submergence, the percentage of decrease in the rachis weight was the highest in Swarna (44.2%) followed by Swarna sub 1 (45.6%) and the least being found in Binadhan 11 (23.4%) over their respective control plants.

4.19 Effect of complete submergence on rachis weight per plant

Genotype	Days to complete submergence			
	0 day (C)	4 day	8 day	Mean
Swarna	0.90	0.60 (-33.1%)	0.50 (-44.2%)	0.67
Swarna sub 1	0.91	0.50 (-44.9%)	0.49 (-45.6%)	0.63
Binadhan 11	0.93	0.80 (-13.7%)	0.71 (-23.4%)	0.81
Mean	0.91	0.63	0.57	0.70
		G	**S**	**G*S**
SEm(±)		0.020	0.020	0.034
CD at 5%		0.06	0.06	0.10
CV%		8.01		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

4.5.10 Harvest Index (%)

Harvest index (HI) was calculated on the basis of economic yield and biological yield and presented in the Table 4.20. Data revealed that the HI was quite similar in all the genotypes under control condition, whereas it declined gradually with the increase in submergence duration. In case of 4 days submergence treated plants, the percentage decrease was minimum in cv. Swarna sub 1 (1.3%) followed c.v. Swarna (4.5%) and cv. Binadhan 11 (7.3%). The same trend was found among the genotypes with 8 days of submergence treatment.

4.20 Effect of complete submergence on harvest index of plants

Genotype	Days to complete submergence			
	0 day (C)	4 day	8 day	Mean
Swarna	43.02	41.09 (-4.5)	28.18 (-34.5)	37.43
Swarna sub 1	42.41	41.85 (-1.3)	37.95 (-10.5)	40.74
Binadhan 11	41.17	38.17 (-7.3)	33.05 (-19.7)	37.46
Mean	42.20	40.37	33.06	38.54
		G	**S**	**G*S**
SEm(±)		0.505	0.505	0.875
CD at 5%		1.50	1.50	2.60
CV%		3.93		

(* C: Control; G: Genotype; S: Stress; RA: 24hr reaeration; Figures in parentheses indicates % increase or decrease over control)

DISCUSSION

Submergence imposes a complex abiotic stress on rice plant, affecting numerous physiological and metabolic processes. The present investigation was carried out during *kharif*, 2018 under factorial completely randomized design to study the **"Physiological and biochemical attributes of rice (*Oryza sativa* L.) genotypes under complete submergence during the vegetative stage"** in the Agronomy Main Research Field of Odisha University of Agriculture and Technology (OUAT), Bhubaneswar. Results obtained on various crop growth parameters, yield attributes and changes in the biochemical functions of different anti-oxidant enzymes, pigments during the crop growth have been recorded. This chapter deals with interpretation and discussion of findings of the present investigation with the support of available literature in the concerned field.

5.1 Effect of complete submergence on morphological parameters

5.1.1 Plant height

In this investigation, complete submergence up to 8 days resulted a significant variation in plant height in all the genotypes. A strong negative correlation found between elongation growth and survival of rice seedlings. Minimum elongation ability recorded in Swarna sub 1 whereas maximum shoot elongation was noticed in Swarna under 4 days and 8 days of submergence. 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. Binadhan 11, a sub 1 introgressed genotype showed more elongation as compared to Swarna sub 1 during the period of submergence as well as reaeration. So the present findings reported that Swarna sub 1 followed by Binadhan 11 showed better performance and survival proficiency than Swarna with respect to shoot elongation.

These findings are in agreement with Singh *et al*., (2001) 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. 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 is concluded that rice plants that exhibit only limited elongation during submergence often show tolerance to complete submergence. In this study Swarna sub 1 showed quiescence strategy where ethylene mediated suppression of stem elongation is key factor.

5.1.2 Leaf area

The effect of submergence on leaf area was prominent in Swarna than other cultivars. From the table 4.2, it was revealed that leaf area increased with a decreasing pattern in all the genotypes under submergence. However, the tolerant cv. Swarna sub 1 was found to maintain higher leaf area than the susceptible cv. Swarna. This corroborates with the findings of Sarkar and Das (2003). They found that the genotypes which showed higher stabilization of yield under submergence, maintained higher leaf area and leaf dry weight.

5.2 Effect of complete submergence on physiological parameters

5.2.1 Leaf pigments (chlorophyll and carotenoid content)

In the present experiment, Chl-a, Chl-b, total chlorophyll and carotenoid content of leaves were found to decrease in all the genotypes during submerged condition. 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 and Sarkar *et al*., 2006). In this study, concentration of these pigments were found to be increased after water level receded, which was reflected after 24 hours of reaeration. Susceptible genotype Swarna showed highest chlorophyll and carotenoid degradation as compared to Swarna sub 1. 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.

5.2.2 Chlorophyll stability index (CSI)

The present study recorded the decrease in CSI with the increase in submergence period. The mean CSI computed after 4 days of submergence was found to be highest in Swarna sub 1 (94.76%) followed by Binadhan 11 (90.7%) and Swarna (72.12%). This corroborates with the findings of Dwibedi *et al.* (2017) who reported that physiological traits like chlorophyll stability, sugar content were higher in tolerant genotypes as compared to susceptible ones during submergence.

5.2.3 Membrane stability index (MSI)

Membrane stability index is important for cellular function, and only cells with stable membrane systems can maintain their normal physiological function. Therefore, membrane stability is usually used for measuring the extent of injury of 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.

Under submergence condition, it was found to reduce with increase in the stress level. However, the reduction was the maximum in Swarna (56.42%) followed by Binadhan 11 (72.33%) whereas minimum reduction was found in Swarna sub 1 (67.25%). 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).

5.3 Effect of complete submergence on biochemical parameters

5.3.1 Proline content

Significant increase in proline accumulation was recorded in the genotypes after 4 days of submergence, where Swarna was found to accumulate higher proline than Swarna sub 1 and Binadhan 11. Alia and Saradhi (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 (Shibasaka and Tsuji, 1988), resulting in accumulation of proline. 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 & 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 Swarna sub 1 and Binadhan 11 than 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*., 2001). 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 Sub 1 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 SUB1A-independent manner.

5.3.2 Protein content

The present investigation indicated that protein degradation was maximum during stressed condition. It was found that under stress condition protein content decreased but during reaeration it was increased as the water level receded and plant exposed to normal environment. Highest protein was measured in Swarna sub 1 (15.55 mg g^{-1} FW) followed by Binadhan 11 (14.67 mg g^{-1} FW) and Swarna (9.59 mg g^{-1} FW) under 4 days of submergence. This is in accordance with the findings of (Dey *et al*., 2017).

5.3.3 Total carbohydrate content

High carbohydrate status during submergence is related to the submergence tolerance of rice crops (Yamada et al., 1955; Pal and Mitra 1985). 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 and chlorophyll fluorescence and low stomatal conductance and inter-cellular CO_2 concentration as well. Moreover, submergence also limits the carboxylation by low/intermediate intercellular CO_2 concentrations which suppress 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. In this present investigation, carbohydrate content in the tolerant c.v. Swarna sub 1 was found to be the highest (53.7 mg g^{-1} dry weight) followed by Binadhan 11 (51.2 mg g^{-1} dry weight) and minimum in Swarna (36 mg g^{-1} dry weight) after 8 days of submergence. These results were in conformity with the earlier findings of Sarkar *et al.* (2006) and Baley-serres *et al.* (2008). 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 sub 1 during reaeration. 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.

5.4 Effect of complete submergence on antioxidants

Submergence and reoxygenation can induce oxidative stress, causing an increased production of reactive oxygen species (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 superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (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, 2000). CAT is considered as a key enzyme removing H_2O_2, and peroxidase has a complementary duty (Malecka *et al*., 2009).

In the present investigation, it was revealed that imposing submergence stress at vegetative stage had caused an increase in the activity of SOD initially in all the cultivars up to 4 days. This result agreed with the reports on the other crops (Ahmed *et al*, 2002). 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. More reduction in SOD activity was noticed in Swarna (30.43%) than that of Swarna sub1 (14.77%) and Binadhan 11 (20.99%). This might be due to the damaged light reaction system and reduced PS II activity under prolonged submergence (Panda *et al*, 2006, 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 reaeration, enzyme activity was found to be increased to scavenge the ROS and this was significant in Swarna sub 1 and Binadhan 11. These findings agree with Panda *et al*., (2012). 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), Swarna failed to encounter oxidative damage efficiently as like Swarna-Sub1 and 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.

5.5 Effect of complete submergence on yield and yield parameters

Yield components that influences grain yield of a cultivar under submergence stress are 1000 grain weight, number of panicles per plant and number of fertile grains per panicle, out of which the last two parameters determine the grain yield of a

cultivar under submerged conditions. Present study revealed that yield and its attributing characters were extremely influenced by complete submergence stress. Tolerant genotypes showed maximum yield in comparison to susceptible one due to translocation of carbohydrates from leaf and stem to the sink after regeneration. Faster recovery of tolerant genotypes was also associated with shorter delay in flowering and maturity. Higher carbohydrate content accompanied by more dry mass production after submergence caused increase in grain yield of tolerant cultivars (Islam *et al.,* 2010).

The attributing factors for augmentation in productivity of sub 1 introgressed genotypes were more number of effective tillers per hill which indicates high survival percentage under submergence and more fertile grains per panicle. Present study revealed that after 8 days of complete submergence, Swarna sub 1 exhibited minimum reduction in effective tillers per plant (10%) followed by Binadhan 11 (13%) whereas the maximum reduction was showed by Swarna (21%) which indicates the more survival percentage of the cultivar during submergence.

Present study revealed the maximum grain yield in Swarna sub 1 followed by Binadhan 11 and Swarna under both 4 and 8 days of complete submergence. Spikelet sterility percentage was found to be the maximum in Swarna, while the minimum percentage was noticed in Swarna sub 1. Number of panicles per plant, grains per panicle and grain filling process were highly affected by complete submergence when occurred for prolonged period of time. Binadhan 11 followed by Swarna sub 1 showed best results with regards to straw yield and test weight under submerged conditions. The highest value of HI was observed in Swarna sub 1. Sub 1 lines recovered faster in terms of higher biomass, larger leaf area and with more productive tillers which resulted in higher yield as compared to susceptible cultivar.

SUMMARY AND CONCLUSION

Rainfed lowland rice ecosystem is affected not only by water deficit but also by excess water leading from partial to complete submergence. Although the rice plant is well adopted to aquatic environment, it is unable to survive if completely submerged in water for several weeks. Damage to rice plants due to excess water has been advocated to occur during submergence and also entry of oxygen after the recession of flood water. Some morpho-physiological and biochemical characters have been reported to improve the performances of rice under submerged condition.

The present experiment was carried out in the Agronomy Main Research Field of the Odisha University of Agriculture and Technology (OUAT), Bhubaneswar during *kharif*, 2018 to study the **"Physiological and biochemical attributes of rice (*Oryza sativa* L.) genotypes under complete submergence during vegetative stage."** Three rice genotypes (cv. Swarna, cv. Swarna sub 1 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 reaeration for a day after 8 days of submergence) in factorial completely randomized design and that were replicated thrice.

From the observations taken, it was revealed that plant height increased in all the genotypes in response to the submergence as compared to control, however with great variations among the genotypes. The highest shoot elongation was observed in cv. Swarna with a tune of 38.50 % & 55.12% followed by cv. Binadhan 11 with 14.26% & 28.39% and cv. Swarna sub 1 with 13.90% & 28.04% after 4 days and 8 days of complete submergence respectively as compared to the control.

Leaf area increased as the duration of submergence increased. Maximum increase in leaf area was observed in Swarna sub 1 followed by cv. Binadhan 11 and cv. Swarna. In cv. Swarna sub 1, the percentage increase in leaf area was 10.7%, 21.1% and 21.4% after 4 days, 8 days and reaeration stage respectively over control.

There was delay in flowering of all the genotypes under complete submergence. Delay in 50% flowering was higher in case of cv. Swarna (5-8days)

followed by cv. Swarna sub 1 (4-7 days) and cv. Bina dhan 11 (3-6 days) after 4 to 8 days of submergence.

Reduction in total chlorophyll content in all the cultivars was observed under submergence stress; however, the degradation of chlorophyll pigment was faster in the susceptible cv. Swarna. After 4 days of complete submergence, maximum chlorophyll was noticed in Swarna sub 1 (3.47mg g^{-1} FW) followed by Binadhan 11 (3.13 mg g^{-1} FW) and the least was recorded in Swarna (2.25 mg g^{-1} FW). But during the re-aeration stage, there was faster increase in total chlorophyll content of cv. Swarna sub 1 and Binadhan 11 as compared to cv. Swarna. Similar trend was also recorded for carotenoid content, where submerged plants were found with decreased carotenoid concentration than respective control plants. The maximum decline of 0.15 to 0.09 mg g^{-1} FW was found in Swarna from 4 to 8 days of submergence minimum decline of 0.38 to 0.30 mg g^{-1} FW rate was noticed in Swarna sub 1.

After 4 and 8 days of complete submergence, cv. Swarna sub 1 showed the maximum CSI with 94.76% and 81.49%, while the lowest CSI was observed in cv. Swarna with 72.12% and 55.13% respectively. At 1 day of reaeration after 8 days of submergence, CSI became significantly high in cv. Swarna sub 1 and all varieties differed significantly.

There was significant decrease in membrane stability index after 4 days and 8 days of complete submergence than control. After 4 days and 8 days of submergence, cv. Binadhan 11 showed the maximum value with a tune of 72.33% and 68.09% followed by cv. Swarna sub 1 with 67.25% and 62.7% respectively.

Protein degradation increased with increase in duration of submergence in all the cultivars. After 8 days of complete submergence, the percentage of degradation was lowest in Swarna sub 1 (47.55%) followed by Binadhan 11 (49.39%) and Swarna (73.95%). But during regeneration period, protein content was increased in all the genotype, fastest in tolerant genotypes.

Significant increase in proline content was observed in all the cultivars under submergence than control. But susceptible cv. Swarna showed the highest accumulation of proline after 4 days of submergence; whereas, Binadhan 11 and

Swarna sub 1 recorded 23.25% and 22.46% proline accumulation respectively over control. However, it gradually decreased with the increase in the submergence period.

Carbohydrate content of all the genotypes decreased due to submergence. After 4 days and 8 days of submergence, the % reduction in carbohydrate content was the highest in cv. Swarna with 35.66% and 46.13% whereas, minimum value of the same was recorded in Swarna sub 1 with 18.60% and 21.95% respectively over control. At the end of the submergence period, Swarna sub 1 and Binadhan 11 were found to maintain higher carbohydrate content showing their tolerance to submergence.

The activity of the antioxidant enzymes (SOD, CAT & GPX) studied in this experiment was found to increase in all the genotypes up to 4 days of complete submergence, beyond which their activity decreased. Swarna Sub 1 at par with Binadhan 11 was found have the highest enzyme activity during submergence stress, while cv. Swarna recorded the lowest.

After 4 days of submergence, highest SOD activity was observed in Swarna sub 1 (3.01 unit g^{-1} FW) and least was recorded in Swarna (2.49 unit g^{-1} FW) over control. On subsequent reaeration for 24 hours after 8 days of inundation, the activity again increased; however, the activity was below the level of non-submerged controlled plants especially in Swarna. The highest CAT activity was found in Swarna sub 1 (3.09 unit min^{-1} g^{-1} FW) after 4 days of submergence followed by Binadhan 11 (2.99 unit min^{-1} g^{-1} FW) and Swarna (2.46 unit min^{-1} g^{-1} FW) compared to their respective control. In terms of GPX activity after 4 days of submergence, Swarna sub 1 showed better performance with 4.58 unit min^{-1} g^{-1} FW followed by Binadhan 11 (4.46 unit min^{-1} g^{-1} FW).

In response to the submergence stress during the vegetative stage of rice genotypes, their grain yield and its contributing characters were found to decrease variably over control. The maximum value for grain yield and its contributing characters (except the sterility percentage) was found in Swarna sub 1 which was at par with Binadhan 11. Swarna performed the worst in response to stress being a susceptible cultivar. After complete submergence for 8 days, the reduction in tiller number was minimum in Swarna sub 1 i.e. 10% over control. Highest straw weight

was recorded in Binadhan 11 (22.00 g/plant) followed by Swarna sub 1 (19.5 g/plant) and Swarna (17.00 g/plant) with 4 days of complete submergence.

Reduction in number of panicles was highest in Swarna with 25% & 38% followed by Binadhan 11 with 11.5% & 26.4% and Swarna sub 1 with 10.83% & 25.00% with 4 days and 8 days of complete submergence respectively. Highest panicle weight was noticed in Swarna sub 1 (12.25 g/plant) followed by Binadhan 11 (12.04 g/plant) over control. With 4 and 8 days of submergence, the highest grain yield was recorded in Swarna sub 1 with 14.60 and 11.45 g/plant followed by Binadhan 11 with 14.25 and 11.00 g/plant respectively whereas, the lowest being recorded in Swarna.

Submergence resulted in increased spikelet sterility percentage in all the genotypes. With 4 and 8 days of submergence, the percentage increase was recorded to be the least in Swarna sub 1 with 39.3% and 82.0% followed by Binadhan 11 with 44.2% and 89.1% whereas, the highest recorded in Swarna. The rate of reduction in test weight was found to be the minimum in cv. Bina dhan 11 with 1.7 % and 4.7% followed by cv. Swarna sub 1 with 3.8 % and 6.5% respectively with 4 and 8 days of complete submergence respectively. Reduction in HI was found highest in Binadhan 11 followed by Swarna and Swarna sub 1.

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 sub 1 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, catalase, peroxidase 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 sub-sequent reaeration 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 sub 1, an already known submergence tolerant cultivar; hence it is likely to be tolerant to complete submergence but for a limited duration. However, for confirmation of the results this warrants further investigation.

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