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**Soumya Mishra**
Department of Plant Physiology, OUAT, Bhubaneswar, Odisha, India

**Manoranjan Kar**
Department of Plant Physiology, OUAT, Bhubaneswar, Odisha, India

**Corresponding Author:**
**Soumya Mishra**
Department of Plant Physiology, OUAT, Bhubaneswar, Odisha, India

# Responses of the antioxidative enzymes of few *Sub1* rice (*Oryza sativa*) genotypes to prolonged submergence stress in Odisha

**Soumya Mishra and Manoranjan Kar**

**Abstract**
The present experiment was conducted in Department of Plant physiology, OUAT, Bhubaneswar during *kharif* 2017 and *kharif* 2019 to screen out the NILs rice genotypes for submergence adaptation traits under coastal regions of Odisha. The potential involvement of activated oxygen species by submergence stress was studied in twenty rice genotypes; thirteen *sub1* NILs, two tolerant checks with six susceptible checks. These rice genotypes were subjected to 17 days of complete submergence. Under 17 days of complete submergence and after the submergence, the genotypes IR-85086-*Sub* 33 -3-2-1 and IR-88760-*Sub* 93-3-3 showed lower lipid Peroxidation in terms of malondialdehyde (MDA) level and also showed lower levels of ACC Oxidase activity (AAO) and presented higher activities of antioxidative enzymes, superoxide dismutase (SOD), catalase (CAT), Peroxidase (POX) when compared to the susceptible checks. The levels of SOD activity indicated that detoxification of $O_2$ - to $H_2O_2$ was maintained at a stable level throughout the submergence stress until up to 17 days in tolerant genotypes. These findings suggested that tolerance to submergence stress in rice might be proven by increased the capacity of antioxidative system. In addition, SOD and CAT activity has much higher affinity for scavenging $H_2O_2$ than POX. The present study evaluated thirteen pairs of *Sub1* near-isogenic lines (NILs) together with FR13A and other check genotypes in pot culture conditions to assess the survival and growth processes occurring during submergence and recovery that are associated with *Sub1*.

**Keywords:** Rice, submergence, genotypes, *Sub1*, AS = After submergence, BS = Before submergence, DS = During submergence, antioxidants, lipid peroxidation

## Introduction
Flooding due to submergence is among significant natural hazard affecting many countries every year. Most of the plants often suffer from anaerobiosis brought by soil flooding and total submergence (Vartapetian and Jackson 1997) [16]. Rice (*Oryza sativa* L.) is the staple food for more than 50% the global population, is a semi aquatic plant, particularly able to survive under the conditions of prolonged oxygen deprivation, at both seedling and adult stages. The submergence-tolerant rice genotype is expected to help rice farmers in Asian countries, where 90% of the world's rice production and consumption takes place. By using the submergence-tolerant rice genotype, the survival rate of rice plants under completely submerged condition is increased. However, the physiology and biochemistry from the use of tolerance genotypes remain applicable limitations in the field. In order to identify the traits required to improve genetic adaptability of rice plants to submergence conditions, it is necessary to properly characterize the floodwater environment and to closely investigate the physiological processes behind the plants. Complete submergence hastens degradation of chlorophyll content in susceptible rice genotypes compared to tolerant ones (Ella *et al*. 2003; Panda *et al*. 2006) [1, 6-8], which also can be used as an indicator of submergence tolerance. The responses to a specific stress may vary with the genotype; nevertheless, some general reactions occur in all genotypes. Most of rice genotypes are flood sensitive and die within a week when they are completely submerged. Only a few genotypes, such as FR13A, can survive for 10–14 days of complete submergence (Fukao and Bailey-Serres 2008) [2]. The submergence tolerance of FR13A is linked to a major quantitative trait locus (QTL), known as Submergence1 (Sub1), on chromosome 9 (Xu and Mackill 1996) [17]. Using marker-assisted backcrossing, a quantitative trait locus (QTL) containing *Sub1* was recently transferred into several popular Asian rice genotypes, already possesing agronomic and quality traits preferred by farmers (Siangliw *et al*.,, 2003; Xu *et al*. 2006; Septiningsih *et al*., 2009, 2013; Singh *et al*., 2010; Thomson *et al*., 2010; Manzanilla *et al*., 2011; Mackill *et al*., 2012; Collard *et al*., 2013) [3, 8-11, 13-15, 17]. By providing options for use of nutrients and other inputs to enhance yields further, these genotypes have provided new opportunities for farmers in submergence-prone areas to secure higher annual productivity (Ismail 2013) [3, 8-10, 14-15].

*Sub1* containing modern genotypes are identical to the original genotypes in nearly all traits (Sarkar *et al*. 2009; Singh *et al*., 2009; Mackill *et al*., 2012) [11, 14]. Consequently, they have been extensively adopted by farmers within few years of their release (Mackill *et al*., 2012; Ismail *et al*., 2013; Singh *et al*., 2013) [3, 8-11, 14, 15]. Being an ethylene-response factor, *Sub1*A is induced at the transcript level by submergence (Fukao *et al*., 2006) [2] and shows no obvious effect under other conditions. Following submergence, survival of the *Sub1* lines is substantially higher than that of non-Sub1 genotypes. This has been consistently reflected in a yield advantage of 1 to 3 t $ha^{-1}$ depending on the stage at which submergence occurred, the duration of submergence and the condition of the flood water (Das *et al*., 2009; Mackill *et al*., 2012; Ismail *et al*., 2013) [3, 8-10, 14-15]. Moreover, *Sub1* genotypes flowered and matured earlier and had better grain filling than the non-*Sub1* genotypes following submergence (Sarkar *et al*., 2009; Singh *et al*., 2009; Manzanilla *et al*., 2011) [3, 11, 14, 15]. Whether the survival of submergence alone is contributing to this yield increase and earliness after submergence, or whether other traits, such as those associated with suppressed production of active oxygen species underwater and earlier and faster recovery, are also regulated by the *Sub 1*A allele is still not known. Other genes carried by FR13A and other tolerant genotypes (Ismail and Mackill 2013) [3, 8-10, 14-15] may also play a role. Additional post-flooding responses may be associated with submergence tolerance. These could include prevention of leaf dehydration (Setter *et al*., 2010) [12] and post-submergence up-regulation of scavengers of reactive oxygen species (Ella *et al*., 2003a) [1]. Earlier studies conducted on the mechanisms of submergence tolerance in rice using the highly tolerant landrace FR13A, and more recently several S*ub1* introgression lines, focused largely on the survival of plants following distinct Periods of inundation. The present study evaluated twenty pairs of rice genotypes which includes thirteen pairs of *Sub 1* near isogenic lines (NILs) together with FR 13A (donor parent) and other check genotypes under pot culture.

## Materials and Methods

The seeds of the twenty rice genotypes were collected from different sources as described in the table no-1

**Table 1:** Name, sources and origin of the genotypes used in the experiment

| Sl. No. | Name of the genotypes | Source | Origin |
|---|---|---|---|
| 1 | IR-85086-*Sub* 33 -3-2-1 | IRRI, Phillipines | IRRI, Phillipines |
| 2 | IR-88760-*Sub* 93-3-3 | IRRI, Phillipines | IRRI, Phillipines |
| 3 | Swarna *Sub*-1 | NRRI, Cuttack | NRRI, Cuttack |
| 4 | Samba mahsuri *Sub*-1 | NRRI, Cuttack | IRRI, Phillipines |
| 5 | Savitri *Sub*-1 | NRRI, Cuttack | IRRI, Phillipines |
| 6 | BR 11 *Sub*-1 | NRRI, Cuttack | IRRI, Phillipines |
| 7 | Ciherang *Sub*-1 | NRRI, Cuttack | IRRI, Phillipines |
| 8 | IR-89246-*Sub* 38-3-2-1 | IRRI, Phillipines | IRRI, Phillipines |
| 9 | TDK *Sub*-1 | IRRI, Phillipines | IRRI, Phillipines |
| 10 | IR 64 *Sub*-1 | IRRI, Phillipines | IRRI, Phillipines |
| 11 | IR-88762-*Sub* 51-3-1-3 | IRRI, Phillipines | IRRI, Phillipines |
| 12 | IR-89262-*Sub* 5-2-3-2 | IRRI, Phillipines | IRRI, Phillipines |
| 13 | PSBRc-68 | NRRI, Cuttack | IRRI, Phillipines |
| 14 | FR 13 A (Tolerant check) | OUAT, Odisha | OUAT, Odisha |
| 15 | Lalat (Susceptible check) | OUAT, Odisha | OUAT, Odisha |
| 16 | Swarna (Susceptible check) | OUAT, Odisha | APAU,Andhra Pradesh |
| 17 | CR-500 (Susceptible check) | NRRI, Cuttack | NRRI, Cuttack |
| 18 | Uphar (Tolerant check) | OUAT, Odisha | OUAT, Odisha |
| 19 | CR- 401 (Susceptible check) | NRRI, Cuttack | NRRI, Cuttack |
| 20 | Pratikshya (Susceptible check) | OUAT, Odisha | OUAT, Odisha |

### Experimental site

The field experiment was conducted in the experimental station (Central Farm), college of Agriculture, OUAT, Bhubaneswar and the pot culture experiment was conducted in Wire house of Department of Plant Physiology, OUAT in which twenty plastic pots of same shape and size were used for the said purpose.

### Sowing and fertilizer application

All the seeds were sown directly in pots containing 8 Kg of farm soil and farm yard manure in a 3:1 ratio. The soil pH ranged from 7.5-7.7 and carbon ranged from 1.0 to 1.8%.

**Table 2:** Date of Sowing

| Year | Date of sowing |
|---|---|
| 2017 | 22/06/2017 |
| 2019 | 26/06/2019 |

### Fertilizer application

Fertilizer for each pot was calculated for 8 kg of soil per pot, considering weight of soil for 1 ha land is equivalent to 2.26 X $10^6$ Kg.

**Table 3:** Fertigation

| Fertilizer | kg/ha | g/pot |
|---|---|---|
| Urea | 130.43 | 0.46 |
| Single Super Phosphate (SSP) | 375.00 | 1.34 |
| Muriate of Potash (MOP) | 67.00 | 0.24 |

### Flood water characteristics

The twenty rice genotypes maintained in pot culture were subjected to 17 days of complete submergence 45 days after sowing (45 DAS) in the integrated farming system (IFS) pond of Agronomy field OUAT during *Kharif* season of 2017 and 2019.The cultured pots were placed in the pond where the water depth was 100 cm, and the depth was maintained for seventeen days due to rainfall. During the entire submergence Period for both the years the flood water characteristics were measured once in three days. Maximum and minimum air temperatures were 38.2 ºC and 34.5.5 ºC, respectively, and

water temperatures at 5, 50 and 75 cm depths averaged about 34.7 ºC, 33.8 ºC and 32.5 ºC, respectively. The warm temperature increased algal growth which reduced light penetration with water depth. pH of floodwater also varied slightly (range of 8.25±8.45) with day time and water depth.

**Enzymatic analysis**

**Superoxide dismutase (SOD) activity**

SOD activity in rice was determined by Beauchamp and Fridovich (1971) method. The SOD activity was recorded at 560 nm as ability to inhibit photochemical reduction of nitro-blue tetrazolium (NBT). The NBT method is based on the principle that NBT undergo photo reduction on exposure to light by suPOXoxide radicals. The reaction mixture was prepared by adding 100 µl of the sample extract to 50mM phosphate buffer (pH=7.8) containing NBT, riboflavin, methionine and EDTA. The reaction mixture without sample was considered as control and kept in light, while blank was kept in the dark. One unit of enzyme activity is required for 50% inhibition of NBT.

**Assay medium**

| Reagent | Final Concentration |
|---|---|
| Phosphate Buffer (pH=7.8) | 50mM |
| EDTA | 2mM |
| Methionine | 9.9mM |
| Nitro-Blue Tetrazolium | 55µM |
| Riboflavin | 1µM |
| Sample extract | 100 µl |

**Preparation**

During preparation, all the samples and the control tubes were placed in dark. The samples were later illuminated with luminescent lamps for 10 mins.

Blank: Kept in the dark – 200 µl extraction buffer + 2.8 ml reaction mixture Control: kept in light – 200 µl extraction buffer + 2.8 ml reaction mixture

Sample: Kept in light – 100 µl sample extract + 100 µl extraction buffer +3 ml reaction mixture Thus, % Inhibition of NBT reduction by SOD

= (Control OD–Treatment OD)/Control OD X 100

= X% Inhibition

**SOD Activity (unit/mg FW) = X × 1/50× $V_E/V_P$ ×1/ $S_{wt}$.**

Where, $V_E$ = Sample enzyme extract = 100 µl

$V_P$ = Volume of total protein extract = 4 ml

$S_{wt}$. = Weight of sample used = 0.5 g

**Catalase (CAT) activity**

Catalase dismutates $H_2O_2$ into water and molecular oxygen, reducing its harmful effects in cells organelle. The reaction mixture consists of 100 µl of sample extract, phosphate buffer (pH=7.0) and $H_2O_2$ solution. The decrease in $H_2O_2$ concentration was read and recorded at 240 nm absorbance for 180 secs (each 30 secs interval). The activity gives the initial rate of disappearance of µmoles of $H_2O_2$ at 240 nm per minute.

**Assay medium**

**Table 4:** Reagents and reaction mixture of CAT

| Reagents | Final concentration | Blank | Sample |
|---|---|---|---|
| Phosphate buffer (pH=7.0) | 50 mM | 1 ml | 1 ml |
| $H_2O_2$ | 10 mM | 200 µl | 200 µl |
| Sample extract | - | 0 µl | 100 µl |
| Extraction buffer | - | 100 µl | 0 µl |
| dd$H_2O$ | - | 1.7 ml | 1.7 ml |
| Total volume | - | 3 ml | 3 ml |

CAT Activity (unit/min/mg protein) = $\Delta OD/\Delta T \times 1/EC \times P_T$

Where, ΔOD = change in OD value

ΔT = change in time (min)

EC = extinction coefficient =39.4$mM^{-1}$ $cm^{-1}$

$P_T$ = total protein in test sample

**Estimation of peroxidase (POX) activity**

**Procedure**

200 mg plant sample was taken and homogenized with 10 ml of Phosphate buffer 0.1 M (pH 6.0) it was centrifuged at 10,000 rpm at $4^0$ C for 30 minutes. The supernatant collected and stored at low temperature. The supernatant was used for enzyme assay and estimated the enzyme activity as given below.

**Table 5:** Reaction mixture of POX

| Reaction mixture | | |
|---|---|---|
| **Test** | **Blank** | **Reagents** |
| 2.0 ml | 2.0 ml | Enzyme extract |
| 2.0 ml | 3.2 ml | Phosphate buffer |
| 1.0 ml | ---- | Pyrogallol |
| 0.2 ml | ---- | $H_20_2$ |
| 5.2 ml | 5.2 ml | |

Shake the mixture well and keep it at 37° C on water bath for 10 minutes for the formation of purpurogallin. Measure the activity at 430 nm and express result as enzyme unit per gram fresh weight or per gram protein basis.

**Ascorbic acid oxidase activity**

Ascorbic acid oxidase activity was assayed according to Olliver (1967). The enzyme was extracted in phosphate buffer (0.05 M, pH 7.4). The reaction mixture consisted of 1 ml crude extract, 2 ml ascorbic acid (2 mM) and 3 ml extracting buffer. It was incubated for 30 min at 37 °C. A blank set was prepared by deducting 1 ml enzyme from the reaction mixture and replacing it with 1 ml of phosphate buffer. The reaction was stopped with 5 ml of 10 % TCA and the reaction mixture was titrated with DCPIP (2,6-dichlorophenol indophenol). The difference between the two readings gave the ascorbic acid oxidase activity.

**Lipid peroxidation (LP)**

The LP occurs in the cell as the result of oxidation of the membrane due to production of reactive oxygen species (ROS) during salinity stress. A standard protocol was adapted to estimate the concentration the MDA (Malondialdehyde) content in leave sample. The test was performed using 100 mg of fresh leave cut into small pieces of 0.5 $cm^2$ each. The samples were homogenized in 0.1% (w/v) of 5 ml trichloroacetic acid (TCA) solution and centrifuged at 12,000 rpm for 20 minutes. The supernatant was collected and further

used for estimation of MDA content. The total reaction mixture consists of 4 ml containing 1 ml of sample extract, 3 ml of 2% (w/v) 2-thiobarbituric acid (TBA) dissolved in 20% TCA. The mixture was boiled at 95°C for 30 mins and then rapidly cooled on ice. The final mixture was again centrifuged at 12,000 rpm for 10 mins to obtain supernatant. The absorbance of the collected supernatant was read at 532 nm. Also, absorbance at 600 nm was recorded to deduct the absorbance due to presence of turbidity in the supernatant. Then, LP was measured and expressed as nmole MDA $g^{-1}$ fresh weight (FW).

LP (nmole MDA $g^{-1}$ FW) = $(A_{532} – A_{600})/EC \times V_{RM} \times 1000$

Where, $A_{532}$ = Absorbance at 532 nm $A_{600}$ = Absorbance at 532 nm

EC = Extinction Coefficient = $155 mM^{-1} cm^{-1}$

$V_{RM}$ = Total volume of reaction mixture = 4 ml

**Statistical analysis**

All the data were recorded, compiled in appropriate tables and analyzed statistically as per the procedure prescribed for Randomized block design. To determine the analysis of variance, standard error of means i.e., SE(m) ± were determined in all the cases, while least significant difference (LSD) at 5 % level of significance was estimated only in cases, where „F" test was found significant.

**Estimation of coefficient of variation (CV)**

A measure of variation which is independent of the unit of measurement and is therefore useful for comparison between different populations is provided by the standard deviation expressed as Percentage of mean. This measure is known as coefficient of variation is given by,

$$CV = (\sigma/\mu) \times 100$$

Where, σ – Standard deviation and μ - Mean of the observation.

**Results and Discussion**

Periodical observations of Biochemical aspects of plants were made at 45 DAS i.e before submergence (BS) and after submergence (AS) i.e 12 days after de-submergence and during submergence (DS) i.e 10 days after submergence.

There was drastic reduction in SOD activity during submergence (55-85 %) in all the genotypes (Table 6), with a maximum amount of reduction i.e., 85.3 % in the susceptible check CR-401 with a value of 19.03 μmol $H_2O_2$ decomposed $min^{-1}$ $g^{-1}$ FW. The maximum amount of SOD was retained in the Sub-1 line IR 85086– Sub 33-3-2-1 with a value of 69.23 m mol $H_2O_2$ decomposed $min^{-1}$ $g^{-1}$ FW during submergence (DS). SOD after submergence increased a little in all tolerant genotypes as compared to the during submergence condition. After re-aeration also, the highest amount of SOD activity was found in IR 85086-sub 33-3-2-1 followed by IR -88760-sub 93-3-3 with a value of 85.27 and 81.47 mmol $H_2O_2$ decomposed $min^{-1}$ $g^{-1}$ FW respectively. The lowest SOD activity was found in CR 401 followed by CR 500 with a value of 10.13 and 10.18 mmol $H_2O_2$ decomposed $min^{-1}$ $g^{-1}$ FW respectively.

Reactions involving $O_2$ free radicals are an intrinsic feature of plant senescence and promote the process of oxidative deterioration that contributes to cell death (del Rio *et al.*, 1998). A number of abiotic stresses, including submergence, lead to overproduction of reactive oxygen intermediates, including $H_2O_2$, causing extensive damage. However, the damage could be reduced in those plants that have well-defined systems to protect against the superoxide radical (O± 2). One protective system involves superoxide dismutase (SOD), converting superoxide radicals to hydrogen Peroxide, which is reduced to water by peroxidases or catalases. It has been reported that tolerant species (Iris pseudacorus) differ from intolerant species (I. germanica and Glyceria maxima) in being able to increase SOD activity during the Period of anaerobic incubation and thus enter the post-anoxic phase well equipped to counteract the potential hazards of superoxide generation (Monk *et al.* 1987) [4, 5]. In the present investigation, however, it was noted that SOD activity decreased under submergence as compared to aerobically grown plants in both tolerant and intolerant rice genotypes.

**Table 6:** Changes in SOD activity (mmol $H_2O_2$ decomposed $min^{-1}$ $g^{-1}$ FW) in leaves of twenty rice genotypes in under submergence conditions

| Sl. No. | Name of genotypes | BS | DS | AS |
|---|---|---|---|---|
| | | Pooled mean | Pooled mean | Pooled mean |
| 1 | IR-85086-Sub 33-3-2-1 | 156.10 | 69.23(-55.6%) | 85.27(+23.16%) |
| 2 | IR-88760-Sub 93-3-3 | 152.00 | 65.91(-56.6%) | 81.47(+23.6%) |
| 3 | Swarna Sub -1 | 148.83 | 61.40(-58.7%) | 77.91(+26.88%) |
| 4 | Samba mahsuri Sub-1 | 150.36 | 56.89(-62.2%) | 75.28(+32.32%) |
| 5 | Savitri Sub-1 | 144.24 | 54.23(-62.4%) | 74.01(+36.47%) |
| 6 | BR-11 Sub-1 | 143.62 | 53.15(-63.0%) | 71.71(+34.92%) |
| 7 | Ciherang Sub-1 | 138.22 | 53.83(-61.0%) | 68.00(+26.32%) |
| 8 | IR-89246-Sub 38-3-2-1 | 136.48 | 49.48(-63.7%) | 45.10(-8.85%) |
| 9 | TDK Sub-1 | 133.21 | 53.97(-59.5%) | 62.84(+16.43%) |
| 10 | IR 64 Sub-1 | 132.05 | 51.74(-60.8%) | 59.57(+15.13%) |
| 11 | IR-88762-Sub 51-3-1-3 | 121.48 | 26.40(-78.2%) | 15.58(-40.98%) |
| 12 | IR- 89262- Sub 5-2-3-2 | 127.11 | 24.55(-80.7%) | 18.57(-24.35%) |
| 13 | PSBRc-68 | 130.21 | 24.49(-53.1%) | 13.17(-46.22%) |
| 14 | FR13A (Tolerant check) | 131.32 | 61.52(-81.2%) | 76.42(+24.21%) |
| 15 | Lalat (Susceptible check) | 122.51 | 22.27(-81.8%) | 11.02(-50.51%) |
| 16 | Swarna (Susceptible check) | 131.20 | 22.64(-82.7%) | 6.97(-69.21%) |
| 17 | CR-500 (Susceptible check) | 125.98 | 23.51(-81.3%) | 10.18(-56.69%) |
| 18 | Uphar (Tolerant check) | 120.25 | 53.00(-55.9%) | 56.68(-2.49%) |
| 19 | CR-401 (Susceptible check) | 129.39 | 19.03(-85.3%) | 10.13(-46.76%) |
| 20 | Pratikshya (Susceptible check) | 127.61 | 35.96(-71.8%) | 37.86(+5.28%) |
| | Total mean | 135.11 | 44.16 | 47.89 |
| | SE(m) | 6.37 | 2.67 | 4.54 |
| | LSD 5% | 18.87 | 7.92 | 13.45 |
| | CV % | - | - | - |

N:B:- Figure in the parentheses indicates percentage of increase or decrease over previous observation, BS = Before Submergence, AS = After Submergence, DS = During Submergence

It can be depicted from Table 7. that CAT activity reduced drastically (85-95%) during submergence (DS) in all the twenty rice genotypes. The highest CAT activity was seen in IR 85086 – Sub 33-3-2-1 and IR 88760-Sub 93-3-3 with a value of 2.54 and 2.3 mmol $H_2O_2$ decomposed $min^{-1}$ $g^{-1}$ FW respectively. The lowest CAT activity (DS) was recorded in the *Sub-1* lines PSBRc-68 and IR -88762-Sub 51-3-1-3 with values of 1.42 and 1.47 mmol $H_2O_2$ decomposed $min^{-1}$ $g^{-1}$ FW respectively. After submergence the catalase activity increases as compared to during submergence in all the genotypes. The maximum amount of CAT activity after submergence was recorded in two *Sub-1* lines IR-85086-Sub 33-3-21 followed by IR 88760-Sub 93-3-3 with values of 4.88 and 4.65 mmol $H_2O_2$ decomposed $min^{-1}$ $g^{-1}$ FW, respectively.

**Table 7:** Changes in catalase activity (mmol $H_2O_2$ decomposed $min^{-1}$ $g^{-1}$ FW) in rice leaves of twenty genotypes under submergence conditions

| Sl. No. | Name of genotypes | BS | DS | AS |
|---|---|---|---|---|
| | | Pooled mean | Pooled mean | Pooled mean |
| 1 | IR-85086-Sub 33-3-2-1 | 24.74 | 2.54 (-89.7%) | 4.88 (+92.12%) |
| 2 | IR-88760-Sub 93-3-3 | 23.99 | 2.30 (-90.4%) | 4.65 (+102.1%) |
| 3 | Swarna Sub -1 | 24.13 | 2.16(-180.9%) | 4.36 (+101.8%) |
| 4 | Samba mahsuri Sub-1 | 23.58 | 2.12 (-91.0%) | 4.24 (+100%) |
| 5 | Savitri Sub-1 | 22.58 | 2.08 (-90.8%) | 4.09 (+96.6%) |
| 6 | BR-11 Sub-1 | 23.36 | 2.09 (-91.0%) | 3.60 (+72.2%) |
| 7 | Ciherang Sub-1 | 22.52 | 1.75 (-92.2%) | 3.45 (+97.1%) |
| 8 | IR-89246-Sub 38-3-2-1 | 22.92 | 1.66 (-92.8%) | 3.23 (+94.6%) |
| 9 | TDK Sub-1 | 23.65 | 1.67 (-93.0%) | 3.02 (+80.8%) |
| 10 | IR 64 Sub-1 | 21.96 | 1.80 (-91.8%) | 2.93 (+62.8%) |
| 11 | IR-88762-Sub 51-3-1-3 | 22.51 | 1.47 (-93.5%) | 1.57 (+6.8%) |
| 12 | IR- 89262- Sub 5-2-3-2 | 22.90 | 1.84 (-92.0%) | 1.63 (-11.4%) |
| 13 | PSBRc-68 | 22.62 | 1.42 (-93.7%) | 1.82 (+28.1%) |
| 14 | FR13A (Tolerant check) | 22.90 | 2.23 (-90.3%) | 4.51(+102.2%) |
| 15 | Lalat (Susceptible check) | 22.37 | 1.54 (-93.1%) | 1.74 (+13%) |
| 16 | Swarna (Susceptible check) | 22.90 | 1.79 (-92.2%) | 1.53 (-14.5%) |
| 17 | CR-500 (Susceptible check) | 22.65 | 1.77 (-92.2%) | 1.69 (-4.5%) |
| 18 | Uphar (Tolerant check) | 22.97 | 2.08 (-90.9%) | 3.05 (+46.6%) |
| 19 | CR-401 (Susceptible check) | 23.10 | 1.75 (-93.0%) | 1.60 (-8.57%) |
| 20 | Pratikshya (Susceptible check) | 24.11 | 1.91 (-92.0%) | 2.61 (-36.6%) |
| | Total mean | 23.12 | 1.90 | 3.01 |
| | SE(m) | 0.72 | 0.09 | 0.32 |
| | LSD 5% | 2.14 | 0.28 | 0.97 |
| | CV% | - | - | - |

N:B:- Figure in the parentheses indicates percentage of increase or decrease over previous observation, BS = Before Submergence, AS = After Submergence, DS= During Submergence

From data presented in Table 8. it is clear that, there was drastic reduction in POX activity during submergence (59-80 %) in all the genotypes, with a maximum amount of reduction i.e., 86.7 % in the susceptible check Swarna with a value of 16.33 changes in O.D. g-1 FW. The maximum amount of POX was retained in the Sub-1 line IR 85086– Sub 33-3-2-1 with a value of 20.67 m mol H2O2 changes in O.D. g-1 FW during submergence (DS). After submergence the Peroxidase activity increases as compared to during submergence in all the genotypes. The maximum amount of POX activity after submergence was recorded in two Sub-1 lines IR-85086-Sub 33-3-21 followed by IR 88760-Sub 93-3-3 with values of 24.39 and 23.38 changes in O.D. g-1 FW respectively. In rice, a group of antioxidative enzymes may be involved in submergence tolerance. In addition to SOD both CAT and POX are also important in protecting from reactive oxygen damage. The involvement of ascorbic acid appeared to be very important in developing a defense system against post-submergence injury in rice, as the tolerant genotype exhibited signicantly lower AAO activity. The activities of CAT, POX and SOD were very similar in the tolerant genotypes

**Table 8:** Changes in peroxidase activity (Changes in O.D. g-1 FW) in leaves of twenty rice genotypes under submergence conditions

| Sl. No. | Name of genotypes | BS | DS | AS |
|---|---|---|---|---|
| | | Pooled mean | Pooled mean | Pooled mean |
| 1 | IR-85086-Sub 33-3-2-1 | 50.56 | 20.67(-59.1%) | 24.39(+18%) |
| 2 | IR-88760-Sub 93-3-3 | 49.45 | 18.61(-62.4%) | 23.38(+25.6%) |
| 3 | Swarna Sub -1 | 48.96 | 17.00(-62.3%) | 23.07(+35.7%) |
| 4 | Samba mahsuri Sub-1 | 52.49 | 15.13(-62.9%) | 22.15(+46.4%) |
| 5 | Saviri Sub-1 | 50.25 | 13.20(-73.7%) | 19.76(+49.7%) |
| 6 | BR-11 Sub-1 | 50.27 | 12.62(-74.9%) | 17.87(+41.6%) |
| 7 | Ciherang Sub-1 | 50.19 | 12.09(-75.9%) | 17.27(+42.8%) |
| 8 | IR-89246-Sub 38-3-2-1 | 45.81 | 10.13(-77.9%) | 15.07(+48.7%) |
| 9 | TDK Sub-1 | 42.19 | 11.83(-72.0%) | 17.27(+46%) |
| 10 | IR 64 Sub-1 | 45.72 | 11.03(-75.9%) | 16.74(+51.7%) |
| 11 | IR-88762-Sub 51-3-1-3 | 46.75 | 8.77(-81.2%) | 7.17(-18.2%) |
| 12 | IR- 89262- Sub 5-2-3-2 | 47.42 | 9.63(-79.7%) | 4.15(-57%) |
| 13 | PSBRc-68 | 40.56 | 8.97(-77.9%) | 3.74(-58.3%) |
| 14 | FR13A (Tolerant check) | 47.92 | 16.52(-65.3%) | 22.34(+35.2%) |

| | | | | |
|---|---|---|---|---|
| 15 | Lalat (Susceptible check) | 44.98 | 9.32(-79.3%) | 3.72(-60%) |
| 16 | Swarna (Susceptible check) | 47.63 | 6.33(-86.7%) | 5.59(-11.6%) |
| 17 | CR-500 (Susceptible check) | 39.84 | 5.96(-85.0%) | 4.85(-18.6%) |
| 18 | Uphar (Tolerant check) | 43.09 | 15.52(-64.0%) | 15.47(-0.3%) |
| 19 | CR-401 (Susceptible check) | 44.58 | 8.33(-81.3%) | 3.90(-53.1%) |
| 20 | Pratikshya (Susceptible check) | 45.16 | 12.77(-71.7%) | 13.59(+6.4%) |
| | Total mean | 46.69 | 12.22 | 14.07 |
| | Sem | 4.05 | 3.06 | 3.74 |
| | LSD 5% | 12.01 | 9.07 | 11.07 |
| | CV% | - | - | - |

N:B:- Figure in the parentheses indicates percentage of increase or decrease over previous observation, BS = Before Submergence, AS = After Submergence, DS = During Submergence

Ascorbic acid oxidase activity data from table 9. Reflects that, in general AAO activity decreased more in all the tolerant genotypes and increased or decreased less in all the susceptible genotypes. Maximum decrease in AAO activity during submergence was recorded in IR-85086-Sub-33-3-2-1 with a reduction Percentage of 48.4 % over before submergence value, while maximum increase of AAO activity was recorded in IR-88762-Sub 51-3-1-3 followed by IR 89262-Sub-5-2-3-2 with the Percentage in increase of 41.4 % followed by 29 % increase respectively. After submergence (AS) or after re-aeration activity of AAO data shows that, its activity decreased more in the genotype IR-85086-Sub 33-3-2-1 followed by IR -88760-Sub 93-3-3 with values of 12.05 and 13.35 µ mol ascorbic acid decomposed 30 min g-1 respectively. The maximum AAO activity was recorded in the susceptible check genotype Lalat followed by Swarna with values of 36.05 and 31.27 µ mol ascorbic acid decomposed 30 min g-1 respectively.

**Table 9:** Changes in ascorbic acid oxidase (AAO) activity (µ mol ascorbic acid decomposed 30 min g-1) in leaves of twenty rice genotypes under submergence conditions

| S. No. | | BS | DS | AS |
|---|---|---|---|---|
| | Name of the genotypes | Pooled mean | Pooled mean | Pooled mean |
| 1 | IR-85086-Sub 33-3-2-1 | 44.92 | 23.17(-48.4%) | 12.05(-48%) |
| 2 | IR-88760-Sub 93-3-3 | 42.04 | 25.00(-40.5%) | 13.35(-46.6%) |
| 3 | Swarna Sub -1 | 39.53 | 28.58(-27.7%) | 14.62(-48.8%) |
| 4 | Samba mahsuri Sub-1 | 31.42 | 31.12(-0.95%) | 15.83(-49.1%) |
| 5 | Savitri Sub-1 | 35.67 | 33.08(-7.26%) | 16.60(-49.8%) |
| 6 | BR-11 Sub-1 | 45.28 | 33.93(-25.0%) | 16.83(-50.3%) |
| 7 | Ciherang Sub-1 | 45.92 | 35.80(-22.0%) | 17.12(-52.1%) |
| 8 | IR-89246-Sub 38-3-2-1 | 30.10 | 37.68(+25.2%) | 18.60(-50.6%) |
| 9 | TDK Sub-1 | 43.82 | 35.53(-18.9%) | 19.17(-46%) |
| 10 | IR 64 Sub-1 | 45.85 | 36.32(-20.8%) | 19.55(-46%) |
| 11 | IR-88762-Sub 51-3-1-3 | 30.10 | 42.57(+41.4%) | 28.28(-33.5%) |
| 12 | IR- 89262- Sub 5-2-3-2 | 33.60 | 43.35(+29.0%) | 28.33(-34.6%) |
| 13 | PSBRc-68 | 43.40 | 43.50(+0.23%) | 30.27(-30.4%) |
| 14 | FR13A (Tolerant check) | 28.27 | 29.00(+2.6%) | 15.32(-47.1%) |
| 15 | Lalat (Susceptible check) | 46.67 | 45.18(-3.2%) | 36.05(-20.2%) |
| 16 | Swarna (Susceptible check) | 48.18 | 42.60(-11.6%) | 31.27(-26.5%) |
| 17 | CR-500 (Susceptible check) | 48.82 | 36.67(-24.9%) | 28.72(-21.6%) |
| 18 | Uphar (Tolerant check) | 44.50 | 36.08(-18.9%) | 17.90(-50.3%) |
| 19 | CR-401 (Susceptible check) | 46.75 | 43.88(-6.1%) | 24.02(-45.2%) |
| 20 | Pratikshya (Susceptible check) | 42.50 | 40.10(-5.6%) | 21.25(-47%) |
| | Total mean | 40.87 | 36.16 | 21.26 |
| | SE(m) | 1.32 | 1.46 | 1.87 |
| | LSD 5% | 3.92 | 4.33 | 5.56 |
| | CV% | - | - | - |

N:B:- Figure in the parentheses indicates percentage of increase or decrease over previous observation, BS = Before Submergence, AS = After Submergence, DS = During Submergence

Malondialdehyde (MDA) activity decreased in all the twenty rice genotypes, but the maximum activity of MDA was recorded in TDK Sub-1 with a value of 5.13 nmol g-1 which is a reduction of 10.3% over the control or before submergence (Table 10). After submergence all the twenty genotypes showed increase in MDA activity. The maximum amount of increase i.e., 188.9 % followed by 145.5 % was recorded in the susceptible check Lalat followed by PSBRc-68 (Sub-1 line) respectively with values of 12.48 n molg-1 and 10.63 nmol g-1. The minimum increase i.e., 6 % in MDA activity was recorded in IR 88760-Sub 93-3-3 and 8.4 % increase was recorded in IR 85086 Sub 33-3-2-1 with Values of 5.6 n mol g-1 and 5.42 n molg-1 respectively. In control conditions MDA activity was at par in all the genotypes.

After exposure of plants to air there might be some Peroxidation of lipids in rice leaves. The product of lipid Peroxidation, i.e. MDA activity, was high in the susceptible check genotype Lalat (12.48 nmol g-1), followed IR-88762-Sub 51-3-1-3 (11.48 nmol g-1). MDA is one of the products of plant lipid Peroxidation, resulting from oxidative stress induced damage to membrane. The MDA content found to be significantly higher in the susceptible and elongating cv. showed more oxidative damage to the membrane was

observed during submergence and subsequent Period of re-aeration (Debabrata Panda *et al*., 2013) [6-8].

**Table 10:** Changes in malondialdehyde (MDA) activity (nmol g-1) in leaves of twenty rice genotypes under submergence conditions

| Sl. No. | Name of genotypes | BS | DS | AS |
|---|---|---|---|---|
| | | Pooled mean | Pooled mean | Pooled mean |
| 1 | IR-85086-Sub 33-3-2-1 | 5.00 | 4.17(-16.6%) | 5.42(+30%) |
| 2 | IR-88760-Sub 93-3-3 | 5.28 | 4.07(-22.9%) | 5.60(+19.1%) |
| 3 | Swarna Sub -1 | 4.87 | 3.87(-20.5%) | 6.03(+55.8%) |
| 4 | Samba mahsuri Sub-1 | 5.38 | 4.32(-19.7%) | 6.47(+49.7%) |
| 5 | Savitri Sub-1 | 5.55 | 4.42(-23.4%) | 6.57(+48.6%) |
| 6 | BR-11 Sub-1 | 5.77 | 4.70(-18.5%) | 6.78(+44.2%) |
| 7 | Ciherang Sub-1 | 5.88 | 4.95(-15.8%) | 7.30(+47.4%) |
| 8 | IR-89246-Sub 38-3-2-1 | 5.92 | 5.10(-13.9%) | 7.52(-+47.4%) |
| 9 | TDK Sub-1 | 5.72 | 5.13(-10.3%) | 7.25(+41.3%) |
| 10 | IR 64 Sub-1 | 5.68 | 4.87(-14.3%) | 7.12(+46.2%) |
| 11 | IR-88762-Sub 51-3-1-3 | 5.38 | 4.42(-17.8%) | 11.40(+158%) |
| 12 | IR- 89262- Sub 5-2-3-2 | 5.20 | 4.78(-8.1%) | 10.70(+123.8%) |
| 13 | PSBRc-68 | 4.33 | 4.20(-3.0%) | 10.63(+153%) |
| 14 | FR13A (Tolerant check) | 4.98 | 4.25(-14.7%) | 6.32(+48.7%) |
| 15 | Lalat (Susceptible check) | 4.32 | 3.70(-14.4%) | 12.48(+237.2%) |
| 16 | Swarna (Susceptible check) | 5.38 | 4.00(-25.7%) | 11.03(+175.7%) |
| 17 | CR-500 (Susceptible check) | 5.37 | 4.62(-14.0%) | 9.77(+111.4%) |
| 18 | Uphar (Tolerant check) | 4.98 | 4.05(-18.7%) | 6.82(+68.3%) |
| 19 | CR-401 (Susceptible check) | 5.30 | 4.08(-23.0%) | 10.12(+148%) |
| 20 | Pratikshya (Susceptible check) | 5.15 | 3.37(-34.6%) | 7.78(+130.8%) |
| | Total mean | 5.27 | 4.35 | 8.16 |
| | SE(m) | 0.25 | 0.24 | 0.50 |
| | LSD 5% | 0.74 | 0.73 | 1.48 |
| | CV% | - | - | - |

N:B: Figure in the parentheses indicates percentage of increase or decrease over previous observation, BS = Before Submergence, AS = After Submergence, DS = During Submergence

## Conclusion

Findings of this study indicate that the group of antioxidants is important for submergence tolerance for rice plants to thrive in severe flooding. In this work, some *sub 1* introgressed lines exhibited higher levels of AOS-detoxifying enzymes (SOD, CAT, POX) and similar patterns of enzymes were observed in some *sub-1* lines such as; IR-85086-Sub 33-3-2-1, IR-88760-Sub 93-3-3, Swarna Sub -1, Samba mahsuri Sub-1 and in two tolerant checks i.e FR 13A and Uphar. Submergence treatment has enhanced the level of POX activity which indicated that the POX activity could be utilized as the selection criteria for evaluating submergence tolerance in rice. It was observed that stress during submergence reduces the activities of all antioxidant enzymes studied. However, after desubmergence the activities of these antioxidant enzymes were higher in the tolerant genotypes than in the susceptible genotypes. In conclusion the two *sub-1* lines; IR-85086-Sub 33-3-2-1, IR-88760-Sub 93-3-3 could be very promising donors for submergence breeding programmes.

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