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Biochemical performance of some *Sub1* rice (*Oryza sativa*) genotypes under submergence stress in Odisha

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Abstract
Recurring floods in Asia cause poor crop establishment. Yields decline drastically when plants are completely submerged for a few days. Traditional rice genotypes predominate because they have acquired moderate tolerance to flooding but they carry the penalty of inherently lower grain yields. Genotypes with tolerance to complete submergence were recently developed in the background of popular genotypes by transferring the submergence tolerance gene SUBMERGENCE1 (*Sub1*) from the highly tolerant Indian landrace FR13A. 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*. 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 present study indicated that among the twenty genotypes, IR-85086-*Sub* 33-3-2-1(4.87 t ha^{-1}), IR-88760-*Sub* 93-3-3 (4.7 t ha^{-1}) and Swarna *Sub* -1 (4.57 t ha^{-1}) contributed highest yield under submergence.

Keywords: Rice, submergence, genotypes, *Sub1*, NSC (Non-structural carbohydrate), antioxidants

Introduction
Rice is the life and life without rice is pedestrian. As a cereal grain, it is most widely consumed staple food for a larger part of the world's human population especially in Asia and Africa. India occupies second position within the world after china in terms of rice production. The total area under rice production is about 433.9 lakh hectares and the total production of rice recorded as 104.3 million tons. The total production of Rice in 2017 was reduced by 1.2 million tons than the production of the preceding year that is 105.5 million tons. So, Rice production in India is marked by low productivity and wide fluctuations in output mainly due to abiotic and biotic stresses. Rice being the staple food for more than 70 percent Indians and a source of livelihood for 120-150 millions rural households, the requirement of rice production by 2030 would be around 145 million tonnes from the present level of 105 million tonnes to sustain self-sufficiency in rice. More than 60% of rice produced in India comes from Eastern regions of India. Out of the 26.8 mha rice area in eastern India, rainfed lowland rice constitutes 39% of the total rice area. About 8.0 mha of rainfed lowland areas are flood/submergence prone. Rainfed lowlands constitute highly fragile ecosystems, always prone to flash-floods and stagnant flooding submergence stress situations. Many *sub1* introgressed lines developed by marker assisted back crossing (MABC) including are valuable addition to the low land rice breeding programme and these genotypes could sustain tolerance to submergence. Since submergence and stagnant flooding stresses are unpredictable, there is a need to develop new varieties with high yield and tolerance to both flash floods and stagnant flooding. Uncertainty of rainfall coupled with water logging or submergence stress is the third major factor affecting the rice yield in India and as well as in Odisha which is one of the important constraints in India, particularly in the eastern Indian states (Sarkar *et al.* 2006 and 2009) [6, 10, 11]. It is estimated that the flood affected area has more than doubled in size from about 5% (19 million hectares) to about 12% (40 million hectares) of India's geographic area. The principal cause of damage to plants grown in submergence soil is inadequate supply of oxygen to the submerged tissues as a result of slow diffusion of gases in water and rapid consumption of O_2 by soil micro-organisms. Oxygen deficiencies in water logged soils occur within a few hours under certain conditions.

Unlike other crop plants, Rice has some adaptive traits for tolerance of submergence. One of the traits is formation of the longitudinal interconnection of gas spaces called arenchyma that enables internal aeration between shoots and roots. The second trait is the "escape strategy". This involves the promotion of elongation of leaves and/or stems by entrapped ethylene. This enables plants to resume aerobic metabolism and photosynthetic fixation of CO_2 by raising their shoots above water. The escape strategy based on elongation by the stem is a prominent characteristic of deep water rice genotypes that are grown where submergence continues for more than one month in water deeper than 50cm. More than 60% of rice produced in India comes from Eastern India. Out of the 26.8 mha rice area in eastern India, rainfed lowland rice constitutes 39% of the total rice area. About 8.0 mha of rainfed lowland areas are flood/submergence prone (Reddy *et al*., 2013) [10, 11]. Rainfed lowlands constitute highly fragile ecosystems, always prone to flash-floods and stagnant flooding submergence stress situations. Since submergence and stagnant flooding stresses are unpredictable, therefore, there is a need to develop new genotypes with high yield and tolerance to both submergence and stagnant flooding for greater stability of production under the diverse rainfed lowland ecosystems of eastern Indian states. The major biochemical submergence tolerant traits is are; less chlorosis, high carbohydrate reserve storage during submergence and prompt re-adaptation to the aerial environment after de-submergence (Setter *et al.*, 1997; Ito *et al.*, 1999; Ram *et al.*, 2002; Jackson and Ram, 2003) [12, 13]. Non-structural carbohydrates (NSC) are the prime substrates for generating energy. Complete submergence causes their rapid consumption and an initiation of protein hydrolysis (Setter *et al*. 1987) [12, 13]. These NSC are utilized during submergence to supply energy for growth and maintenance metabolism (Sarkar *et al*. 1996) [6, 10, 11]. To date, the most significant finding in flood-tolerance rice research is the identification of the *Sub1*A gene on chromosome 9, as the major determinant of submergence tolerance in FR13A and its derived progenies (Xu and Mackill 1996) [10, 14]. 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 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: Fertilizer Application

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.

Biochemical analysis

Estimation of chlorophyll content

Total chlorophyll content in the leaves were 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 grams 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 filterate was used to record the absorbance (OD) at 645 nm and 663 nm. The respective chlorophyll content was calculated using the following formula and expressed as mg g^{-1} FW leaf.

$$\text{Chlorophyll-a} = (12.7 \times OD663 - 2.69 \times OD645) \times \frac{V}{1000 \times W_f}$$

$$\text{Chlorophyll-b} = (22.9 \times OD645 - 4.68 \times OD663) \times \frac{V}{1000 \times W_f}$$

$$\text{Total Chlorophyll} = (20.2 \times OD645 - 8.02 \times OD663) \times \frac{V}{1000 \times W_f}$$

Where,
OD645 = OD value at 645 nm
OD663 = OD value at 663 nm
V= Total volume of extract (ml)
W_f = Fresh weight of leaf (g)

Total soluble sugar (TSS)

Total soluble sugar (TSS) was estimated by anthrone method (Dubois *et al*., 1951). About 100 mg sample was hydrolyzed in boiling water bath for 3 hours with 5 ml of 2.5N hydrochloric acid (HCl). The extract obtain is neutralized with solid sodium carbonate until the effervescence ceases, then made up to 100 ml and centrifuged at 5,000 rpm for 5 mins. The supernatant was collected and 1 ml of aliquots was used for analysis. One ml aliquot mixed with 4 ml anthrone reagent to dehydrate glucose to hydroxymethylfurfural. The mixture was heated for 8 mins in a boiling water bath and cooled rapidly. After the development of the dark green color, the absorbance was recorded at 630 nm. The total soluble sugar was estimated by standard graph drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0 and 1 ml of the glucose with '0' serving as blank and make up the volume to 1 ml by adding distilled water.

$$\text{TSS (mg/g FW)} = \frac{\left[\left(C_{ccurve}\right) \times 1000\right]}{\left(S_{wt}\right)}$$

Where,
C_{ccurve} = TSS content derived from standard curve (µg/ml)
S_{wt}. = Weight of sample used = 0.1 g

Carbohydrate estimation

Carbohydrate (NSC) content of plant samples was determined by following procedure (Yosidha *et al*., 2005). 100 mg of powdered dry sample was taken into in powder form and extracted using 80% ethanol (v/v).The exert was then used for sugar analysis by adding anthrone reagent, followed by measurement of absorbance at 630 nm using a spectophotometre.in case of simple carbohydrate estimation the sample was kept in a boiling tube and hydrolyzed by keeping it in boiling water for three hours with 5 ml of 2.5 N HCl and then cooled to room temperature. The sample was neutralized with sodium carbonate till the effervescence ceases and then transferred it to 100ml volumetric flask and the volume was made up to 100 ml. 10ml of this was taken in a centrifuged tube and was centrifuged for 10 minute. The supernatant was collected and 0.2 to 0.3 aliquots were taken for analysis. 12 ml of anthrone reagent was added and heated for eight minutes in a boiling water bath. The absorbance (OD) of the filtrate was recorded at 630 nm. The quantity of glucose was calculated from the standard curve prepared from glucose stock solution.

Amount of carbohydrate present in 100 mg of the sample = [(mg sugar from graph/ml of aliquot sample) × (Total volume of extract in ml of sample in mg)] ×100

Estimation of proline

Proline protects membranes and proteins against the adverse effects of high concentrations of inorganic ions. A standard protocol was adopted for proline estimation (Bates *et al*., 1973). About 200 mg fresh leaf tissues were homogenized using 4 ml of 3% (w/v) aqueous sulfosalicylic acid and filtered through Whatman paper no. 2. About 2 ml of the homogenized extract added with 2 ml of ninhydrin acid and 2 ml of glacial acetic acid and boiled at 100 °C for 1hour. The reaction was quenched by putting the tubes in ice, rapidly. The reaction mixture is extracted with 2 ml toluene, mixed vigorously and kept at room temperature for 30 mins until separation of the two phases. The chromophore-containing toluene (1 ml, upper phase) is warmed to room temperature and its optical density (OD) was measured at 520 nm using toluene as a blank. The proline concentration was estimated with reference to calibration curve and expressed as µmoles/g fresh weight.

$$\text{Proline content} = \frac{\left[\left(C_{ccurve}\right) \times \left(\frac{T_{Ext.}}{115.5 \mu g / \mu \text{mole}}\right)\right]}{\left(\frac{S_{wt}}{5}\right)}$$

Where, C_{ccurve} = Proline content derived from standard curve (µg proline/ml)
$T_{Ext.}$ = Toulene added = 2 ml
S_{wt}. = Weight of sample used = 0.2 g

Protein extraction

About 500 mg of leave sample were powdered in mortal pestle with the use of liquid nitrogen. Total protein was extracted by homogenizing these leaf tissue in 4 ml of extraction buffer made up of 50mM phosphate buffer (pH=7.8) containing 1mM EDTA (Ethylene diamine tetra acetic acid), 2% PVP (Poly vinyl pyrrolidone, w/v) and 0.1%

(v/v) triton X-100.The homogenate was centrifuged at 10,000 rpm for 30 mins at 4 °C. The supernatant obtained was stored in 2 ml eppendrof tubes at -20°C and labeled properly for future use.

Protein estimation

A 1 ml aliquot of the supernatant was used to determine the total protein content in the samples through Lowry *et al.* (1951) method utilizing bovine serum albumin (BSA) as the standard.

Lowry reagent

Mix solution A and solution B in 50:1 ratio, just prior to use.
Solution A: 2% sodium carbonate in 0.1N NaOH.
Solution B: 0.5% copper sulfate solution in 1% sodium potassium tartarate solution (to be prepared fresh)
A series of tubes were prepared with '0' (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 ml of working standard BSA (200 mg/ml). The total volumes of all tubes were made up to 1ml with addition of distilled water. In another set of tubes, 1ml of each unknown protein sample was taken. Then, 5ml of the alkaline-copper sulfate solution (Lowry Reagent) was added in all tubes and mixed well. Allow the tubes to stand at room temperature for 10 to 15 mins. Add 0.5 ml of diluted Folin-Ciocalteau reagent into each tube and mixed rapidly. The tubes were finally incubated in dark for 30 mins for the blue color development. The absorbance was measured at 700 nm. A calibration curve was prepared with concentration (mg) of protein on X-axis and OD on Y-axis to determine the amount of protein present in the unknown samples. The protein (mg/g fresh weight) was calculated using the linear equation: y = 0.0024x + 0.013.

$$\text{Protein content (mg/g FW)} = (C_{ccurve}) \times V_t/V_a \times 1/S_{wt.} \times 1/1000$$

Where

C_{ccurve} = TSS content derived from standard curve (µg /ml)
V_t = Total protein extract = 4ml
V_a = Volume of aliquot used for analysis = 1ml
$S_{wt.}$ = Weight of sample used = 0.5 g

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.

Test of significance of correlation coefficient

The degree of correlation between different plant characters were measured in terms of correlation coefficient values. These correlation coefficients were estimated following the standard techniques as outlined in the above reference book.
The observed value of correlation coefficient is compared with the tabulated value for (n-2) degree of freedom. If the observed value is more than the tabulated one, the correlation coefficient is said to be 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).
It was revealed from Table 4. that there was reduction in chlorophyll –a, chlorophyll b, and total chlorophyll in all genotypes. Maximum amount of total chlorophyll (2.3, 2.2 and 2.18 mg g^{-1} FW) was retained in the genotypes IR 85086-Sub 33-3-2-1 followed by FR13A (Tolerant check) followed by IR 88760-Sub93-3-3- respectively. Maximum reduction (89.8% and 8.8%) in total chlorophyll was seen in one Sub-1 NIL IR-89262-Sub-5-2-3-2 followed by CR-500 (susceptible check) respectively. The chlorophyll reduction is accompanied with carbohydrate reserves before the submergence in the shoot which helps in minimum shoot elongation and regulation of plant hormones like GA and ethylene. The ethylene triggered the gene expression and chlorophyllase enzyme activity which reduced the chlorophyll contents. Chlorophyll reduction was less in submergence tolerant genotypes due to reduction in ethylene production (Das *et al*., 2005, Sarkar *et al*., 2006) [6, 10, 11].

Table 4: Pooled mean of chlorophyll-a, chlorophyll-b and total chlorophyll (mg g^{-1} FW) of twenty rice genotypes in response to before submergence (45 DAS) and after submergence (after 7 days of de-submergence)

Sl. No.	Name of genotypes	Chlorophyll before submergence (BS)			Chlorophyll after submergence (AS)		
		Chl-a	Chl-b	Total Chl	Chl-a	Chl-b	Total Chl
1	IR-85086-Sub 33-3-2-1	1.82	0.69	2.67	1.75	0.64	2.30 (-13.8%)
2	IR-88760-Sub 93-3-3	1.58	0.62	2.46	1.60	0.54	2.18 (-11.4%)
3	Swarna Sub-1	1.66	0.60	2.40	1.46	0.49	2.14 (-10.8%)
4	Samba mahsuri Sub-1	1.58	0.59	2.48	1.41	0.42	2.08 (-16.1%)
5	Saviri Sub-1	1.35	0.65	2.38	1.37	0.46	2.01 (-15.5%)
6	BR-11 Sub-1	1.57	0.63	2.28	1.50	0.47	1.92 (-15.7%)
7	Ciherang Sub-1	1.64	0.58	2.36	1.46	0.41	1.88 (-20.3%)
8	IR-89246-Sub 38-3-2-1	1.55	0.47	2.32	1.40	0.30	1.61 (-30.6%)
9	TDK Sub-1	1.64	0.55	2.28	1.26	0.27	0.96 (-88.2%)
10	IR 64 Sub-1	1.37	0.52	2.33	1.13	0.32	1.48 (-36.5%)
11	IR-88762-Sub 51-3-1-3	1.13	0.62	2.23	0.60	0.16	0.20 (-91%)
12	IR- 89262- Sub 5-2-3-2	1.33	0.56	2.26	0.58	0.14	0.23 (-89.8%)
13	PSBRc-68	1.35	0.51	2.20	0.19	0.12	0.65(-70.5%)
14	FR13A (Tolerant check)	1.43	0.59	2.22	1.54	0.47	2.20 (-0.9%)
15	Lalat (Susceptible check)	1.37	0.60	2.26	0.13	0.17	0.52 (-77%)

16	Swarna (Susceptible check)	1.13	0.39	1.87	0.38	0.13	0.73 (-61%)
17	CR-500 (Susceptible check)	1.07	0.39	2.03	0.23	0.14	0.24 (-88.17%)
18	Uphar (Tolerant check)	1.24	0.37	2.51	1.44	0.36	1.74 (-30.7%)
19	CR-401 (Susceptible check)	1.17	0.33	2.02	0.62	0.14	0.60 (-70.3%)
20	Pratikshya (Susceptible check)	1.15	0.38	1.90	0.99	0.20	1.05 (-44.7%)
	Total mean	1.40	0.53	2.27	1.05	0.32	1.34
	SE(m)	0.09	0.020	0.09	0.08	0.03	1.10
	LSD 5%	0.27	0.080	0.28	0.26	0.11	0.32
	CV%						

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

It can be depicted from Table 5. that in general the starch content of shoot has decreased in all the 30 rice genotypes but, maximum amount of starch was retained in the genotypes IR -86086-Sub 33-3-2-1 (30.98 mg g-1 FW) followed by IR -88760 Sub 93-3-3 (31.04 mg g-1 FW). Data reflected in Table 5. shows that total soluble sugar (TSS) increased in nine Sub-1 lines, with maximum increase i.e., 30.71% in FR13A followed by Samba Mahsuri Sub-1 with an increase of 27.15% over the control. The maximum amount of TSS was retained by IR 85086-Sub 33-3-2-1 followed by IR-88760-Sub 93-3-3 i.e., 32.91 and 31.04 mg g-1 FW respectively.

From data presented in Table 5 it is clear that non-structural carbohydrate (NSC) increased in tolerant checks. Maximum NSC content was found i.e., (63.89, 58.97 and 56.2) mg g-1 FW in IR-85086-Sub 33-3-2-1 followed by IR-88760-Sub-93-3-3 followed by Swarna Sub-1 respectively. In control conditions also highest starch and TSS content was observed in IR 85086-Sub 33-3-2-1 i.e., (34.98, 26.03) mg g-1 FW respectively. Data reflected in Table 5. it is depicted that total carbohydrate content of shoot decreased in all the 20 rice genotypes but maximum shoot carbohydrate content i.e. 14.68 mg g-1 FW was retained in the Sub-1 line, IR 85086 Sub- 33-3-2-1 followed by IR 88760-Sub 93-3-3 with a value of 13.66 mg g-1 FW. In control conditions the tolerant check FR 13A had higher shoot carbohydrate content (16.65 mg g-1 FW) as compared to all other genotypes. High carbohydrate status during submergence is related to submergence tolerance of rice crops (Yamada *et al*., 1955; Pal and Mitra 1985) [15]. In the present search it was evident that higher levels of initial carbohydrate act as buffer stock and its continued slow availability is critical for the survival and growth of rice under submergence stress. The metabolic energy required by the plant during submergence is primarily supplied from stored carbohydrate present in the tissue in non-stressed condition. The present findings indicated that irrespective of genotypes there was reduction in carbohydrate content of shoot after submergence. It is obviously 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 CO2 concentration as well. Moreover, submergence also limits the carboxylation by low/intermediate intercellular CO2 concentrations which suppress the RUBISCO activity, vis-a-vis enhancing the oxygenation process (Buchanan *et al*., 2004) [3]. This deviation ratio to oxygenation under submergence is more serious for switching over the tissues to make it more prone to photorespiration. Under submerged conditions when the leaves, stem and roots are completely submerged the rate of depletion of carbohydrate is very slow in tolerant genotypes than the susceptible genotypes. Drastic reduction in carbohydrate leads to high rate of anaerobic fermentation and production of ethanol at toxic level (Setter *et al*. 1988 b) [12, 13]. The ability of rice coleoptiles to grow under strict anoxia during submergence was related to induction of α-amylase causing break down of starch reserves (Perata *et al*., 1992) [8].

Table 5: Pooled mean values of total carbohydrate and non-structural carbohydrate (NSC = Starch + TSS) in mg g-1 FW components of shoot of twenty rice genotypes under submergence conditions

Sl. No.	Name of genotypes	Before submergence (BS)				After submergence (AS)			
		Starch	TSS	NSC	Carbohydrate	Starch	TSS	NSC	Carbohydrate
1	IR-85086-Sub 33-3-2-1	34.98	26.03	61.01	16.65	30.98	32.91	63.89 (+4.72%)	14.68
2	IR-88760-Sub 93-3-3	32.30	25.20	57.50	15.58	27.93	31.04	58.97 (+2.55%)	13.66
3	Swarna Sub -1	31.70	24.39	56.09	15.39	25.40	30.8	56.2 (+0.19%)	13.45
4	Samba mahsuri Sub-1	25.78	23.20	48.98	14.93	23.66	29.5	53.16 (+8.53%)	13.26
5	Savitri Sub-1	25.51	24.69	50.21	14.41	22.92	27.79	50.71 (+0.99%)	12.76
6	BR-11 Sub-1	25.63	22.70	48.33	14.27	22.25	27.95	50.2 (+3.86%)	11.40
7	Ciherang Sub-1	24.5	22.41	46.91	13.93	21.63	27.4	49.03 (+4.51%)	11.24
8	IR-89246-Sub 38-3-2-1	22.93	23.14	46.07	14.45	21.53	26.53	48.06 (+4.31%)	10.42
9	TDK Sub-1	22.25	21.20	43.45	13.13	21.00	21.67	42.67 (-1.79%)	9.60
10	IR 64 Sub-1	19.31	20.63	39.94	12.70	18.73	20.9	39.63 (-0.77%)	9.48
11	IR-88762-Sub 51-3-1-3	17.15	21.51	38.66	13.42	15.87	10.11	25.98 (-32.79%)	8.98
12	IR- 89262- Sub 5-2-3-2	15.26	20.96	36.23	12.45	14.10	11.33	25.43 (-29.8%)	3.39
13	PSBRc-68	14.83	20.12	34.95	14.08	9.42	11.11	20.53 (-41.2%)	2.66
14	FR13A (Tolerant check)	28.38	23.05	51.43	16.67	24.78	30.13	54.91 (+6.76%)	13.62
15	Lalat (Susceptible check)	18.33	21.7	40.03	13.05	10.42	12.94	23.36 (-41.6%)	2.63
16	Swarna (Susceptible check)	16.70	19.30	36.00	14.58	9.31	13.98	23.29 (-35.3%)	3.15
17	CR-500 (Susceptible check)	17.61	18.5	36.11	12.12	6.96	9.5	16.46 (-54.4%)	2.955
18	Uphar (Tolerant check)	15.28	18.61	33.8	12.97	20.48	23.48	43.96(+30.05%)	10.25
19	CR-401 (Susceptible check)	15.70	18.91	32.61	12.83	7.95	10.44	18.39(-43.6%)	3.38
20	Pratikshya	18.46	19.96	38.43	12.9	12.60	14.87	27.47(-28.5%)	8.05
	Total mean	22.13	21.80	43.94	14.04	18.39	21.22	39.03	8.95
	SE(m)	1.77	1.01	-	0.66	0.06	1.20	-	0.37
	LSD 5%	3.48	3.00	-	1.98	1.78	3.57	-	1.11

N:B:- Figure in the parentheses indicates percentage of increase or decrease over previous observation

From data presented in Table-6 it was revealed that proline content increased in most of the genotypes except the genotypes which did not survive till maturity. Maximum proline content i.e., 13.37 µg g^{-1} FW was found in IR-85086-Sub 33-3-3-2-1 followed by IR-88760 Sub 93-3-3 with a value of 12.84 µg g^{-1} FW, which is almost at par with the tolerant check FR13A with a value of 12.79 µg g^{-1} FW. The lowest amount of proline was found in the susceptible check Swarna with a value of 4.12 µg g^{-1} FW showing almost 52.3% of reduction over control.

Table 6: Proline content (µg g^{-1} FW) in twenty rice genotypes under submergence conditions

Sl. No.	Name of genotypes	Before submergence (BS)			After submergence (AS)		
		Kharif 2017	Kharif 2019	Pooled mean	Kharif 2017	Kharif 2019	Pooled mean
1	IR-85086-Sub 33-3-2-1	9.7	11.31	10.53	13.40	13.35	13.37 (+27.0%)
2	IR-88760-Sub 93-3-3	10.13	10.47	10.3	12.70	12.97	12.84 (-24.7%)
3	Swarna Sub -1	10.28	11.00	10.64	11.36	12.59	11.97 (+12.5%)
4	Samba mahsuri Sub-1	10.43	11.75	11.09	10.56	12.98	11.77 (+6.1%)
5	Savitri Sub-1	9.53	11.35	10.44	10.30	12.1	11.2 (+7.3%)
6	BR-11 Sub-1	9.4	8.4	8.9	10.34	12.19	11.26 (+26.5%)
7	Ciherang Sub-1	8.45	9.8	9.12	10.07	10.91	10.49 (+15.0%)
8	IR-89246-Sub 38-3-2-1	9.21	9.24	9.22	10.19	10.83	10.55 (+14.4%)
9	TDK Sub-1	8.36	9.15	8.76	9.37	8.53	8.95 (+2.2%)
10	IR 64 Sub-1	8.5	8.17	8.33	9.90	10.61	10.25 (+23.0%)
11	IR-88762-Sub 51-3-1-3	7.56	7.75	7.65	9.72	9.29	9.5 (+24.2%)
12	IR- 89262- Sub 5-2-3-2	8.03	9.84	8.94	6.31	6.19	6.24 (-30.2%)
13	PSBRc-68	8.12	8.77	8.44	5.53	6.77	6.15 (-27.1%)
14	FR13A (Tolerant check)	10.57	9.58	10.07	12.46	13.11	12.79 (+27.0%)
15	Lalat (Susceptible check)	8.13	9.72	8.92	3.97	5.36	4.66 (-47.7%)
16	Swarna (Susceptible check)	8.89	8.4	8.64	3.29	4.94	4.12 (-52.3%)
17	CR-500 (Susceptible check)	7.83	7.86	7.84	6.24	5.06	5.65 (-27.9%)
18	Uphar (Tolerant check)	7.63	8.21	7.92	10.36	10.7	10.53 (+32.9%)
19	CR-401 (Susceptible check)	8.35	9.09	8.72	6.78	5.9	6.34 (-27.3%)
20	Pratikshya (Susceptible check)	7.92	7.77	7.85	7.18	9.42	8.3 (+5.7%)
	Total mean	8.85	9.38	9.12	9.00	9.24	9.35
	SE(m)	0.26	0.44	0.43	0.36	0.32	0.50
	LSD 5%	0.77	1.27	1.29	1.03	0.92	1.49
	CV%	5.26	8.23	-	7.43	6.03	-

N:B: Figure in the parentheses indicates percentage of increase or decrease over previous observation

Sarkar *et al.* (2001) [6, 10, 11] inferred that accumulation of proline is maximum in tolerant genotype under submerged condition. So proline accumulation is considered as an indicator of submergence injury where the concentration of proline builds up under stress due to hydrolysis of proteins. Submergence caused the plants to accumulate proline. The accumulation of proline in a wide variety of species under various types of abiotic stresses is well known. Proline content increased under flooding and was highest at 17 days submergence, as compared to non-submerged control condition. This may be due to the de novo synthesis of proline under induction of excess water stress, possibly because of involvement in adaptive mechanism to maintain normal osmoregulation as also reported by Chen and Kao (1993) [4, 5]. Production and accumulation of proline by plant tissues during water stress is an adaptive response. Proline has been proposed to act as a compatible solute that adjust the osmotic potential in the cytoplasm (Cabellero *et al.*, 2005).

Table 7. reveals that total protein content decreased in all the 20 genotypes, but the minimum amount of reduction i.e., 13.5% was found in the *Sub1* line IR -85086-Sub 33-3-2-1 with a value of 23.95 mg g^{-1} FW after submergence. The maximum amount of total protein following 17 days of submergence was obtained from the tolerant check FR 13A with a value of 25.01 mg g^{-1} FW. Lowest protein content was found in the susceptible check genotype CR 401 with a value of 9.1 mg g^{-1} FW with almost a reduction of 64% over the control. Submergence inhibits protein synthesis and increases denaturation of protein in rice, but submergence tolerant genotypes somehow manages to decrease the protein denaturation. Inhibition of protein synthesis and activation of protein degradation can explain in part enhanced accumulation *Sub1*A regulates post-submergence recovery in rice leaves of amino acids under submergence and oxygen deficiency. However, the results of individual amino acid quantification reflect drastic alterations in biosynthesis and degradation of particular amino acids under the stress (Jasper Benedict Alpuerto *et al.*, 2016) [1]. In the present investigation highest amount of total protein was retained in the tolerant genotype IR-85086-Sub 33-3-2-1 (23.95 mg g^{-1} FW) as compared to other genotypes, but In general there was a reduction in total protein content in all the twenty genotypes.

Table 7: Total protein content in rice genotypes under submergence condition

Sl. No.	Name of genotypes	Before submergence (BS)			After submergence (AS)		
		Kharif 2017	Kharif 2019	Pooled mean	Kharif 2017	Kharif 2019	Pooled mean
1	IR-85086-Sub 33-3-2-1	29.06	26.30	27.68	25.23	22.66	23.95 (-13.5%)
2	IR-88760-Sub 93-3-3	30.26	28.46	29.36	25.00	22.32	23.66 (-19.4%)
3	Swarna Sub -1	27.56	27.23	27.40	22.56	20.36	21.46(-21.7%)
4	Samba mahsuri Sub-1	26.70	30.46	28.58	20.53	21.46	21.00 (-26.5%)
5	Savitri Sub-1	29.33	25.73	27.53	22.36	20.56	21.46 (-22.0%)
6	BR-11 Sub-1	25.63	28.26	26.95	18.43	19.60	19.01 (-29.5%)
7	Ciherang Sub-1	29.63	30.33	29.98	20.23	20.60	20.41 (-31.9%)
8	IR-89246-Sub 38-3-2-1	24.50	27.76	26.13	17.36	17.83	17.60 (-32.6%)
9	TDK Sub-1	22.56	25.30	23.93	15.66	15.56	15.61 (-34.7%)

10	IR 64 Sub-1	29.46	31.56	30.51	21.38	20.43	20.90 (-31.5%)
11	IR-88762-Sub 51-3-1-3	28.56	29.36	28.96	14.43	18.36	16.40 (-43.4%)
12	IR- 89262- Sub 5-2-3-2	32.20	28.32	30.28	18.33	15.33	16.83 (-44.4%)
13	PSBRc-68	28.30	31.86	30.08	13.70	9.43	11.56 (-61.6%)
14	FR13A (Tolerant check)	31.46	29.53	30.50	25.53	24.50	25.01 (-18.0%)
15	Lalat (Susceptible check)	25.40	29.60	27.50	16.53	15.40	15.96 (-42.0%)
16	Swarna (Susceptible check)	30.50	29.43	29.96	13.43	16.63	15.03 (-49.9%)
17	CR-500 (Susceptible check)	24.86	21.43	23.15	16.83	18.43	17.63 (-23.8%)
18	Uphar (Tolerant check)	32.56	32.40	32.48	23.52	20.30	21.91 (-32.5%)
19	CR-401 (Susceptible check)	28.40	22.20	25.30	9.30	8.90	9.10 (-64.0%)
20	Pratikshya (Susceptible check)	31.46	29.23	30.35	19.36	18.33	18.85 (-37.9%)
	SE(m)	0.83	0.85	1.06	1.06	0.60	1.06
	LSD (5%)	2.38	2.43	3.16	0.58	1.71	3.16
	CV%	5.07	5.23	-	5.30	5.67	-

Data reflected in Table 8. shows that maximum yield after submergence was recorded in IR-85086-Sub 33-3-2-1 followed by IR 88760-Sub 93-3-3 followed by Swarna Sub-1 with values of 4.87, 4.70, 4.5 t ha^{-1} respectively. In control conditions the highest yield was recorded in the susceptible check Pratikshaya followed by IR-85086-Sub 33-3-2-1 with values of 6.4 t ha^{-1} and 6.21 t ha^{-1} respectively. The genotype Pratikshya recorded the lowest yield after submergence i.e., 0.93 t ha^{-1} followed by TDK-Sub-1 with a yield of 1.34 t ha^{-1}. The minimum amount of reduction in yield recorded in BR-11 Sub-1 with a reduction percentage of 10.8% over the control (Table 8).

Table 8: Yield (t ha^{-1}) under control (C) conditions and submerged conditions (S)

Sl. No.	Name of genotypes	Yield					
		Control (C)			Submerged (S)		
		Year 1	Year 2	Pooled mean	Year 1	Year 2	Pooled mean
1	IR-85086-Sub 33-3-2-1	6.40	6.21	6.30	4.81	4.93	4.87 (-22.7%)
2	IR-88760-Sub 93-3-3	6.10	6.16	6.13	4.60	4.80	4.70 (-23.3%)
3	Swarna Sub -1	5.98	6.06	6.02	4.51	4.63	4.57 (-24.0%)
4	Samba mahsuri Sub-1	5.00	5.20	5.10	4.30	4.47	4.38 (-14.1%)
5	Saviri Sub-1	4.80	4.90	4.85	4.10	4.14	4.12 (-15.0%)
6	BR-II Sub-1	5.00	5.20	5.10	4.00	4.07	4.03 (-21.0%)
7	Ciherang Sub-1	4.30	4.40	4.35	3.90	3.87	3.88 (-10.8%)
8	IR-89246-Sub 38-3-2-1	4.90	5.03	4.96	2.82	2.88	2.85 (-42.5%)
9	TDK Sub-1	4.70	4.80	4.75	2.50	2.60	2.55 (-46.3%)
10	IR 64 Sub-1	4.40	4.60	4.50	1.24	1.44	1.34 (-70.2%)
11	IR-88762-Sub 51-3-1-3	4.33	4.43	4.38	0.00	0.00	0.00 (-100%)
12	IR- 89262- Sub 5-2-3-2	4.20	5.25	4.72	0.00	0.00	0.00 (-100%)
13	PSBRc-68	5.33	5.46	5.40	0.00	0.00	0.00 (-100%)
14	FR13A (Tolerant check)	4.60	4.50	4.55	2.27	2.60	2.43 (-46.0%)
15	Lalat (Susceptible check)	4.00	3.93	3.97	0.00	0.00	0.00 (-100%)
16	Swarna (Susceptible check)	6.00	6.20	6.10	0.00	0.00	0.00 (-100%)
17	CR-500 (Susceptible check)	3.33	3.20	3.26	0.00	0.00	0.00 (-100%)
18	Uphar (Tolerant check)	6.20	6.37	6.28	3.68	4.01	3.85 (-38.7%)
19	CR-401 (Susceptible check)	3.80	3.76	3.78	0.00	0.00	0.00 (-100%)
20	Pratikshya (Susceptible check)	6.50	6.30	6.40	0.98	0.88	0.93 (-85.5%)
	Total mean	4.99	5.10	5.04	2.19	2.27	2.23
	Sem	0.08	0.09	0.12	0.06	0.06	0.04
	LSD 5%	0.23	0.27	0.38	0.18	0.17	0.13
	CV%	2.87	3.27	-	5.24	4.65	-

Conclusion

The grain yield among the genotypes varied from 0.93 t ha^{-1} in Pratikshya to 4.87 t ha^{-1} in IR-85086-Sub 33-3-2-1. The variation of grain yield of all the genotypes followed the sequence of IR-85086-Sub 33-3-2-1 > IR-88760-Sub 93-3-3 > Swarna Sub -1 > Samba mahsuri Sub-1 > Savitri Sub-1> BR-11 Sub-1 > Ciherang Sub-1>Uphar > IR-89246-Sub-38-3-2-1> TDK Sub-1 > FR13A > IR64 Sub-1 > Pratikshya. In general, the higher yield was obtained due to its greater number of effective tillers, 1000 seed weight, and better biochemical traits. IR-85086-Sub 33-3-2-1, IR-88760-Sub 93-3-3 and Swarna Sub-1 contributed highest yield due to their tolerance under submerged condition which was mainly due to substantial amount of carbohydrate reserve and less chlorophyll and protein disintegration before and after submergence. Among the tolerant checks Uphar exhibited highest yield, and rest of the four susceptible genotypes Lalat, Swarna, CR-500, CR-401 and the three *Sub-1* lines namely IR-88762-Sub 51-3-1-3, IR-89262-Sub 5-2-3-2, PSBRc-68 didn't survive till maturity, which reveals that there might be some additional QTLs in FR13A which helped in the better survival of the same in submergence conditions as compared to some *Sub-1* lines. However, for confirmation of the results this warrants further investigation.

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