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Physiological validation of CO43 *Sub1* for flooding stress tolerance

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ABSTRACT

The present study was aimed at developing submergence tolerant version of a popular rice variety of Tamil Nadu namely CO43 through marker assisted introgression of Sub 1 locus from submergence tolerant FR 13A. Efforts were made to continue the previous work done in our group. In the present study, BC_1F_1 generation was forwarded upto BC_4F_1 and selfed progenies of BC_2 generations were phenotyped for submergence tolerance. Two molecular markers linked to the Sub 1 locus viz., one SSR namely RM219 and another InDel marker namely ART5 were found to be polymorphic between CO 43 and FR13A and used for Foreground Selection (FGS). Seventy six SSR markers covering all 12 chromosomes of rice genome were found to be polymorphic between CO 43 and FR13A which were used in back ground selection (BGS). Advanced backcross progenies (BC_2F_1, BC_3F_1 and BC_4F_1) harboring Sub1 locus (from FR13A) was generated by repeated backcrossing with the recurrent parent CO 43. About 130 BC_2F_1 plants were raised under greenhouse conditions and genotyped using RM219 linked to Sub1 locus. After foreground selection , background selection using 25 genome wide SSR markers, BC_2F_1 plants having more than 85 – 90% recurrent parent genome recovery were identified. Three BC_2F_1 plants viz., # 57, 61 and 84 found to possess >90% recurrent parent (CO 43) genome. Superior BC_2F_1 plants were back crossed with CO 43 to develop BC_3F_1. Totally twenty two BC_3F_1 plants were generated and raised under greenhouse conditions. Foreground selection using RM219 and ART5 resulted in the identification of five positive plants which were subjected to BGS using 37 SSR markers (12 additional markers). Selected ten plants were then subjected to background selection with 29 SSR markers. Results revealed three plants (Line no # C20, C22 and C31) were found to have more than 90% of CO 43 genome. The seeds of these three lines (Line no # C20, C22 and C31) were selfed to generate BC_2F_4 progenies. Submergence tolerance screening of three BC_2F_4 progenies namely (C20, C22 and C31) and four superior progenies of BC_2F_3 selected from the field namely (23-66; 23-62; 61-24; 61-19) along with their parents revealed that all positive BC_2F_3 and BC_2F_4 progenies were found to be superior in terms of recovery after desubmergence. All the survived plants were evaluated for agronomic and physiological traits namely plant height, TDMP, stem and leaf carbohydrates, chlorophyll, chlorophyll fluorescence and other gas exchange parameters (Photosynthetic rate, stomatal conductance and transpiration rates) before and after flooding.

Keywords: CO43 Sub1, flooding stress tolerance, BGS

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INTRODUCTION

Rice is one of the most important food crops in the world, crucial for the food security of many Asian countries [1]. Flash floods leading to complete submergence of rice plants for 10–15 days are one of the major recurring problems for rice production, mainly in rainfed lowland areas. In India, 30% of the rice growing area (15-16 M ha) is prone to flash flooding with average productivity of only 0.5–0.9 t ha^{-1} [2]. Most dryland cereals, such as maize, wheat, and barley, are sensitive to waterlogging, causing up to 20% yield losses in irrigated areas, and even greater losses in rainfed ecosystems exceeding 40% [3]. Waterlogging hampers root growth and function because of oxygen shortages that restrict root respiration. The concentrations of potentially toxic compounds increase in anoxic soils, and these can enter through roots, damaging both root and shoot tissues [4]. There has been a steady and significant

increase in paddy cultivation and yields per unit area between 2013 and 2015 and a sharp increase in paddy sales during 2016 [5].

In Tamil Nadu, Cauvery delta zone is facing serious problems frequently due to flash flooding during the monsoon period. About 3 lakhs ha of paddy area is being affected severely every year due to submergence/flooding. Flash floods may completely submerge the plant, restricting gaseous exchange and thereby hindering growth processes leading to its decay and death. Most aerobic crops cannot withstand standing water or water logging but rice thrives in shallow standing water because it can maintain oxygen supplies to the root through its extensive aerenchyma. The increase in plant length had already started even during the shortest period of flooding, indicating rapid growth from the upper part of the seedlings in order to escape from the submergence condition and reach for the oxygen above the water surface. This escape strategy through shoot elongation has long been reported in many studies [6, 7, 8 & 9].

Nipponbare genome was showed to be intolerant to submergence stress based on the absence of the Sub1A locus in its genome though the basis for the submergence tolerance have been well established as being associated with high initial and after submergence non-structural carbohydrate contents, high levels of chlorophyll retention, and minimum elongation under submergence (10,11, 12 & 13]. Maintenance of high levels of stored carbohydrates in the seedlings prior to submergence coupled with minimum shoot elongation and retention of chlorophyll are all desirable traits for submergence tolerance [14].

Submergence induced alteration of photo-system II (PS II) structure and function was probed using fast O-J-I-P chlorophyll a fluorescence transient and CO2 photo-assimilation rate [15]. Hence the study was designed to investigate the generation of advanced backcross progenies (BC_2F_1, BC_3F_1 and BC_4F_1) harboring *Sub1* locus (from FR13A) by repeated backcrossing with the recurrent parent CO 43 through Marker Assisted Selection(MAS) and physiological evaluation of superior lines for submergence tolerance related traits such as plant height, chlorophyll content, chlorophyll fluorescence and stored carbohydrate.

MATERIAL AND METHODS

Genotypes used

Two rice genotypes namely, CO 43 (recurrent parent) and FR13A (Flood Resistant 13A) were used. CO 43 is a long duration rice variety released from Paddy Breeding Station, TNAU, Coimbatore and it is popularly grown in irrigated areas of Tamil Nadu. It is derived from a cross between Dasal X IR 20 and known for its high level of salinity tolerance. FR13A is a photoperiod-sensitive and highly submergence tolerant rice genotype but possessing undesirable agronomic traits viz., low yield, awns and poor cooking quality [16]. Seeds of FR13A were obtained from Central Rice Research Institute, Cuttack and seeds of CO 43 were obtained from Paddy Breeding Station, Tamil Nadu Agricultural University (TNAU).

Previous work done

CO43 is a long duration variety popularly grown in the Cauvery delta region of Tamil Nadu which is known for its superior level of salinity tolerance but susceptible to submergence or flash flooding. Hence efforts were made to improve the submergence tolerance of CO 43 through marker assisted introgression of *Sub1* locus from the submergence tolerant FR13A. Efforts were made to develop F_1s between CO 43 and FR13A and the developed F1s were used for backcrossing with CO 43 to develop BC_1F_1. On the other hand, seeds collected from F_1s were used for rising F_2 generation. A total of 256 F_2 seedlings were raised and evaluated for the morphological traits namely, days to flowering, plant height, number of tillers/hill, number of panicles/plant, panicle length, number of grains per panicle, 100 grain weight and grain yield per plant. Through genotyping using SSR markers linked to *Sub1* locus, contrasting F_2 plants differing for their possession of *Sub1* locus (homozygote FR13A allele of RM219, homozygote CO 43 allele of RM219 and heterozygote's were identified and the effect of introgression of *Sub1* locus on submergence tolerance was confirmed. In this study, efforts were made to carry forward these genetic materials to develop submergence tolerant version of CO 43 through marker assisted back crossing. The developed BC_1F_1 plants were utilized in this study to develop further back cross materials.

Generation of BC_2F_1 generation

About 130 BC_2F_1 plants were raised under greenhouse conditions during Rabi'2012 season and genotyped using RM219 linked to *Sub1* locus. SSR genotyping was done using the genomic DNA isolated from all the 130 BC_2F_1 plants and screened for the presence of *Sub 1* locus from FR 13A. The positive heterozygous plants of BC_2F_1 generations were subjected to BGS using 25 genome wide SSR markers (Table.1). Lines exhibiting maximum recovery of CO 43 genomes were used for further back crossing.

Generation of BC_3F_1 generation

Three superior BC_2F_1 plants viz., # 57, 61 and 84 were used for further back crossing to generate BC_3F_1 generation. Totally twenty two BC_3F_1 plants were generated and raised under greenhouse conditions during summer 2012-13. All the plants were subjected to Foreground selection using RM219 and an Indel marker ART5. The positive BC_3F_1 plants were subjected to background selection using 37 SSR markers (12 new SSR markers in addition to the 25 SSRs used for genotyping of BC_2F_1 generation) (Table 2.).

Generation and Evaluation of BC_4F_1 generation

The superior BC_3F_1 plant (# 7) was having >92% recovery of CO 43 genome was used for backcrossing with CO 43 to develop BC_4F_1 plants. A total number of 28 BC_4F_1 plants were generated and raised under greenhouse conditions and subjected to FGS using RM219 and ART5. The positive BC_4F_1 plants were subjected to BGS using 37 - 40 SSRs (3 new SSR markers in addition to the 37 SSRs used in BGS of BC_3F_1 plants) covering all 12 chromosomes (Table 3).

Physiological evaluation of superior BC_2F_3 lines

Three superior progenies of BC_2F_4 generation namely (C20, C22 and C31) and four superior progenies of BC_2F_3 selected from the field namely (23-66; 23-62; 61-24; 61-19) along with their parents were subjected to flooding stress for two weeks. Physiological and biochemical basis of flooding tolerance was studied by assessing key physiological traits namely plant height, chlorophyll, chlorophyll fluorescence and stem, leaf carbohydrates before and after flooding. These plants were grown in submergence tanks for 21 days (Fig.1**)** and then completely submerged for 13 days. Chlorophyll fluorescence and other parameters were measured 2 days before submergence while chlorophyll contents stem and leaf carbohydrates were analyzed one day before submergence and plant height was assessed on the day of submergence. After 13 days of submergence, tanks were de-submerged and allowed for recovery for 2 weeks. Survival of plants was scored 14 days after de-submergence (calculated as a percentage for confirmation of the presence of the *Sub1* locus.

Plant height

Plant height was measured on the day of imposing stress and again after 14 days of desumbergence. Plant height was measured from the ground level to the tip of the primary panicle and expressed in centimeters (Table 4).

Chlorophyll contents

Contents of chlorophyll 'a', 'b' and total were estimated in a fully expanded young leaf one day before flooding and 14 days after de-submergence and expressed in mg g^{-1} fresh weight [17]. Three replicants were taken in the all the lines and error degrees of freedom were calculated (Table 5).

Chlorophyll fluorescence (Fv/Fm)

Chlorophyll fluorescence measurements were recorded using Plant Efficiency Analyzer (Hansatech, UK) following the method advocated [18]. Measurements were made on intact leaves, which were dark adapted for 30 min prior to measurement. The minimal fluorescence level (F_0) with all PS II reaction centers open was assessed by measuring the modulated light, which was sufficiently (low < 0.1 µmol m^{-2} s^{-1}) not to induce any significant variable fluorescence. The maximal fluorescence level (Fm) with all PS II reaction centers closed were determined by a 0.8 saturating pulse at 8000 µmol m^{-2} s^{-1} in dark adapted leaves [19]. Using light and dark fluorescence parameters, the maximal efficiency of PS II photochemistry in the dark adapted state, Fv/Fm = (Fm-Fo) / Fm [20] was calculated. Three measurements taken in the same leaf consisted of one replication, likewise three replications were taken and error degrees of freedom were calculated (Table 6).

Estimation of total carbohydrates in rice shoots and leaves

Leaf and stem tissues of the parents and backcross progenies were collected one day before flooding and 14 days after desubmergence and dried at 70ºC for 48 hrs and ground in a mortar and pestle (Table 7). Total carbohydrate contents of the above samples were estimated in replicates of three as described [21].

Carbohydrate estimation

About 100 mg of ground leaf/stem samples were taken into boiling test tubes (50ml). The hydrolysis was done by keeping the tubes in water bath for 3 hours with 5ml of 2.5 N HCl and cool down to room temperature. The hydrolyzed samples were neutralized with solid sodium carbonate until the effer**v**escence ceases. And the volume was made upto 100 ml using distilled water. 10 ml was taken from the sample solution and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected in a falcon tube and 0.1 ml and 0.2 ml aliquots were taken for analysis. Standards were prepared by taking working standard at different concentrations- 0 ml as blank, 0.2 ml, 0.4 ml. 0.6 ml, 0.8 ml and 1 ml in test tubes. Volume was made upto 1 ml using distilled water and 4 ml Anthrone reagent was added in each tube (The contents of all the tubes were cooled on ice before adding ice –cold Anthrone reagent). All the test tubes were heated for eight minutes in boiling water bath. Test tubes were rapidly cooled to room

temperature and the absorbance of standards and samples were measured using a spectrophotometer at 630 nm (Table 7).

Amount of carbohydrates present in 100 mg of leaf sample = $\frac{\text{mg of glucose}}{\text{Volume of test sample}}$ x 100 ml

RESULTS

Generation of advanced backcross progenies (BC_2F_1, BC_3F_1 and BC_4F_1)

Agronomically superior BC_1F_1 plants harboring Sub1 locus from FR13A and exhibiting background genome recovery (CO 43 genome) of >70% were used for further backcrossing with CO 43 to develop BC_2F_1 population.

Generation of BC_2F_1 generation

About 130 BC_2F_1 plants were raised under greenhouse conditions and genotyped using RM219 linked to *Sub1* locus. Foreground selection of BC_2F_1lines using RM219 and ART 5 resulted in the identification of 20 positive BC_2F_1 plants harboring *Sub1* locus from FR13A.These positive plants were subjected to BGS using 25 genome wide SSR markers. Background genotyping of these 21 BC_2F_1 plants resulted in the identification of 10 superior BC_2F_1 plants (# 15, 23, 26, 36, 55, 57, 61, 84, 91 and 108) possessing >80% of CO43 genome (Table.1). Three BC_2F_1 plants viz., # 57, 61 and 84 were found to possess >90% recurrent parent (CO 43) genome. Superior BC_2F_1 plants were back crossed with CO 43 to develop BC_3F_1.

Generation of BC_3F_1 generation

Totally twenty two BC_3F_1 plants were generated and raised under greenhouse conditions. All the plants were subjected to Foreground selection using RM219 and ART5 which resulted in the identification of 5 BC_3F_1 plants harboring *Sub1* locus from FR13A. These five positive BC_3F_1 plants were subjected to background selection using 37 SSR markers (12 new SSR markers in addition to the 25 SSRs used for genotyping of BC_2F_1 generation) covering all the 12 chromosomes. Background recovery analysis of all the 5 BC_3F_1 plants revealed that excepting one plant (# 5) all the other four BC_3F_1 plants had more than 83% background genome recovery. One of the BC_3F_1 plants (# 7) was found to have shown about 92% CO 43 genome recovery (Table 2). Superior BC_3F_1 plants were used for backcrossing to develop BC_4F_1 generation.

Generation and Evaluation of BC_4F_1 generation

A total number of 28 BC_4F_1 plants were raised under greenhouse conditions and subjected to FGS using RM219 and ART5 which resulted in the identification of 10 BC_4F_1 plants harboring *Sub1* locus from FR13A.These 10 positive BC_4F_1 plants were subjected to BGS using 37 - 40 SSRs (3 new SSR markers in addition to the 37 SSRs used in BGS of BC_3F_1 plants) covering all 12 chromosomes. Background genome recovery analysis revealed that all the 10 BC_4F_1 plants were found to possess more than 90% of CO 43 genome. Four BC_4F_1 plants were found to have 100% of CO 43 genome (Table.3).

Physiological evaluation of superior BC_2F_3 and BC_2F_4 lines

Plant height (cm)

Plant height ranged between 54-83 cm among the BC_2F_3 and BC_2F_4 individuals and their parents before flooding. Maximum plants were found to possess the plant height between 61-66 cm before flooding. Generally the plant height increased after flooding with FR13A recording a maximum height of 92.1cm followed by BC_2F_4 generation namely C22, C31 and C20 lines recording 90, 91.7 and 94cm respectively. The BC_2F_3 lines 23-62, 23-66, 61-19 and 61-24 showed shorter plants compared to BC_2F_4 lines. (Table.4)

Chlorophyll contents

The Chlorophyll contents (Chlorophyll a, Chlorophyll b and total chlorophyll) generally decreased after flooding irrespective of the parents and BC_2F_3 & BC_2F_4 (Table.5). The BC_2F_4 lines namely C22, C31 and C20 were able to retain their pigment composition and greenness during the recovery period. Total chlorophyll was 1.207, 1.037 and 1.198 mg/g in C22, C31, and C20 respectively. Chlorophyll a, chlorophyll b and total chlorophyll contents were much higher in the parent compared to the BC_2F_3 progenies after flooding.

Chlorophyll fluorescence (fv/fm)

FR13A recorded maximum chlorophyll fluorescence (fv/fm values) which reflects the efficiency of photosystem II .The BC_2F_3 progenies selected from the field namely 23-62, 61-24,61-19 recorded higher chlorophyll fluorescence values of 0.702 ,0.651, 0.641 respectively (Table.6).

Total carbohydrates

The total carbohydrate content (both stem and leaf carbohydrates) in the plants before submergence showed an increasing trend compared to the contents after submergence. In the submerged plants, the total carbohydrate content was found to be consumed very rapidly which resulted in the reduced level of

total carbohydrates. The consumption rate of total carbohydrates in the tolerant FR13A was found to be lower (Table.7). Though Co 43 recorded higher carbohydrate contents before flooding compared to the BC_2F_4 individuals, the entire carbohydrates were utilized during the period of submergence and hence could not tolerate the stress and were dead after 14 days of submergence.

After de-submergence, the tolerant FR13A plants were able to synthesize and accumulate carbohydrates. The BC_2F_3 and BC_2F_4 progenies also survived the stress by minimum utilization of carbohydrates under stress. The BC_2F_4 lines C22, C20 and BC_2F_3 lines 23-66 recorded 86.0, 87.0, 84.33 and 83.03 mg /g of stem carbohydrates after flooding. Similarly the leaf carbohydrates ranged from 80.0 to 85.0 mg/g in the BC_2F_3 individuals selected from the field during the recovery period.

DISCUSSION

The present study was aimed at developing submergence tolerant version of a popular rice variety of TN namely CO43 through marker assisted introgression of *Sub 1* locus from a submergence tolerant FR 13A. Efforts were made to continue the previous work done in our group. In the present study, BC_1F_1 generation was forwarded upto BC_4F_1 and selfed progenies of BC2 generations were phenotyped for submergence tolerance.

Two molecular markers linked to the *Sub 1* locus viz., one SSR namely RM219 and another InDel marker namely ART5 were found to be polymorphic between CO 43 and FR13A and used for Foreground Selection (FGS). Seventy six SSR markers covering all 12 chromosomes of rice genome were found to be polymorphic between CO43 and FR13A which were used in BGS. Advanced backcross progenies (BC_2F_1, BC_3F_1 and BC_4F_1) harboring *Sub1* locus (from FR13A) were generated by repeated backcrossing with the recurrent parent CO43. About 130 BC_2F_1 plants were raised under greenhouse conditions and genotyped using RM219 linked to *Sub1* locus.

Table 1. List of SSR markers used for background selection of BC_2F_1 generations

BC_2F_1 progenies	Total number of primers surveyed	No. of CO 43 alleles	No. of FR13A alleles	No. of Heterozygotes	% Recovery of Co 43 genome
12	25	17	0	8	68
13	25	17	0	8	68
23	**25**	**21**	**0**	**4**	**84**
26	**25**	**20**	**0**	**5**	**80**
31	25	16	0	9	64
36	**25**	**21**	**0**	**4**	**84**
40	25	15	1	9	60
43	25	18	0	7	72
55	**25**	**21**	**0**	**4**	**84**
57	**25**	**25**	**0**	**0**	**100**
61	**25**	**25**	**0**	**0**	**100**
84	**25**	**23**	**0**	**2**	**92**
91	**25**	**22**	**0**	**3**	**88**
96	25	14	0	11	56
108	**25**	**20**	**0**	**5**	**80**
121	25	18	0	7	72
125	25	14	1	4	56

After foreground selection and background selection using 25 genome wide SSR markers, BC_2F_1 plants having more than 85 – 90% recurrent parent genome recovery were identified. Three BC_2F_1 plants viz., # 57, 61 and 84 found to possess >90% recurrent parent (CO 43) genome Superior BC_2F_1 plants were back crossed with CO43 to develop BC_3F_1. Totally twenty two BC_3F_1 plants were generated and raised under greenhouse conditions. Foreground selection using RM219 and ART5 resulted in the identification of five positive plants which were subjected to BGS using 37 SSR markers (12 additional markers). Background Selection using 37 SSR markers resulted in the identification of one plant (# 7) to have about 92% CO43 genome. This plant was used to generate 28 BC_4F_1 plants. FGS using RM219 and ART5 resulted in the identification of 10 BC_4F_1 plants harboring *Sub1* locus from FR13A. These plants were subjected to BGS

using 37- 40 SSR markers lead to identification of four plants (# 9-2; 9-4; 9-5 and 0-7) to have about 100% CO3 genome. The effect of introgression of *Sub1* locus in terms of tolerance against submergence was studied in BC_2F_3 and BC_2F_4 progenies under simulated submergence conditions. Ten BC_2F_3 progenies were found to recover successfully on par with the tolerant parent FR13A by developing new leaves and tillers. Selected ten plants were then subjected to background selection with 29 SSR markers. Results revealed three plants (Line no # C20, C22 and C31) were found to have more than 90% of Co 43 genome. The seeds of these three lines (Line no # C20, C22 and C31) were selfed to generate BC_2F_4 progenies. Submergence tolerance screening of three BC_2F_4 progenies namely (C20, C22 and C31) and four superior progenies of BC_2F_3 selected from the field namely (23-66; 23-62; 61-24; 61-19) along with their parents revealed that all positive BC_2F_3 and BC_2F_4 progenies were found to be superior in terms of recovery after desubmergence. All the survived plants were evaluated for agronomic and physiological traits namely plant height, stem and leaf carbohydrates, chlorophyll and chlorophyll fluorescence before and after flooding. Superior progenies of CO43 improved for their submergence tolerance have been developed which will be further tested for their agronomic performance and efforts will be taken for release.

Table 2. List of SSR markers used for background selection of BC_3F_1 generation

BC_3F_1 progeny	Total primers surveyed	No. of CO43 allele	No. of FR13A allele	No. of heterozygotes	% Co43 genome recovery
5	37	28	5	4	75.7
6	37	31	0	6	83.7
7	37	34	2	1	91.8
9	37	32	1	4	86.5
18	37	32	1	4	86.5

Table 3. List of markers used for background selection of BC_4F_1 generation

BC_4F_1 progeny	Total No. of SSRs surveyed	No. of markers possessing homozygote CO 43 allele	No. of markers possessing heterozygote allele	% of CO 43 genome recovery
6-7	40	37	3	92.50
6-8	40	36	4	90
6-9	37	34	3	91.89
9-2	37	37	0	100
9-4	37	37	0	100
9-5	39	39	0	100
0-1	37	36	1	97.3
0-2	37	36	1	97.3
0-3	37	36	1	97.3
0-7	37	37	0	100

Table 4. Changes in Plant height (cm) in the BC_2F_3 and BC_2F_4 generations of CO43XFR13A before and after submergence

Plant height					
Genotypes	**BS**			**AS**	
FR13A	63.9	±	1.2	92.1	± 1.2
C043	54.8	±	0.9	-	
23-66	64.5	±	1.1	71.7	± 1.3
23-62	61.8	±	0.9	79.9	± 0.7
61-24	66.2	±	1.2	81.4	± 0.3
61-19	64.3	±	1.2	81.1	± 0.8
C22	65.5	±	1.2	70.0	± 0.8
C31	65.7	±	1.2	91.7	± 0.7
C20	83.8	±	1.8	94.0	± 1.0

BS: 0 day before submergence

AS: 14 day after submergence

Table.5 Changes in Chlorophyll content (mg/ g^{-1}) in the Backcross generations of BC_2F_3 & BC_2F_4 CO43XFR13A subjected to 13 days of submergence.

Genotype	Chlorophyll a		Chlorophyll b		Total Chlorophyll	
	BS	AS	BS	AS	BS	AS
FR13A	0.853 ± 0.064	0.703 ± 0.053	1.101 ± 0.083	0.644±0.049	1.938 ± 0.146	1.360 ± 0.103
CO43	1.116 ± 0.084	-	0.629 ± 0.047	-	1.765 ± 0.133	-
23-66	0.923 ± 0.070	0.331 ± 0.025	0.510 ± 0.039	0.286 ± 0.022	1.429 ± 0.108	1.133 ± 0.086
23-62	1.117± 0.084	0.361 ± 0.025	0.612 ± 0.046	0.311 ± 0.024	1.732 ± 0.131	0.941 ± 0.071
61-24	1.885 ± 0.142	0.420 ± 0.032	0.976 ± 0.074	0.39 ± 0.030	2.913 ± 0.220	1.120 ± 0.085
61-19	1.272 ± 0.096	0.184 ± 0.014	0.595 ± 0.045	0.05 ± 0.004	1.961 ± 0.148	0.450 ± 0.034
C22	1.235 ± 0.093	0.472 ± 0.036	0.594 ± 0.045	0.30 ± 0.023	1.955 ± 0.148	1.207 ± 0.091
C31	1.184 ± 0.089	0.393 ± 0.030	0.686 ± 0.052	0.35 ± 0.027	1.874 ± 0.141	1.037 ± 0.078
C20	0.977 ± 0.074	0.450 ± 0.034	0.548 ± 0.041	0.448 ± 0.034	1.547 ± 0.117	1.198 ± 0.090

BS: 1 day before submergence AS : 14 day after de-submergence

Table 6. Changes in the Chlorophyll fluorescence (Fv/Fm) in the BC_2F_3 and BC_2F_4 generations of CO43XFR13A subjected to 13 days of submergence

Chlorophyll Fluorescence		
Genotypes	**BS**	**AS**
FR13A	0.594 ± 0.03	0.644 ± 0.041
CO43	0.542 ± 0.03	-
23-66	0.411 ± 0.05	0.567 ± 0.03
23-62	0.402 ± 0.04	0.702 ± 0.05
61-24	0.496 ± 0.04	0.651 ± 0.01
61-19	0.542 ± 0.01	0.641 ± 0.01
C22	0.438 ± 0.07	0.538 ± 0.07
C31	0.484 ± 0.11	0.584 ± 0.11
C20	0.476 ± 0.03	0.553 ± 0.01

BS : 2 day before submergence AS : 14 days after submergence

Table.7 Changes in the Total Carbohydrate content (mg/g) in the BC_2F_3 and BC_2F_4 generations of CO43XFR13A subjected to 13 days of submergence

Genotype	Stem carbohydrates		Leaf carbohydrates	
	BS	AS	BS	AS
FR13A	128.33 ± 9.49	81.33 ± 0.88	117.67 ± 4.63	82.53 ± 1.43
CO43	112.6 ± 4.04	-	132.67 ± 5.04	-
23-66	147.67 ± 9.39	83.03 ± 2.88	135.00 ± 6.11	85.33 ± 2.33
23-62	104.6 2 ± 6.74	80.67 ± 1.20	128.67 ± 2.60	80.17 ± 0.60
61-24	140.67 ± 6.98	81.33 ± 2.85	133.00 ± 3.61	82.77 ± 0.96
61-19	96.00 ± 3.79	79.00 ± 1.73	126.67 ± 3.18	80.47 ± 1.18
C22	98.33 ± 4.98	86.67 ± 2.33	111.33 ± 2.40	77.83 ± 0.93
C31	110.67 ± 2.73	79.00 ± 2.65	80.67 ± 30.87	77.00 ± 1.32
C20	153.67 ± 0.88	84.33 ± 2.91	132.33 ± 6.64	79.57 ± 1.29

BS : 1 day before submergence; AS : 14 day after submergence

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