

Original Research Article
Evaluation of genotypes against water stress and salinity in Rice (*Oryza sativa* L.)

Abstract

Abiotic stresses poses significant challenges to growth, yield and productivity thus threatening food security. Hence, it is essential to develop climate resilient rice varieties that mitigate the impact of abiotic stress. A study on physiological characterization for water and salinity stress was conducted in twenty-eight rice genotypes during *kharif* 2023 at Regional Agricultural Research Station, Maruteru. Among all the treatments, root length, shoot length, root and shoot dry weight and chlorophyll content was maximum under control followed by 1% mannitol, 2% mannitol and salinity stress. Visual scoring for water stress at seedling stage revealed that under mild water stress (1% M) more than 90% of plants survived in genotypes viz., AC-35678, FL 478, Ravana, Rahaspunjar, AUS 63, AUS 73, Vandana, NICRA 16, NICRA 17, CR-3439-4-E-17-2-1-B-1-S-1, CR-4215-2-5-2-M-4-SUB-2-5-1. Under severe water stress (2% M) more than 70% of the seedlings survived in NICRA 16, CR-4215-2-5-2-M-4-SUB-2-5-1, CRAC-4423-3, IET -18727, IC-516 149, IET-18716, AC-35678, FL 478, AC 85, Rahaspunjar, AUS 100, NICRA 17, CR-3439-4-E-17-2-1-B-1-S-1. Under salinity stress the score 3 was recorded in FL 478, and 5 in Rahaspunjar, IET -18727, NICRA 16, Ravana, NICRA 17. For both combined stresses, FL 478, Rahaspunjar, NICRA 16 and NICRA 17 were identified as tolerant and can be identified as donors for development of rice varieties having climate resilience.

Keywords: Rice, Salinity, SES, Visual scoring, Water stress

Introduction

Rice is an important cereal crop and is widely cultivated around the globe. The present production of rice is not sufficient to meet the needs of the growing population. Besides biotic stresses and abiotic stresses pose a significant pressure which results in the yield penalty. Under the changing climate scenario, abiotic stresses have become a major concern and crops are expected to experience combination of stresses rather than challenged by a single abiotic stress [1].

Rice is mainly cultivated in tropical climate which is mainly affected by drought and salinity and these cause drastic reduction in yield and quality [2]. Rice is relatively tolerant to salinity during germination stage, but becomes very sensitive during the early seedling stage [3]. When seedlings are exposed to these abiotic stresses it leads to poor establishment of crop, reduction of leaf area as well as root to shoot length, reduction in biomass that may even cause death of the seedlings [4]. This makes seedling stage tolerance an essential trait for the abiotic stresses that enables a better establishment of the plant resulting in good vegetative growth and which in turns yields higher.

Water stress effect the rice production and productivity [5]. It affects germination and growth of the seedling as well as chloroplast development and reduces the leaf chlorophyll content [6]. Rice is basically a glycophytic plant and it is most susceptible to salinity among the cereal crops. It has been reported that salt stress reduced germination percentage that leads to reduction in the shoot length, root length and dry weights [7]. Plant physiological processes such as net photosynthetic rate and pigment composition such as chlorophyll content, anthocyanins and carotenoids are affected [8].

In view of appearance of more than one abiotic stress in fields simultaneously tolerance to multiple abiotic stresses especially at the seedling stage is imperative for breeding programmes. Under laboratory conditions studies on germination and seedling development have been accepted for testing the response to abiotic stresses. With this background, the major objective of the present study was to identify rice genotypes that can tolerate both water stress as well as salinity stress which can act as a donor for further crop improvement programmes.

Materials and methods

Plant material

Twenty-eight rice genotypes were tested for their multiple stress tolerance during *kharif* 2023 at Regional Agricultural Research Station, Maruteru (Table 1). The seeds of rice genotypes were surface sterilized with 70% ethanol solution for 5 min followed by thorough washing for two-three times with sterilized distilled water. Pre-germinated seeds of each variety were taken and placed in hydroponic setup in 4 sets with 3 replications. The experiment was laid down in CRD. The plants were initially grown in 2-3 days in fresh water. Later it was shifted to Hoagland's solution containing the essential macro and micro nutrients.

Imposition of stress

At 3-4 leaf stage, stress was imposed by addition of Mannitol to the solution for 1% and 2% imposing mild and severe water stress treatments respectively. The requirement of mannitol was calculated based on the quantity of the nutrient solution taken in the tray (~5 lit). For salinity stress, sodium chloride was added. An EC of 6 was maintained for first 3 days till the plants were acclimatised. The EC was increased to 12 and maintained till the end of the experiment. The nutrient solutions were changed and was replaced by fresh solution every 2-3 days by draining the old solution completely followed by rinsing three-four times with fresh solutions so as to avoid increased stress level due to Mannitol and sodium chloride. The pH of the solution was adjusted daily to 5.5.

Observations recorded

Visual scoring under mannitol and salinity stress was taken. Shoot length, root length, shoot dry weight, root dry weight and chlorophyll content were recorded at the end of the experiment. Shoot and roots were separated at the shoot-root junction and the length of both shoot and root was measured. Both were expressed in cm. The samples were oven dried for 48 h at 60°C and the weight was recorded as shoot and root dry weight and it was expressed in g.

Chlorophyll content in the samples were recorded at the end of the experiment. 25 mg of leaf sample was taken and placed in 80 % acetone [9]. Using a UV-VIS spectrophotometer, absorbance of chlorophyll a and chlorophyll b were measured at 663.2 nm and 646.8 nm

respectively and the chlorophyll content was expressed in mg g^{-1} fresh weight (mg g^{-1} FW). Chlorophyll a content, chlorophyll b content and the total chlorophyll content [10].

Statistical analysis

Two-way analysis of variance (ANOVA) was performed using Statistix 8.1 package. Statistical significance of the parameter means was determined by performing Fisher's LSD test to test the statistical significance.

Results and Discussion

Visual scoring under water and salinity stresses

Visual scoring for water stress at seedling stage revealed that under mild water stress (1% M) 11 genotypes viz., AC-35678, FL 478, Ravana, Rahaspunjar, AUS 63, AUS 73, Vandana, NICRA 16, NICRA 17, CR-3439-4-E-17-2-1-B-1-S-1 and CR-4215-2-5-2-M-4-SUB-2-5-1 recorded more than 90% of survival. Under severe water stress (2% M) almost more than 80% of the seedlings survived in 3 genotypes (NICRA 16, CR-4215-2-5-2-M-4-SUB-2-5-1 and CRAC-4423-3). 70%-80% survival under 2% Mannitol was noted in IET -18727, IC-516 149, IET-18716, AC-35678, FL 478, AC 85, Rahaspunjar, AUS 100, NICRA 17 and CR-3439-4-E-17-2-1-B-1-S-1 (Table 2 and 3). Under salinity stress the score 3 was recorded in FL 478, and 5 in Rahaspunjar, IET -18727, NICRA 16, Ravana and NICRA 17 (Table 4).

Shoot and Root length

Water stress and salinity stress resulted in reduction of shoot and root lengths in all the tested genotypes. Shoot length under control conditions varied from 12.4cm (AUS 131) to 16.7 cm (CRAC-4423-3). Under mild and severe water stress (1% M and 2% M), shoot length was maximum in CRAC-4423-3 (15.6 and 14.5 cm respectively) followed by IET -18727 (14.5 cm) and IET-18716 (14.4 cm) under 1% M and CR-4215-2-5-2-M-4-SUB-2-5-1 (13.8 cm) and NICRA 17 (13.1 cm) under 2% M treatment. AUS 131 recorded lowest shoot length under 1% M (10.8 cm) and 2% M (10.2 cm). Under salinity stress shoot length was maximum in FL 478 (13.1cm) followed by CR-4215-2-5-2-M-4-SUB-2-5-1 (12.7 cm) and CRAC-4423-3 (12.1 cm). Minimum shoot length under salinity was in IC-516 149 (9.3 cm) followed by Naveen (9.4 cm) (Table 5).

In control conditions the range of root length recorded was from 7.7 cm (CRAC-4423-3) to 4.8 cm (Rahaspunjar). Under 1% M and 2% M, CRAC-4423-3 (7.1 cm and 7.0 cm) followed by IET 18727 (6.9 cm and 6.4 cm) recorded maximum root length. Minimum was in CR-3439-4-E-17-2-1-B-1-S-1 (4.4 cm and 4.1 cm). Under salinity stress, FL 478 had maximum root length of 6.1 cm followed by CRAC-4423-3 (5.9 cm) and IET -18727 (5.4 cm) while minimum was in CR-3439-4-E-17-2-1-B-1-S-1 (3.4 cm) followed by IR 29 (3.8 cm) (Table 5).

Water stress when imposed at early seedling stage results in inhibition of the growth of rice seedlings reflected in the form of reduced length of shoot and root [11]. Water stress induced a decrease in root length by 3.4% [12]. In a study conducted a reduction of 22.7% and 8.9 % of mean shoot and root length respectively in the rice seedlings when exposed to water stress [13]. Imposition of water stress for 10 days in 5 rice genotypes resulted in mean reduction of shoot and root length by 18.71% and 35.39% respectively [14]. In this experiment also a significant reduction under mild and severe water stress was noted in the tested rice genotypes. The impact of salinity stress (120mM NaCl) was assessed in 35 rice genotypes at germination stage and reported a reduction in root and shoot length in all the tested cultivars [15]. With increase of salinity stress, reduction of root length and shoot length was observed in rice seedlings [16]. The above results are in agreement with the results in this study.

Shoot and Root Dry weight

Imposition of water stress and salinity resulted in reduction of shoot and root dry weight. The mean shoot dry weight reduced from 1.48 g in control to 1.40 g in 1% M, 1.33 g in 2% M and 1.24 g under salinity. Shoot weight varied from 1.63 g in IET -18727 to 1.27 g in AUS 131 in control conditions. Under 1% M and 2% M, NICRA 17 (1.55 g and 1.51 g) followed by CR-4215-2-5-2-M-4-SUB-2-5-1 (1.54 g and 1.50 g) recorded highest shoot weight. Lowest under 1% M was in AUS 131 (1.22 g) followed by Ravana and Morishal (1.28 g). Under 2% M in AUS 131 (1.14 g) followed by Morishal (1.16 g). Under salinity highest shoot weight was in FL 478 (1.45 g) followed by CR-4215-2-5-2-M-4-SUB-2-5-1 (1.44 g), CRAC-4423-3 (1.41 g) and NICRA 17 (1.40 g). Lowest was in AUS 131 (1.02 g) followed by AC-35678 (1.03 g) and Morishal (1.04 g) (Table 6)

Under control environment, root weight varied from 0.53 g (IC-516 366 and CRAC-4423-3) to 0.36 g (IR 29). Under mild and severe water stress (1% M and 2% M) genotypes CRAC-4423-3

(0.5 g and 0.45 g respectively), IC-516 366 (0.49 g and 0.44 g respectively) and NICRA 17 (0.45 g and 0.42 g respectively) had the maximum root weight. Minimum was in IR 29 (0.32 g) followed by NDGR 201 (0.33 g) under mild stress and in IC-516 149 (0.28 g) and IR 29 (0.28 g) each under severe water stress conditions. Naveen (0.41 g) followed by IC 516366 (0.40 g) and NICRA 17 (0.37 g) recorded maximum root weight under salinity conditions and minimum was in AUS 131 (0.24 g). (Table 6).

Reduction in the biomass is an immediate response when a plant faces stress conditions. Water stress induced a decrease in root dry weight by 26.5% in rice [12]. Reduction in dry weight was also evident in experiment conducted in rice [11]. With increase of salinity stress, reduction of root and dry weight of shoot was observed [16]. Similarly, reduction in biomass of rice seedlings was evident in an experiment conducted [17]. These findings are in tune with the results of this study where under stress both root and shoot biomass reduced in all the genotypes tested.

Chlorophyll content

Chlorophyll content reduced by 13.6% under 1% M, 21.7% under 2% M and 35.6% under salinity stress. The Chlorophyll content in control was highest in NICRA 16 (2.14 mg/g FW) and lowest was in NDR 9930111 (1.49 mg/g FW). Highest chlorophyll content under 1% M was in CR-4215-2-5-2-M-4-SUB-2-5-1 (1.68 mg/g FW) followed by NICRA 16 (1.66 mg/g FW), FL 478 (1.64 mg/g FW), IET- 18727 (1.63 mg/g FW) and NICRA 17 (1.61 mg/g FW). Similarly, under 2% M highest was in NICRA 17 (1.59 mg/g FW) followed by NICRA 16 (1.55 mg/g FW), FL478 (1.54 mg/g FW) and IET-18716 (1.51 mg/g FW). Highest chlorophyll content under salinity stress was noted in FL 478 (1.52 mg/g FW) followed by Ravana (1.33 mg/g FW), NICRA 17 and NICRA 16 (1.3 mg/g FW). Lowest was noted in IC-516 366 (0.27 mg/g FW) (Fig 1).

Chlorophyll is an important pigment responsible for photosynthetic activity in the plant. Any alteration in chlorophyll content leads to disruption of photosynthesis leading to reduced yields. When subjected to water stress a reduction in chlorophyll accumulation was substantially

declined in developing rice seedlings [18]. Similarly, in PEG induced rice seedlings noticed a significant reduction in chlorophyll content compared to control [6]. In this study also the chlorophyll content reduced by 13.6% under mild water stress and 21.7% under severe water stress. The main reason being that imposition of stress leads to production of reactive oxygen species that results in lipid peroxidation and damage to the chlorophyll pigment [19]. Besides an elevated activity of chlorophyllase enzyme leads to chlorophyll degradation and hinders biosynthesis of new pigment.

Salinity results in the damage to the photosynthetic apparatus leading to chlorophyll bleaching [20]. A reduction in chlorophyll content when rice seedlings were exposed to salinity stress at seedling stage [13]. The impact of salinity stress was studied on 12-d old rice seedling exposed to 150mM of NaCl and reported a reduction in both chlorophyll a and b content [21]. These results are in agreement with this study.

Conclusion

Abiotic stress in rice is a major constraint that restricts its yield potential. In the scenario of changing climate, it is imperative to identify genotypes that can tolerate multiple stresses at a time rather than a single stress. In view of this in this study genotypes FL 478, Rahaspunjar, NICRA 16 and NICRA 17 were identified tolerant to both water stress as well as salinity and can be identified as physiological donors in the breeding programmes.

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Table 1: List of genotypes used for phenotyping against water (mild and severe) and salinity stress conditions

S.no	Genotypes
1	IET -18727
2	IC-516 149
3	IET-18716
4	AC-35678
5	FL 478
6	IC-516 366
7	AC 85
8	Ravana
9	Morishal
10	AUS 131
11	Rahaspunjar
12	AUS 63
13	AUS 103
14	AUS 100
15	AUS 73
16	NICRA 16
17	Vandana
18	NICRA 17
19	CR-3439-4-E-17-2-1-B-1-S-1
20	CR-4215-2-5-2-M-4-SUB-2-5-1
21	NDR 9930111
22	NDGR 201
23	NAVEEN
24	CRAC-4423-3
25	CRAC-4423-14
26	IR 29
27	IET -18727
28	IC-516 149

Table 2: Visual scoring for water stress (1 % Mannitol) at seedling stage

Observation	Genotypes
Almost all plants dead	-
<25% survival	-
25-50% survival	-
51-80% survival	IET-18716, AUS 100, CRAC-4423-14
81-90% survival	IET -18727, IC-516 149, IC-516 366, AC 85, Morishal, AUS 131, AUS 103, NDR 9930111, NDGR 201, Naveen, CRAC-4423-3, IR 29
More than 90%	AC-35678, FL 478, Ravana, Rahaspunjar, AUS 63, AUS 73, Vandana, NICRA 16, NICRA 17, CR-3439-4-E-17-2-1-B-1-S-1, CR-4215-2-5-2-M-4-SUB-2-5-1

Table 3: Visual scoring for water stress (2 % Mannitol) at seedling stage

Observation	Genotypes
Almost all plants dead	AUS 103
<40% survival	Ravana, Morishal, AUS 131, AUS 63, NDGR 201
41%- 70% survival	IC-516 366, AUS 73, Vandana, NDR 9930111, Naveen, CRAC-4423-14, IR 29
70%-80% survival	IET -18727, IC-516 149, IET-18716, AC-35678, FL 478, AC 85, Rahaspunjar, AUS 100, NICRA 17, CR-3439-4-E-17-2-1-B-1-S-1
More than 80%	NICRA 16, CR-4215-2-5-2-M-4-SUB-2-5-1, CRAC-4423-3

Table 4: Modified standard evaluation score (SES) for salinity stress at seedling stage

Score	Observation	Tolerance	Genotypes
1	Normal growth, no leaf symptoms	Highly tolerant	-
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant	FL 478
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant	Rahaspunjar, IET -18727, NICRA 16, Ravana, NICRA 17
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible	Morishal, AUS 103
9	Almost all plants dead or dying	Highly Susceptible	IC-516 149, IET-18716, AC-35678, IC-516 366, AC 85, AUS 131, AUS 63, AUS 100, AUS 73, Vandana, CR-3439-4-E-17-2-1-B-1-S-1, CR-4215-2-5-2-M-4-SUB-2-5-1, NDR 9930111, NDGR 201, NAVEEN, CRAC-4423-3, CRAC-4423-14, IR 29

Table 5: Impact of water stress (1% M and 2% M) and salinity stress on shoot and root length in rice genotypes

Entry	Shoot length (cm)					Root length (cm)				
	C	1% M	2% M	NaCl	Mean	C	1% M	2% M	NaCl	Mean
IET -18727	15.3	14.5	11.6	10.4	15.3	7.1	6.9	6.4	5.4	7.1
IC-516 149	13.5	11.3	10.6	9.3	13.5	6.7	5.2	4.6	4.0	6.7
IET-18716	15.6	14.4	12.1	11.4	15.6	7.5	6.1	5.8	5.1	7.5
AC-35678	14.3	12.5	12.2	11.5	14.3	6.4	5.4	4.6	4.2	6.4
FL 478	14.0	13.4	13.0	13.1	14.0	6.7	6.1	6.0	6.1	6.7
IC-516 366	14.2	13.2	12.3	12.0	14.2	6.1	5.6	5.1	4.5	6.1
AC 85	12.6	12.3	12.1	11.0	12.6	5.7	5.1	4.7	4.1	5.7
Ravana	15.2	14.1	11.8	11.6	15.2	6.9	5.8	5.5	5.1	6.9
Morishal	12.9	11.7	11.1	10.4	12.9	6.0	5.3	5.1	4.8	6.0
AUS 131	12.4	10.8	10.2	9.9	12.4	5.7	5.0	4.6	4.3	5.7
Rahaspunjar	12.8	12.1	11.3	10.4	12.8	4.8	4.5	4.2	4.0	4.8
AUS 63	14.4	13.9	10.8	10.0	14.4	6.4	5.6	5.2	4.7	6.4
AUS 103	13.5	11.9	10.5	9.7	13.5	5.3	4.6	4.4	4.0	5.3
AUS 100	14.5	13.8	12.9	11.5	14.5	6.5	5.4	4.9	4.4	6.5
AUS 73	13.2	12.4	11.2	10.7	13.2	5.3	4.9	4.4	4.0	5.3
NICRA 16	14.5	13.9	11.9	10.0	14.5	6.4	5.7	5.0	4.8	6.4
Vandana	14.2	13.6	12.5	10.9	14.2	6.1	5.5	4.9	4.4	6.1
NICRA 17	13.4	13.2	13.1	11.6	13.4	5.6	5.4	5.3	4.6	5.6
CR-3439-4-E-17-2-1-B-1-S-1	14.4	13.1	12.8	10.5	14.4	5.5	4.4	4.1	3.4	5.5
CR-4215-2-5-2-M-4-SUB-2-5-1	14.2	14.0	13.8	12.7	14.2	5.7	5.5	5.1	4.5	5.7
NDR 9930111	13.6	11.6	10.9	10.3	13.6	5.2	4.9	4.4	3.9	5.2
NDGR 201	12.6	11.5	10.8	10.2	12.6	6.0	5.4	4.9	4.0	6.0
NAVEEN	13.3	11.2	10.6	9.4	13.3	6.7	5.2	4.6	4.0	6.7
CRAC-4423-3	16.7	15.6	14.5	12.1	16.7	7.7	7.1	7.0	5.9	7.7
CRAC-4423-14	13.3	12.2	11.0	9.8	13.3	5.4	5.0	4.6	4.1	5.4
IR 29	14.2	12.8	13.0	11.1	14.2	5.4	4.7	4.4	3.8	5.4
Mean	13.9	12.8	11.8	10.8		6.1	5.3	5.0	4.4	
LSD (T)			0.27					0.11		
LSD (V)			0.71					0.31		
LSD (TxV)			1.43					0.62		
CV (%)			7.7					8.0		

Table 6: Impact of water stress (1% M and 2% M) and salinity stress on shoot and root dry weight in rice genotypes

Entry	Shoot dry weight (g)					Root dry weight (g)				
	C	1% M	2% M	NaCl	Mean	C	1% M	2% M	NaCl	Mean
IET -18727	1.63	1.48	1.30	1.27	1.63	0.52	0.40	0.36	0.30	0.52
IC-516 149	1.54	1.44	1.40	1.24	1.54	0.43	0.34	0.28	0.25	0.43
IET-18716	1.41	1.31	1.27	1.20	1.41	0.49	0.43	0.38	0.36	0.49
AC-35678	1.50	1.29	1.18	1.03	1.50	0.47	0.44	0.38	0.24	0.47
FL 478	1.53	1.43	1.37	1.45	1.53	0.46	0.45	0.41	0.41	0.46
IC-516 366	1.55	1.49	1.45	1.40	1.55	0.53	0.49	0.44	0.40	0.53
AC 85	1.47	1.44	1.39	1.27	1.47	0.51	0.42	0.40	0.35	0.51
Ravana	1.39	1.28	1.27	1.21	1.39	0.41	0.36	0.32	0.26	0.41
Morishal	1.32	1.28	1.16	1.04	1.32	0.46	0.43	0.38	0.34	0.46
AUS 131	1.27	1.22	1.14	1.02	1.27	0.38	0.34	0.31	0.27	0.38
Rahaspunjar	1.51	1.43	1.41	1.25	1.51	0.41	0.37	0.35	0.25	0.41
AUS 63	1.52	1.48	1.28	1.20	1.52	0.46	0.40	0.36	0.25	0.46
AUS 103	1.48	1.39	1.34	1.22	1.48	0.42	0.36	0.30	0.26	0.42
AUS 100	1.49	1.43	1.34	1.31	1.49	0.50	0.44	0.40	0.35	0.50
AUS 73	1.48	1.39	1.33	1.21	1.48	0.41	0.37	0.32	0.25	0.41
NICRA 16	1.48	1.42	1.22	1.13	1.48	0.46	0.40	0.36	0.25	0.46
Vandana	1.44	1.32	1.27	1.24	1.44	0.41	0.37	0.34	0.29	0.41
NICRA 17	1.59	1.55	1.51	1.40	1.59	0.48	0.45	0.42	0.36	0.48
CR-3439-4-E-17-2-1-B-1-S-1	1.38	1.31	1.27	1.22	1.38	0.39	0.35	0.30	0.25	0.39
CR-4215-2-5-2-M-4-SUB-2-5-1	1.56	1.54	1.50	1.44	1.56	0.42	0.38	0.32	0.26	0.42
NDR 9930111	1.44	1.35	1.30	1.25	1.44	0.39	0.35	0.31	0.26	0.39
NDGR 201	1.40	1.32	1.27	1.21	1.40	0.37	0.33	0.29	0.27	0.37
NAVEEN	1.54	1.46	1.40	1.24	1.54	0.43	0.34	0.29	0.25	0.43
CRAC-4423-3	1.58	1.50	1.44	1.41	1.58	0.53	0.50	0.45	0.37	0.53
CRAC-4423-14	1.50	1.41	1.38	1.30	1.50	0.45	0.40	0.34	0.28	0.45
IR 29	1.39	1.32	1.29	1.19	1.39	0.36	0.32	0.28	0.25	0.36
Mean	1.48	1.40	1.33	1.24	1.48	0.44	0.39	0.35	0.29	0.44
LSD (T)			0.021					0.012		
LSD (V)			0.056					0.034		
LSD (TxV)			0.112					0.068		
CV (%)			5.5					12.2		

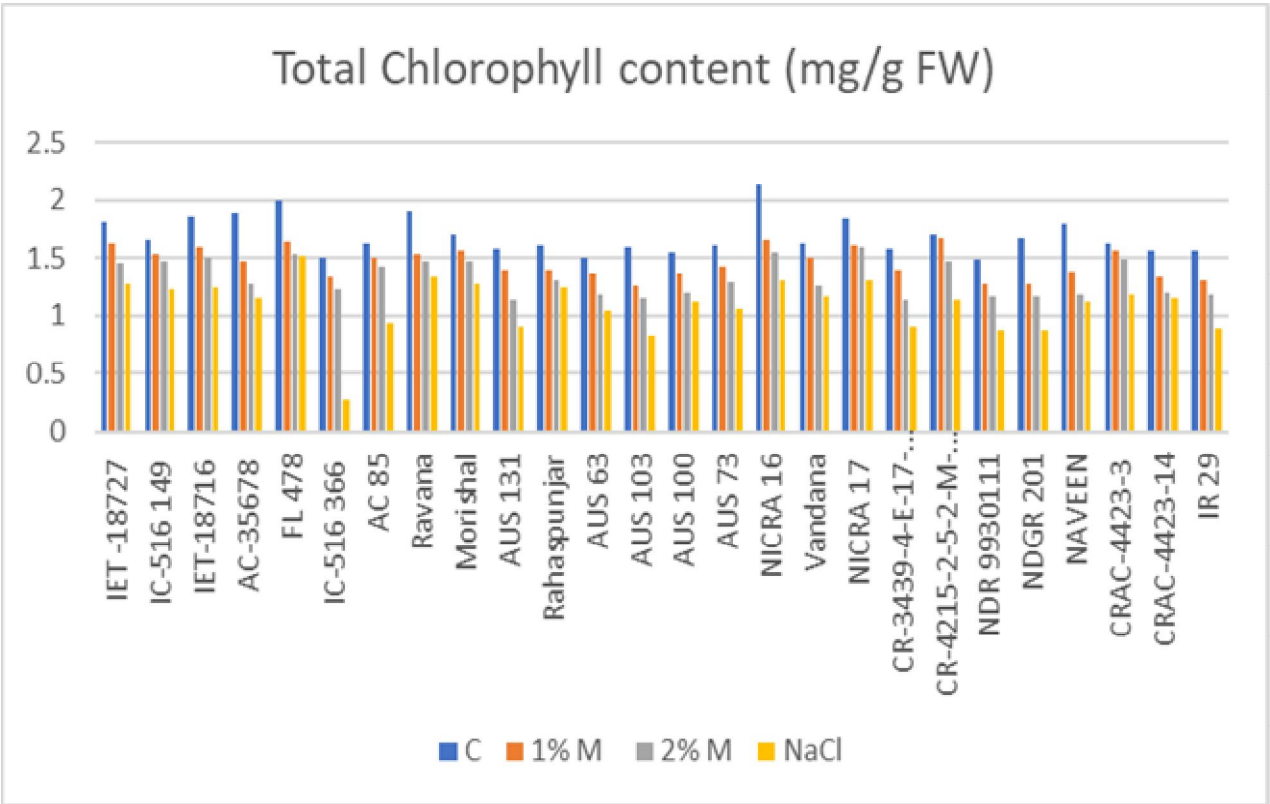


Fig 1: Impact of water stress (1% M and 2% M) and salinity stress on total chlorophyll content (mg/g FW) of rice genotypes.

UNDER PUBLICATION