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ORIGINAL ARTICLE

Physio-Biochemical assessment of Varied Rice *(Oryza sativa* L.) Genotypes Under Salinity Stress at Reproductive Stage

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ABSTRACT
This work has been done to study the relationship between various physio-biochemical traits in paddy under salinity stress at reproductive stage. Total of 150 rice genotypes including 10 checks was studied under non-stress and saline stress in micro plots. The experiment was formulated in Randomized Block Design (RBD) with two replicates. It was observed that yield declined in genotypes which have reduced chlorophyll content, lower proline content, lower sugar accumulation, lesser relative water content, decreased root-shoot fresh and dry weight. Characterization and grouping of various genotypes under this study grouped under four major category (T-Tolerant, MT-Moderately tolerant, S-Sensitive and VS-Very sensitive) on the basis of physiological and biochemical traits. Hence, tolerant genotypes the outcome of these findings could be useful in breeding programme to develop salt tolerant rice varieties. These lines could be used as donor to improve salinity tolerance in paddy and furthermore used for commercial cultivation.
Key Words*: Physio-Biochemical Traits, Salinity, Rice (ORYZA SATIVA L.), Reproductive Stage.*

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INTRODUCTION

Salinity stress decreases growth, yield and development of agricultural crops reported by many [1-4]. Screening of the genotypes under salt stress on the basis of Physio-biochemical parameters would assist in classifying the salt tolerant genotypes for future breeding program to develop tolerant rice varieties. Hence, objective of this study was to find the genotypes which could work as a reliable indicator of salt stress and eradicate the problem of crop productivity loss under stress at reproductive stage. Several physio-biochemical processes such as solutes accumulation viz., proline [5-7] soluble sugars [8][9] within a plant undergoes to compete with the stress. Apart from this exclusion of ions such as Na^+ [10][11] prevents the toxicity caused by salt stress [12]. Moreover, by maintaining the desired potassium concentration in the cell is essential for undergoing metabolic activities under stress [13] Furthermore, it is undeniable that calcium plays prominent role in stress [14].Rice is adversely affected under salinity stress [15] at vegetative, reproductive stage and seedling stage reported by [16-17]. Whilst, this study focused on reproductive stage salinity tolerance of rice genotypes

MATERIAL AND METHODS

Plant Materials and experimental design

150 genotypes of rice (*O. sativa)* were developed at CSSRI Karnal used to assess for their responses to salinity stress under reproductive stage along with 10 checks in normal irrigated and saline stress. The experiment was conducted at Central Soil Salinity Research Institute (CSSRI), Karnal (Latitude: 29° 43' N, Longitude: 76° 58' N).

Phenotyping of rice genotypes for salinity tolerance at reproductive stage

The research study was laid down in randomized block plan with 2 replicate. Each plant genotype was sown in one row of 2 m with 15cm spacing. Healthy viable seeds were sterilized using sterilization of hypochlorite solution. The rice genotypes were grown in (pH~8) and evaluated for salinity tolerance at the reproductive stage in micro plot (6m X 3m).

Statistical Analysis

Pearson correlation coefficient has been done with IBM-SPSS Software package v 16.0 for windows, Graph pad prism and DARwin for unweighted neighbor joining.

Determination of chlorophyll content

Chlorophyll pigments were determined using DMSO method (Hiscox *et al.,* [18].Leaf samples of similar sizes were cut into pieces, Homogenized with (5ml) of reagent and centrifuged at10, 000rpm. The pellets were resuspended in acetone than heated in water bath at 65̊ C (30min) for complete extraction of chlorophyll pigments. Absorbance of supernatant was recorded at 663 and 645 nm on spectrophotometer and calculated by (Arnon equation, 1949).

Determination of ions and sugar

0.01g of sample (leaf or stem) was put in closed vessel (_conical flask of 50ml volume) which contains 3 parts nitric acid and one parts perchloric acid and kept this (sample+diacid mixture in volumetric flask) overnight. And keep this flask on hot plate after 12h and samples were boiled till the transparency reached. After the complete drying of the samples the volumetric flask kept for cooling and filtered using Whatman

Paper 1 and reading were taken in flame photometer instrument after making final volume up to 100ml. The digested samples (diacid mixture) were used for calcium and magnesium determination by mixing 1ml of digested sample solution with 1ml of (1% Lanthanum) with 8ml of water was used for the determination of both the ions and readings were taken in Atomic adsorption spectrophotometer (AAS).

Soluble Sugar Estimation

100mg of leaf sample was taken and crushed with pestle and mortar than add (5ml) of ethanol and kept it to evaporate till dryness and further add 10ml of distilled water. The aliquot was later on taken for soluble sugar estimation in which absorbance taken at 625nm in spectrophotometer (Yemm, E. and Wills [19]).

Proline Content

Proline in leaf samples was estimated (spectrophotometer) by the method Bates *et al.,* [20]. 100mg of leaf sample were used and crushed with 5ml of 3% (SSA, Sulphosalicylic acid) in pestle and mortar and centrifuge for 15min at (5000rpm) and 2 ml of supernatant were taken in test tube and add 2ml of acetic acid, 2ml of ninhydrin reagent and 2ml glacial acid was added. Than keep the test tubes in water bath at 100̊ C (1hour).Add 4ml of toluene to this reaction mixture and absorbance was taken at 520nm and measured the red color intensity.

Hydrogen Peroxide (Alexieva *et al.,* [21])

Take 0.5g of tissue (ice cold) and crush it in pestle and mortar in 2.5ml of 0.1% of tri-chloro- acetic acid and centrifuged at 10000 rpm at 15min, keep the supernatant in dark room and after 1hour mix with buffer and potassium iodide (0.5ml: 1ml). Then take the absorbance at 390nm.

Relative water content (Barr and weatherley, [22])

$$\text{RWC \%} = \frac{\text{FW (Fresh weight)} - \text{DW (Dry weight)}}{\text{TW (Turgid weight)} - \text{DW (Dry weight)}}$$

RESULTS

CHLOROPHYLL

Sodium ions hinder the photosynthetic activity in rice by affecting the chlorophyll pigments both at seedling and reproductive stage especially in sensitive cultivars [16]. Chloroplast is the primary organelles which hides the light capturing chlorophyll pigments (both chl a and chl b). The chlorophyll a under normal and stress environment was found to be significantly high in tolerant and moderately tolerant groups as compared with the other groups and similar results were shown by the chlorophyll b (Fig 1). The chlorophyll b was maximum under stress in these genotypes designated as, CSR-2711-140, CSR-2711-177, CSR-1148-157, CSR-2711-81, and CSR-2748-4441-107. The chlorophyll a was maximum in the following genotypes CSR-2748-100, CSR-2711-183, CSR-2748-4441-104, CSR-2711-5 and CSR-2748-49. While the sensitive genotypes along with sensitive checks IR29 and PUSA 44 showed a dramatic decrease in both the chlorophyll pigment under stress.

PROLINE

Significant variation among the 150 genotypes was observed,CSR-KR-50(Tolerant), CSR-2711-178(Tolerant),CSR-2711-177(Moderatelytolerant), CSR-2711-171(Moderately tolerant) showed high proline content along with the tolerant checks CSR36 and CSR43.The high proline content in tolerant genotypes and moderately tolerant might be accumulated to contribute towards osmotic adjustment.

RELATIVE WATER CONTENT

The relative water content decreased with the increasing salinity in almost all the genotypes but the %reduction in the sensitive genotypes were found to be highest among the genotypes under study. CSR-2748-206 (Moderately tolerant), CSR-2748-57 (Tolerant), CSR-1148-102 (Tolerant), CSR-2711-178 (Tolerant) showed high relative water content along with the tolerant check CSR36 under saline stress.

HYDROGEN PEROXIDE

The sensitive and very sensitive cultivars CSR-KR-4, CSR-C27SM-83, CSR-CPB-8, CSR-TPB-116 and CSR-C27SM-103 along with the sensitive check PUSA44 showed maximum hydrogen peroxide accumulation under salinity stress. Whilst, the lower amount were found in tolerant genotypes CSR-C27SM-62, CSR-TPB-69, CSR-KR-79, CSR-1148-28 and CSR-1148-177 with tolerant check CSR27.

IONIC CONTENT

The sodium ions under normal condition was found to be lesser in concentration in leaves and stem but it was significantly increased in all the genotypes under stress but most prominent were evident in case of sensitive cultivars CSR-C27SM-67, CSR-TPB-238 , CSR-C27SM-123, CSR-TPB-1, CSR-TPB-215 along with sensitive check Swarna. Unlike sodium ions, potassium was found to be increased in only tolerant and moderately tolerant genotypes CSR-1148-31, CSR-CPB-39, CSR-1148-130, CSR-2748-136 and CSR-KR-49 under salt stress. While calcium and magnesium ions were found to be decreased in almost all the rice genotypes under stress except a few tolerant genotypes. These ions were found higher in concentration among those genotypes in which Na^+/k^+ ratio were lower. Hence, might play a role in the maintenance of Na^+/k^+ homeostasis in plant to cope with salinity stress. The maximum, minimum and standard error values for each trait observed under salinity are shown in the table 2 and dendrogram (fig2) showing the clustering and grouping of rice genotypes under four groups (T-Tolerant CSR-1148-31, CSR-2711-176, CSR-2711-178, CSR-2711-164, CSR-2711-78, CSR-1148-130, CSR-C27SM-22,CSR-CPB-39, CSR-2711-186, CSR-2748-136, CSR-2748-146 and CSR-2748-57 with tolerant check CSR36, MT-Moderately tolerant CSR-KR-16, CSR-KR-44, CSR-KR-56, CSR-KR-62, CSR-KR-79, CSR-KR-90, CSR-KR-109, CSR-KR-190, CSR-1148-172, CSR-1148-75, CSR-1148-157, CSR-1148-177, CSR-1148-122, CSR-1148-32, S-Sensitive CSR-KR-152, CSR-1148-5, CSR-1148-25, CSR-2748-58, CSR-C27SM-49, CSR-C27SM-52, CSR-C27SM-54, CSR-C27SM-55, CSR-C27SM-19, CSR-C27SM-27 and CSR-C27SM-153 with sensitive check PUSA44,VS-Very sensitive CSR-KR-2, CSR-KR-4, CSR-KR-5, CSR-KR-6, CSR-KR-27, CSR-KR-40, CSR-KR-46, CSR-KR-54, CSR-KR-75, CSR-KR-86, CSR-KR-100) on the basis of various traits under study.

Table1: Pearson correlation coefficient showing the relationship of various traits under study for 150 rice genotypes under salt stress.

TRAITS	SS	Pro	Na+/k+	Ca^{2+}/Mg^{2+}	Fr.Wt	D.Wt	H_2O_2	RWC	Cl^-
SS	1	-.569**	.206**	-.476**	-.560**	0.072	.679**	-.578**	.701**
Pro		1	-.209**	.455**	.397**	-.317**	-.392**	.479**	-.665**
Na+/k+			1	-0.105	-.241**	0.059	.289**	-.375**	.368**
Ca^{2+}/Mg^{2+}				1	.367**	-0.057	-.346**	.366**	-.482**
Fr.Wt					1	-0.054	-.493**	.576**	-.657**
D.Wt						1	0.037	-.189*	.296**
H_2O_2							1	-.515**	.516**
RWC								1	-.747**
Cl^-									1

**. Correlation is significant at the 0.01 level

*. Correlation is significant at the

Note: SS-soluble sugars (µmol/g), Pro-Proline (mg/g FW), Na^+-sodium (mmol/g), K^+ -Potassium (mmol/g),Ca2+-calcium(mmol/g), Mg2+ - magnesium(mmol/g), Fr.Wt-Fresh weight, D.Wt, H_2O_2 (µmol/g), RWC-Relative water content(%),Cl-(chloride)

Table2: Descriptive statistics of various traits under study

Traits	Range	Minimum	Maximum	Mean	Std.Error	Std. Deviation
SS	17.6	0.52	18.12	6.5908	0.39711	5.02313
Pro	0.62	0.04	0.66	0.2427	0.01119	0.14148
Na+/k+	14.41	0.49	14.9	1.0545	0.10404	1.31604
Ca^{2+}/Mg^{2+}	78.98	2.23	81.21	20.1678	1.12673	14.25209
Fr.Wt	8.58	1.2	9.78	4.251	0.14513	1.83573
D.Wt	9.9	0	9.9	2.2169	0.09414	1.19077
H_2O_2	1.66	0.09	1.76	0.5413	0.03095	0.39151
RWC	90.16	9.8	99.96	51.2338	1.83258	23.18048
Cl^-	6.18	0.58	6.75	4.2166	0.1306	1.65197

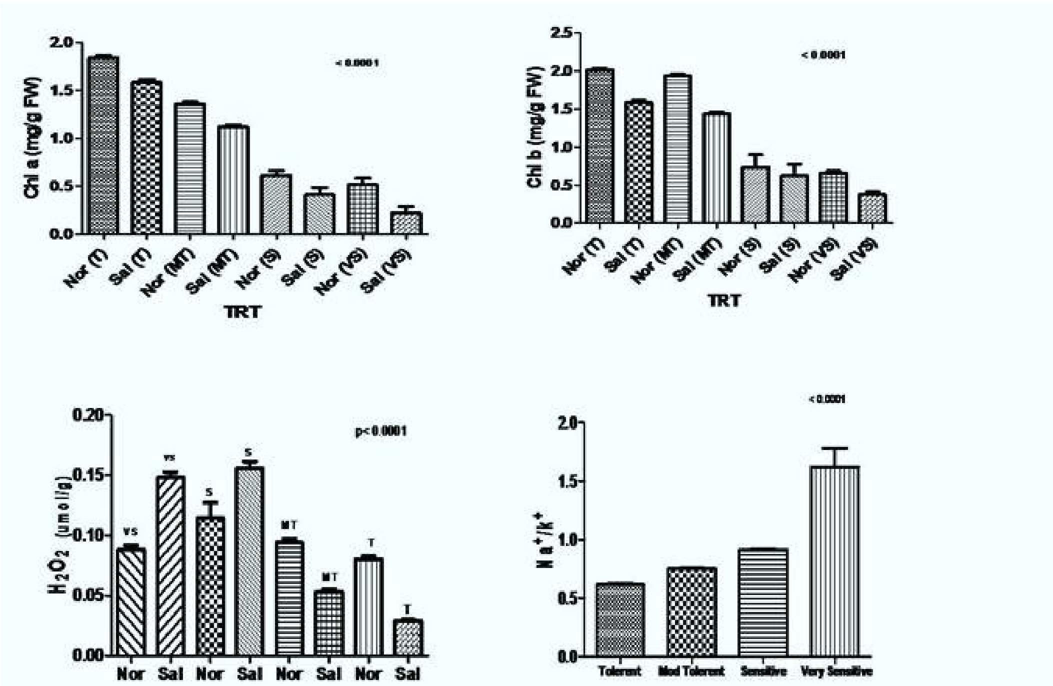

Fig1: Effect of Nacl on Chlorophyll a (chl a (mg/g FW), Chlorophyll b (chl b (mg/g FW), H_2O_2 content (µmole/g) and Na^+/k^+ ratio.

DISCUSSION

Reproductive stage is very sensitive stage under salinity [23-24].Due to salinity stress decrease in the chlorophyll pigments(both chla and chlb) in sensitive cultivars are apparent as shown in fig1 which in turn affects the photosynthetic rate also reported earlier by [25]. Our findings shows that the sodium accretion are decreased in tolerant genotype at Reproductive Stage., whilst prominent increase in potassium ions was significant for maintaining the homeostasis and proper functioning of the metabolism, hence showed decline in Na^+/K^+ ratio (fig 1),the results are in accordance with [26-27]. Sensitive and very sensitive genotypes showed significant increase in Na^+/K^+ ratio than the tolerant genotypes (fig 1) mainly due to higher Na^+ accumulation and these results are in accordance with [28]. It has been well established that salt stress at the reproductive stage has substantial effect on proline in sensitive cultivars [25]. The genotypes under sensitive and very sensitive category in this study also showed the similar pattern. The relative water content is negatively correlated with Na^+/K^+ (table 1) similarly observed by [30] as well as with H_2O_2 content which is harmful for cell integrity as it is oxidizing compound causes damages to the cellular parts [33]. The Pierson correlation coefficients (table 1) showed a negative correlation between $Ca^{2+}$and$Mg2^+$ with total soluble sugars and the decrease in calcium and magnesium ions similar with the result of [31] particularly in sensitive cultivars and lesser amount of chloride found in tolerant genotypes. All these results suggest that influx and out flux of ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-) in various genotypes might be required for proper maintenance of ionic homeostasis especially in tolerant and moderately tolerant genotypes by imposing restricting to the entry of sodium [32]as well as chloride and permitting the entry of potassium and calcium. Hence tolerant genotypes shows lower Na^+/K^+ ratio. The shoot fresh weight and dry weight (table1) decreased in all the rice genotypes evident with the findings of [34] and shoot fresh weight (Fr.Wt) is positively correlated with relative water content.

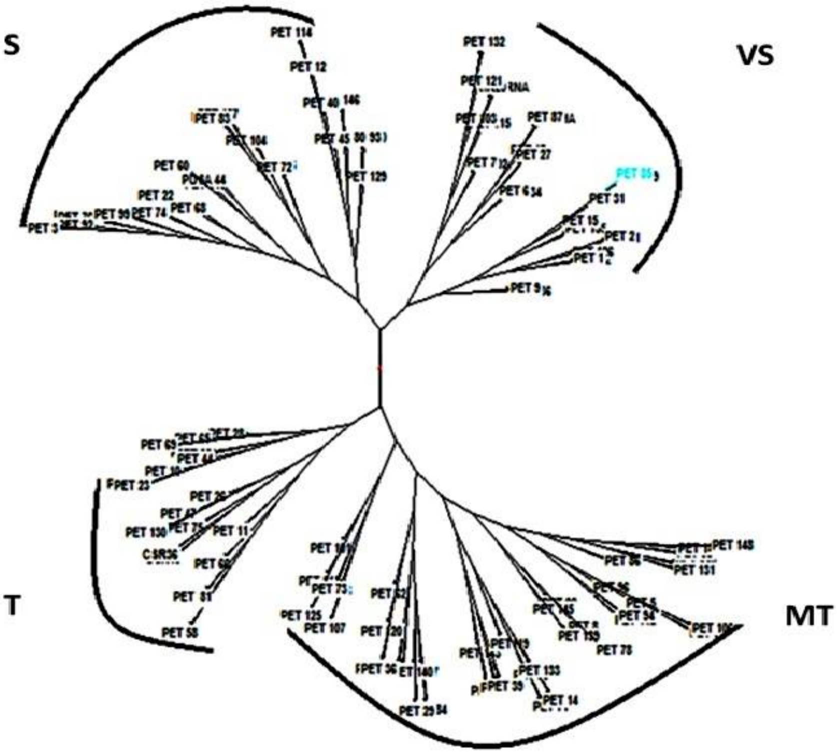

Fig2: Dendrogram prepared using DARwin software showing grouping of rice genotypes under study into four major groups T-Tolerant, MT-Moderately tolerant, S-Sensitive and VS-Very sensitive.

CONCLUSION

In conclusion,the present study has been done in microplot in which various rice genotypes were characterized into four major groups (T-Tolerant, MT-Moderately tolerant, S-Sensitive and VS-Very sensitive) on the basis of various physiological and biochemical traits and among these four groups the tolerant genotypes showed lesser damages to the above mentioned traits.Hence, these salt tolerant lines could be further used in field trials and later on used as a powerful tool for commercial cultivation of rice cultivars that are resistant to salt stress.

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