Plant Archives Vol. 26, No. 2, 2026 pp. 1130-1136 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.2.153

PHYSIOLOGICAL AND BIOCHEMICAL TRAIT SCREENING OF RICE (*Oryza sativa* L.) GENOTYPES AT SALINE FIELD CONDITION

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(Date of Receiving-31-05-2026; Date of Revision-15-08-2026; Date of Revision-28-08-2026)

ABSTRACT

Rice crop grown highly in peninsular India which is inundated with salt water from oceans during extreme environmental conditions. The present investigation deals with tolerance of rice genotypes grown under saline lands in coastal areas. The selected genotypes previously screened hydroponically both seedling and reproductive stages. At field condition under high salinity stress photosynthetic, biochemical and yield characters were positively and Na/K ratio and reproductive score positively correlated with each other and negatively correlated with Na/K ratio and reproductive score. In the PCA and cluster analysis revealed that CSR27, AC39416A, considered highly tolerant among tolerant genotypes.

***Key words*:** Physiological, Biochemical, Screening, Saline field, Rice genotypes

Introduction

Climate change and increasing temperature due to global warming leads to high evapotranspiration causes salt remains on the surface of soil mainly because of evaporation mostly in arid soils increasing the soil salinity (Khamidov *et al.*, 2022). High tidal flooding causes Inundation of brackish water with high salt content of soluble salt ions mainly Na^+ and Cl^- ions contaminate soil and water sources nearby coastal areas. Saline areas categorised into non saline (0-1.9 dS/m), slightly saline (2-4 dS/m), moderately saline (4.1 to 8 dS/m), saline (8.1 to 16) and highly saline >16 dS/m) (Rahman *et al.*, 2011). High salinity causes hyper ionic salt stress leads to oxidative stress to plant produce reactive oxygen species (ROS) that effects metabolism both anabolic activities ie photosynthesis and catabolic activities ie respiration through damaging membrane system and interfering electron transporting chains (Ullah *et al.*, 2021). Significant reduction in plant growth, tiller number, biomass accumulation, spikelet per panicle, no of panicles, grain filling percentage, grain yield under high saline conditions. Stomatal closure is a type physiological adaptation of rice plants to preserve cellular dehydration by reducing transpiration rate leads to reduction in photosynthetic activity and also maintain homeostasis by ion exclusion and compartmentalization of toxic ions in addition where as proline maintains osmotic adjustment. In addition salinity triggers antioxidant enzymes *viz* CAT and SOD to defend against the activity of reactive oxygen species and nullify its effect on crop yield (Xu *et al.*, 2024). The method of evaluation of tolerant genotypes under saline field conditions were more realistic than laboratory conditions where soil heterogeneity and other biotic and abiotic factors that effect the plant growth and yield (Krishnamurthy *et al.*, 2016). In addition to morphological and yield parameters biochemical parameters including Na/K ratio, Proline, CAT, SOD and physiological parameters including Stomatal conductance, transpiration rate and photosynthetic rate were investigated in this experiment. The findings of the study for identification

of donar plants for future breeding programs

Materials and methods

Plant materials

Ten genotypes seeds of rice (FL478, CSR27, MCM147-1-1-2-1, MCM253-8-2-2, MCM151-3-2-1-1, IR18T1045, MCM208-12-1-1, IR100120-B-B-B-11, MCM159-3-1-1-1 and AC39416A were selected from reproductively tolerant genotypes which were screened with the procedure given by (Singh *et al.*, 2021) and evaluated under Randomized block design with three replication at saline field conditions from Fig. 1. The Ec of the field range from 10.32 to 15.28 and pH range from 8.11 to 8.4 during crop growth period at ARS Machilipatnam, Rudravaram region. The parameters were recorded *viz* Proline content was estimated by the modified procedure of (Bates *et al.*, 1973). Approximately 0.5 g fresh weight of leaf tis sues was homogenized using 10mL of 3% aqueoussulfosalicylic acid. 2 mL of extract was used in a reaction with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid and boiled at 100 C for 1 h. The reaction was quenched by putting the tubes rapidly on ice. The resulting solutions were extracted with toluene, and the absorbance of the toluene fraction was measured at 520 nm. Proline concentration was estimated with reference to calibration curve and expressed µ g^{-1} fresh weight. The activity of superoxide dismuatse (SOD) was assayed using slightly modified procedure (Madamanchi *et al.*, 1994); 1g of fresh and cleaned leaf sample was homogenized in 10 mL of 50mMM phosphate buffer with pH(7.8). and prepare 3ml of cocktail solution contains 50 µL of enzyme extract, 0.3 mL of NBT solution, 0.39 mL of Methionine , 0.06 mL of EDTA solution. 0.0006 mL of riboflavin solution was added just before illuminating reaction mixture and Preparation without enzyme extract and NBT serve as a blank to calibrate the spectrophotometer then read absorbance (OD) at 560 nm and the activity was calculated by using the following formula

SOD activity: (Maximum absorbance – Minimum absorbance) × 60 × 2

Catalase (CAT) activity was assayed by measuring the rate of disappearance of H2O2 using the method of (Luck, 1974). The reaction mixture contained 2 mL of 50mM buffer solution, 0.95 mL of 0.3 % hydrogen peroxide and 0.1 mL of enzyme extract were added to a cuvette and the contents were mixed well and placed in a spectrophotometer to record the absorbance using kinetics method at 240 nm and the activity was calculated by using the following formula

Catalase activity: (Maximum absorbance – Minimum absorbance) × 60 × 2 (units min^{-1} g^{-1})

Na^+ concentration (ppm) estimated by using one gram of powdered plant sample was taken in 150 mL Erlenmeyer flask and digested with diacid mixture (HNO_3 and $HClO_4$ in 9: 4 ratio) and Na+ and K+ concentrations were determined by Flame Photometer as per the method of d (Jackson 1973)

Quantitative analysis of total chlorophyll and carotenoid content was determined according to a modified method outlined by (Koleyoreas, 1958). Leaf samples (0.1 g) were extracted by soaking them in 10 mL of DMSO solution for overnight in the dark. Total chlorophyll and carotenoid content was measured with a spectrophotometer at 652, 663, 645, 480 and 510 nm and was then calculated using the following equation:

$$\text{Total chlorophyll content (mg g}^{-1}) = \frac{\text{OD at 652 nm} \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

$$\text{Carotenoid content (mg g}^{-1}) = 7.6\,(D480) - 1.49\,(D510) \times \frac{V}{1000 \times W}$$

V= Volume of DMSO taken (mL)

W= Fresh weight of the sample (g)

A mature young leaf has been selected from non-destructive samples and the net photosynthetic rate, stomatal conductance and transpiration rate was recorded between 10:00 AM to 12:00 Noon with the help of Infra-Red Gas Analyser (IRGA model TPS-2.) (Hernández *et al.*, 2019). The yield and its attributes measured at harvest stage,the number of days taken from date of sowing to complete exertion of the panicle in 50 per cent of plants in a plot was recorded as days to 50% flowering. The plant height was measured in centimetres from the ground level in the field to the tip of the panicle at the time of harvest. Total number of ear bearing tillers in each plant was counted at maturity. The length from base of the panicle to the tip was measured in centimetres. The number of filled grains per panicle were counted manually and recorded after harvesting. The matured panicles from each plant were harvested, threshed, cleaned and seed dried to 12% moisture level. The grain yield per plant was recorded in grams. Spikelet fertility was calculated as proportion of the total number of filled grains to the total number of grains per panicle and expressed in percentage.

$$\text{Spikelet fertility} = \frac{\textit{Number of filled grains per panicle}}{\textit{Total number of grains per panicle}} \times 100$$

Harvest index (HI) is defined as the ratio of economic yield to total biological yield (Donald and Hamblin 1976) and expressed either in percentage or in number.

Table 1: Morphological and photosynthetic parameters at reproductive stage under saline field conditions.

S.No.	Designation	Genotype	RS	PH	TP	DW	NPR	SC	TR
1	CPST6	FL478	7	54.52	3.82	15.7*	26.5	68	6.39
2	CPST8	CSR27	3	40.29	4.57	15.98	29.42	66.17	7.18**
3	CPST9	MCM147-1-1-2-1	5	36.83	2	11.59	26	69.33	6.35
4	CPST11	MCM253-8-2-2	7	52	3.08	10.52	31.67**	71	6.55
5	CPST15	MCM151-3-2-1-1	5	55.32	4.35	28.13*	24.73	60.67	5.65
6	CPST16	IR18T1045	3	54.47	5.37	26.23*	27.2	70	6.52
7	CPST17	MCM208-12-1-1	7	49.05	3.3	18.62*	20.3	66.17	6.27
8	CPST18	IR100120-B-B-B-11	5	50.65	3.21	20.43*	23.98	70.67	6.58
9	CPST19	MCM159-3-1-1-1	5	48.77	5.94	27.15*	25.82	66.67	6.63
10	CPST20	AC39416A	3	87.67*	10.15	29.18*	26.43	72.67*	6.67
	Mean			52.96	4.58	20.35	26.21	68.13	6.48
	SEm±			2.83	0.21	1.01	1.56	1.8	0.233
	CD (0.05)		0.204	8.41	0.64	2.97	4.65	5.5	0.69
	CV%		5.01	9.26	8.25	8.51	10.35	6.1	6.2

RS: Reproductive score; PH: Plant height(cm); TP: Tillers (plant^{-1}); DW: Dry weight (g plant^{-1}); NPR: Net Photosynthetic rate (µ. mole CO_2 m^{-2} s^{-1}); SC: Stomatal conductance (mole H_2O m^{-2} s^{-1}); TR: Transpiration rate (mmole H_2O m^{-2} s^{-}

$$HI = \frac{Economic\ Yield\ (\ grain\ yield\ )}{Biological\ Yield\ (\ grain\ and\ straw\ yields\ )} \times 100$$

Transplanting in Saline field at Rudravaram

Statistical analysis

Analysis of variance was carried out as per the method outlined by Panse and Sukhatme (1965) in RBD method. PCA analysis and hierarchical clustering were used for evaluating tolerant genotypes.

Result and discussion

The results of morphogical and hysiological parameters displayed in Table 1 where as biochemical and yield parameters revealed in (Table 2 and Table 3).

Based on SES score at Seedling stage and Reproductive stage CPST8 (salt tolerant check CSR27), CPST20(AC39416A), CP19(MCM159-3-1-1-1, CPST15 (MCM151-3-2-1-1), CPST18(IR100120-B-B-B-11), CPST16 (IR18T1045) were found to be salt tolerant at saline field conditions. Visual symptoms of salinity stress include reduced leaf area, whitish appearance of lower leaves, leaf tip death and leaf rolling. The modified standard evaluation system (SES) was used in rating the visual symptoms of salt toxicity (IRRI 2014)

AC39416A and IR18T1045 had shown better performance in terms of plant height. Similar findings were reported by Sultana *et al.*, (2022) when the experiment was conducted at 6, 9 and 12 dSm^{-1} salinity levels and all these entries expressed the salinity tolerant score "3". The standard evaluation score (SES) based on visual salt injury at reproductive stage has given clear indication regarding the tolerance levels of different entries under test. Higher number of tillers recorded in AC39416A. Such variation among different genotypes was noticed by Hasan *et al.*, (2020) under saline conditions. This indicates the inherent ability of the genotypes to respond under abiotic stress conditions and CSR27 and higher dry matter production is noticed in AC39416A due to the presence of more number of leaves. (Sultana *et al.*, 2022) reported similar performance of leaf production by different genotypes under varied salinity levels (6, 9 and 12 dSm^{-1}). Production and maintenance of more number of leaves is important for dry matter production and yield asper findings of Uzzaman *et al.*, (2015). Higher photosynthetic rate was recorded in MCM253-8-2-2 and CSR27. The genotypic variation observed in photosynthetic traits might be related to their origin and genetics of the varieties. Huanhe *et al.*, (2024) reported that high yielding genotypes had higher photosynthetic rate which is positively correlated with total biomass production and none of the other gas exchange traits had significant relationship with either total dry matter or with seed yield. Higher stomatal conductance and transpiration rate in AC39416A and CSR27. Higher stomatal conductance improves transpiration rate .while water diffusing out and CO_2 diffuses into mesophyll cells of leaves there might be increases CO_2 fixation. Similar findings were previously

Fig. 1: Salinity symptoms at field condition.

Table 2: Biochemical parameters, proline and Na/K ratio at reproductive stage under saline field conditions.

Sr.	Desig-nation	Genotype	Na/K	Proline (µg g^{-1})	Catalase (units min^{-1} g^{-1})	SOD (OD min^{-1} g^{-1})	Chlorophyll content (mg g^{-1})	Carotenoid content (mg g^{-1})
1	CPST6	FL478	2.57	3.68	98.99	45.86	0.83	0.26
2	CPST8	CSR27	1.13*	1.95	120.82**	58.32**	1.27**	0.35
3	CPST9	MCM147-1-1-2-1	1.56*	4.03	49.58	50.81	0.76	0.32
4	CPST11	MCM253-8-2-2	1.82*	3.29	80.98	48.79	0.95	0.3
5	CPST15	MCM151-3-2-1-1	1.56*	4.22**	39.92	45.77	1.1**	0.37
6	CPST16	IR18T1045	1.34	3.32*	58.77	46.67	0.97	0.32
7	CPST17	MCM208-12-1-1	2.11*	4.38**	48.15	54.82**	1.03	0.32
8	CPST18	IR100120-B-B-B-11	1.72*	3.64*	50.97	53.8**	0.5	0.3
9	CPST19	MCM159-3-1-1-1	1.53*	3.15*	56.54	52.38**	1.34**	0.27
10	CPST20	AC39416A	1.35	2.56*	76.5	49.82	1.43**	0.31
	Mean		1.67	3.42	68.12	50.7	1.02	0.31
	SEm±		0.086	0.16	2.88	2.08	0.077	0.014
	CD (0.05)		0.25	0.48	8.56	6.19	0.22	0.041
	CV%		8.9	8.27	7.32	7.12	11.9	7.8

reported by (Ueda *et al.*, 2013) in an experiment conducted at different salinity levels.

At reproductive stage, high proline content recorded in MCM208-12-1-1 and MCM147-1-1-2-1.hence Proline acts as an osmoprotectant, a protein stabilizer, a metal chelator (Dar *et al.*, 2016). Lower Na/K ratio in AC39416A and CSR27 and contributes the spikelet fertility (Ahmadizadeh *et al.*, Salt tolerance in FL478 and CSR27 is due to high catalase enzyme activity at reproductive stage . Catalase contain heme-group that catalyses the conversion of H_2O_2 into H_2O and O_2 Similar findings were reported by (Kordrostami *et al.*, (2017) and high SOD activity in MCM208-12-1-1 and CSR27. SOD dismutates O_2 • into less toxic H_2O_2, thereby

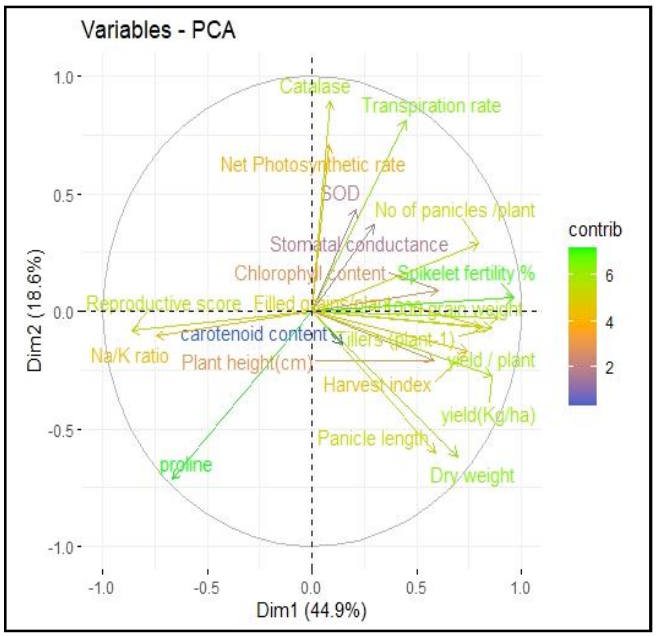

Fig. 2: Variables PCA

preventing formation of more harmful •OH via Haber-Weiss reaction (Gill and Tuteja 2010).

In case of MCM159-3-1-1-1 and MCM151-3-2-1-1, the salt tolerance can be attributed to higher chlorophyll and carotenoid content respectively at reproductive stage. Accumulation of proline in the leaves under saline conditions was reported by Batra, *et al.*, (2023) and this higher accumulation can be attributed to osmotic regulation of the cell under abiotic stress conditions.

Carotenoids defend against ROS species particularly in chloroplast there by increase photosynthetic activity (Sofo *et al.*, 2015. Higher number of panicles in AC39416A and IR100120-B-B-B-11 higher panicle length in MCM151-3-2-1-1and IR18T1045. Similar genotypic differences for panicle length at 10 dSm^{-1} salinity stress was reported by Jahan *et al.*, (2023) under field conditions and higher spikelet fertility% , 1000 grain weight and harvest index in AC39416A. Similar findings were previously reported by Pranaya *et al.*, (2024), Pirdashti and Yaghoubian (2024) and Cheng *et al.*, (2024). under saline field conditions might have contributed to higher yields under saline conditions. Salt tolerance in rice is influenced by several physiological and molecular mechanisms and tolerant genotypes better performed at yield parameters (Ali *et al.*, 2013)

In the present study, expression of salt tolerance varied with genotypes based on physiological and biochemical mechanisms viz; high photosynthetic rate, stomatal conductance and transpiration rate, high SOD, catalase, Proline content, high chlorophyll and carotenoid content of the leaf, low Na/K Ratio. The salt tolerance traits of these genotypes reflected on morphological

Table 3: Parameters contributing yield and its attributes at saline field conditions.

S. No.	Designation	Genotypes	5F	NPP	PL	FGP	SF	GW	HI	YP	YK
1	CPST6	FL478	90	5.87	11.25	37.03	10.18	6.76	0.11	1.56	518.89
2	CPST8	CSR27	91	14.25	12.56	86.4	34.29	15.26	0.2	4.01	1335.83
3	CPST9	MCM147-1-1-2-1	118.	6.29	17.86*	115.4	23.7	9.23	0.15	2.61	871.11
4	CPST11	MCM253-8-2-2	105	6.78	10.35	45.31	12.92	7.41	0.13	1.98	658.89
5	CPST15	MCM151-3-2-1-1	119	7.92	19.34*	43.15	18.53	11.39	0.15	3.55	1184.44
6	CPST16	IR18T1045	101.00	11.44	21.63*	354.03	38.28*	10.45	0.2	6.23*	2075.34*
7	CPST17	MCM208-12-1-1	122.	7.42	12.1	49.04	15.77	8.21	0.28	3.03	1010
8	CPST18	IR100120-B-B-B-11	91.	12.25	20.19*	135.41	25.62	13.79	0.25	4.85*	1616.5*
9	CPST19	MCM159-3-1-1-1	96.	7.97	22.23*	123.81	28.93	20.65*	0.22	6.03*	2009.89*
10	CPST20	AC39416A	93	12.85	19.13*	901.19	48.98*	24.94	0.37	5.45*	1815.56*
	Mean		106	9.3	16.66	109.95	25.72	12.81	0.2	3.93	1309.64
	SEm±		4.16	0.43	1.22	9.68	1.25	0.72	0.009	0.22	73.9
	CD(0.05)		12.36	1.27	3.62	8.8	1.77	2.14	0.026	0.65	219.7
	CV%		7.03	8.06	11.99	28.7	8.46	9.7	8.15	9.7	9.7

5F: 50% Flowering; NPP: No of panicles (plant^{-1}); PL: Panicle length (cm); FGP: Filled grains plant^{-1}; SF: Spikelet fertility %; GW: 1000 grain wt (g); HI: Harvest index; YP: Yield(plant^{-1}); YK: Yield(kg /ha)

parameters viz plant height, no of tillers and dry matter production and yield attributing parameters viz: number of panicles, panicle length, spikelet fertility%, harvest index, 1000 grain weight and ultimately yield under high soil salinity stress.

The distribution of traits drawn by X and Y axis respectively using PCA biplot and each trait indicated by a vector. The variability of trait indicated by relative length of vector represented on biplot (Al Naggar *et al*., 2020). The vector length of catalase, transpiration, Spikelet fertility, 1000 grain weight ,proline dry weight, panicle length and Na, K ratio contribution towards maximum divergence. Angle 0 indicates positive correlation (+1), 90 indicates no correlation, <90 indicates positive correlation (0-1), >90 indicates negative correlation (-1 to 0) and 180 indicates perfect negative correlation (Fig. 2) and the the distribution of both characters (in the form of vectors) and genotypes (as dots). The genotypes which are closer to the trait vectors represent more variability with respect to those traits (Barathi *et al.*, 2024). The distribution of both characters (in the form of vectors) and genotypes (as dots). The genotypes which are closer to the trait vectors represent more variability with respect to those traits. Tolerant genotypes displayed on right upper side of PCA biplot (Alsajri *et al.*, 2019) from Fig 3.

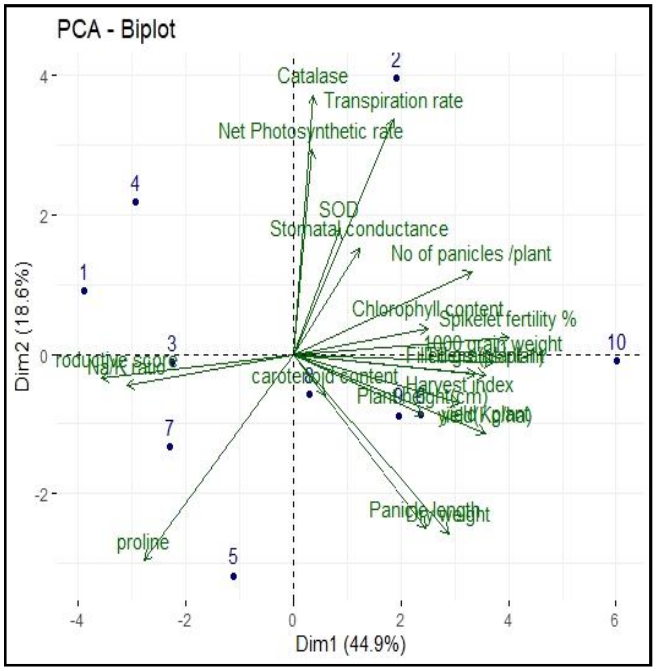

Fig. 3: PCA Biplot.

Hierarchical clustering into two group Cluster I and II ; Cluster II further divided into cluster IIA and Cluster IIB; cluster I includes AC39416A whereas Cluster IIA includes IR100120-B-B-B-11, MCM159-3-1-1-1, IR18T1045, MCM151-3-2-1-1, FL478, MCM253-8-2-2, MCM208-12-1-1 and MCM147-1-1-2-1 and Cluster IIB includes CSR27. Hence tolerance level of CSR27 and AC39416A different from other genotypes (Ghazy *et al.*, 2023).presented in Fig. 4.

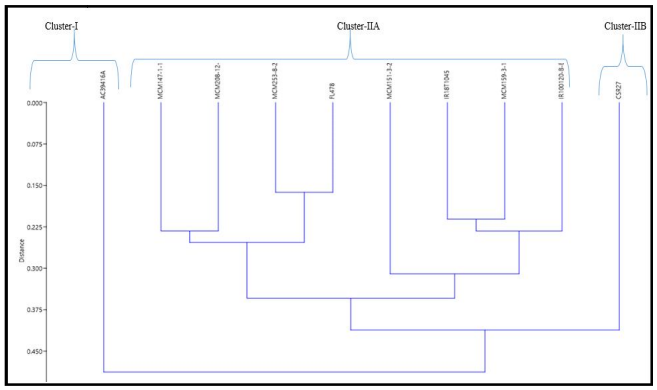

Fig. 4: hierarchical clustering of genotypes.

Conclusion

In undulation of saline water increases the salinity in coastal areas. Evaluating of genotypes in highly heterogeneous saline soils shows better results by examining individual genotypes under such salinity yield better when compared to susceptible. Most salinity tolerance contributing parameters are ion homeostasis like compartmentation and exclusion plays a important role with support of proline in the cytoplasmic level and antioxidant defence mechanisms against Reactive oxygen specie at level of electron chain transports in cells at reproductive have better yield parameters. Evaluation of genotypes physiologically as well as biochemically under field condition leads to identification of donor plants that helpful for future breeding programs.

Acknowledgement

I am very greatful to my chairperson (Dr. S. Ratna Kumari) and advisory committee members (Dr. M. Girija Rani, Dr. K. Suneetha , Dr. G.Vijaya Kumar, Dr. I. Usha Rani) especially iam very thankful one of my member Dr. M.Girija Rani madam principle scientist at ARS Machilipatnam for providing saline field, labours, field operations and equipments like EC and pH meter for this study.

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