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Research Article

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Screening of Rice Genotypes for Salinity Stress Tolerance

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Abstract

Experiments were conducted at Unit of Crop Physiology, Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai to study the effect of salinity on growth of various genotypes of rice. The varieties (ASD 16 MDU 6 and ADT 45) and landraces (Kuliyadichan, Kallurundaikar and Norungan) were taken for this study. Germination and seedling characters were measured at seventh day after inducing abiotic stress conditions such as, salinity by application of NaCl at 200mM concentration. Physiological and biochemical parameters were estimated at the twentieth day after inducing the salinity stress conditions. The number of seeds germinated, shoot and root length, vigor index, nitrate reductase, α-amylase content and the soluble protein content were studied under the laboratory conditions in comparison to the control. Analyses of variance, revealed significant differences for germination percentage, shoot and root length, vigor index, nitrate reductase, α-amylase and protein contents under salinity. It was evident from the data that increased levels of sodium chloride decreased the germination percent, shoot and root lengths and hence the vigor index nitrate reductase, α- amylase and protein contents linearly.

Keywords: Salinity Stress, Rice Varieties, Morphology, Physiological Characters

1. Introduction

Rice is the seed of the grass species *Oryza sativa* (Asian rice) or *Oryzaglaberrima*(African rice). As a cereal grain, rice, a monocot, is normally grown as an annual plant, although in tropical areas it can survive as a perennial and can produce a ratoon crop for up to 30 years. The rice plant can grow to 1 to 1.8 m tall, occasionally more depending on the variety and soil fertility. It has long, slender leaves 50 to 100 cm long and 2 to 2.5 cm broad. The small wind-pollinated flowers are produced in a branched arching to pendulous inflorescence 30 to 50 cm long. The edible seed is a grain, a fruit called caryopsis 5 to 12 mm long and 2 to 3mmthick. Rice cultivation is well-suited to countries and regions with low labor costs and high rainfall, as it is labor intensive to cultivate and requires ample water. However, rice can be grown practically anywhere, even on a steep hill or mountain area with the use of water- controlling terrace systems. The traditional method for cultivating rice is flooding the fields while or after setting the young seedlings. This simple method requires sound planning and servicing of the water damming and channeling, but reduces the growth of less robust weed and pest plants that have no submerged growth state, and deters vermin. While flooding is not mandatory for the cultivation of rice, all other methods of irrigation require higher effort in weed and pest control during growth periods and a different approach for fertilizing the soil. Water management : Uniform leveling of field and proper drainage are most essential for an effective water management in irrigated field. Efficient water-management facilitates, good tillering and better nutrient uptake and helps in reducing weed population. In India rice is grown in 43.86 million ha, the production level is 104.80 million tones and the productivity is about 2390 kg ha^{-1} (Agricultural Statistics at a glance, 2015). The rice production has registered an appreciable increase from 20.58 million tons in 1950-51to 104.86 million tons during 2014-15, which is nearly 5 times. The yield was 668 kg ha-1in 1950-51which has increased to 2390 kg ha-1 during 2014-15. Major share of rice production is in Kharif season. It is grown in almost all the states in the country however the major 5 states in

rice production are West Bengal, UP, Andhra Pradesh, Punjab and Tamil Nadu.

It is one of the most important food crops and feeds more than 60 per cent population of India. Though the area in production of rice is high compared to that of china, the production and productivity of rice is low in India. And also the productivity of countries like Indonesia, Bangladesh and Vietnam is high to that of India. On the whole, Indian agriculture does not show high efficiency or productivity, though there is an improvement since independence. The reasons and constrains of the above said results may have connections like., population pressure, uneconomic holdings, uncertain monsoons and inadequate irrigation facilities, subsistence nature of farming, decline in soil fertility, lack of support services, poor organization of resources and lack of entrepreneurship and also poor crop plant population in case of broadcast sowing method resulting in uneven germination (upland and direct seeded lowland). Delay in monsoon onset often results in delayed and prolonged transplanting and sub-optimum plant population (mostly in rainfed low lands). Therefore, the productivity is low. In the high rainfed regions, the rain water is lost rapidly through deep percolation, because of the upland location and loose texture of the soil. In these soils the plant nutrient applied through fertilizers are lost rapidly and investment of fertilizer become risky. Further, low water retention capacity by the soil due to high permeability brings in moisture stress conditions quickly after cessation of rains. Such situation contributes low productivity. In the low rainfall regions, the crops suffer from iron and zinc deficiency in some soils. Among the above said problems and constrains, the increasing areas under salinity are the major problems leading to severe yield reduction. Abiotic stress, which includes salinity, heat and cold, critically threatens crop production and causes significant yield loss in large areas [1,2]. Among these, soil salinity is the second major environmental constraint, to crop production and is expected to increase due to global climate changes and as a consequence of many irrigation practices and depleting water resources respectively. Plant growth and developmental processes in terms of biochemical, physiological and morphological characteristics are inhibited by both water deficit and salt stresses [3-5].

Salinity is a common abiotic stress that severely limits crop growth and development, productivity and causes the continuous loss of arable land, which results in desertification in arid and semi-arid regions of the world [6]. It is estimated that more than 800 million hectares of land throughout the world are adversely affected by high salinity [7]. Ali et al opined that saline soils are characterized by excess of sodium ions with dominant anions of chloride and sulfate resulting in higher electrical conductivity (>4 dS m1) [8]. In general, salinity stress induces an initial osmotic stress and subsequent toxicity as a consequence of the accumulation of ions. However, damage can also ensue as a result of excessive reactive oxygen species (ROS) such as superoxide radicals (O2), hydrogen peroxide (H2O2) and hydroxyl radicals (OH) produced at a high rate commonly accumulated in plant tissues due to ion imbalance and hyper osmotic stresses. ROS accumulation leads to lipid oxidation and has a negative effect on cellular metabolism and physiology, thus adversely ruining the membrane integrity [9]. Salinity tolerance in glycophytic crops including rice is predominantly associated with the maintenance of ion homeostasis, particularly low Na^+/K^+ or high K^+/Na^+ ratios, through exclusion, compartmentation, and partitioning of Na^+ [10]. In addition to ion homeostasis strategies, many plants have evolved mechanisms to regulate the synthesis and accumulation of compatible solutes such as proline and glycine betaine, which function as osmoprotectants and have a crucial role in plant adaptation to osmotic stress through stabilization of the tertiary structure of proteins [5]. Rice is the most important global food crop that feeds over half of the world population and still getting a major proportion of their energy requirement from rice and its derived products with the demand for food expected to increase by another 38% within 30 years [11]. However, rice productivity in many areas is affected by salinity stress, which originates from the accumulation of underground salt and is exacerbated by salt mining, deforestation and irrigation [12]. Rice is generally characterized as a salt sensitive crop but the extent of its sensitivity varies during different growth and developmental stages. It is tolerant to salinity stress during germination and active tillering, whereas it displays more sensitivity during early vegetative and reproductive stages [13].

Attempts to measure the tolerance capacity in a genotypes or hybrid with single parameter have limited value because of the multiplicity of the factors and their interactions contributing to salinity tolerance. Different researchers used different traits to appraise genetic variances in salinity tolerance. Hence, in the present study, rice genotypes and hybrids have been selected to assess their tolerant capacity under salinity and the various traits contributing to salinity tolerance during germination and seedling establishment stage of the crop with the following objectives viz., To investigate the germination, physiological and biochemical responses of rice genotypes to salinity stress at germination level and To screen the rice genotypes that are tolerant and perform good to the induced salinity stress conditions.

2. Materials and Methods

A laboratory study was conducted in the Unit of Crop Physiology, Agricultural College and Research Institute, Madurai with the objective to screen the rice genotypes for salinity stress tolerance at seed germination level of selected rice genotypes. A brief account of the materials used and methodologies followed in the present study are presented in this chapter. Three varieties and three landraces were choosen for this experiments with two treatments T1-Control (Water only used) and T2 – NaCl @200mM withfour replications.

2.1 Varietal Details

The experiment was conducted with six rice genotypes. It includes 3 landraces (Kuliyadichan, Kallurundaikar, NORUNGAN) ,3 varieties (ADT 45, MDU6, ASD16) .The seeds for the experiment were obtained from Department of plant breeding & genetics , Agricultural College and Research Institute, Madurai.

2.2 Methodology

The experiment was laid out under laboratory with 6 rice genotypes with 4 replications. NaCl stress treatment was imposed. The standardized protocols using NaCl stress (200mM equivalent to -1.26 Mpa water potential). Change solutions once in every two days for NaCl stress. Drain completely rinses three to four times with fresh solutions if possible so as to avoid increased stress level due to NaCl. Experiment was continued at lab for one month and seedling vigour, germination and other related parameters were observed against control.

2.2.1 Screening for Salinity Tolerance

• Screening for salinity tolerance need to be carried out hydroponically at early vegetative stage of the plants that is when the plant reaches 3 to 4 leaf stages

• For this floating styrofoam panel as shown in figure need to be prepared as per the size of the tray using thermocool sheet, mosquito nets and Adhesive. The styroform panel should have 6* 4 holes

• Individual accession need to be grown as single line along with standard check lines and 6 replications

• The tray need to be filled with a nutrient solution comprising of both macro and micronutrients and should have pH of 5.5

• Preparation of stock solution Preparation of working solution

• During the experiment the solution need to be checked every day for maintaining the pH of the solution if the pH shifts to far then it is better to replace the solution

Beforestartingtheexperimenttheseedsofeachgenotypesneedstobepreheatedinhot air oven for 3 to 5 days at 50 degree centigrade to break seed dormancy(if any)

• The surface sterilized seeds need to be replaced in dishes with moistured filter papers and incubator at 30 degree centigrade for 48 hours to germinate

• These pre-germinated seeds should be placed in the individual holes of the styrofoam panel two seedlings per hole

• Initially the Seedling should be kept in normal water for 2 to 3 days.

• After that it need it to be kept in nutrient solution

• After 5 days of growth in nutrients solution salinity stress need to be imposed in one set of the .Other set would be kept as search for control

• Salt stress need to be imposed as 6ds m-1 NaCl solution (approximately 60 mM NaCl i. e. 3.0-gram NaCl per liter solution would be required level of EC) for initial 2 days. After 2 days it should be increases to 12 ds m-1 NaCl solution(approximately 120 mm NaCl i.e. 6.0-gram NaCl per liter solution)

• Visual scoring of genotype should be started as soon as the appearance of the saltspecific symptoms. Scoring should be continued until 60% of plants of most suspectable genotypes reaches the score of "9" .Genotype should be ranked based on the final scoring at the stage

Preparation of Stock Solution

Element (macronutrient)	Reagent(ARgrade)	Preparation(g/250mlsolution)
N	Ammoniumnitrate(NH_4NO_3)	22.85
P	Sodiumphosphate,monobasicmonohydrate ($NaH_2PO_4.H_2O$)	8.9
K	Potassiumsulphate(K_2SO_4)	17.85
Ca	Calciumchloride,Dihydrate($CaCl_2.2H_2O$)	29.3
Mg	Magnesium sulphate, 7-hydrate ($MgSO_4.7H_2O$)	81

Micro nutrient dissolve each reagent separately & mix in 250 ml of distilled water, then add 50 mL of conc. H_2SO_4 and makeup volume to 7 lit.

Element(micro nutrient)	Reagent(ARGRADE)	Preparation (g/250ml solution)
Mn	ManganousChloride,4hydrate($MnCl_3.4H_2O$)	0.375
Mo	Ammoniummolybdate,4-hydrate[$(NH_4)^6$ $Mo_7O_{24}.\ 4H_2O$]	0.0185
Zn	Zincsulphate,7-hydrate($ZnSO_4.\ 7H_2O$)	0.00875
B	Boric acid (H_3BO_3)	0.2335
Cu	Cupricsulphate,5-hydrate($CuSO_4.5H_2O$)	0.0075
Fe	Ferricchloride6-hydrate($FeCl_3.6H_20$)	1.925
	Citricacid, monohydrate ($C_6H_8O_7.H_2O$)	2.975

Element	**Reagent**	**ml of stock required solution/1Lof working nutrient solution**	**Concentration of element in ppm**
Macro nutrient			
N	NH_4NO_3	1.25	40
P	$NaH_2PO_4.H_2O$	1.25	10
K	K_2SO_4	1.25	40
Ca	$CaCl_2.2H_2O$	1.25	40
Mg	$MgSO_4.7H_2O$	1.25	40
Micro nutrient			
Mn	$MnCl_34H_2O$	1.25	0.50
Mo	$(NH_4)^6$ $Mo_7O_{24}.4H_2O$	1.25	0.05
Zn	$ZnSO_4.7H_2O$	1.25	0.01
B	H_3BO_3	1.25	0.20
Cu	$CuSO_4.5H_2O$	1.25	0.01
Fe	$FeCl_3.6H_2O$	1.25	2.00

2.2.2 Observations Recorded

The morphological, physiological and biochemical parameters were recorded at different date of stress imposition of rice seeds. Three plants in each replication were selected at random as sample seedlings and tagged to record observations in salinity stress. The methods followed in recording each of these parameters are described below.

2.3 Morphological and Growth Characters

2.3.1 Germination Percentage

Seed germination tests were carried out and performed with three replications of 5 seeds each holes. The mean values were expressed in percentage.

2.3.2 Root Length
The root length was measured and expressed as cm.

2.3.3 Shoot Length
The shoot length was measured and expressed as cm.

2.3.4 Vigour Index
The Vigour Index was computed as per the procedure suggested by Abdul-Baki and Anderson, (1973) by using the following formula. (Shoot length + Root length) x Germination percentage

2.3.5 Stress Tolerance Index (STI)
The Stress Tolerance Index was computed as per the procedure suggested by Dhopte and Livera, (1989) by using the following formula.

$$STI = \frac{\text{Vigour Index (Treated)}}{\text{Vigour Index (Absolute control)}} \times 100$$

2.4 Physiological and Biochemical Parameters
2.4.1 Soluble Protein Content
Soluble protein content of the leaf was estimated by following the procedure described by Lowry *et al*. and expressed as mg g^{-1} of fresh weight [14].

2.4.2 Determination of Soluble Protein Content
Proteins were estimated using method.0.50 g of seed sample is macerated with 10ml of phosphate buffer (pH 7.0) [15]. The extract was centrifuged at 3000 rpm for 10 minutes and the supernatant was collected. One ml of the supernatant is transferred to a tube and 5ml of alkaline copper tartarate (ACT) reagent is added. The solution is kept as such for 30 minutes for color development. Then, 0.5ml of phenol reagent is added and the OD value of the sample for the intensity of blue color is read at 660nm in spectrophotometer. The protein content is expressed as mg g-1 of sample. Protein concentration was measured using bovine serum albumin as standard.

2.4.3 Alpha Amylase Content
Alpha amylase content of the germinated seedlings was estimated by following the procedure described by Miller, (1959) and expressed as enzyme unit g-1 fresh weight.

2.4.4 Determination of Alpha Amylase Content
One g of sprouting cereal (here rice) is milled in 5ml of prechilled 0.05M citrate buffer, pH 6.0; the resulting homogenate is centrifuged at 10,000rpm for 10 minutes. Enzyme activity is assayed in the supernatant as yield of crude enzyme. 0.1ml of this supernatant is pipette into a separate test tube and 0.9ml of 2%soluble starch is added and is incubated in shaking water bath at 50OC for 30 minutes. The reaction is stopped by adding DNSA reagent and boiled for at least 3 minutes for color development. Absorbance is read at 550nm against blank. Standard glucose curve is prepared from a series of glucose concentrations.

2.4.5 Nitrate Reductase Activity
Nitrate reductase activity in young leaves was estimated as per the method described by Nicholas et al. and the enzyme activity was expressed as µg NO2 g^{-1} h^{-1}.

2.4.6 Determination of Nitrate Reductase Activity
A seedling of one g weight is taken in a test tube and 5ml of assay medium is added.Thisiskeptinsidethevacuumdesiccatorfor5minutes,undisturbed.2mlof the supernatant is pippeted out into a test tube and 1ml of 1M zinc acetate is added followed by 1ml of 70% ethanol. This is filtered using watman filter paper. To the filtrate 1ml of 1% sulphanilamide and 1ml of 0.02% NEDD is added. The OD Value is read at 540nm after the development of pink color using the spectrophotometer.

3. Result and Discussion
3.1 Effect of Salinity on Morphological Characters
3.1.1 Germination Percentage (%)
The germination percentage was reduced by the salinity compared to control. Significant difference was noticed in all the treatments with respect to seed germination. Control recorded the highest germination percentage (92 to 100%) and salinity (40 to 80%) recorded least germination percentage (Table 1).

Varieties & Landraces	Seed germination(%)		Shoot length (cm)	
	Control	Salinity	Control	Salinity
Kuliyadichan	100.0	80.0	16.2	15.9
Kallurundaikar	100.0	80.0	17.8	16.0
Norungan	100.0	100.0	19.3	17.7
ASD16	98.0	60.0	12.8	13.3
MDU6	100.0	40.0	15.3	9.5
ADT45	92.0	80.0	13.5	7.9
Mean	**98.3**	**73.3**	**15.8**	**11.8**
SEd	**1.91**	**1.71**	**0.20**	**0.12**
CD(0.05)	**3.95**	**3.53**	**0.43**	**0.24**

Table 1. Screening of rice genotypes on seed germination and shoot length for salinity stress tolerance

Among the rice genotypes, ADT45 recorded the minimum germination percentage (40%) followed by kuliyadichan (96%) under controlled conditions. The lowest rate of reduction in germination percentage in the genotype Kuliyadichan and Norungan under salinity stress imposed. Under salinity (NaCl 200 mM) conditions, the lowest rate of reduction in germination percentage recorded in the genotyes 3 land races Kuliyadichan, Kallurundaikar and Norungan. Highest reduction was observed in ASD16, ADT45 and MDU6 among the genotypes studied. Seed germination is very important stage for the victorious establishment of vigorous seedlings. This germination stage is very sensitive to salinity as compared to other stages. Salinity accumulates the toxic ion in plant cells causing a membrane damage and mineral imbalance. The most evident effect of salinity to the seed germination of rice was reducing the osmotic potential of soil which makes decline in water imbibition by seed to the creation of ionic toxicity which alters enzymes action involved in nucleic acid metabolism [16]. Other impacts of salt stress on seed germination include change in metabolism of protein [17]. In this present study, a significant increase was observed in germination percentage under controlled conditions. However, a considerable reduction could also be noticed in germination percentage due to the influence of sodium chloride treatments. The genotypes land races Kuliyadichan, Kallurundaikar and Norungan maintained its superiority with about 10 to 12 percent reduction, whereas, all the other genotypes showed about 25 to 30 percent reduction due to salinity.

3.1.2 Shoot and Root Length (Cm)

The results of shoot and root length showed a significant reduction under salinity stress compared to controlled condition. Among the rice genotypes, Norungan recorded the maximum shoot length (17.7cm) followed by Kallurundaikar (11.8cm) and Kuliyadichan (15.9cm) under controlled conditions. The lowest rate of reduction in shoot length due to the influence of sodium chloride in the genotypes of Norungan, Kuliyadichan and Kallurundaikar had lowest reduction in shoot length under salinity stress imposed and the highest reduction was observed in ASD16, MDU6 and ADT45 among the rice genotypes and hybrids studied. The root length was reduced by the influence of sodium chloride compared to control. Significant differences were noticed in all the treatments and genotypes with respect to root length. The control was recorded the maximum mean root length (17.9m) and sodium chloride 200mM recorded the minimum mean root length (7.4cm, 8.9cm and 4.0cm) (Table 2).

Varieties & Land races	Root length (cm)		Seedling vigour	
	Control	Salinity	Control	Salinity
Kuliyadichan	9.9	8.15	2610	1924
Kallurundaikar	9.7	6.2	2750	1776
Norungan	10.3	7.9	2960	2560
ASD16	9.3	5.8	2165.8	1146
MDU6	11.2	7.9	2650	1392
ADT45	9.6	6.3	2125.2	568
Mean	**10.0**	**7.04**	**2543.5**	**1561**
SEd	**0.23**	**0.07**	**42.45**	**15.88**
CD	**0.47**	**0.15**	**87.43**	**32.72**

Table 2. Screening of rice genotypes on root length and seedling vigour for salinity stress tolerance

Among the rice genotypes, Kuliyadichan, Kallurundaikar, Norungan, ASD16, MDU6 and ADT45 recorded the maximum root length (15 to 17 cm) under salinity conditions and genotype kuliyadichan, kallurundaikar and MDU6 had maximum root length under salinity stress imposed. The rate of reduction in root length was the lowest in the genotypes Norungan, ASD16 andADT45 under the highest reduction was observed in ASD16 and ADT 45among the genotypes and hybrids studied.

The main functions of root are absorption of water and inorganic nutrients and anchoring of the plant body to the ground. Many researchers reported that with an increase in salinity there was a decrease in the development of the xylem and phloem. Many studies have shown that the fresh and dry weights of the shoot system are affected, either negatively or positively, by changes in salinity concentration, type of salt present, or type of plant species [18]. Root length was more suppressed than shoot by salinity at each specific salt concentration level. According to Rahman et al. who stated that, the gradual decrease in root length with the increase in salinity as observed might be due to more inhibitory effect of NaCl salt to root growth compared to that of shoot growth. In this present study, a lesser reduction was observed in the genotype of kuliyadichan and Norungan under salinity stress (NaCl) imposed showed about 11 to 15 percent and the highest reduction was observed in ASD16 and ADT45 with about 22 to 31 percent reduction due to the impact of salinity.

3.1.3 Vigour Index

Significant differences were noticed in all treatments and genotypes with respect to vigour index. The highest mean vigour index was recorded in control (2960) and lowest mean vigour index was recorded by salinity (568). Among the rice genotype, Norungan, Kuliyadichan and Kallurundaikar recorded the maximum vigour index (2560,1924 and 1776) under salinity stress (NaCl 200mM) imposed. The rate of reduction in vigour index was the lowest in the genotypes ASD16, MDU6, ADT45 and the highest reduction was observed in ADT45 among the genotypes and hybrids studied.

3.1.4 Stress Tolerance Index

The highest mean Stress Tolerance Index was recorded in Norungan (86.4)and lowest mean Stress Tolerance Index was recorded by salinityADT45(26.7) (Table 3). Among the rice genotypes, Norungan recorded the maximum Stress Tolerance Index (86.4%) followed by kuliyadichan (73.7) and kallurundaikar (64.5) had maximum Stress Tolerance Index under salinity stress (NaCl 200mM) imposed among the genotypes and hybrids studied.

3.2 Effect of Salinity Stress on Physiological and Biochemical Parameters

3.2.1 Soluble Protein Content (mg/g)

The data on soluble protein content was reduced by the influence of sodium chloride compared to control. The control was recorded the maximum mean soluble protein content (22.0mg g-1) and the sodium chloride 200 mM recorded the minimum mean soluble protein content (8.8mg g^{-1}, 8.7mg g^{-1} and 7.8mg g^{-1}).

Varieties & Land races	Stress tolerance index (%)	Soluble protein (mg g^{-1})	
	Salinity	Control	Salinity
Kuliyadichan	73.7	18.2	8.8
Kallurundaikar	64.5	18.6	9.6
Norungan	86.4	22.0	7.8
ASD16	52.9	21.2	11.7
MDU6	52.5	19.7	11.7
ADT45	26.7	18.5	8.7
Mean	**59.45**	**19.7**	**9.71**
SEd	**0.73**	**0.38**	**0.14**
CD	**0.51**	**0.79**	**0.29**

Table 3. Screening of rice genotypes on stress tolerance index and soluble protein content for salinity stress tolerance

Among the rice genotypes, Lalat recorded the genotypes Norungan, MDU6, ASD16 and kallurundaikar had maximum soluble protein content (11.7 mg g^{-1}) under salinity stress imposed. The rate of reduction in soluble protein content was the highest reduction was observed in ADT45 and Norungan among the genotypes and hybrids studied. The soluble protein content of the rice seedlings, being a measure of RuBP carboxylase activity was considered as an index for photosynthetic efficiency. There were reports that RuBP-case enzyme forms nearly 80% of the soluble proteins in leaves of many plants [19]. Diethelm and Shibles opined that the RUBISCO content per unit leaf area was positively correlated with that of soluble protein content of the leaf [20]. In the present study, salinity caused a significant reduction in soluble protein content of seedlings of all the rice genotypes. Among the genotypes studied, MDU6, ASD16 and kallurundaikar maintained its superiority with about 8 to 12 percent reduction in soluble protein content due to the influence of sodium chloride. These findings were in accordance with the results of who observed a significant reduction in soluble protein content of rice plants grown under salinity stress conditions [21]. Besides these results, Martignone et al. observed

that in soybean soluble protein content was the first nitrogenous compound affected under abiotic stress conditions, which at severity got denatured and lost the activity [22].

3.2.2 Nitrate Reductase Enzyme Activity(µgg^{-1}d^{-1})

The result on nitrate reductase enzyme activity was reduced by the influence of sodium chloride compared to control. The control was recorded the maximum mean nitrate reductase enzyme activity (167.1µgg^{-1}d-1) and the sodium chloride 200mM recorded the minimum mean nitrate reductase enzyme activity (141.1µg g^{-1} d-1, 138.1µg g^{-1} d^{-1}and 135.7µg g^{-1} d^{-1}) (Table 4).

Among the rice genotypes, Lalat, kallurundaikar and kuliyadichan had maximum nitrate reductase enzyme activity (74.6 to 68.1µg g^{-1} d^{-1}) under salinity stress imposed. The highest reduction was observed in ASD16and Norungan among the genotypes and hybrids studied. Nitrate reductase is the important key enzyme of the nitrogen assimilation pathway in plant system. Its catalytic action in tissue is subjected to complex regulation in response to different abiotic factors. Salinity is one of the most important abiotic factors that restrictions growth and development of plants. It is well known that initial steps of nitrogen metabolismin plants (NO3−ions uptake, reduction of nitrates and assimilation of ammonium) aresensitive to salt stress [23]. The plants are treated with NaCl reduces the level of nitrate ions in the cytoplasms incite so inhibits nitrate uptake by plants [24]. In the present study, salinity caused a significant reduction in nitrate reductase enzyme activity of seedlings of all the rice genotypes. Among the genotypes studied, kallurundaikar (74.6), kuliyadichan (68.1), ADT45 (64.8) maintained its superiority with about 10 to 14 percent reduction in nitrate reductase enzyme activity due to the influence ofsodium chloride. These findings were in accordance with the results of Rao and Gnaham, who stated that, the nitrate uptake and nitrate reductase activity (NRA) decrease in plants under salinity stress [25].

3.2.3 Alpha Amylase Enzyme Activity (enzyme unitg-1 fresh weight)

The data on alpha amylase enzyme activity was reduced by the influence of sodium chloride compared to control. The control was recorded the maximum mean alpha amylase enzyme activity (15.1) and the sodium chloride 200 mM recorded the minimum mean alpha amylase enzyme activity (12.7, 12.5 and 12.3).

Varieties & Land races	Nitrate reductase activity (µg g^{-1} d^{-1})		Alpha amylase (enzyme unit g^{-1} fresh weight)	
	Control	Salinity	Control	Salinity
Kuliyadichan	141.1	68.1	12.7	6.2
Kallurundaikar	144.7	74.6	13.0	6.7
Norungan	167.1	34.2	15.1	3.1
ASD16	135.7	42.4	12.3	3.8
MDU6	148.2	43.8	13.4	3.9
ADT45	138.1	64.8	12.5	5.8
Mean	**145.8**	**54.65**	**13.16**	**4.9**
SEd	**2.97**	**1.11**	**0.27**	**0.10**
CD	**6.12**	**2.12**	**0.55**	**0.21**

Table 4. Screening of rice genotypes on NRase and alpha amylase enzyme activity for water and salinity stress tolerance.

Among the rice genotypes, the genotypes kallurundaikar, kuliyadichan, ADT45 had maximum alpha amylase enzyme activity (6.7, 6.2 and 5.8) under salinity stress imposed. Higher accumulation of alpha-amylase during grain development, especially the filling stage, could result in rice grains with reduced quality, chalky, and with few stored starch grains. In rice, seed germination is dependent on the degradation of storage reserves in mature seeds, and the sugars from starch hydrolysis are the major source of energy for seedling emergence [26]. α-Amylase is the important enzyme implicated in starch mobilization; thus, α-amylase enzyme activity is an important factor in seed germination [27]. In this study, salinity caused a significant reduction in alpha-amylase enzyme activity of seedlings of all the rice genotypes. Among the genotypes studied, kallurundaikar, kuliyadichan and ADT45 maintained its superiority with about 12 to 17 percent reduction in alpha-amylase enzyme activity due to the influence of sodium chloride. These findings were in accordance with the results of who found that, positive relationships between bioactive GA content and α-amylase activity and between α-amylase activity and the rice seed germination rate and the seeds are growing under NaCl induced bioactive GA deficiency inhibits seed germination by decreasing α- amylase activity [28-92].

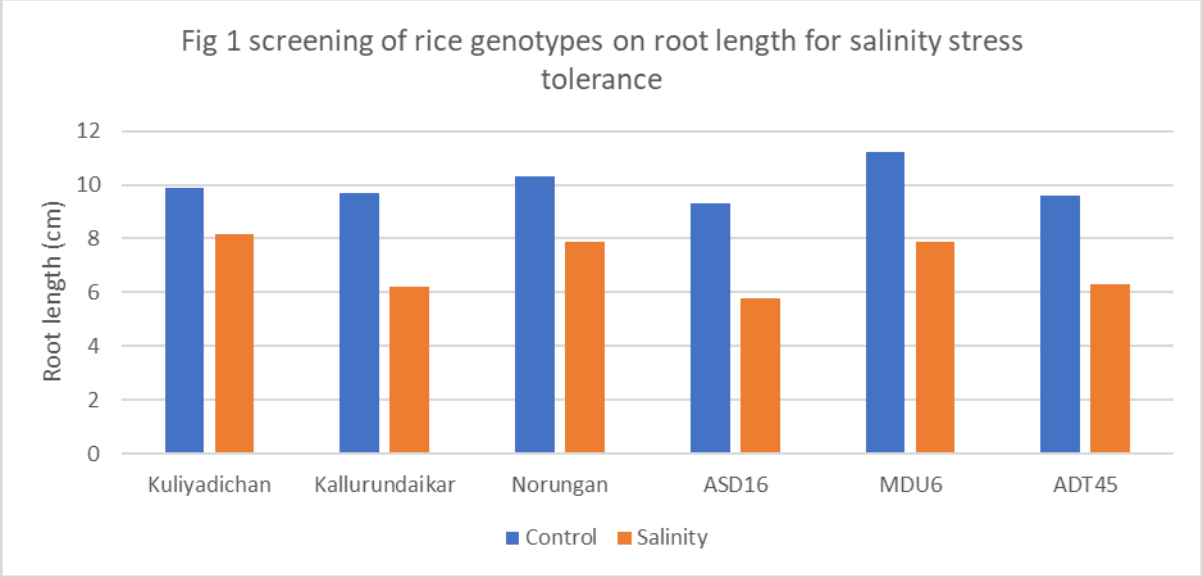
Fig 1 screening of rice genotypes on root length for salinity stress tolerance
Root length (cm)
12
10
8
6
4
2
0
Kuliyadichan
Kallurundaikar
Norungan
ASD16
MDU6
ADT45
Control
Salinity

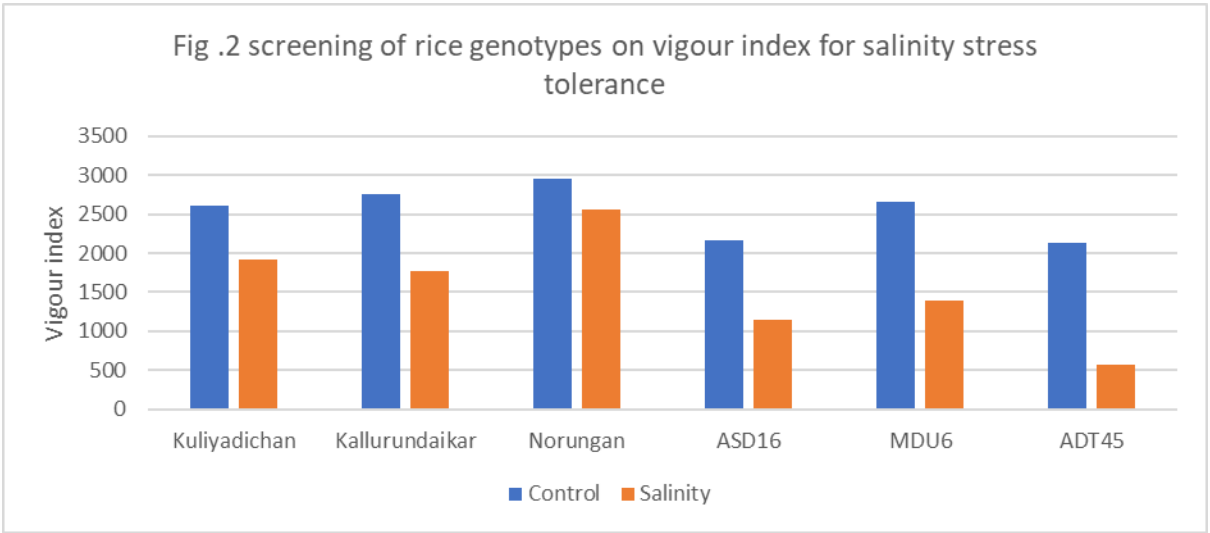
Fig .2 screening of rice genotypes on vigour index for salinity stress tolerance
Vigour index
3500
3000
2500
2000
1500
1000
500
0
Kuliyadichan
Kallurundaikar
Norungan
ASD16
MDU6
ADT45
Control
Salinity

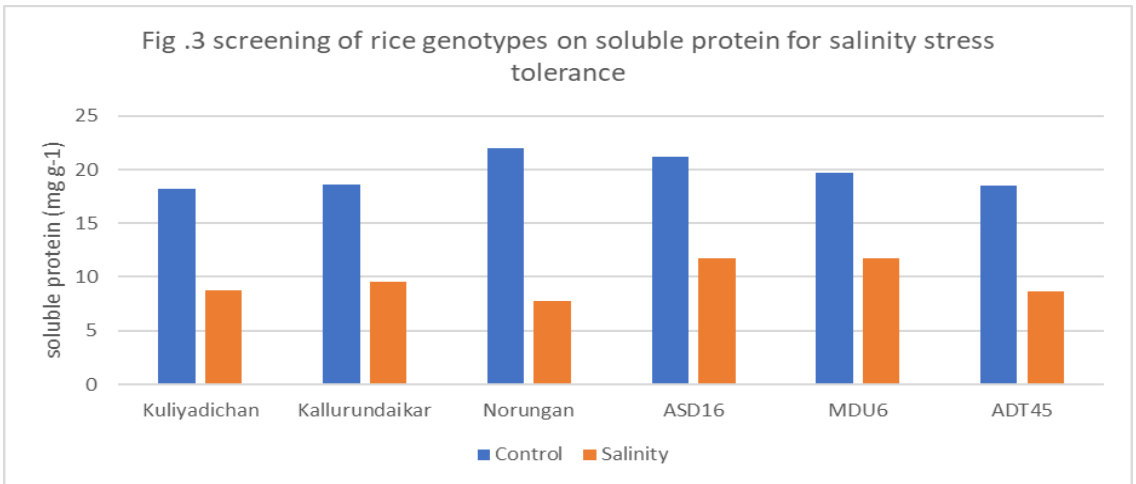
Fig .3 screening of rice genotypes on soluble protein for salinity stress tolerance
soluble protein (mg g-1)
25
20
15
10
5
0
Kuliyadichan
Kallurundaikar
Norungan
ASD16
MDU6
ADT45
Control
Salinity

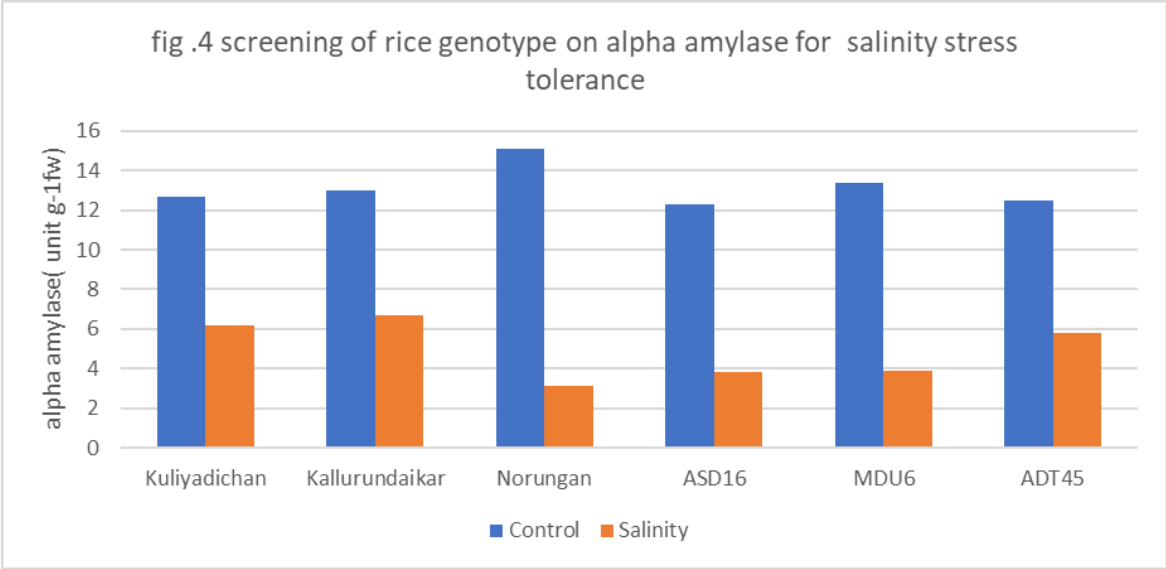
fig .4 screening of rice genotype on alpha amylase for salinity stress tolerance
alpha amylase(unit g-1fw)
16
14
12
10
8
6
4
2
0
Kuliyadichan
Kallurundaikar
Norungan
ASD16
MDU6
ADT45
Control
Salinity

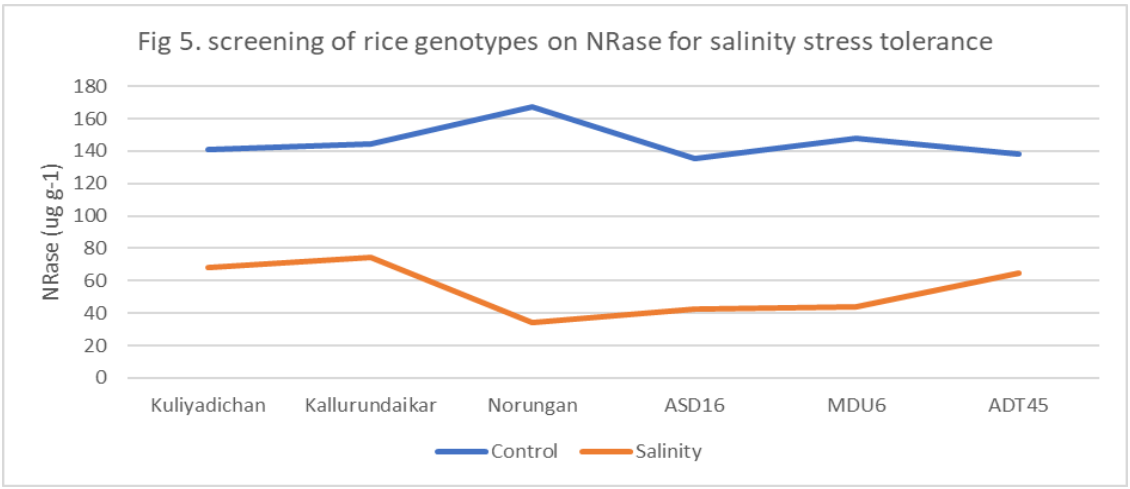
Fig 5. screening of rice genotypes on NRase for salinity stress tolerance
NRase (ug g-1)
180
160
140
120
100
80
60
40
20
0
Kuliyadichan
Kallurundaikar
Norungan
ASD16
MDU6
ADT45
Control
Salinity

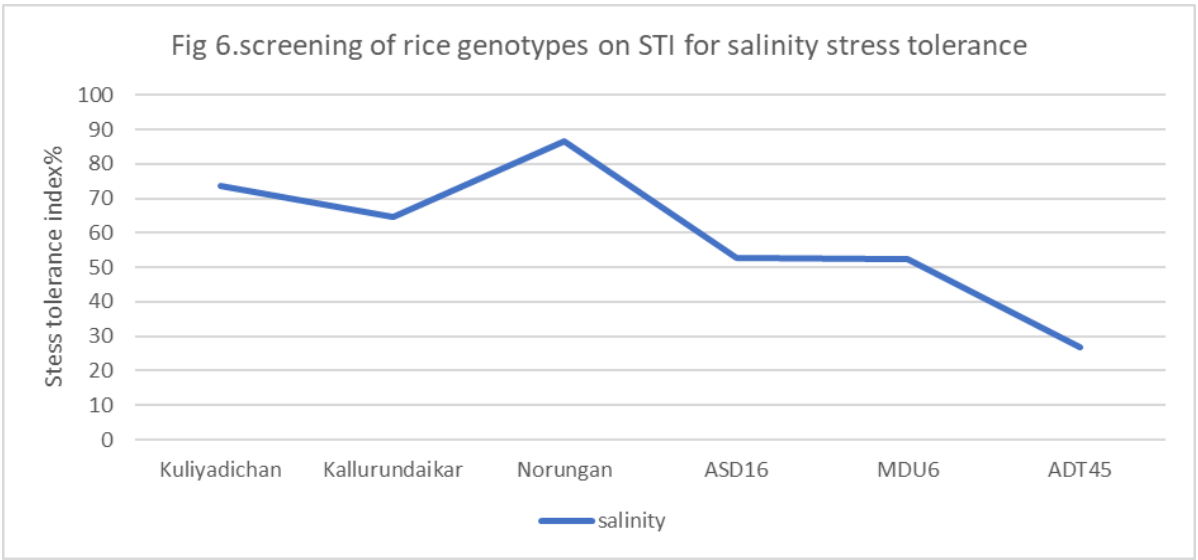
Fig 6.screening of rice genotypes on STI for salinity stress tolerance
Stess tolerance index%
100
90
80
70
60
50
40
30
20
10
0
Kuliyadichan
Kallurundaikar
Norungan
ASD16
MDU6
ADT45
salinity

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