BIOCHEMICAL CHARACTERIZATION AND EFFECTS OF OSMOPROTECTANTS UPON SODIUM CHLORIDE SALINITY STRESS IN RICE CULTIVARS

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ABSTRACT

Salinity is one of the severe abiotic stresses among the different stresses of paddy crop. Four varieties of paddy procured from Main Rice Research Station, Anand Agricultural University, Nawagam were : Jaya, Dandi, CSR-27 – all tolerant and GR-3 – susceptible. These varieties were differing in their degree of salt tolerance. All the varieties were grown in earthen pots up to 15 days following the normal practice of pot culture. Fifteen DAG seedlings after gently uprooting were treated with 100, 150 and 200 mM sodium chloride solution for 24, 48 and 72 h time intervals. Various physiological and biochemical observations were recorded for germination percentage, root length, shoot length, seedling vigour index, chlorophyll, total soluble sugars, proteins, SDS-PAGE, proline content, antioxidants enzymes such as super oxidase dismutase, peroxidase, polyphenol oxidase, catalase, esterase and their isoenzymes and sodium, potassium ions. In another experiment to study the

effects of osmoprotectants in imparting salt tolerance in paddy seedlings of 15 DAG were treated with osmoprotectants like proline and trehalose of 1 mM concentration for six hours and then to see the effects of osmoprotectants to counteract the salinity, these seedlings were retreated in 100, 150 and 200 mM sodium chloride solution for 72 h. These osmoprotectants and saline treated seedlings were analyzed for biochemical parameters.

Genotype CSR-27 recorded the highest germination percentage and GR-3 recorded the minimum germination percentage whereas Jaya and Dandi both remained at par. Better growth vigour index was observed in Jaya and Dandi as compared to GR-3 and CSR-27.

GR-3 recorded the highest chlorophyll content and Jaya recorded the highest total soluble sugar. Among the different levels of NaCl, chlorophyll content increased up to 150 mM but it decreased gradually with time intervals. Total soluble sugar content increased towards increasing salinity levels and also with time intervals in all the varieties.

Protein content also increased up to 150 mM NaCl level then decreased at 200 mM level. Tolerant varieties recorded the highest protein content at all three time intervals. However, proline which serve as osmoprotectants increased continuously up to 200 mM NaCl treatment in all the varieties. The trend also remained similar with different time intervals. Dandi recorded the maximum protein content at 24 h, 48 h time intervals whereas GR-3 recorded maximum proline content at 72 h time period.

Study on antioxidantive enzymes susceptible variety GR-3 recorded the maximum SOD activity where CSR-27 recorded the maximum activities of both catalase and peroxidase. Dandi recorded the highest esterase activity. Polyphenol oxidase activity did not follow such trend up to 24 h. Jaya recorded the highest value whereas at 48 h CSR-27 and at 72 h Dandi recorded the highest PPO activity.

Sutdy on effects of ionic imbalance due to saline treatment revealed the highest sodium and potassium levels in Jaya. Whereas tolerant variety CSR-27 recorded the highest potassium content (except 48 h).

The study on changes in isoenzyme banding pattern of antioxidants enzymes showed some differences based on their intensity. The protein profile by SDS-PAGE observed the presence of some inducible proteins due to salinity.

Treatment with osmoprotectants like proline and trehalose also showed the positive effect to counteract the saline stress in sensitive cultivar GR-3.

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CERTIFICATE

This is to certify that the thesis entitled "**BIOCHEMICAL CHARACTERIZATION AND EFFECTS OF OSMOPROTECTANTS UPON SODIUM CHLORIDE SALINITY STRESS IN RICE CULTIVARS**" submitted by **Shri Mungara Balakrishna** in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (Agriculture)** in **Biochemistry** to the Anand Agricultural University is a record of bonafide research work carried out by him under my guidance and supervision and the thesis has not been previously formed the basis for the award of any degree, diploma or any other similar title.

Place : Anand **(Y. M. Shukla)**
Date : May , 2006 **Major Advisor**

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ABBREVIATIONS

AAU	Anand Agricultural University
Anon.	Anonymous
ANOVA	Analysis of variance
AOS	Activated oxygen species
APEDA	Agricultural and Processed Food Products Export Development Authority
APOX	Ascorbic peroxidase
APS	Ammonium per sulphate
$CaCl_2$	Calcium chloride
CAT	Catalase
CD	Critical difference
Cm	Centimeter
CV	Coefficient of variation
cv.	Cultivar
DAG	Days after germination
°C	Degree centigrade
DMSO	Dimethyl sulphoxide
dSm	Desi Siemens per mole
EC	Enzyme code
EC	Electric conductivity
EDTA	Ethylene diamine tetra acetic acid
EU	Enzyme unit
Fig	Figure
g	Gram
GDW	Glass distilled water
glu	Glucose
GPOX	Guiacol peroxidase
h	Hour
H_2O_2	Hydrogen peroxide
ha	Hectare
HCl	Hydrochloric acid
$HClO_4$	Perchloric acid
HNO_3	Nitric acid
k. Cal	Kilo calories
%	Percentage
KCl	Potassium chloride
KCN	Potassium cyanide
kDa	Kilodalton

L	Linn
µg	Microgram
µl	Microlitre
mA	Milli ampere
ME	Mercapto ethanol
mg	Milligram
$MgCl_2$	Magnesium chloride
Mha	Million hectare
min	Minutes
ml	Millilitre
mM	Millimolar
MT	Million tones
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NBT	Nitroblue tetrazodium
nm	Nanometer
O.D.	Optical density
O_2	Oxygen
PAGE	Polyacrylamide gel electrophoresis
pH	Negative logarithm of hydrogen ion concentration
POX	Peroxidase
ppm	Parts per million
PPO	Polyphenol oxidase
R_m	Relative mobility
R_{ef}	Retardation factor
ROS	Reactive oxygen species
rpm	Revolution(s) per minute
S	Susceptible
S	Salinity stress
S.Em.	Standard error of mean
SDS	Sodium dodecyl sulphate
Sec	Second
SOD	Supper oxide dismutase
T	Tolerant
TEMED	N,N,N^1,N^1 Tetra methyl ethylene diamine
Tris	2-amino-2-hydroxymethyl propane-1,3-diol
TSS	Total Soluble Sugar
V	Variety
Vg	Vigour index
viz.,	Like
Vol.	Volume
Wt.	Weight

I. INTRODUCTION

"Rice is life" reflects the importance of rice as primary food source, and is drawn from an understanding that rice based systems are essential for food security, poverty alleviation and improved livelihoods. Rice (*Oryza sativa* L.) is the staple food of 60% of the world population (2.4 billion people), the number will increase to 4.6 billion people by 2050 (Mishra, 1998). The crop is grown in 152 million hectares in the world with the production of 586 MT (Mahadevappa, 2004). India stands first in area and second in production as well as consumption next to China. In India, rice is grown in 43.92 million hectares with the production of 91.61 MT and 2000 kg/ha productivity (Singhal, 2003). Major rice growing states in the country are West Bengal, Andhra Pradesh, Chhattisgarh, Tamilnadu, Karnataka, Assam, Maharashtra,Orissa,Punjab and Gujarat. In Gujarat, the rice growing area is about 6.55 lakh hectares with the production of 1.01 MT and productivity is 1553 kg/ha (Anon., 2003). Cultivation of rice is affected by various biotic and abiotic stresses, among the abiotic stresses salinity is a severe threat, from the total cultivable land, 8.57 million hectares are salt affected of which 1.21 million hectares is in Gujarat (Anon., 2004). In Gujarat, rice crop is grown in Surat, Valsad, Navsari, Kheda, Anand, Vadodara, Panchmahals and Ahmedabad districts.

Plants frequently encounter stresses, external conditions that adversely affect growth, development, or productivity. Stresses can be biotic or abiotic.

Environmental conditions that responsible for abiotic stresses are low temperatures, inadequate mineral nutrients in the soil and too much or too low light. High salinity in the soil is a common environmental problem; and affects all most all plant functions. Salinity in soil or water is of increasing importance to agriculture because it causes a stress conditions to crop plants affect the field severely. It is the one of the important constraints and better understanding of the mechanisms that enable plants to adapt to salinity stress and maintain growth will ultimately help in the selection of stress tolerant cultivars for exploiting saline soils.

Salinity is a major problem in agriculture today. Soil salinization and alkalization are serious land degradation problems affecting approximately 10% (about 952 Mha) of the total land surface of the globe (Singhal, 2003). The problem occurs in varying intensities in more than 20 countries extending to all the countries and is more prominently witnessed in the arid and semi-arid regions. Many coastal areas in the humid climate are also affected due to the ingress of sea water. This problem also occur in India particularly in the Indo-Gangetic plains, arid and semi-arid regions of Rajasthan and Gujarat, Maharashtra, Karnataka, Andhra Pradesh, Tamilnadu and coastal belts (Yadav, 1993).

High concentrations of salts like $MgCl_2$, NaCl, $CaCl_2$, and KCl are hazardous to agricultural crops because of some constraints over agricultural lands *viz.*, improper drainage, poor infiltration etc. These salts accumulate in

the top layer of the soil and inhibit the proper growth of the plants at initial stages. Thereby resulting poor establishment of plants in the field. Among various salts present in the cultivated lands, sodium chloride is the most prominent one both in quantity as well as in severity. Which effects crop plants adversely right from germination to ultimate crop growth stages.

Salinity is one of the most important abiotic stresses especially for rice, which is mostly grown under irrigated conditions. Ability of rice to grow on saline soils has been proved in many parts of the world. Rice is a species whose recent evolutionary history has been in fresh water marshes, it can be adapted to water logged condition, possessing a well developed root oxidation properties (Yoshida, 1981). Many researchers consider rice to be a crop with medium salt resistance and since water reduces salt concentration, plant growth is not inhibited. But high concentration of salts causes hyper osmotic and ionic stresses which in turn generate secondary stresses such as oxidative stress, ionic imbalance and ultimately cell death (Rana, 1988).

Host plants adopt various physiological salt tolerance mechanisms such as accumulation of ions, osmotic solutes, ion selectivity, osmotic adjustment and water use efficiency. Besides this, biochemical and molecular approaches also helps to counteract the effects of salinity remarkably.

Accumulation of osmotically active compounds called osmolytes lower the osmotic potential. These compatible solutes however do not apparently

interfere with normal cellular metabolism, but eventually could stabilize membranes loss and other damages to macro molecular structures.

Plants growing in a saline environment may be affected by the lower water potential in the environment and plant cell, causing reduced salt uptake or by increased salt uptake caused by high external ion concentrations. Plants may adjust to this situation by accumulating organic solutes where salt uptake is reduced by controlling high levels of salt uptake, so that plant cells osmotically adjust to external environment and thus maintain high turgor. However, plant growth is reduced where cell turgor can not be maintained or where internal salt concentrations become toxic to the normal cell metabolism (Johansen, 1987). Processes such as seed germination, seedling growth, vegetative growth, flowering and fruit set adversely affected by high salt concentrations ultimately causing diminished economic yield and also quality of produce.

Relative contribution and accumulation of high concentration of either inorganic ions or low molecular weight organic solutes play an important role in higher plants grown under saline conditions. The compatible osmolytes generally found in higher plants are low molecular weight sugars, organic acids, polyols and nitrogen containing compounds such as amino acids, amides, imino acids, protein and quaternary ammonium compounds (Ashraf and Harris, 2004).

The most apparent biochemical changes occurring when plants are subjected to deleterious stress condition in the production of activated oxygen species (AOS). The chloroplasts and mitochondria of the plant cells are important intracellular generators of activated oxygen species. Electrons released from electron transport chains can react with O_2 during normal abiotic metabolism of characteristics of adaptive value.

Study for the response of plants/crops to salinity under naturally saline conditions is not feasible due to extreme variability in soil salinity both spatially and temporarily. To avoid this problem, comparative differences for salt tolerance among crops/varieties have been studied under artificially salinized control conditions.

The mechanism behind the salt tolerance is not understood clearly. But different researchers have done the work regarding salt tolerance mechanism in various crop plants. Based on their investigations, some osmoprotectants like proline and trehalose are used to improve salinity tolerance in rice.

Since salt damage has a broad physiological spectrum affecting many metabolic processes, it is difficult to assess the contribution of individual processes to plant death to be final damage done to the plant. One approach for evaluating the contribution of individual process to salt damage has been to compare crop varieties that show differential responses to salt stress. Studies with closely related varieties, differing in salt tolerance can also contribute greatly for the elucidation.

The tolerant plant possesses specific characteristics, which provide resistance against high concentration of salts and sodium. The specificity of plant exclude salts or their ability to osmoregulate or stability of membrane and enzyme system against high concentration of salts has close relationship with their salt tolerance.

Salinity affects the plants at all stages of development and in some cases sensitivity varies with the growth stage of crops. Rice has been found to be tolerant during germination, very sensitive at early seedling growth and more tolerant at vegetative growth stage (Kadlag *et al*., 1993). Detail information available for biochemical and molecular characterization at seedling stage of different cultivars of crop growth is limited and hence, keeping this in view, present study has been undertaken to differentiate the tolerant varieties at different time intervals with the following objectives:

- To study the response of different level of salinity in different varieties of rice differing in salt tolerance.
- To study the physiological and biochemical marker for salt tolerance.
- Development of suitable marker in the form of isozymes or specific protein for identification of tolerant lines.
- Determination of Na^+/K^+ ratio as a marker for salt tolerance.
- To elucidate the possible role of enzymes in salt tolerance.
- To study and reveal the effects of osmoprotectants towards salt tolerance.

II. REVIEW OF LITERATURE

Origin of rice

The primary centre of origin of *Oryza sativa* L is large belt extending from northeast hills in India to the mountain ranges of the mainland Southeast Asia and South West-China (Chopra, 2001).

Importance of rice

It is the second largest cereal crop after wheat, which is a staple food for fifty percent of world's population. In Asia, rice supplies 30 to 80 percent of daily calories consumed (Narciso and Housain, 2002). Rice is an anomaly among the domesticated cereals, a C_3 grass that evolved in a semi aquatic, low radiation habitat. It can be grown in almost all climatic conditions range from temperate to humid tropics. It is a rich source of carbohydrates as well as proteins. It can be used in different forms depending upon the food habits of the people, rice bran oil is less fat containing edible oil is also an important as nutritional point of view. The dried grass also useful as animal feed. From export point of view, agricultural export is 17 percent of India's total exports. The rice as such has become one major exportable commodity with its share forty-two percent of APEDA (Agricultural and Processed Food Products Export Development Authority) total exports.

Table 1 : Nutritional composition and nutritional value of rice

Nutritional composition		**Nutritional value**	
Major Nutrient	**Percentage**		
Carbohydrates	79 - 91.4	Available carbohydrates	64.4 %
Protein	6.7 - 8.2	Energy (Kcal/kg)	1610
Crudelipids	2.4 - 2.8	Digestible energy	96.3 %
Ash	0.4 - 0.9	**Major vitamins**	**(mg/100g)**
Crude fiber	0.2 - 0.4	Thiamin	0.29
Minerals	**ppm**	Riboflavin	0.04
Calcium	7.4 - 17.4	Niacin	4.0
Phosphorus	170.2 - 397.2	**Major amino acids**	**(mg/100g)**
Iron	2.4 - 8.5	Lysine	3.8
		Thereonine	3.6
		Methionine & cysteine	3.9
		Tryptophan	1.1
		Protein quality	**(per cent)**
		True digestibility	99.7
		Biological value	74.0
		Net protein utility	73.8
		Utilization protein	5.4
Chavan and Kadam (1989)		Grist (1986)	

Constraints over rice production

The yield of rice is reduced due to both biotic as well as abiotic stresses nearly about 55 to 60 percent. Stem borer, plant hoppers and gall midge are the most important pests and blast and leaf spots are the major diseases. Various abiotic stresses limit rice production, which comprise about 45 percent of the global rice area. Important abiotic stresses include water deficit, submergence, salinity and mineral deficiencies of phosphorus and zink. Among these, salt stress is one of the major stresses affecting the crop production significantly.

2.1 SALT STRESS

Salt stress is due to accumulation of high concentration of salts in soil. The major salts are sodium chloride (NaCl), sodium sulphate (Na_2SO_4), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$) and potassium chloride (KCl). These salts not only lead excess salinity in soil but also affect crop growth severely. In Asia alone, 21.5 million ha are affected of which 12 million ha are saline and 9.5 million ha are alkaline/sodic (Anon., 2004).

Rice is a salt sensitive crop, but it is the only cereal that has been recommended as a desalinization crop because of its ability to grow well under flooded conditions and because the standing water in rice fields can help to leach the salts from the top soils to a level enough for subsequent crops (Bhumbla and Abrol 1978). Despite its high sensitivity to salinity,

considerable variation in tolerance was observed in rice (Akbar *et al.,* 1972; Flowers and Yeo, 1981). Crop plants in natural environments are exposed to various levels of salinity. One-third of the land being irrigated worldwide is affected by salinity, but salinity also occurs in non-irrigated land (Allen *et al.,* 1994). The irrigation water is also responsible for salinity which prominently contains salts rich in calcium, magnesium, and sodium (Serrano *et al.,* 1999). Evaporation and transpiration of calcium and magnesium ultimately leaves sodium dominance in the soil. In fields, the salt levels fluctuate seasonally and spatially, and variation will occur due to the circumstances influencing each particular plant. The uptake of ground water by plant roots can increase the salinity of ground water and the soil around the roots due to the exclusion of salt (Niknam and McComb, 2000).

These variable conditions make research difficult, and this is compounded by the fact that each species has its own level of salt tolerance. Together, it will be a complicated process to match plants with their optimal growing conditions. The response of plants to salt stress is based on the transcriptional action of many defense proteins, and research has not discovered the basis for them all (Serrano *et al*. 1999). Osmotic stress and ion toxicity are the problems stemming from salt stress and the resulting decrease in chemical activity causes cells to lose turgor. Cell growth depends on turgor to stretch the cell walls and lack of turgor

implies danger for cell survival. The plant's defense against this salinity attack requires osmotic adjustment and to a certain degree, this can be done through synthesis of intracellular solutes. Salinity creates the ion toxicity. High salt concentrations inhibit enzymes by impeding the balance of forces controlling the protein structure. As the toxic effects of salt can occur at relatively low concentrations, depending on the plant species the homeostasis of sodium is important for the tolerance of organisms to salt stress (Serrano *et al.,* 1999). The stress caused by ion concentrations allows the water gradient to decrease making it more difficult for water and nutrients to move through the root membrane. In turn, the water uptake slows, and as the osmotic effect spreads from the root membrane to the internal membranes, the ion concentration inside the plant alters the solute balances. Once high concentrations of salt have reached the inside of the plant; tissue and organs development is altered. The salt causes a slower rate or shorter duration of expansion of cells and this compromise the size of the leaves. The overall effect of salinity on plants is the eventually leads shrinkage of leaf size and subsequently the plant death (Volkmar *et al.,* 1998). Salinity may also cause reduced Adenosine tri phosphate (ATP) and growth regulators in plants (Allen *et al.,* 1994).

2.1.1 Morphological effects of salinity

Morphological symptoms such as wilting and drooping of seedlings and appearance of white patches on the leaves are the indications of the

injurious effects of salt stress. The extent of inhibitory or adverse effects can be known only by making critical comparisons with plants growing under comparable conditions in normal soils. Salinity may directly or indirectly inhibit cell division and enlargement in the plants growing point. Reduced shoot growth caused by salinity originated from growing tissues not in mature photosynthetic tissues (Munns *et al*., 1982). As a result, leaves and stems of the affected plants appear stunted. Chloride also induces elongation of the palisade cells which leads to succulents leaves. Salt stress reduces dry matter content increases root to shoot ratio and diminishes leaf size, and as a result grain yield is reduced. (Mass and Poss, 1989).

2.1.2 Physiological effects of salinity

Physiological process such as photosynthesis, photorespiration, mobilization of food reserves, respiration, growth and development are severely disturbed due to excess salt in the soil. These effects adversely affect plant growth either through osmotic inhibition of water uptake by roots or by specific ion effects (Bernstein and Hayward, 1958). Specific ion effects may cause direct toxicity or alternatively the insolubility or competitive absorption of ions. Photosynthesis is reduced because it is affected by leaf expansion rate, leaf area and leaf durations as well as photosynthesis and respiration per unit leaf area. This decrease may be due to stomatal closure or the direct effects of salt on the photosynthetic

apparatus. Transport of photosynthates in the phloem is also inhibited (Passera and Albuzio, 1997). Mineral uptake by roots is affected as a result of imbalance in the availability of different ions (Munns and Ternatt 1986).

2.1.3 Germination

In entire crop period germination is the first critical phase on which success of the crop depends. The reasons for the reduction in germination percentage is because of accumulation of high concentrations of salt which reduces the synthesis of seed reserve and enhances hydrolysis of protein scarcity (Uprety and Sarin, 1974).

Dajanaguiraman *et al*. (2003) observed that the germination percentage decreased with increased salt concentration, among the salinity levels the highest germination was observed in 100 mM (83.65) and the lowest in 300mM (67.41). Pokkali is a highly tolerant variety with ninety percent above germination where as IR50 with below seventy percent germination showing the susceptible nature.

Tirumeni *et al*. (2001) also reported the similar observations in fifteen cultivars of rice germinated in 0, 4, 8 and 12 EC solution for fourteen days at room temperature.

Sugimoto (1986) observed the decrease in germination for *Indica* rice with 0.2 percent NaCl. Mandal *et al.* (1988) also reported decrease in

germination percentage with increase in salinity levels in twelve cultivars of rice germinated in 5, 10 and 15 dSm^{-1} EC solution for seven days.

Punyavardena and Dharmasri (1989) compared the salt tolerance of rice cv. BG-350, incubating seeds in solution of 0.25, 1, 2, 3, 4, 6, 8, 10 and 12 dSm^{-1} for five days and observed similar response in germination up to 8 dSm^{-1}. Seeds of five rice cultivars, germinated at 0.2, 4, 8, 12, 16 and 20 dSm^{-1} salinity levels in moist sand by Ahemd *et al*. (1990) and found out decreased emergence with increased salinity. Gupta (1993) revealed significant reduction in germination percentage in ten selections of rice were significantly reduced when salinity was increased from 3 to 7.8 dSm^{-1}.

Rao et *al.* (1995) observed higher germination percentage and better root growth in GR-11, CSR-10and SLR than GR-3, GR-4 varieties of rice which showed higher sensitivity.

2.1.4 Seedling growth

The differential response of rice varieties to salinity clearly pronounced in terms of seedlings height and physiological appearance. Mass screening techniques are more effectively applied to vegetative growth. Reduction in plant growth has been observed with change in the turgidity of the cell (Johansen, 1987). Seedling growth decreased in rice with increase in salinity level from 0.5 to 22.7 dSm^{-1} (Babu, 1985).

Krishnamurthy *et al*. (1987) showed high salinity indices in salt tolerant cultivars (AV1, CO-43, CSC-1) and less reduction of seedling growth than salt sensitive cultivars. They identified early wilting in sensitive cultivars than tolerant cultivars.

Seedling growth declined with salt concentration from 0 to 12 dSm^{-1} in all the tolerant as well as susceptible varieties (Tirumeni *et al.,* 2001). They noticed that high concentration of salts disturb the normal cellular metabolism there by decreasing in the growth.

Djanaguiraman *et al*. (2003) observed that the root length, shoot length and vigour index decreased with increased salt concentration

Natarajan *et al*. (2004) observed that fresh and dry weights of shoot and shoot length were significantly affected under saline hypoxic conditions. Shoot and root length and fresh and dry weights decreased due to increased level of salinity in all the rice varieties

2.2 BIOCHEMICAL ALTERATIONS WITH SALINITY

Chlorophyll a, chlorophyll b, total chlorophyll, amino acids, proteins, total soluble sugars and enzymes are the major biochemical parameters, which are severely affected during saline stress. These constituents can also serve as a marker for salt tolerance. Salinity changes the levels of plant hormones, such as abscisic acid and cytokinin (Moorby and Besford, 1983). It has been suggested that salt affects cellular and nuclear volume, includes nucleic acid and protein synthesis (Leopold and

Willing 1984). Several steps involved in protein synthesis are very sensitive to changes in the ionic environment and may result in impairment of protein metabolism. (Wynjones *et al.,* 1979) The effects on seedling growth have been attributed to variations in biochemical parameters. Sodium chloride inhibit radical emergence by adversely affecting water absorption (Gill and Singh, 1985) and mobilization of reserves from storage organs (Gomes *et al.,* 1983).

Salt stress situations cause increase production of toxic oxygen derivatives. Antioxidantive defense system counteracts the toxicity of active oxygen species (Sairam and Tyagi, 2004). Accumulation of organic and inorganic solutes also serves as potential indicators of salt tolerance.

2.2.1 Accumulation of osmolytes

Osmotic adjustment in plants subjected to salt stress can occur by the accumulation of high concentrations of either inorganic ions or low molecular weight organic solutes. These solutes of low molecular weight are nontoxic even at high concentrations. Osmoprotectants therefore, play important roles in adaptation against several environmental stresses such as temperature, salt, drought and chilling. Although both of these play an important role in higher plants grown under saline conditions their relative contribution varies among species and cultivars. The compatible osmolytes generally found in higher plants are low molecular weight sugars, organic acids, polyols and nitrogen containing compounds, imino acids, proteins

and quatenanary ammonium compounds (Ashraf and Harris, 2004).

2.2.2 Mobilization of seed reserves

Mobilization of seed reserves like sugars, proteins and fats during early seed germination is crucial because it supplies substrates for the proper functioning of different metabolic processes that are essential for growth of embryonic axis. The mechanism of NaCl may inhibit mobilization of reserves (Prakash and Prathapasenan, 1988).

Gill and Singh (1985) studied the effect of salinity on carbohydrate metabolism during paddy seed germination under salt stress conditions and recorded that salt tolerant varieties of paddy (Jaya and PR-106) had higher rate of water absorption followed by rapid decrease in starch and greater release of soluble sugars than sensitive varieties (M-48,Palman-579 and Basmati-370).

2.2.3 Chlorophyll contents

Pushpam and Sreerangasamy (2000) studied the effects of salinity on seven rice cultivars with different levels of NaCl and noticed that in all the varieties increase in salt concentration increased the chlorophyll content up to one percent. Similar results were reported on rice by Gill and Dutt (1987) and Pandey and Srivastava (1987). Krishnaraj *et al*. (1992) reported increase in total chlorophyll, chlorophyll a, chlorophyll b in wheat as a result of salt stress. The increase was more in tolerant variety than in the susceptible variety.

Govindaraju and Balakrishnan (2002) conducted an experiment with three levels of salinity *viz*. EC < 1, 1-4, > 4 dSm^{-1} with four rice varieties and noticed that chlorophyll content was decreased with salinity and reduction was more in the susceptible varieties than the resistant varieties.

Pandey and Srivastava (1987) demonstrated the two sodium chloride salinity regimes in rice. The adverse effect of salinity was exposed mainly through their influence of photosynthesis rather than chlorophyll. Sahu and Mishra (1987) observed that decreased in chlorophyll content with acceleration of salt stress.

Saxena and pandey (1987) observed soil salinization to 10 dSm^{-1} decreased chlorophyll content and photosynthetic rate. Kang and Titus (1987) indicated that NaCl and KCl enhanced the degradation of chlorophyll and protein in detected rice leaves. At high salinity the degradation of chlorophyll was somewhat lower in the salt tolerant cv. Kharchia and Sakha-8 than in salt sensitive cultivars cv.Sakha-69 and T-79 of wheat. The chlorophyll a / chlorophyll b ratio increased from 2.6 to 4.1 considerably more in sensitive cultivars than in the tolerant one (Salama *et al.,* 1994).

Ashraf and Ali (1998) studied the effect of salinity on growth, chlorophyll and flag leaf area of rice genotypes and concluded that

chlorophyll content (a, b and total) of rice was generally reduced by salinity.

2.2.4 Soluble sugars

According to Cram (1976) of the various osmotica, sugars contribute up to 50 percent of the total osmotic potential in glycophytes subject to saline conditions. The accumulation of soluble carbohydrates in plant has been widely reported as a response to salinity or drought. In the case of salt (Gill and Singh, 1985), adaptation to these stresses has been attributed to the stress induced increase in carbohydrate levels.

Prakash and Prathapasenan (1988) recorded higher concentration of sucrose and lower concentration of starch and protein in rice plant exposed to NaCl than in control.

The effect of osmotic stress on germination, growth and soluble sugar content in S*orghum bicolor* (L) cv. CSH-9 seeds and seedling component during early germination was investigated by Gill *et al*. (2002). A considerable increase in sugar levels in both embryo and endosperm was detected under stress conditions. Total sugar and reducing sugar content increased remarkably after 14 hours of stress imposition. However, the level of glucose and sucrose was higher in the embryo and endosperm after stress treatments than in the controls.

2.2.5 Soluble proteins

Several salt induced proteins have been identified in plant species and have been classified into two distinct groups; salt stress proteins which accumulate only due to salt stress and stress associated proteins which also accumulate in response to heat, cold, drought and high and low mineral nutrient. Proteins that accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is reutilized when stress is over (Singh *et al.,* 1987) and may play a role in osmotic adjustment.

Pushpam and Sree Rangasamy (2000) identified that protein content in rice seedlings decreased with increased salt concentration. Similar results were also reported by Reddy and Vaidyanath (1986) and Daniel and Sree Rangasamy (1991). They revealed that salt ions interfere with protein synthesis mechanism and obstruct the consequent steps there by net protein content decreased towards salinity.

Dubey and Rani (1989) observed that salt tolerant cultivars (CSR-1 and CSR-3) maintained higher protein and amino acid than the susceptible cv. Ratnanol and Jaya whereas the protein and amino acid concentrations of root and shoot increased with salinity level in both type of cultivars.

Krishnamurthy and Bhagwat (1989) measured the protein and total nitrogen levels in shoots and roots of salt tolerant and salt sensitive rice cultivars under NaCl-stress (100 mM NaCl equivalent to EC of 9.35

mmhos/cm) during different stages of seedling growth. Salt tolerant cultivar CO-43 was highly efficient in maintaining high levels of insoluble protein and total nitrogen in the shoot and root systems and experienced less reduction of shoot and root growth than the salt sensitive culture TKM-9 during seedling stage.

The stress resulted from dehydration can denature many proteins and membranes. Plant exposed to these stresses induce many different types of gene expressions and the majority of new gene induced compensate for denature proteins or to repair injuries. The proteins made during stress serve as osmoprotectant. Salinity stress induced eight proteins in the roots of salt sensitive rice Var. Taichung Native-I (Claes *et al.,* 1990). One of them was found to have molecular mass of 15 kDa and an isoelectric point of 5.5. During the investigation of osmo defense processes in rice Garcia *et al*. (1997) noted the same 15 kDa protein induced reproducibly in double strength MS medium supplemented with one percent NaCl, one percent KCl.

Shirata and Takagishi (1990) showed the presence of a 26 kDa and a 27 kDa protein in rice cell cultured in medium containing 10 and 20 g NaCl but it was absent in control.

2.2.6 Proline content

Prolines may be synthesized *de novo* in response to salt stress or may be present constitutively at low concentration and increase when plants are exposed to salt stress (Pareek *et al.,* 1997).

Proline, which occurs widely in higher plants, accumulates in larger amount than other amino acids in salt stressed plants. Proline accumulation is one of the common characteristics in many monocotyledons under saline conditions (Storey *et al.,* 1977; Pushpam and Sree Rangasamy (2000); Stewart and Lee (1974) and Dubey and Rani (1989). noticed that in rice varieties proline content increased towards salinity.

Water loss and proline accumulation under water and salt stress were accelerated more in the salt sensitive cultivar Iri-348 than in the salt tolerant cultivar Jinju in rice plant. Stress conditions induced a high ratio (5.6 to 7.2) of low molecular organic solutes to crude protein in Jinju (Park, 1982). Nyan and Shyon (1984) found the free proline content increased by 336-415 percent with sodium compared to control. The proline content increased more in susceptible (IK-8) than resistant (TDN) cultivar. Krishnamurthy *et al*. (1987) recorded the cultivar TKM-9 and CO-43 to accumulate high levels of sodium and exhibited less reduction of vegetative growth, free protein and bound proline in shoot and root under saline condition.

Seedlings of ten rice cultivars when treated with 0, 0.2, 0.4, 0.6 or 0.8 percent NaCl treatment, differences in salt injury between cultivars were clear at twenty five days after treatment with 0.6 percent NaCl (Lee *et al.,* 1992). Basu *et al*. (2002) observed the similar results in unadapted and NaCl adapted callus of a salt sensitive (Basmati-370) and salt tolerant (SR-26 B) cultivar of rice after a NaCl shock.

The salt induced accumulation of nitrogen compounds such as free amino acids, and ammonia in shoots of eight rice cultivars differing in salt tolerance was investigated. Salt treatment of 100 mM for 6 days significantly increased the proline content of shoots but appeared to be a reaction to stress damage and not associated with salt tolerance (Nguyen *et al*., 2003).

Raja *et al*. (2003) attempted to study the free proline content of rice seedlings grown under stress conditions and the salt tolerance rice (CO-43 and TRY-1) and salt sensitive (ADT-36 and White Ponni) were subjected to salt stress *in vitro* by growing in Hoagland nutrient solution added with 50 mM of NaCl solution. Higher proline content was recorded in salt sensitive cultivars at 7 and 21 days after treatment as compared to salt tolerant cultivars.

2.3 ANTIOXIDANT ENZYMES AND THEIR ISO-ENZYMES

For plants to survive under salt stress conditions, it is important that antioxidants work in co-operation. During salinity stress generation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl molecules cause rapid cell damage by triggering reduction type chain reaction. To counteract this effect antioxidant enzymes like superoxidedismutase (SOD), catalases (CAT), peroxidase (POX) are induced towards stress situations (Srivalli *et al.,* 2003).There is conclusive evidence that production of activated oxygen species is enhanced in plants in response to different environmental stresses such as salinity, drought, water logging or mineral nutrient deficiency (Wise and Naylor, 1987). The increase in the activity of antioxidant enzymes is due to the higher levels of O_2 production under stress. This shows that an efficient defense mechanism might be involved in the increase of the antioxidant levels.

Plants containing high concentration of antioxidants show considerable resistance to the oxidative damage caused by the activated oxygen species. Djanaguiraman *et al.* (2003), Wang *et al.* (1990), Fadzilla *et al.* (1997), Dionisio and Tobita (1998) and Khan *et al*. (2002) have worked for comparing the mechanism of antioxidants production and their role in salt tolerant and salt sensitive plants.

Djanaguiraman *et al.* (2003) and Badiani *et al*. (1990) reported that peroxidase as well as catalase activities increased with increased salinity levels in both tolerant and sensitive cv. of rice.

Wang *et al*. (1990) studied decrease in the activities of superoxide dismutase (SOD) and catalase (CAT) in rice seedling cv. 80-85 (salt tolerant) and cv. 83-51 (salt tolerant) with 0.2, 0.5 and 0.8 percent NaCl in hydroponic culture. Mittal and Dubey (1992_b, 1995) recorded association of salt tolerant ability with higher catalase activity in embryo axes of germinating rice under salinization. Catalase activity was higher in seedlings of salt tolerant cv. CSR-1 and CSR-3 than in sensitive cv Jaya and Ratna. Salinity stress during early germination caused greater membrane deterioration by lipid peroxidation in cv. Ratna than Co-6. Hamilton and SR-26B (Bhattacharjee and Mukherjee, 1997). When shoot cultures derived from salt sensitive rice cv. Taipei-39 grown in medium containing 0.35 M NaCl, overall levels of Mn-superoxide dismutase activity, Cu, Zn-SOD and H_2O_2 were significantly elevated (Fadzilla *et al.,* 1997).

Dionisio-sese and Tobita (1998) reported a decline in SOD activity and an increase in peroxidase activity in the salt sensitive rice varieties, Hitomebore and IR-28, in response to salt stress. In contrast, salt tolerant cv. Pokkali showed only a slight increase in SOD but a slight decrease in

peroxidase activity. Khan *et al*. (2002) studied NaCl-salinity stress induced oxidative reaction in root tissue of rice seedlings. It revealed decrease activities of CAT, guaiacol peroxidase (GPOX) and SOD with the increasing NaCl concentrations. The activities of catalase, guaiacol peroxidase and superoxide dismutase increased in the salt treated leaves of salt sensitive *indica* rice (Panda and Khan, 2003).

Swapna (2003) studied salt stress induced changes on esterase, superoxide dismutase, peroxidase and catalase activities during different developmental stages of rice. An increase in activity of SOD and peroxidase was observed during different developmental stage under stress but there was a fluctuation in catalase activity under NaCl stress during developmental stage in all varieties.

The activities of some antioxidative enzymes in mangrove, *Bruguiera parviflora* subjected to varying levels of NaCl under hydroponic culture. In the leaves, salt treatment preferentially enhanced the content of H_2O_2 as well as activity of ascorbate peroxidase (APOX), guaiacol peroxidase (GPOX) and superoxide dismutase (SOD) whereas decreased the catalase (CAT) activity (Parida *et al*., 2004).

Development of suitable marker-system for identification of tolerant lines is a pre-requisite in the form of isozymes and specific proteins which correlate with stress adaptation and stress tolerance in

different plant systems including rice (Claes *et al.,* 1990; Moons *et al.,* 1995).

Isoenzymatic patterns of several important stress related enzymes can show variable responses to stress inducing factors.

Peroxidase isoenzyme characteristics *in situ* and *in vitro* were compared in four rice cultivars with different degree of salt tolerance by Mittal and Dubey (1995). After 96 h of NaCl treatment under low concentration (90 mM), it was observed that more tolerant cv. showed similar isoemzymatic pattern (5 bands). The highest salt concentration in the medium (148 mM) the lowest the intensity in the first three bands. Basu *et al*. (1997) observed the profiles of isozymes (peroxidase and esterase) of unadapted, NaCl (128 mM) and mannitol adapted (247 mM) calli in two varieties of rice (Basmati-370 and SR-26 B). Appearance of two new isoperoxidase bands (R_{nf} 0.6 and 0.75) and one esterase band (R_{nf} 0.68) was unique and common in NaCl-adapted callus of both the varieties.

Wei *et al*. (1995) characterized superoxide dismutase (SOD) using electrophoresis and recorded similar patterns of multiple superoxide dismutase isoenzymes in the leaves of rice given different salt stress treatments. Three rice cultivars. (salt sensitive, salt tolerant and intermediate tolerant) were studied using peroxidase (POX) isoenzyme

analysis by Wang and Zheng (2000). Under rho (NaCl) = 10 g/l stress, a new band POD 4 was induced in salt tolerant lines No. 779. In the salt sensitive line ZooHua No. 2, three POX bands POX1, POX2, POX3 disappeared. They suggested the relation between changes of POX bands and salt tolerance in rice.

In order to analyze the changes of antioxidant enzyme isoforms against salt stress in the rice cv. Dangjin, plant extracts were subjected to native page by Lee *et al*. (2001). Leaves of rice plant had two isoforms of Mn SOD and five isoforms of Cu/Zn-SOD. Fe-SOD isoform was not observed in the activity gel. Expression of Cu/Zn-1, -2 and Mn-SOD-2 isoforms was preferentially enhanced by salt stress. Seven ascorbate peroxidase (APOX) and glutathione reductase (GR) isoforms were presented and results were concluded that SOD leads to the over production of hydrogen peroxide in the leaves of rice plants subjected to salt stress and the over production of hydrogen peroxide functions as the signal of salt stress, which induced the induction of specific APOX isoforms but not specific isoforms under catalase deactivation.

2.4 MINERAL IONS

During salinity stress levels of sodium, calcium, magnesium, chlorides and ionic composition is imbalanced. There exists an inverse relationship between salt tolerance and sodium (Na) accumulation in non-

halophytes and it may be used as an index of characterizing salt tolerant genotypes (Greenway and Munnus, 1990).

Tripathy and Kar (1995) revealed that sodium content increased with increasing level of salinity while potassium content decreased with increasing salinity levels.

Dutt and Bal (1985) analyzed plant tissue for accumulation of Na^+ at peak tillering and concluded that rice varieties that accumulate less Na^+ in the shoots at peak tillering stage were more tolerant to salinity than the varieties that accumulate higher amount of Na^+. Zheng and Yan (1996) studied the distribution of Na^+ and Cl^- in root of six rice cultivars differing in salt tolerance using X-ray microanalysis. Under salinity stress, Na^+ content was highest in the stele parenchyma of salt tolerant cultivars and was evenly distributed throughout the roots in salt sensitive cultivars. Salt tolerant rice cultivars Pusa 221 and Saket-4 showed lower levels of sodium and higher levels of potassium in rice seedlings than the salt sensitive cv. Kamini and Sugandha when subjected to salt stress (EC-12 and EC-16) treatment. The sodium: potassium ratio was lower in tolerant cultivars and higher in sensitive cultivars (Mandal *et al*, 1999). Accumulation of Na^+ and K^+ in unadapted and NaCl adapted callus of a salt sensitive (Basmati-370) and a salt tolerant (SR-26 B) cultivar of rice was co-relatable to support differential growth in tissue after NaCl shock (Basu *et al.,* 2002).

Pandey and Sharma (2002) tested twelve genotypes of paddy under pot culture for their salinity tolerance at 10 and 15 dSm^{-1}. Salt tolerance genotypes *viz.,* Kalaratta, Damodar and RPA-5929 have relatively lower Na:K and Na:Mg ratio as compared to salt sensitive genotypes.

2.5 EFFECTS OF OSMOPROTECTANTS

Plants accumulate a number of osmoprotective substances in response to sodium chloride stress. Some of the proteins made during stress synthesize substances believed to serve as osmoprotectants (Delauney and Verma, 1990; Bartels *et al*., 1991). Sodium chloride stressed rice (*Oryza sativa* L.) accumulates polyamines (Krishnamurthy and Bhagwat, 1989) and proline (Alia Saradhi, 1993). When administered externally, these molecules have been found to protect plants from some of the damage that is caused by drought or excess salinity (Kavi kishor, 1989; Genard *et al.,* 1991; Krishnamurthy, 1991). Other plants have been found to accumulate common sugars (Kameli and Lorel, 1993), polyols (Loescher *et al*., 1992; Alexander *et al.,* 1994), or in some cases less common sugars such as trehalose (Fougere *et al.,* 1991; Drennan *et al.,* 1993). It is generally assumed that proline, polyols and sugars serve as physiologically compatible solutes that increase as to maintain a favorable osmotic potential between the cell and its surroundings (Pollard and Wynjones, 1979). There is additional evidence that high concentrations of these substances stabilize some macro molecules or molecular assemblies,

thus decreasing the loss of either enzymatic activity or membrane integrity that occurs when water is limiting (Schwab and Gaff, 1990; Genard *et al.,* 1991).

Osmoprotective substances did not accumulate through out the plant and are needed to protect different types of molecules or cellular components. Therefore structurally different osmoprotectants choose for their effects on salinity stress in rice (Garcia *et al*., 1997).

Proline and trehalose both osmolytes are strucrally as well as functionally different and effect on a number of sodium chloride sensitive physiological and biochemical processes in rice. Garcia *et al.* (1997) studied the effects of proline and trehalose on different physio and biochemical processes, by giving treatment separately to the sodium chloride stressed rice seedlings and noticed that trehalose protects rice seedlings better than proline. Proline promotes leaf growth only fifteen percent where as trehalose forty five percent. They identified trehalose as a potential osmoprotectant and compared the effect of proline and trehalose for their role in salt stress. They found that proline either has no effect or in some cases exasperates the effect of NaCl on growth inhibition, chlorophyll loss and induction of a highly sensitive marker for salt stress, by contrast low to moderate concentrations of trehalose reduced Na^+ accumulation and growth inhibition. Some what higher concentration (10 mM) prevent NaCl induced loss of chlorophyll in blade, prevents root

integrity and enhance growth. Finally they concluded that during osmotic stress trehalose or carbohydrates might be more important for rice than proline.

III. MATERIALS AND METHODS

The present study was conducted at the Department of Biochemistry, B.A.College of Agriculture, Anand Agricultural University, Anand.

3. 1 EXPERIMENTAL MATERIALS

Experiment was conducted during the year 2005 in the department of Biochemistry under controlled environmental conditions. Four varieties of rice *viz* Jaya, CSR-27, Dandi and GR-3 differing in degree of susceptibility to salt stress were procured from Main Rice Research Station, AAU, Navagam, Gujarat.

3.1.2 Glassware and polyware

All glassware used was obtained from Corning/Borosil and disposable plastic wares from Tarsons Ltd. The glassware were washed thoroughly with the detergent (Lab. wash, S.D. Fine Chem. Pvt. Ltd., Boisar) followed by tap water and finally rinsed with distilled water. The glasswares were dried in oven before use.

3.1.3 Chemicals and solvents

The chemicals used in the experiments were of analytical grade and were obtained from standard manufacturers through local dealers.

Equipments

Weighing balance	Bp 210D, Sartorius, Germany
Distillation unit (Water)	Millipore
Variable volume micropipettes	Finnpipette Lab. systems, Finland, Japan
Centrifuge	Sigma 2-16K, Germany
Waterbath	Yorko YSI-413, York Scientific Ind. Ltd., India
Spectrophotometer	Specord 200, Analytikjena, Germany

Gel electrophoresis unit

Vertical slab gel	Bangalore Genei Ltd., India
Sub Merine	AHO, Japan
Power pac	E 8750 AHO, Japan
Microwave oven	Kenstar, India

3.1.4 Preparation of stoke solutions

3.1.4.1 *For Native and SDS PAGE*

a) **Stock Acrylamide solution (30%)**

29.2 g Acrylamide

0.8 g N, N'-Methylene bisacrylamide

Final volume made upto 100 ml with distilled water.

b) **Stock – 1.5 M Tris - HCl**

Tris buffer 91.816 g was dissolved in 60 ml distilled water. pH was adjusted to 8.8 with 3 N HCl and final volume was made up to 100 ml with distilled water.

c) **Stock 0.5 M Tris-HCl**

3 g of Tris buffer was dissolved in 35 ml of distilled water. pH was adjusted to 6.8 with 3N HCl and final volume made up to 50 ml with distilled water.

d) **Electrode buffer pH (8.3)**

Tris buffer (3 g) and 14.4 g glycine were dissolved in distilled water and finally adjusted to 1000 ml. For SDS-PAGE 10 ml of 10% SDS was added and finally volume was made up to 1000 ml.

e) **10 % Ammonium per sulphate (APS)**

APS (100 mg) was dissolved in 1 ml of distilled water prepared fresh at the time of gel casting.

f) N, N, N'N'-Tetramethyl lenediamine (TEMED)- Readily available

g) 10 % Sodium Dodecyl sulphate (SDS)

SDS 10 g was dissolved and final volume was to 100 ml with distilled water.

h) **Gel loading dye**

Bromophenol blue	0.5 %
Glycerol	10 %
Sucrose	0.5 % (for isoenzymes)

i) **Preparation of 10 % running gel for isozymes (Native PAGE)**

6.6 ml stock solution of acrylamide (30 %)
3.5 ml stock 1.5 M Tris HCl (pH 8.8)
10.0 ml distilled water mixed and degassed
100 µl APS (10 %)
10 µl TEMED

j) **Preparation of 4 % stacking gel**

1.5 ml stock solution of acrylamide (30 %)
1.5 ml stock 0.5 M Tris-HCl pH (6.8)
7.0 ml distilled water were mixed and degassed
150 µl APS (10 %)
20 µl TEMED

k) **12.5% running gel for protein (SDS-PAGE)**

12.5 ml stock solution of acrylamide (30 %)
5.0 ml stock 1.5 M Tris-HCl pH 8.8
13.2 ml distilled water mixed and degassed
250 µl APS
300 µl SDS
20 µl TEMED

3.1.4.2 *Preparation of osmoprotectant solutions*

1 mM L-Proline

Dissolved 115.13 mg of L-Proline in distilled water and final volume make up to one liter.

1 mM of D-Trehalose

Dissolved 378.33 mg of D-Trehalose in distilled water and final volume make up to one liter.

3.2 EXPERIMENT DETAILS

The experiment was studied in two parts. First part of the experiment was carried to study the effects of salinity at seedling stage and the second part was related to counteract the influence of salinity through the treatments of osmoprotectants. Details of the experiments have been described as under.

Experiment - I

Biochemical characterization of rice to salinity tolerance

Crop **:** Rice

Variety **:** Jaya (Tolerant)
Dandi (Tolerant)
CSR – 27 (Tolerant)
GR – 3 (Susceptible)

Treatments: Seedlings (15 DAG) of above four varieties were treated with 100mM, 150 mM and 200 mM levels of sodium chloride solution for 24, 48 and 72 h. For control no saline solution was applied.

Sample collection: Samples were collected at 24, 48 and 72 h for below mentioned physiological and biochemical analysis.

Study on physiological observations

1) Germination percentage
2) Shoot length
3) Root length
4) Vigor index

Analysis of Biochemical Parameters

1) Chlorophyll contents
2) Soluble protein and protein characterizations
3) Proline content
4) Oxidative and anti oxidant enzymes and their isoenzymes
   - i) Super oxide dismutase (SOD)
   - ii) Catalase
   - iii) Peroxidase (Guaiacol peroxidase)
   - iv) Polyphenol oxidase
   - v) Esterase

Ion analysis

Sodium (Na^+)
Potassium (K^+)

Experiment - II

Effects of osmoprotectants upon NaCl salinity stress in rice

Seedlings (15 DAG) uprooted gently and thoroughly cleaned were treated for 6 h. with 1mM conc. of two osmoprotectants solutions i) proline and ii) trehalose. After incubation period treated seedlings were removed and were kept for 72 h in 100, 150 and 200mM NaCl solution. For control no treatment of osmoprotectans was given. After 72 h seedlings were washed thoroughly with tap water followed by distilled water wash and were analyzed for following biochemical parameters.

Biochemical parameters

1) Chlorophyll contents
2) Proline content
3) Total soluble sugars
4) Enzymes and isoenzymes
    i) Superoxide dismutase (SOD)
    ii) Catalase
    iii) Peroxidase (Guaiacol peroxidase)

3.3 RAISING OF SEEDLINGS FOR PHYSIOLOGICAL OBSERVATIONS

For physiological observations fifty healthy uniform size paddy seeds of four varieties were allowed to germinate at 25°C in the 15 cm petri plates with NaCl salinity levels of 100, 150 and 200 mM for control treatment no

saline solution was used. Germinated seedlings were gently removed at 15 DAG were and analyzed further for physiological observations.

3.3.1 Treatment of seedlings with saline solution for biochemical study

For biochemical study, the seeds were sown in normal soil containing earthen pots of 30 cm dm. Fifteen days old seedlings were gently removed, washed thoroughly. These seedlings were allowed to establish at room temperature for 5 min. and the salinity treatments were applied by immersing the seedlings in saline water with salt concentration of 100, 150 and 200 mM. The treated seedlings were removed at 24, 48 and 72 h, for biochemical analysis. For control seedlings were immersed in distilled water.

3.3.2 Procedure

3.3.2.1 *Estimation of chlorophyll*

Total chlorophyll in leaves of rice seedlings was determined as per the method described Hiscox and Israelstam (1979).

To a 50 mg of finely cut rice leaf, 10 ml of dimethyl sulphoxide (DMSO) was added in a test tube. The tubes were incubated at 65°C for three h. After incubation the tubes were cool down at room temperature and the chlorophyll extracted was measured at 663 and 645 nm in spectrophotometer. The amount of chlorophyll a, b and total chlorophyll present in the sample were calculated using the following formula.

Chlorophyll a (mg/g fresh tissue) = 2.7(O.D at 663) - 2.69 (O.D. at 645)
Chlorophyll b (mg/g fresh tissue) = 22.9 (O.D at 645) - 4.68 (O.D at 663)
Total chlorophyll (mg/g fresh tissue) = 22.2 (O.D at 645) + 8.02 (O.D at 663)

3.3.2.2 ***Total soluble sugars content***

Total soluble sugars from the rice seedlings were determined by phenol sulphuric acid method as described by Dubois *et al*. (1956).

One hundred milligram of the fresh leaf sample was extracted for soluble sugars in 25ml of 80 percent ethanol. 5 millilitre (ml) of extract was evaporated to dryness on waterbath and residues were dissolved in 25 ml of hot distilled water. One ml of diluted sample was pipetted in 30 ml test tube and to this, 1 ml of 5 percent freshly distilled phenol solution followed by direct addition of and 5 ml of concentrated sulphuric acid was added. The contents in the test tubes were shaken and placed on an ice bath for 20 min. Intensity of red colour developed was recorded at 490 nm in spectrophotometer. In a similar way 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard glucose solution containing (20-100 µg glucose) was pipette out into a series of test tubes. The volume in each test tube was made up to 1 ml with distilled water. In a blank 1 ml of distilled water taken. Sugar content was determined using following calculation formula

Total soluble sugar (g/100 g fresh wt.) = Sample O.D x Graph factor x Dilution factor

3.3.2.3 *Estimation of crude protein*

For crude protein estimation method described by Lowry *et al*. (1951) was followed. Crude protein was extracted in tissue homogenizer from 100 mg fresh leaf tissue in 0.1M phosphate buffer pH 6.8. Homogenate was centrifuged at 10,000 rpm for 4°C for 10 min. Clear supernatant obtained was precipitated with chilled acetone and after centrifugation precipitates were dissolved in 1 ml of 0.01N NaOH for protein analysis. The intensity of blue colored was read at 660 nm wave lengths in spectrophotometer.

A standard solution of protein was prepared by dissolving 50 mg of bovine serum albumin (fraction) in 50 ml of distilled water. Ten ml of stock solution of was diluted to 50 ml with distilled water. From this working standard in the range of 20 – 200 µg was prepared and procedure was carried out as mentioned before.

The amount of protein content in the sample was calculated as:

Protein (mg/g fresh wt.) = Sample O.D x Graph factor x Dilution factor

Protein characterizations by protein banding pattern (SDS-PAGE)

To determine the stress protein characterizations study of protein banding pattern through SDS PAGE was carried out following the method of Laemmli (1970). The seedlings (200 mg) were homogenized at in 1 ml of 0.1 M phosphate buffer pH 7.2 containing 30 mM mercaptoethanol (ME) and 2 percent SDS. The extract was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant containing 50 µg protein was loaded for electrophoresis run.

Standard molecular wt. protein markers were also run along with samples. The electrophoresis was carried out on vertical SDS-PAGE (12 %) at 30 mA. The gel was washed with acetic acid 4 percent to remove excess SDS and stained for protein.

3.3.2.4 *Estimation of Proline*

The proline content from seedlings was estimated as per the method described by Malik and Singh (1980).

Rice seedlings (200 mg) were homogenized with 5 ml of one percent sulphosalicylic acid. The crude homogenate was centrifuged at 2000 rpm for 15 min. Volume of clear supernatant collected was adjusted to 5 ml with distilled water. To this, five ml of glacial acetic acid and 1 ml of acid ninhydrin was added and mixed well. The tubes were placed on boiling water bath for one h. The mixture was extracted with addition of toluene. The absorbance of toluene layer was recorded at 520 nm using spectrophotometer. Blank was run simultaneously with 5 ml of one percent sulphosalicylic acid. Standard curve of proline was prepared in the range of 10-50 µg of proline. The amount of proline in the sample was calculated as:

Proline (%) = Sample O.D x Standard factor x Dilution factor

3.3.3 Assay of enzymes and isoenzymes

Oxidative enzymes such as catalase, peroxidase, superoxidedismutase, polyphenoloxidase and esterase were determined as described bellow.

Table 2 : Assay procedure of enzymes from rice seedlings

Steps	Guiacol Peroxidase	Catalase	Superoxide dismutase	Esterase	Poly phenol oxidase
Extraction buffer (Tissue/ buffer) (Wt./Vol.)	75 mM Sodium phosphate buffer 2 ml pH 7.0	75 mm sodium phosphate buffer 2.9 ml pH 7.0	75 mM sodium phosphate buffer 2ml pH 7.0	0.3 M Potasium phosphate bufferp 0.5 ml pH 7.2	0.1 M sodium phosphate buffer 1 mlpH6.0
Other reagents added to extraction media	Poly vinyl pyrollidine 1 %	Poly vinyl pyrollidine 1 %	Poly vinyl pyrollidine 1 %	Poly vinyl pyrollidine 1 %	-
Enzyme aliquot taken ml	0.1 ml	0.05 ml	0.05 ml	1 ml	0.1 ml
Substrate	1% H_2O_2	0.03% H_2O_2	-	Ethyl butyrate	0.1 M catechol
Reagents/dye added to initiation of reaction	1 mM EDTA 100 Mm Guaiacol	-	75 µm NBT 2µm Riboflavin 0.1 mM EDTA	Distilled water	-
Initiation of reaction	Enzyme addition	Enzyme addition	Kept in light	Enzyme addition	Enzyme addition
Blank preparation	No substrate	No substrate	Kept in dark	No substrate	No substrate
Final volume	3 ml	3ml	3 ml	4.5 ml	3.1 ml
Activity reading time/ Interval	0- 60 sec : 15 sec	0- 60 sec : 15 sec	0- 60 sec : 15 sec	0- 60 sec : 15 sec	3 min: 15 sec
Δ O.D at nm	470	240	560	490	490
Enzyme activity / unit	Change in O.D / min/ g fresh tissue	Change in O.D / min/ g fresh tissue	Change in O.D / min/ g fresh tissue	Change in O.D / min/ g fresh tissue	Change in O.D / min/ g fresh tissue
EC number	1.11.1.7	1.11.1.6	1.15.1.1	3.1.1.11	1.14.18.1
Reference	Upadhyay *et al* .(1985)	Aebi, 1984	Salin and Lyon, 1983	Sadasivam and Manickam ,1992	Malik and khan 1943

3.3.3.1 *Enzyme extraction*

Three hundred mg of rice seedlings were crushed in prechilled mortar and pestle with 3 ml of 0.05 M phosphate buffer (pH 7.2) containing one per cent polyvinyl pyrolydine (PVP). The extract was centrifuged at 10000 rpm for 15 min at 4°C and the supernatant was used for assay of peroxidase, super oxide dismutase and esterase, no PVP was added in extraction media for polyphenol oxidase assay.

3.3.3.2 *Peroxidase* (EC 1.11.1.7)

Upadhyay *et al*. (1985) have described the method for Guaiacol peroxidase activity by, monitoring the increase in absorption at 470 nm due to guaiacol oxidation (1 EU = 1 μmol guaiacol oxidized in 1 min).

The 3ml reaction mixture consists of 2 ml of 75 mM phosphate buffer pH 7.0, and 0.3 ml of 0.1% H_2O_2, 0.3 ml of 0.25% guaiacol, 0.3 ml of 1 mM EDTA and 0.1 ml of enzyme extract. In blank no substrate was added.

Enzyme activity = Change in O.D/min/ g fresh tissue.

3.3.3.3 *Catalase* (EC 1.11.1.6)

Procedure described by Aebi (1984) for assay of Catalase activity was followed. The reaction mixture containing 2.9 ml of 50 mM phosphate buffer pH 7.0, 0.95 ml of 0.03% H_2O_2 and 0.05 ml enzyme extract. The decomposition of H_2O_2 was followed at 240 nm.

Enzyme activity = Change in O.D/min/ g fresh tissue.

3.3.3.4 *Superoxide dismutase (SOD)* (EC 1.15.1.1)

Superoxide dismutase activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), 2 µM riboflavin, 0.1 mM Ethylene diamine tetra acetate (EDTA) and 200µl enzyme extract in a total volume of 3 ml. Riboflavin was added last and tubes were shaken and placed below a light blank consisting of 15 watt white fluorescent lamp. The reaction was initiated by switching on the light and was allowed to react for 10 min. The reaction was stopped by switching off the lights and the tubes were covered with a black cloth. The absorbance of the reaction mixture was read at 560 nm. A non-irradiated reaction mixture did not develop color, serve as a control. The reaction mixture lacking enzyme develop maximum color and color intensity decreased with decrease in volume of enzyme extract added. Enzyme activity was expressed as:

$$1 \text{ unit of SOD} = \frac{\text{O.D. of control}}{\text{O.D. of sample}} - 1$$

3.3.3.5 *Esterase* (EC 3.1.1.9)

The reaction mixture contained 1 ml of absolute ethyl butyrate and 0.5 ml of M/30 potassium phosphate buffer (pH7.0) in 3 ml of distilled water and 1 ml of enzyme extract .The contents were mixed by shaking and kept in an incubator at 37°C for 24 h. At the end of incubation period 5 drops of one percent phenolphthalein was added. The solution was titrated against N/20 NaOH till a permanent faint pink colour appeared.For blank no enzyme was added. The enzyme activity was expressed as:

Enzyme activity = Change in O.D/min/ g fresh tissue.

3.3.3.6 *Polyphenoloxidase (PPO)* (EC 1.14.18.1)

The reaction mixture contained 3.0 ml of 0.01 M catechol in 0.1 M phosphate buffer (pH 6.0) and 100 µl of enzyme extract. The reaction was inhibited in the blank with enzyme without the substrate. The colour change in oxidised catechol (light green colour) was read at 490 nm up to 3 min interval of 15 seconds. The enzyme activity expressed as change in O.D/min/g fresh tissue.

Enzyme activity=Change in O.D/min/g fersh tissue.

3.3.4 Isoenzyme analysis

For visualization of isoenzymes native Poly Acrylamide Gel Electrophoresis (PAGE) was carried out. 20 µl of extracted sample containing 50 µg protein was loaded in each well. After the completion of run isoenzymes were studied using the below referred staining dye.

3.3.4.1 *Peroxidase*

The gel was incubated in dark for 10 min in the staining solution consisting of 100 ml 0.01 M phosphate buffer (pH 6.0), 20 mg orthodianisidine (dissolved in minimum amount of methanol) and 1 % hydrogen peroxide.Visualizationof dark brown bands was recorded for each sample.

3.3.4.2 *Esterase*

Staining solution used for visualization of isoenzymes banding pattern consist of sodium dihydrogen phosphate (2.8 g), disodium hydrogen phosphate (1.1 g), fast blue RR salt (0.2 g), 2-napthyl acetate (0.03 g) and water to 200 ml. The gel was incubated at 37°C in dark. The reaction was stopped by the addition of methanol (10): water (10): acetic acid (2): ethyl alcohol (1): Light blakish colour bands appeared was recorded

3.3.4.3 *Catalase*

Woodbury *et al*. (1971) developed a procedure for catalase isoenzyme analysis. The gel after electrophoretic run was first washed three times with distilled water for 15 min. Then the gel was incubated in 0.003% hydrogen peroxide for 10 minute and was again washed with distilled water. The gel was then incubated in 1 % ferric chloride and 1% potassium ferricyanide sodium (prepared separately and mixed fresh before using) for 10 min. with shaking to detect green yellowish color bulbs of catalase isozymes.

3.3.4.4 *Superoxide dismutase*

Salin and Lyon (1983) prepared a suitable method for isoenzyme analysis of super oxide dismutase. The gel was soaked in 50 ml 50 mM sodium phosphate buffer pH 7.8 containing 0.24 mM NBT and 28 μM riboflavin for 20 minutes in the dark, followed by immersing in 50 mM sodium phosphate buffer pH 7.8 containing 28 mM TEMED. Then the gel was exposed to light source at room temperature until opaque band appeared on blue background. Different

isoforms were differentiated by performing activity staining in the gels previously incubated for 20 min at 25°C in 50 mM sodium phosphate pH 7.8 containing 3 mM KCN or 5 mM H_2O_2.

3.3.5 Estimation of sodium and potassium

The procedure outlined by Jackson (1973) was followed for determination of Na and K ions. Seedlings were dried in the oven at 75°C for three days and the dried material was powdered. One gram of powder was digested in diacid mixture of Nitric acid (HNO_3): Perchloric acid ($HClO_4$) (Ratio 2:1). The final volume of digested acid extract was made to 50 ml with distilled water. The ionic concentrations of sodium and potassium were recorded on flame photometer. Comparing the standard graph values for Na and K calculation was done.

3.4 STATISTICAL ANALYSIS

Observations for all the physiological and biochemical parameters were taken in three replications, analyzed by Factorial Complete Randomized Design. Pooled analysis was also carried out and compared the genotype and treatment effects with time intervals.

IV. RESULTS AND DISCUSSION

The results of present investigation on "Biochemical characterizations and effects of osmoprotectants upon sodium chloride salinity stress in rice cultivars" are presented and discussed for two experiments in this chapter.

EXPERIMENT- I

BIOCHEMICAL CHARACTERIZATIONS OF RICE TO SALINITY TOLERANCE

4.1 PHYSIOLOGICAL STUDY OF RICE SEEDLINGS WITH SALINITY

Physiological approach is a preliminary marker to know the effects of salinity tolerance in crops.

4.1.1 Germination percentage

The study on germination in rice varieties treated with different levels of sodium chloride salinity was carried out as mentioned earlier. Data on changes in per cent germination have presented in Plate I, Table 3 and Figure 1.

As seen in Plate I, the germination was more in control as compared with other salinity levels. Germination percentage decreased with increased salinity levels. Among the four rice varieties studied CSR-27 which is a tolerant variety to salinity recorded significantly highest (79.33%) germination percentage. Jaya and Dandi both were at par with each other. GR-3 salinity susceptible variety recorded significantly lowest germination percentage (62.04%).

Table 3 : Changes in per cent germination in rice varieties with different levels of sodium chloride salinity

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
V_1	85.16	81.33	74.33	65.50	76.70
V_2	85.50	81.00	72.83	63.33	75.66
V_3	94.16	89.73	71.66	64.16	79.33
V_4	68.16	63.33	60.00	56.66	62.04
Treatment mean	83.25	78.97	69.97	62.41	
	V		S		V x S
S.Em.	0.84		0.84		1.67
C.D.	2.42		2.46		4.84
C.V. %	3.94				

There were significant differences among the different levels of sodium chloride.100 mM NaCl treatment level recorded the highest percent of germination than remaining NaCl levels in all the varieties. Among different combinations of V x S, CSR-27 at control level recorded significantly the highest germination percentage (94.16) over the rest of treatment combinations Interaction between varieties (V) and salinity levels (S) were found to be significant as shown in the Table 3. Salinity effects were clearly noticed in germination percentage of both tolerant and susceptible cultivars.

These results are also in agreement with the findings of Djanguiraman *et al*. (2003) and Tirumeni *et al*. (2001). The reduction in germination percentage was due to the adverse effects of excess sodium as well as chloride ions on the mobilization of seed reserves and inactivation of hydrolyzing

enzyme systems during germination hence radicle emergence could not occurred.

4.1.2 Seedling growth

Study on seedling growth in rice varieties treated with different levels of sodium chloride salinity was carried out and data on changes in seedling growth have been presented in Plate II, Table 4 to 6 and in Figure 2.

The seedlings which were grown in control found to be healthy in terms of rootlengh as well as shootlengh than different levels of sodium chloride.

Root length was also reduced with increasing levels of salinity (Fig.2a and Table 4). The root growth was more sensitive to salinity, than shoot. The maximum root length was observed in Jaya (9.61) for control treatment. The similar pattern was observed in all the varieties. GR-3 showed maximum shoot length (12.21 cm) in control and Jaya showed minimum shoot length (9.00 cm) at 200 mM level. The shoot length significantly decreased with the increase in salt concentration as shown in Fig.2b and Table 5. Among the four varieties Jaya showed maximum decrease in shoot length of about 16 percent as compared to control. The interaction VxS remained significant for shoot and root length. The lower concentration of salt did not have much effect on the length of root and shoot, as observed earlier in rice. Ahmed *et al*. (1987) and Kumar *et al*. (1998) have also reported similar observations for rice and barley respectively.

Table 4 : Changes in root length in rice varieties during germination with different levels of sodium chloride salinity

Root length (cm)

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
V_1	9.61	8.12	8.03	7.95	8.43
V_2	8.86	8.24	8.17	8.01	8.32
V_3	9.18	7.82	7.35	7.00	7.84
V_4	7.81	7.34	7.06	6.73	7.23
Treatment mean	8.37	8.30	7.73	7.42	
	V		S	V x S	
S.Em.	0.09		0.09	0.19	
C.D.	0.26		0.26	0.55	
C.V. %	4.05				

Table 5 : Changes in shoot length in rice varieties during germination with different levels of sodium chloride salinity

Shoot length (cm)

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
V_1	10.66	10.29	9.63	9.00	9.89
V_2	11.12	11.01	10.44	9.81	10.62
V_3	12.02	11.74	10.95	10.82	11.38
V_4	12.21	12.10	11.68	10.76	11.69
Treatment mean	11.47	11.34	10.67	10.10	
	V		S	V x S	
S.Em.	0.07		0.07	0.13	
C.D.	0.19		0.19	0.29	
C.V. %	2.05				

The decrease in plant height towards increase levels of salinity was due to the effects of NaCl which diversified the stored energy and showed the adverse effects on growth and metabolism (Reddy and Vaidyanath, 1986; Tirumeni *et al*., 2001).

These results suggested that the salt stresses not only affected germination but also the growth of the seedlings. The reduction in growth was more at higher salinity levels (150, 200 mM).

Amzallag *et al* (1994), Gill and Singh (1992) have also reported the similar results in rice varieties. The results indicated a decrease trend in shoot height and root length as salinity increased.

4.1.3 Seedling vigour

The data on seedling vigour has been presented in Table 6 and Fig 2c. The vigour index gradually decreased under salinity ranging from 100mM to 200 mM. Among the four varieties Dandi recorded significantly the highest vigour index (1457.08) than the rest of the varieties. Where as CSR-27 and GR-3 both were at par with each other. There were significant differences among different levels of sodium chloride concentrations.

Jaya at control level recorded significantly the highest vigour index (1871.52) over the rest of the treatment combinations.

Table 6 : Changes in vigour index in rice varieties during germination with different levels of sodium chloride salinity

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
V_1	1871.52	1434.98	1198.38	981.40	1371.57
V_2	1801.37	1562.83	1346.59	1117.55	1457.08
V_3	1570.30	1265.53	1012.48	934.07	1165.59
V_4	1575.45	1283.93	1001.61	805.01	1166.50
Treatment mean	1704.66	1386.82	1139.76	959.50	
	V		S		V x S
S.Em.	41.14		41.14		82.29
C.D.	118.82		118.82		236.58
C.V. %	10.98				

The variations observed were due to the varietal differences and salinity levels which remained significant for germination percentage, seedling growth and seedling vigor. The varieties Jaya, Dandi and CSR-27 found more tolerant where as GR-3 was sensitive to salt stress.

Physiological observations serve as a better marker for salinity tolerance in rice because the adverse effects of salinity were clearly visible and easy to notice. All the physiological parameters, which were studied, are reduced with increasing salt concentrations; the reduction was more in high salinity levels. All the varieties responded in a positive manner with salinity treatments. Tolerant varieties resisted better at NaCl treatment than susceptible variety.

4.2 BIOCHEMICAL STUDIES WITH SALINITY

Physiological approaches are just not satisfactory in deciding the salinity tolerance so far. Salinity is a complex phenomenon, which is governed by polygenic traits. Combination of both physiolocal and biochemical approaches are required for identification of suitable markers for salt tolerance. The various biochemical parameters studied have been described below.

4.2.1 Changes in biochemical attributes with salinity

Study on biochemical parameters in rice varieties treated with different levels of sodium chloride salinity was carried out and the results are summarized in the following manner.

4.2.1.1 *Total chlorophyll*

Data on total chlorophyll content have been presented in Table 7 and Fig. 3. All the varieties showed significant differences for total chlorophyll content. However, maximum total chlorophyll content was observed in Jaya (4.27 mg g^{-1}) followed by GR-3 (3.78 mg g^{-1}) at 24 h salinity treatment period. The effect of salt concentration on total chlorophyll was found to be significant. The treatment of 150 mM NaCl registered the highest value of total chlorophyll followed by 100 mM NaCl (Fig. 3a).

At 48 h salinity treatment period GR-3 recorded maximum chlorophyll content (3.99 mg g^{-1}) which was at par with Jaya (Fig. 3b). Dandi and CSR-27 both are at par with each other. Jaya and Dandi recorded the highest chlorophyll content at 100 mM where as CSR-27 and GR-3 recorded the

Table 7 : Changes in chlorophyll content in seedlings of rice varieties treated with different levels of sodium chloride salinity at different time intervals

Chlorophyll (mg 100 g^{-1})

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	24 hour				
V_1	3.89	5.72	3.14	2.36	4.27
V_2	2.72	3.28	3.01	2.61	2.91
V_3	2.59	3.14	3.86	2.27	2.97
V_4	3.15	4.36	5.80	3.76	3.78
Treatment mean	2.73	3.59	4.60	2.15	
	V		S	V x S	
S.Em.	0.07		0.07	0.14	
C.D.	0.20		0.20	0.41	
C.V. %	7.43				
	48 hour				
V_1	3.23	3.92	5.99	2.68	3.89
V_2	2.81	3.37	3.43	2.58	3.04
V_3	2.76	3.01	3.75	2.12	2.91
V_4	2.90	4.57	5.58	2.50	3.99
Treatment mean	2.93	3.71	4.69	2.47	
	V		S	V x S	
S.Em.	0.25		0.11	0.22	
C.D.	0.67		0.32	0.65	
C.V. %	11.24				
	72 hour				
V_1	2.69	4.28	5.88	2.05	3.53
V_2	2.71	3.16	3.77	2.27	2.98
V_3	2.45	3.07	3.91	1.99	2.85
V_4	3.10	3.89	4.84	2.28	3.75
Treatment mean	2.73	3.61	4.60	2.15	
	V		S	V x S	
S.Em.	0.07		0.07	0.14	
C.D.	0.20		0.20	0.41	
C.V. %	7.43				

highest at 150 mM NaCl level. In CSR-27 with 200 mM NaCl level registered the lowest chlorophyll content (1.99 mg g^{-1}) at 72 h salinity treatment period (Fig 3c). In all the cultivars, the total chlorophyll content decreased at 200 mM of NaCl concentration. These results showed that the pigment like chlorophyll can withstand up to certain levels of salinity because of its sensitivity to excess amount of sodium as well as chloride ions.

Similar results also observed during 48 h (Fig. 3b) as well as 72 h (Fig. 3c) salinity treatment. But the total chlorophyll content decreased towards the exposing time of salinity. The interaction between varieties and salt levels was significant as shown in Table 7.

Pooled analysis on chlorophyll (Table 9) content revealing that the content was found to be significant among the varieties, treatments as well as h interaction between varieties, treatments and time intervals.

These results are also in agreement with the findings of Pushpam and Sree Rangasamy (2000) and Gill and Dutt (1987). The increase in chlorophyll content was more in tolerant varieties (Jaya, Dandi, and CSR-27) than susceptible variety (GR-3). Contrary to this, decrease in chlorophyll content also was reported by Pandey and Saxena (1987) and Sudhakar *et al*., (1991) in rice. The reduction in leaf chlorophyll under salinity has been reported and attributed to the destruction of the chlorophyll pigments and the instability of the pigment protein complex. Salinity was found to enhance the chlorophyllase activity which resulted in lowering the chlorophyll content of leaves and has

been described to be an effect, associated with increase in chloride content in plants.

It is seen from the results that low levels of chlorophyll caused by salt stress could be partly due to interference of salt ions with the synthesis of proteins, the structural component of chloroplast rather than the break down of chlorophylls.

4.2.1.2 *Total soluble sugars (TSS)*

The data on changes in total soluble sugar content have been presented in Table 8, Fig 4. Among the four varieties studied, Jaya recorded the highest total soluble sugar (4.75 g 100 g^{-1}). GR-3 showed the lowest TSS content (1.86 g 100 g^{-1}) as observed in Table 8. Sugar content increased with salinity up to 150 mM thereafter it decreased (Fig. 4a) except in case of CSR-27 up to 200 mM concentration TSS content increased. TSS content decreased with salinity treatment periods. Consistent results were noticed at 48h (Fig. 4b) and 72 h periods (Fig. 4c). The treatment as well as varietal effects were significant with time intervals.

The interaction between varieties (V) and salinity levels (S) was significant as mentioned in Table 8. In all the varieties sugar content increased up to 150 mM there after it decreased. All the varieties showed the highest TSS content at 150 mM NaCl level. This may be due to over come the effects of excess salinity conditions, plants utilized their food reserves it may be one of the reasons for initial increase and subsequent decrease in sugar levels.

Table 8 : Changes in total soluble sugar content in seedlings of rice varieties treated with different levels of sodium chloride salinity at different time intervals

Total soluble sugar (g 100 g^{-1})

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	24 hour				
V_1	3.00	4.18	6.12	5.69	4.75
V_2	2.12	3.46	4.13	5.15	3.71
V_3	2.86	3.43	4.25	5.42	3.99
V_4	1.75	2.11	2.47	1.09	1.86
Treatment mean	2.43	3.29	4.24	4.34	
	V		S	V x S	
S.Em.	0.06		0.06	0.13	
C.D.	0.19		0.19	0.38	
C.V. %	6.34				
	48 hour				
V_1	2.93	3.32	4.10	3.07	3.36
V_2	2.06	2.42	1.51	2.03	2.00
V_3	2.58	3.49	1.47	3.35	2.72
V_4	1.52	2.19	2.27	1.38	1.84
Treatment mean	2.27	2.86	2.34	2.46	
	V		S	V x S	
S.Em.	0.05		0.07	0.11	
C.D.	0.16		0.20	0.32	
C.V. %	7.76				
	72 hour				
V_1	2.53	0.88	3.05	2.83	2.32
V_2	1.79	2.03	2.42	2.77	2.02
V_3	1.45	1.53	2.16	2.93	2.25
V_4	1.22	1.96	2.34	1.23	1.60
Treatment mean	1.75	1.60	2.49	2.44	
	V		S	V x S	
S.Em.	0.04		0.05	0.09	
C.D.	0.16		0.16	0.27	
C.V. %	7.84				

Table 9 : Pooled analysis of chlorophyll and total soluble sugar contents in rice varieties

	Chlorophyll (mg 100 g^{-1})				Total soluble sugar (g 100 g^{-1})			
V_1	2.92				2.15			
V_2	3.81				2.58			
V_3	4.41				3.02			
V_4	2.46				3.08			
S.Em.±	0.19				0.30			
C.D.	0.64				0.60			
T_1	3.90				3.48			
T_2	2.97				2.66			
T_3	2.91				2.91			
T_4	3.82				1.79			
S.Em.±	0.11				0.32			
C.D.	0.38				0.64			
P_1	3.45				3.58			
P_2	3.48				2.48			
P_3	3.27				2.07			
S.Em.±	0.04				0.03			
C.D.	0.12				0.08			
Interaction T x V	V_1	V_1	V_2	V_3	V_4	V_2	V_3	V_4
T_1	3.05	2.82	2.80	4.43	3.87	4.28	5.41	2.85
T_2	2.75	1.99	2.64	2.68	3.32	3.25	3.40	2.49
T_3	2.60	2.29	2.82	2.63	3.90	3.07	3.84	2.13
T_4	3.27	1.49	2.09	2.36	1.24	4.64	5.01	2.36
S.Em.±	0.35				0.29			
C.D.	0.24				0.86			
Interaction P x V	V_1	V_1	V_2	V_3	V_4	V_2	V_3	V_4
P_1	2.93	2.43	3.30	4.24	4.34	3.71	4.69	2.47
P_2	3.09	2.27	2.86	2.34	2.46	4.13	3.95	2.75
P_3	2.73	1.75	1.60	2.49	2.44	3.60	4.60	2.15
S.Em.±	0.09				0.06			
C.D.	0.24				0.16			
Interaction P x T	T_1	T_1	T_2	T_3	T_4	T_2	T_3	T_4
P_1	3.89	4.75	3.71	3.99	1.86	3.04	2.91	3.96
P_2	4.27	3.36	2.00	2.72	1.84	2.91	2.97	3.78
P_3	3.53	2.32	2.25	0.17	1.69	2.98	2.85	3.72
S.Em.±	0.09				0.06			
C.D.	0.24				0.16			
PxTxV	Sig.				Sig.			
C.V. %	8.78				7.22			

Gill *et al*. (2002) determined total soluble sugar content in *sorghum bicolar* (L.) cv. CSH-9, which increased towards stress conditions; the sugar content increased remarkably after 14 hours of salt stress imposition.

Ashraf and Tufail (1995) elucidated the total soluble sugar content in five sunflower accessions differing in salt tolerance and observed that sugar content was increased significantly in all five accessions with increasing salt concentration in the growth medium. The tolerant lines had generally greater soluble sugars than the salt sensitive ones. Sairam and Tyagi (2004) demonstrated that biosynthesis and accumulation of carbohydrates in plants are correlated with salt stress tolerance. These solutes (osmolytes) are widely believed to function as a protectors or stabilizer of enzymes or membrane structures during stress conditions.

Similar results were also observed in present study at 48 h and 72 h salinity treatments but decrease in TSS content when the salinity exposing time was increased.

Pooled analysis data from the Table 9 indicated that treatments were effective with varieties as well as with time periods .The interaction between time period, varieties and treatment levels was also significant.

4.2.1.3 *Total soluble protein content*

Changes in soluble protein content has been shown in Table 10 and Fig. 5. Among the four varieties studied CSR/27 recorded maximum protein content (68.59 mg g^{-1}) at 24 h salinity treatment period (Fig. 5a). Maximum

Table 10 : Changes in total soluble protein content in seedlings of rice varieties treated with different levels of sodium chloride salinity at different time intervals

Total Soluble Protein (mg g^{-1})

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	24 hour				
V_1	87.63	73.13	89.56	81.64	59.26
V_2	45.25	77.95	63.72	52.12	59.76
V_3	62.62	58.65	79.55	73.56	68.59
V_4	44.38	32.60	23.51	16.53	34.52
Treatment mean	59.98	60.63	64.21	55.95	
	V		S		V x S
S.Em.	1.34		1.34		2.67
C.D.	3.85		3.85		7.78
C.V. %	7.68				
	48 hour				
V_1	90.88	80.71	98.62	74.37	90.14
V_2	54.79	87.36	67.48	47.27	86.15
V_3	79.89	70.32	83.16	71.87	76.14
V_4	47.10	38.77	29.66	19.16	39.29
Treatment mean	65.67	70.13	69.55	53.19	
	V		S		V x S
S.Em.	1.35		1.35		2.78
C.D.	3.90		3.99		7.82
C.V. %	7.26				
	72 hour				
V_1	97.41	82.24	102.54	78.71	61.73
V_2	59.14	92.70	71.29	61.71	71.26
V_3	76.29	65.57	85.87	71.10	75.19
V_4	50.06	43.16	32.35	14.31	36.96
Treatment mean	71.14	70.91	73.03	58.47	
	V		S		V x S
S.Em.	1.27		1.27		2.59
C.D.	3.68		3.68		7.36
C.V. %	6.45				

variation in protein content was observed with regards to salinity stress in all three tolerant varieties than sensitive variety.

Among the salt levels 150 mM NaCl recorded the maximum (64.21 mg g^{-1}) protein content followed by 100 mM NaCl (60.63 mg g^{-1}). The salt doses had distinct effects.

At 48 h treatment period Jaya recorded highest protein content (90.14 mg g^{-1}) followed by Dandi (86.15 mg g^{-1}) (Fig. 5b). Where as at 72h treatment period CSR-27 recorded the highest protein content (75.19 mg g^{-1}) followed by Dandi (71.26 mg g^{-1}) (Fig. 5c). The interaction between varieties and salt doses showed significant effects (Table 10). The treatment effect showed significant differences. The interaction between varieties and treatments was also significant.

These results also agree with the findings of Pushpam and Sree rangasamy (2000) and Reddy and Vaidyanath (1986).

This reduction in protein content may be due to the diversion of some quantum of energy for growth and metabolism to over come the stress situations.

From the pooled analysis (Table 11) it was clear that protein content showed significant difference with varieties, treatment periods but, it was non significant with treatment periods with respect to varieties and treatment levels.

Table 11 : Pooled analysis of protein and proline contents in rice varieties

	Protein (%)				**Proline (mg g^{-1})**			
V_1	65.60				0.80			
V_2	67.23				1.32			
V_3	68.93				1.66			
V_4	55.84				1.96			
S.Em.±	1.61				0.05			
C.D.	5.57				0.18			
T_1	33.58				1.74			
T_2	64.30				1.16			
T_3	73.31				0.82			
T_4	86.45				2.02			
S.Em.±	1.29				0.06			
C.D.	4.47				0.21			
P_1	60.20				1.35			
P_2	64.64				1.41			
P_3	68.39				1.54			
S.Em.±	0.66				0.01			
C.D.	1.86				0.05			
Interaction T x V	V_1	V_1	V_2	V_3	V_4	V_2	V_3	V_4
T_1	47.19	0.89	1.68	1.95	2.42	39.29	28.47	19.37
T_2	49.73	0.65	1.03	1.35	1.61	86.00	67.74	53.72
T_3	73.60	0.77	0.61	0.86	1.05	64.85	82.61	72.17
T_4	91.88	0.90	1.98	2.46	2.76	78.75	96.91	78.24
S.Em.±	1.53				0.15			
C.D.	4.29				0.45			
Interaction P x V	V_1	V_1	V_2	V_3	V_4	V_2	V_3	V_4
P_1	59.99	0.84	1.26	1.51	1.81	60.63	64.22	55.96
P_2	65.67	0.79	1.28	1.63	1.95	70.13	69.55	53.19
P_3	71.14	0.78	1.43	1.83	2.13	70.92	73.03	58.47
S.Em.±	1.32				0.03			
C.D.	3.71				0.09			
Interaction P x T	T_1	T_1	T_2	T_3	T_4	T_2	T_3	T_4
P_1	29.26	1.53	1.10	0.69	2.09	59.89	68.59	83.05
P_2	34.53	1.75	1.11	0.87	1.92	61.74	76.14	86.15
P_3	36.96	1.93	1.28	0.91	2.06	71.26	75.20	90.15
S.Em.±	1.32				0.03			
C.D.	3.71				0.09			
PxTxV	NS				Sig.			
C.V. %	7.11				7.82			

4.2.1.4 *Characterization of proteins by SDS PAGE*

Study on protein banding pattern was carried out in SDS PAGE to characterize the proteins induced due to salinity stress.

Protein banding pattern of rice seedlings at 24 h salinity treatment period (Plate V) showed the presence of four bands of which first band (R_m 0.28, 97 KD) showed maximum intensity than remaining bands. The appearance of this band was maximum in Dandi followed by CSR-27.Where as second protein band ($R_{m0.}$44, 46 KD). This protein band was absent in control and remaining treatments. Variation was also noted in second protein band with respect to intensity.

At 48 h third and fourth (R_m 0.56 & 0.69) protein bands corresponded to the ≅27-30 KD molecular weight protein marker. More number of protein bands were observed in case of GR-3 at 100, 150, 200 mM NaCl level where this was not seen in control of the same variety. The intensity as well as number of protein bands increased during this treatment period (Plate VI). But no variation was found in banding pattern among the varieties as well as treatment levels. The protein bands which appeared during this treatment period were same in molecular weight basis as well as intensity basis. First group of bands were corresponding in between 97 and 72 KD protein molecular weight markers having the R_m (0.28 & 0.31). The third and fourth group of protein bands were corresponding to 46, 27 KD molecular weight protein markers respectively. Fifth band showed maximum intensity as compared to remaining four bands.

Study on changes in protein profile at 72 h showed the protein bands increased during this salinity treatment period. Among all the varieties Jaya CSR-27 showed maximum protein bands and also showed variation in banding pattern. First group of protein bands were corresponding to 97 molecular weight protein marker.(R_m 0.21-0.26) In case of 100, 200 NaCl levels of CSR-27 this group of protein bands were not seen. Variation in protein banding pattern was clearly seen in all the varieties. Maximum protein bands were corresponding to 27 KD molecular weight protein markers.

Some stress protein bands were inducted which were due to salinity treatments; the tolerant varieties may able to gain more proteins during salinity stress conditions hence the tolerant cultivars showed maximum intensity as well as more protein bands than the sensitive cultivar GR-3.

4.2.1.5 *Proline*

Results on changes in proline content has been presented in Table in 12. Among the different varieties at 24 h period proline content was high in Dandi (2.09 mg g^{-1}) and least in GR-3 (0.69 mg g^{-1}). Variation was noted due to the differences in degree of tolerance. Salinity stress creates a maximum demand for proline during stress conditions in tolerant varieties to withstand against salinity stress than sensitive cultivars.

There was a significant difference between varieties and salinity levels; proline content was increased with increasing salinity levels (Table 12). It was maximum at 200 mM salinity level as compared to control in all the varieties.

Table 12 : Changes in proline content in seedlings of rice varieties treated with different levels of sodium chloride salinity at different time intervals

Proline (mg g^{-1})

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	24 hour				
V_1	0.77	1.49	1.64	2.27	1.52
V_2	1.57	2.06	2.31	2.43	2.09
V_3	0.66	0.95	1.23	1.54	1.09
V_4	0.39	0.54	0.85	0.98	0.69
Treatment mean	0.83	1.25	1.50	1.80	
	V		S		V x S
S.Em.	0.03		0.03		0.07
C.D.	0.09		0.09		0.19
C.V. %	8.79				
	48 hour				
V_1	0.29	0.38	0.45	0.56	0.41
V_2	0.58	0.56	0.60	0.68	0.60
V_3	0.33	0.55	0.56	0.68	0.53
V_4	0.39	0.42	0.49	0.48	0.44
Treatment mean	0.39	0.48	0.52	0.59	
	V		S		V x S
S.Em.	0.01		0.01		0.02
C.D.	0.03		0.03		0.06
C.V. %	7.34				
	72 hour				
V_1	1.14	1.87	2.21	2.57	1.93
V_2	0.73	1.16	1.56	1.68	1.28
V_3	0.59	0.76	0.97	1.29	0.91
V_4	0.66	1.99	2.59	2.97	2.06
Treatment mean	0.78	1.43	1.83	2.13	
	V		S		V x S
S.Em.	0.03		0.04		0.07
C.D.	0.10		0.10		0.20
C.V. %	7.90				

Similar trend of results observed in 48 h (Fig. 6b) and 72 h salinity treatment periods (Fig. 6c). But maximum proline accumulation was seen at 24 h and 72h salinity treatment periods than 48 h. Under 72h treatment period GR-3 recorded maximum proline accumulation by Dandi. The increase in proline content was nearly twice in all the varieties at 200 mM NaCl level than the control.

With increase in the level of salt stress, an increase in the level of proline was observed in all the varieties. These results indicated that proline has a positive relation with salt tolerance.

Data on pooled analysis on proline content revealed significant difference for VxT, PxT, VxP, PxVxT interaction (Table 11).

An increase in the proline content was observed with increasing sodium chloride concentration (Fig. 6), which implies that proline accumulates as a response to salt stress. Which acts as an inorganic nitrogen reserve and helps in recovery during stress situations (Mansour, 1998). It may also work as an intra cellular osmolytes (Basu *et al*., 1996). Proline will act as a potential biochemical marker for assessing the salinity tolerance both in tolerant as well as sensitive cultivars. Still more detail study is required to reveal the actual mechanism which impart stress situations. .

Similar results were reported by Stewart and Lee (1974) and Dubey and Rani (1989).

From these results it is understood that proline might serve as nitrogen source for growth and survival under saline conditions. Thereby inducing salinity resistance to rice cultivars. Salt tolerant cultivars thus maintain higher level of free proline than the salt sensitive cultivars when grown in a saline medium.

4.2.2 Enzyme activity and isoenzyme analysis with different levels of salinity in rice

Salinity induces significant changes in the activities of various oxidative enzymes and hydrolytic enzymes in plants (Srivalli *et al*., 2003).

4.2.2.1 *Super oxide dismutase (SOD)*

This enzyme showed significant differences among all the rice varieties, salinity levels where as the interaction between varieties and salinity levels were non-significant (Table 13).

From the Figure 7, it is seen that susceptible variety GR-3 recorded the highest SOD (39.65 unit min^{-1} g^{-1}) activity at 72 h salinity treatment as compared to rest of the varieties. Whereas Jaya recorded minimum SOD activity (13.50 unit min^{-1} g^{-1}) at 24 h salinity treatment, the SOD activity increased with salinity. Rice seedlings under 200 mM NaCl showed maximum SOD activity as compared to control in all the varieties (Fig. 7). Stress especially increased the content of H_2O_2 as well as the activities of SOD in the leaves of rice plant during salinity stress (Lee *et al*., 2001).

Table 13 : Changes in super oxide dismutase (SOD) activity in seedlings of rice varieties treated with different levels of sodium chloride salinity at different time intervals

SOD (Unit min^{-1} g^{-1})

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	24 hour				
V_1	10.45	12.98	8.13	22.44	13.50
V_2	12.58	18.15	14.60	26.99	18.08
V_3	21.38	25.27	19.49	34.25	25.10
V_4	25.72	32.91	20.97	41.72	30.33
Treatment mean	17.53	22.33	15.80	31.35	
	V		S	V x S	
S.Em.	0.66		0.66	1.32	
C.D.	1.84		1.84	3.68	
C.V. %	10.60				
	48 hour				
V_1	12.33	15.75	10.13	26.95	16.29
V_2	17.60	24.10	17.08	30.66	22.36
V_3	24.91	32.62	24.18	38.47	30.05
V_4	30.36	20.08	27.18	49.45	31.77
Treatment mean	21.30	23.14	19.64	36.38	
	V		S	V x S	
S.Em.	0.82		0.82	1.64	
C.D.	2.27		2.27	4.54	
C.V. %	11.32				
	72 hour				
V_1	18.42	24.33	15.99	31.82	22.64
V_2	23.95	31.93	20.26	39.64	28.95
V_3	28.71	40.02	28.21	43.61	35.14
V_4	34.94	41.39	30.34	51.93	39.65
Treatment mean	26.51	34.42	23.70	41.75	
	V		S	V x S	
S.Em.	0.66		0.66	1.32	
C.D.	1.84		1.84	3.68	
C.V. %	7.34				

A reduced activity of SOD leading to accumulation of O_2^- has been shown to flooding stress in maize ,whereas no significant differences in the activity have been observed in two cultivars of rice differing in sensitivity to chilling (Saruyama and Tahida, 1995).

An increase in total SOD activity has also been detected in wheat roots under anoxia but not under hypoxia and the increase correlated with the duration of anoxia (Biemelt *et al*., 2000). SOD activity decreased during water stress in rice (Bod and Jung, 1999) but increased under salt stress in a tolerant cultivar of pea (Hernandez *et al*., 1995).

The differences in the activities were identified due to the differences in the enzyme protein level of the different isoenzymes in rice (Ushimaru *et al*., 1995).

4.2.2.2 *Isoenzyme pattern of SOD with salinity*

1) 24h salinity treatment

The isozyme pattern of SOD is presented in Plate VIII with different levels of NaCl salinity treatment at 24 h salinity treatment period.

SOD showed the presence of two different isoforms having R_m values ranges from 0.63 to 0.87 (Table 14) with different intensity. All treatment combinations showed isoforms of SOD with out having any differences .How ever, Jaya showed maximum intensity followed by Dandi.

Table 14 : RM values of SOD in rice at 24 h salinity treatment

Parameters / Band No.		1	2
Varieties (V)	**Salinity levels (S)**		
Jaya (T)	Control	0.63^{M}	0.87^{D}
	100 mM (S1)	0.63^{D}	0.87^{D}
	150 mM (S2)	0.63^{L}	0.87^{L}
	200 mM (S3)	0.63^{D}	0.87^{L}
Dandi (T)	Control	0.61^{D}	0.87^{M}
	100 mM (S1)	0.61^{L}	0.87^{L}
	150 mM (S2)	0.61^{D}	0.87^{M}
	200 mM (S3)	0.61^{M}	0.87^{M}
CSR-27 (T)	Control	0.61^{L}	0.88^{M}
	100 mM (S1)	0.61^{L}	0.88^{L}
	150 mM (S2)	0.63^{M}	0.88^{L}
	200 mM (S3)	0.63^{M}	0.88^{M}
GR-3 (S)	Control	0.61^{D}	0.88^{L}
	100 mM (S1)	0.61^{L}	0.88^{L}
	150 mM (S2)	0.61^{D}	0.88^{D}
	200 mM (S3)	0.61^{L}	0.88^{L}

L = Light, M = Medium, D = Dark

2) 48 h salinity treatment

Two isoforms of SOD were identified with R_m values of 0.58 and 0.69 (Plate IX). First band showed polymorphism. This isoform was absent at control stage in Dandi as well as 150 mM NaCl level and also at 150 mM NaCl level in GR-3 (Table 15).

Second isoform showed maximum intensity. The intensity was more in Dandi at 100 mM NaCl level. Polymorphism was seen in intensity only in second isoform. Intensity was increased towards salinity.

3) 72 h salinity treatment

Three different isoforms of SOD were noticed with R_m values of 0.56 to 0.86 (Plate X). Variation was also noticed among 3 isoforms in terms of intensity. First isoform (R_m – 0.56) was absent at 150 mM NaCl level in Dandi as well as at 200 mM in CSR-27 and also at 100, 150 mM NaCl levels of GR-3. (Table 16) The intensity increased towards salinity.

SOD represented the presence of Mn SOD isoforms (R_m 0.63 to 0.87) because the photograph was taken after performing the activity and the Mn SODs are resistant to the inhibitors. H_2O_2 and potassium cyanide (Salin and Lyon, 1983). The intensity increased towards salinity 150 and 200 mM NaCl levels showed maximum intensity. Polymorphism was observed in intensity only. Scandalior (1993) identified genetically and biochemically distinct isoenzymes of SOD in maize.

Table 15 : RM values of SOD in rice at 48 h salinity treatment

Parameters / Band No.		1	2
Varieties (V)	**Salinity levels (S)**		
Jaya (T)	Control	-	0.69^{M}
	100 mM (S1)	0.58^{D}	0.69^{D}
	150 mM (S2)	0.58^{M}	0.69^{M}
	200 mM (S3)	0.58^{M}	0.69^{D}
Dandi (T)	Control	0.58^{D}	0.69^{D}
	100 mM (S1)	0.58^{M}	0.69^{M}
	150 mM (S2)	0.58^{M}	0.69^{D}
	200 mM (S3)	0.58^{D}	0.69^{M}
CSR-27 (T)	Control	-	0.69^{D}
	100 mM (S1)	0.56^{D}	0.69^{D}
	150 mM (S3)	-	0.69^{L}
	200 mM (S3)	0.56^{M}	0.69^{M}
GR-3 (S)	Control	0.56^{L}	0.69^{D}
	100 mM (S1)	0.56^{D}	0.68^{M}
	150 mM (S2)	-	0.68^{D}
	200 mM (S3)	0.56^{D}	0.68^{M}

L = Light, M = Medium, D = Dark

Table 16 : RM values of SOD in rice at 72 h salinity treatment

Parameters / Band No.		1	2	3
Varieties (V)	**Salinity levels (S)**			
Jaya (T)	Control	-	0.63^{M}	0.86^{D}
	100 mM (S1)	0.56^{D}	0.63^{D}	0.86^{D}
	150 mM (S2)	0.56^{M}	0.63^{M}	0.86^{L}
	200 mM (S3)	0.56^{D}	0.63^{D}	0.86^{L}
Dandi (T)	Control	0.56^{D}	0.63^{M}	0.86^{M}
	100 mM (S1)	0.56^{L}	0.63^{L}	0.86^{L}
	150 mM (S2)	-	0.63^{D}	0.86^{D}
	200 mM (S3)	0.56^{M}	0.63^{M}	0.86^{D}
CSR-27 (T)	Control	0.56^{M}	0.63^{M}	0.86^{M}
	100 mM (S1)	0.56^{M}	0.63^{M}	0.86^{D}
	150 mM (S2)	0.56^{L}	0.63^{L}	0.86^{L}
	200 mM (S3)	-	0.63^{M}	0.86^{L}
GR-3 (S)	Control	0.54^{L}	0.63^{L}	0.86^{M}
	100 mM (S1)	-	-	0.86^{L}
	150 mM (S2)	-	0.63^{D}	0.86^{D}
	200 mM (S3)	0.54^{M}	0.63^{L}	-

L = Light, M = Medium, D = Dark

4.2.2.3 *Catalase*

Among the four varieties studied, CSR-27 recorded the highest catalase activity (16.69 unit min^{-1} g^{-1}) at 72 h salinity treatment. Whereas minimum activity recorded in GR-3 (3.23 at 24 h salinity treatment) (Fig 8). Salinity caused a significant increase in the catalse activity. The increase was more in CSR-27 followed by rest of the varieties. Effects of treatments, varieties and interaction VxS were significant (Table 17). Pooled analysis for catalase activity also remained significant (Table 18).

The observed increase in catalase activity over control was in accordance with Badiani *et al*. (1990) in wheat and also with the results of the Djanaguiraman *et al*. (2003).

The increase in activity was positively correlated with salt concentrations (Fig 8). Highest enzyme activity was recorded in 200 mM NaCl concentration over all the varieties. The percentage increase was more in tolerant cultivars showing that it has the inherent capacity to with stand the stress full conditions. These results were at par with the results of Zhang and Kirkham (1994). The main function of catalase is the dismutation of hydrogen peroxide in to water and oxygen. Catalase is indispensable for ROS detoxification during stress,due to the fact that there is proliferation of peroxisomes during stress. Which might help in scavenging of H_2O_2 which can diffuse from the cytosol (Lopez- Huertas *et al*., 2000).

Table 17 : Changes in catalase (CAT) activity in seedlings of rice varieties treated with different levels of sodium chloride salinity at different time intervals

CAT (Changes in OD min^{-1} g^{-1})

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	24 hour				
V_1	3.23	3.86	5.17	6.55	4.70
V_2	4.03	5.46	6.83	8.42	6.19
V_3	6.24	7.82	10.49	13.26	9.45
V_4	2.37	2.88	3.54	4.17	3.24
Treatment mean	3.69	5.01	6.51	8.09	
	V		S	V x S	
S.Em.	0.04		0.04	0.08	
C.D.	0.11		0.11	0.22	
C.V. %	2.25				
	48 hour				
V_1	4.11	5.46	6.59	8.09	6.06
V_2	5.67	6.38	6.16	9.99	7.05
V_3	7.23	9.56	10.12	13.27	10.05
V_4	3.97	4.63	5.57	6.72	5.22
Treatment mean	5.24	6.51	7.11	9.52	
	V		S	V x S	
S.Em.	0.09		0.09	0.19	
C.D.	0.27		0.27	0.57	
C.V. %	4.53				
	72 hour				
V_1	5.62	7.35	8.74	9.89	7.90
V_2	7.66	8.79	9.97	12.52	9.73
V_3	10.98	15.72	18.75	21.33	16.69
V_4	4.17	6.44	7.55	8.64	6.70
Treatment mean	7.10	9.58	11.25	13.10	
	V		S	V x S	
S.Em.	0.04		0.04	0.08	
C.D.	0.12		0.12	0.24	
C.V. %	1.40				

Table 18 : Pooled analysis of superoxidedismutase and catalase activities in rice varieties

	Super oxide dismutase (Unit min^{-1} g^{-1})				CAT (Changes in OD min^{-1} g^{-1})			
V_1	17.46				5.44			
V_2	23.12				7.03			
V_3	30.09				8.29			
V_4	33.92				10.24			
S.Em.±	0.42				0.32			
C.D.	1.17				1.10			
T_1	21.76				6.22			
T_2	26.63				7.66			
T_3	19.70				12.06			
T_4	36.50				5.05			
S.Em.±	0.98				0.74			
C.D.	3.38				2.55			
P_1	21.74				5.89			
P_2	25.12				7.10			
P_3	31.58				10.26			
S.Em.±	0.36				0.03			
C.D.	1.02				0.09			
Interaction T x V	V_1	V_2	V_3	V_4	V_1	V_2	V_3	V_4
T_1	13.67	18.04	25.00	30.34	4.32	5.56	6.84	8.18
T_2	17.69	24.73	32.64	31.46	5.79	6.88	7.66	10.31
T_3	11.42	17.27	23.96	26.16	8.15	11.04	13.12	15.95
T_4	27.07	32.43	38.78	47.70	3.50	4.65	5.55	6.51
S.Em.±	1.66				0.33			
C.D.	NS				0.99			
Interaction P x V	V_1	V_2	V_3	V_4	V_1	V_2	V_3	V_4
P_1	13.50	18.04	25.10	30.33	3.97	5.01	6.51	8.10
P_2	16.29	22.36	30.05	31.77	5.24	6.51	7.11	9.52
P_3	22.60	28.95	35.14	39.65	7.11	9.58	11.25	13.10
S.Em.±	0.72				0.06			
C.D.	NS				0.18			
Interaction P x T	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
P_1	17.53	22.33	15.76	31.35	4.70	6.19	9.45	3.24
P_2	21.30	23.14	19.64	36.38	6.06	7.05	10.05	5.22
P_3	26.46	34.42	23.70	41.75	7.90	9.74	16.70	6.70
S.Em.±	0.72				0.06			
C.D.	2.03				0.18			
PxTxV	Sig.				Sig.			
C.V. %	9.58				2.80			

4.2.2.4 ***Isozyme pattern of catalase with salinity***

The isozyme pattern of catalase presented in Plates XI, XII, XIII with different time interval of salinity treatment. Catalase showed the presence of single isoform with different intensity.

The intensity of catalase varies with varieties and salinity levels. Maximum intensity was observed in Jaya as compared to CSR-27 at 24, 48 salinity treatment periods. Whereas at 72 h salinity treatment, Dandi showed maximum intensity followed by GR-3. At 150 mM NaCl level maximum intensity was observed in all the varieties.

Ota *et al*. (1992) noticed two isoforms of catalase in bean and also Frugoli *et al*. (1996) observed six catalase isoforms in *Arabidopsis*.

4.2.2.5 *Peroxidase*

Among the four varieties CSR-27 recorded highest guaiacol peroxidase activity (34.18 unit min^{-1} g^{-1}) at 72 h salinity treatment period whereas minimum activity was observed in GR-3 (7.08 unit min^{-1}- g^{-1}) during 24 h salinity treatment (Fig 9). Increase in enzyme activity was more in tolerant varieties than susceptible variety. The percentage of increase was more in CSR-27 followed by Dandi (Table 19). The interaction between V x S was also significant among all the varieties.

Peroxidases are present in most of the plant tissues which are thermally stable present as soluble, ionically-bound or covalently bound forms (Ingham *et al*., 1998). The enhanced activity due to *de novo* synthesis has been observed

Table 19 : Changes in peroxidase (POX) activity in seedlings of rice varieties treated with different levels of sodium chloride salinity at different time intervals

POX (Changes in OD min^{-1} g^{-1})

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	24 hour				
V_1	8.75	10.96	16.44	17.57	13.42
V_2	11.62	15.14	19.21	25.29	17.81
V_3	16.64	20.72	26.44	30.79	23.64
V_4	4.38	6.60	8.00	9.37	7.08
Treatment mean	10.34	13.35	17.52	20.75	
	V		S		V x S
S.Em.	0.10		0.10		0.21
C.D.	0.30		0.30		0.60
C.V. %	2.34				
	48 hour				
V_1	10.36	13.24	19.79	22.32	16.43
V_2	16.46	20.03	24.23	25.24	21.49
V_3	21.78	26.25	33.33	36.73	29.52
V_4	6.76	8.55	9.95	10.64	8.97
Treatment mean	13.84	17.01	21.82	23.73	
	V		S		V x S
S.Em.	0.05		0.05		0.09
C.D.	0.13		0.13		0.27
C.V. %	0.84				
	72 hour				
V_1	15.14	17.34	23.20	25.27	20.24
V_2	18.69	25.41	30.35	36.52	27.74
V_3	26.44	29.07	38.26	42.94	34.18
V_4	7.02	9.36	11.24	13.39	10.25
Treatment mean	16.83	20.29	25.76	29.53	
	V		S		V x S
S.Em.	0.08		0.08		0.16
C.D.	0.24		0.24		0.47
C.V. %	1.23				

in rice seedlings under anoxia (Lee and Lin, 1995) and low temperature stress (Oidaira *et al*., 2000).

Lee *et al*. (2001) also observed that enhanced activity of ascorbate peroxidase activity (APOX) in rice seedlings under salinity stress. These results were also in agreement with Panda and Khan (2003) and Djanagunraman *et al*., (2003).

The increase in the activity of antioxidant enzymes due to higher levels of O_2 production under stress. This shows that an efficient defense mechanism might be involved in the increase of the anti-oxidant levels.

Pooled analysis of peroxidase activity with different time intervals upon sodium chloride treatment was found significant. The treatments are very effective with the time intervals as well as with the varieties.

4.2.2.6 *Isozyme pattern of peroxidase with salinity*

1) 24 h salinity treatment

The peroxidase showed different isoforms having the R_m values of 0.11 to 0.80 at 24 h salinity treatment (Table 20) whereas 7 isoforms having R_m values of 0.23 to 0.98 at 48 h salinity treatment (Table 21, Plate XVI) and also 8 different isoforms with R_m values of 0.04 to 0.92 with 72 h salinity treatment (Plate XVI).

At 24 h salinity treatment level isoforms 3, 4 and 5 showed polymorphism (Plate XV) remaining 1, 2, 5 and 6 does not showed polymorphism in appearance but polymorphism was seen in terms of intensity.

Table 20 : RM values of peroxidase in rice at 24 h salinity treatment

Parameters / Band No.		1	2	3	4	5	6
Varieties (V)	**Salinity levels (S)**						
Jaya (T)	Control	0.11^{L}	0.51^{D}	0.67^{L}	0.68^{L}	0.72^{L}	0.80^{D}
	100 mM (S1)	0.11^{D}	0.51^{D}	0.67^{L}	0.68^{L}	0.72^{L}	0.80^{M}
	150 mM (S2)	0.11^{M}	0.51^{L}	0.67^{M}	0.68^{L}	0.72^{L}	0.80^{D}
	200 mM (S3)	0.11^{D}	0.51^{M}	0.67^{M}	0.68^{M}	0.72^{D}	0.80^{D}
Dandi (T)	Control	0.11^{D}	0.51^{D}	-	0.68^{M}	0.72^{M}	0.80^{M}
	100 mM (S1)	0.11^{L}	0.51^{M}	-	-	-	0.80^{D}
	150 mM (S2)	0.11^{M}	0.51^{D}	0.67^{M}	0.68^{D}	0.72^{D}	0.80^{D}
	200 mM (S3)	0.11^{D}	0.51^{D}	-	0.68^{M}	-	0.80^{D}
CSR-27 (T)	Control	0.11^{D}	0.51^{M}	-	0.68^{L}	-	0.80^{L}
	100 mM (S1)	0.11^{D}	0.51^{D}	0.67^{D}	0.68^{D}	0.72^{D}	0.80^{D}
	150 mM (S2)	0.11^{M}	0.51^{M}	-	0.68^{M}	0.72^{M}	0.80^{M}
	200 mM (S3)	0.11^{L}	0.51^{M}	-	0.68^{M}	0.72^{L}	0.80^{M}
GR-3 (S)	Control	0.11^{M}	0.51^{L}	-	0.68^{L}	0.72^{L}	0.80^{L}
	100 mM (S1)	0.11^{D}	0.51^{M}	-	0.68^{M}	0.72^{M}	0.80^{M}
	150 mM (S2)	0.11^{M}	0.51^{D}	-	0.68^{D}	0.72^{M}	0.80^{M}
	200 mM (S3)	0.11^{D}	0.51^{D}	-	0.68^{M}	0.72^{D}	0.80^{D}

L = Light, M = Medium, D = Dark

The isoform 3 (R_m 0.67) showed polymorphism in Jaya which was absent in CSR-27 and GR-3. In case of isoform 4 (R_m – 0.68) was absent at 100 mM salinity level in Dandi variety. Isoform 3 totally absent in GR-3. Isoform 5 was absent in 150 mM salinity level in. Dandi and Control, 100 mM salinity level in CSR-27. The isoform intensity was more at 200 mM NaCl level (Broetto *et al*., 1997).

Broetto *et al*. (1997) also reported the isoenzymic polymorphism of peroxidases of common bean under saline stress. Parida *et al*. (2004) also found similar results in mangrove.

2) 48 h salinity treatment

Seven different peroxidase isoforms were observed after 48 h salinity treatment period (Plate XVI). The peroxidase isoforms having R_m values of 0.23 to 0.98. Isoforms 3, 4, 5, 6 were did not show polymorphism but slight polymorphism observed in terms of intensity. The intensity was more at 150, 200 mM salinity levels in all the varieties. Whereas in case of Jaya and CSR-27 even control also showed maximum intensity, which showed the tolerant nature of these varieties.

Isoforms 1 and 2 showed polymorphism where some treatment combinations did not showed the peroxidase isoforms. In Dandi 150, 200 mM salinity level, in CSR-27 100 mM salinity level, in GR-3 100 mM did not showed isoforms.

Remaining isoforms 3, 4, 5, 6, 7 (R_m value 0.83, 0.86, 0.92, 0.96, 0.98) polymorphism observed only with intensity (Table 21).

3) 72 h salinity treatment

The peroxidase showed 8 different isoforms with R_m values ranging from 0.04 to 0.92 (Table 22). Isoforms 3 and 7 showed polymorphism in terms of presence as well as intensity. In case of isoforms (Plate XVI) 1, 2, 4, 5 polymorphism was observed in terms of intensity only. When compared to 24 h, 48 h, the intensity of bands was more in 72 h salinity treatment period among all the varieties. The intensity was more in tolerant varieties than susceptible variety GR-3.

4.2.2.7 ***Polyphenol oxidase activity***

Polyphenol oxidase (PPO, o-diphenol : O_2 oxido-reductase) also known as phenolase, phenol oxidase, catechol oxidase and tyrosinase. PPO's are involved in i) in phenol oxidation, ii) oxidation of IAA. This enzyme indirectly influences the activity of peroxidases by oxidation of phenolic compounds.

Among the four varieties CSR-27 recorded highest PPO activity (0.76 unit min^{-1} g^{-1}) at 72 h salinity treatment period. Whereas the activity was least in GR-3 (0.07 unit min^{-1} g^{-1}) at 24 h salinity treatment (Fig 10).

The varieties, salinity levels were found to be significant in all the varieties. The activity was increased with reference to salinity levels. However, the decrease was more in tolerant varieties than sensitive variety.

Table 21 : RM values of peroxidase in rice at 48 h salinity treatment

Parameters / Band No.		1	2	3	4	5	6	7
Varieties (V)	**Salinity levels (S)**							
Jaya (T)	Control	0.23^{D}	0.71^{M}	0.83^{D}	0.86^{D}	0.92^{D}	0.96^{D}	0.98^{D}
	100 mM (S1)	0.23^{L}	-	0.83^{L}	0.86^{L}	0.92^{L}	0.96^{L}	0.98^{L}
	150 mM (S2)	0.23^{M}	0.71^{L}	0.83^{M}	0.86^{M}	0.92^{M}	0.96^{M}	0.98^{M}
	200 mM (S3)	0.23^{D}	0.71^{D}	0.83^{D}	0.86^{D}	0.92^{D}	0.96^{D}	0.98^{D}
Dandi (T)	Control	0.23^{M}	0.71^{M}	0.83^{M}	0.86^{M}	0.92^{D}	0.96^{D}	0.98^{D}
	100 mM (S1)	-	-	0.83^{L}	0.86^{L}	0.92^{L}	0.96^{L}	0.98^{L}
	150 mM (S2)	-	0.71^{M}	0.83^{M}	0.86^{M}	0.92^{M}	0.96^{M}	0.98^{M}
	200 mM (S3)	0.23^{D}	0.71^{D}	0.83^{D}	0.86^{M}	0.92^{D}	0.96^{D}	0.98^{D}
CSR-27 (T)	Control	0.23^{D}	0.71^{D}	0.83^{D}	0.86^{D}	0.92^{D}	0.96^{D}	0.98^{D}
	100 mM (S1)	-	-	0.83^{L}	0.86^{L}	0.92^{L}	0.96^{L}	0.98^{L}
	150 mM (S2)	0.23^{M}	0.71^{L}	0.83^{L}	0.86^{M}	0.92^{M}	0.96^{M}	0.98^{M}
	200 mM (S3)	0.23^{M}	0.71^{D}	0.83^{D}	0.86^{D}	0.92^{D}	0.96^{D}	0.98^{D}
GR-3 (S)	Control	0.23^{D}	0.71^{M}	0.83^{M}	0.86^{D}	0.92^{D}	0.96^{D}	0.98^{D}
	100 mM (S1)	-	0.71^{L}	0.83^{M}	0.86^{L}	0.92^{L}	0.96^{L}	0.98^{L}
	150 mM (S2)	0.23^{M}	0.71^{D}	0.83^{D}	0.86^{M}	0.92^{M}	0.96^{M}	0.98^{M}
	200 mM (S3)	0.23^{D}	0.71^{D}	0.83^{D}	0.86^{M}	0.92^{D}	0.96^{D}	0.98^{D}

L = Light, M = Medium, D = Dark

Table 22 : RM values of peroxidase in rice at 72 h salinity treatment

Parameters / Band No.		1	2	3	4	5	6	7	8
Varieties (V)	**Salinity levels (S)**								
Jaya (T)	Control	0.04^{M}	0.10^{M}	-	0.38^{M}	0.57^{M}	0.63^{M}	0.77^{L}	0.92^{M}
	100 mM (S1)	0.04^{M}	0.10^{M}	-	0.38^{M}	0.57^{M}	0.63^{M}	0.77^{M}	0.92^{M}
	150 mM (S2)	0.04^{D}	0.10^{D}	0.36^{D}	0.38^{D}	0.57^{D}	0.63^{D}	0.77^{D}	0.92^{D}
	200 mM (S3)	0.04^{M}	0.10^{M}	0.36^{M}	0.38^{M}	0.57^{M}	0.63^{M}	0.77^{M}	0.92^{M}
Dandi (T)	Control	0.04^{D}	0.10^{D}	0.36^{D}	0.38^{M}	0.57^{M}	0.63^{M}	0.77^{M}	0.92^{M}
	100 mM (S1)	0.04^{M}	0.10^{D}	0.36^{M}	0.38^{D}	0.57^{D}	0.63^{D}	0.77^{M}	0.92^{D}
	150 mM (S2)	0.03^{D}	0.10^{D}	0.36^{L}	0.38^{D}	0.57^{M}	0.63^{M}	0.77^{L}	0.92^{L}
	200 mM (S3)	0.03^{M}	0.09^{L}	0.36^{L}	0.38^{L}	0.57^{L}	0.63^{L}	0.77^{L}	-
CSR-27 (T)	Control	0.03^{L}	0.09^{L}	0.36^{M}	0.38^{L}	0.57^{L}	0.63^{L}	0.77^{L}	-
	100 mM (S1)	0.04^{M}	0.09^{M}	0.36^{D}	0.38^{D}	0.57^{D}	0.63^{D}	0.77^{M}	0.92^{L}
	150 mM (S2)	0.04^{M}	0.09^{M}	0.36^{D}	0.38^{M}	0.57^{M}	0.63^{M}	0.77^{D}	0.92^{M}
	200 mM (S3)	0.04^{D}	0.09^{D}	0.36^{M}	0.38^{M}	0.57^{D}	0.63^{D}	0.77^{M}	0.92^{L}
GR-3 (S)	Control	0.03^{M}	0.09^{M}	0.36^{M}	0.38^{M}	0.57^{D}	0.63^{D}	0.77^{L}	0.92^{L}
	100 mM (S1)	0.03^{D}	0.09^{D}	0.36^{D}	0.38^{D}	0.57^{M}	0.63^{M}	0.77^{L}	0.92^{L}
	150 mM (S2)	0.03^{M}	0.09^{D}	0.36^{D}	0.38^{D}	0.57^{M}	0.63^{M}	0.77^{L}	0.92^{M}
	200 mM (S3)	0.03^{M}	0.09^{M}	0.36^{D}	0.38^{M}	0.57^{L}	0.63^{L}	-	-

L = Light, M = Medium, D = Dark

The PPO activity was minimum in control and maximum in 200 mM NaCl level. These results were similar in all most all varieties (Table 23).

The interaction between VxS was also significant. The decrease was more in 200 Mm NaCl level as compared to 100 mM NaCl level.

4.2.2.8 *Esterase activity in rice seedlings*

Esterases are the hydrolases which comprises of different group of enzymes like lipases, chlorophyllase, phosphatase, nucleases, phorphorylases, ATPases, enolases, Tranaphosphorylases, which having a wide range of functions according to the nature of the substrates.

This enzyme also serves as a suitable biochemical marker for salinity tolerance in rice.

Among the four varieties studied Dandi recorded maximum esterase activity (0.73 unit min^{-1} g^{-1}) at 48 h salinity followed by CSR-27, whereas minimum activity was observed in GR-3 (0.45 unit min^{-1} g^{-1}) at 24 h salinity period, which was nearly at par with Jaya (0.48 unit min^{-1} g^{-1}) (Table 25).

The enzyme activity was increased towards salinity (Fig. 11). The varieties and salinity levels were significant among all the varieties among different salinity levels 200 mM registered maximum activity (0.696 unit min^{-1} g^{-1}) at 24 h salinity treatment period and control recorded the minimum enzyme activity (0.41 unit min^{-1} g^{-1}).

Table 23 : Changes in polyphenol oxidase activity (PPO) in seedlings of rice varieties treated with different levels of sodium chloride salinity at different time intervals

PPO (Changes in OD min^{-1} g^{-1})

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	24 hour				
V_1	0.09	0.16	0.13	0.19	0.14
V_2	0.08	0.12	0.11	0.16	0.12
V_3	0.08	0.14	0.12	0.17	0.13
V_4	0.07	0.10	0.08	0.12	0.09
Treatment mean	0.16	0.13	0.11	0.16	
	V		S	V x S	
S.Em.	0.002		0.002	0.004	
C.D.	0.006		0.006	0.012	
C.V. %	6.02				
	48 hour				
V_1	0.28	0.38	0.41	0.53	0.41
V_2	0.56	0.56	0.59	0.67	0.60
V_3	0.33	0.55	0.56	0.68	0.53
V_4	0.39	0.42	0.49	0.48	0.44
Treatment mean	0.39	0.48	0.52	0.59	
	V		S	V x S	
S.Em.	0.01		0.01	0.02	
C.D.	0.03		0.03	0.06	
C.V. %	7.34				
	72 hour				
V_1	0.37	0.41	0.48	0.59	0.46
V_2	0.55	0.61	0.70	0.79	0.67
V_3	0.34	0.57	0.66	0.76	0.58
V_4	0.36	0.42	0.48	0.56	0.46
Treatment mean	0.41	0.50	0.58	0.68	
	V		S	V x S	
S.Em.	0.008		0.008	0.01	
C.D.	0.02		0.02	0.04	
C.V. %	5.39				

Table 24 : Pooled analysis of polyphenol oxidase (PPO) and peroxidase activities in rice varieties

	Polyphenol oxidase activity (Changes in OD min^{-1} g^{-1})				POX (Changes in OD min^{-1} g^{-1})			
V_1	0.32				13.67			
V_2	0.37				16.89			
V_3	0.41				21.70			
V_4	0.45				24.67			
S.Em.±	0.05				0.41			
C.D.	NS				1.43			
T_1	0.34				16.70			
T_2	0.46				22.35			
T_3	0.41				29.12			
T_4	0.33				8.77			
S.Em.±	0.03				1.02			
C.D.	NS				3.51			
P_1	0.12				15.49			
P_2	0.50				19.10			
P_3	0.54				23.10			
S.Em.±	0.00				0.04			
C.D.	0.01				0.11			
Interaction T x V	V_1	V_1	V_2	V_3	V_4	V_2	V_3	V_4
T_1	0.28	11.42	13.85	19.81	21.72	0.32	0.36	0.40
T_2	0.42	15.59	21.19	24.60	29.02	0.43	0.47	0.52
T_3	0.28	21.62	25.35	32.68	36.82	0.42	0.45	0.51
T_4	0.29	6.06	8.17	9.73	11.13	0.31	0.35	0.37
S.Em.±	0.02				0.64			
C.D.	0.04				1.90			
Interaction P x V	V_1	V_1	V_2	V_3	V_4	V_2	V_3	V_4
P_1	0.16	10.35	13.36	17.52	20.75	0.13	0.11	0.08
P_2	0.39	13.84	17.02	21.82	23.73	0.48	0.52	0.59
P_3	0.41	16.83	20.29	25.76	29.53	0.50	0.58	0.68
S.Em.±	0.01				0.08			
C.D.	0.02				0.23			
Interaction P x T	T_1	T_1	T_2	T_3	T_4	T_2	T_3	T_4
P_1	0.14	13.43	17.82	23.65	7.09	0.12	0.13	0.93
P_2	0.41	16.43	21.49	29.52	8.97	0.60	0.53	0.44
P_3	0.46	20.24	27.74	34.18	10.25	0.67	0.58	0.46
S.Em.±	0.01				0.08			
C.D.	0.02				0.23			
PxTxV	Sig.				Sig.			
C.V. %	7.06				1.46			

Table 25 : Changes in esterase activity in seedlings of rice varieties treated with different levels of sodium chloride salinity at different time intervals

Easterase (Changes in OD min^{-1} g^{-1})

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	24 hour				
V_1	0.36	0.43	0.51	0.60	0.48
V_2	0.49	0.61	0.71	0.79	0.65
V_3	0.42	0.55	0.65	0.75	0.60
V_4	0.33	0.41	0.49	0.55	0.45
Treatment mean	0.40	0.50	0.58	0.67	
	V		S	V x S	
S.Em.	0.008		0.008	0.02	
C.D.	0.02		0.02	0.04	
C.V. %	5.23				
	48 hour				
V_1	0.48	0.47	0.57	0.69	0.54
V_2	0.61	0.70	0.80	0.79	0.73
V_3	0.51	0.54	0.75	0.78	0.65
V_4	0.32	0.39	0.45	0.52	0.42
Treatment mean	0.48	0.52	0.63	0.69	
	V		S	V x S	
S.Em.	0.009		0.009	0.02	
C.D.	0.02		0.02	0.05	
C.V. %	5.06				
	72 hour				
V_1	0.39	0.43	0.50	0.60	0.48
V_2	0.47	0.62	0.70	0.76	0.64
V_3	0.50	0.55	0.61	0.76	0.61
V_4	0.34	0.41	0.49	0.55	0.45
Treatment mean	0.42	0.50	0.59	0.67	
	V		S	V x S	
S.Em.	0.01		0.01	0.02	
C.D.	0.03		0.03	0.06	
C.V. %	6.67				

In Jaya a slight decrease in the activity of enzyme at 72 h salinity treatment period was noted, whereas in Dandi and CSR – 27 notable decrease in the enzyme activity was observed at 72 h salinity treatment. In susceptible variety GR-3 the activity decreased at 48 h which again increased at 72 h salinity treatment.

From the pooled analysis it was noticed (Table 29) that esterase enzyme activity was significant with time intervals and also with the treatment combinations.

4.2.2.9 *Isozyme Pattern*

1) 24 h salinity treatment

Plate XVIII showed 4 different isoforms with R_m values ranging from 0.25 to 0.66 (Table 26). Among these 4 isoforms 4^{th} isoforms showed variation in Dandi where the isoform absent at control and 100 mM salinity level. Remaining all the treatment combinations showed variations in terms of intensity only. The intensity increased with higher salinity level and also the more intensity was noticed in tolerant varieties.

2) 48 h salinity treatment

Plate XIX showed five different isoforms of esterase were noticed at 48 h salinity treatment period. The R_m values ranges from 0.30 to 0.84 (Table 27) during the treatment polymorphism noticed only in terms of intensity. All the treatment combinations showed esterase isoforms but the intensity was clearly more in high treatment levels in all the varieties.

Table 26 : RM values of esterase in rice at 24 h salinity treatment

Parameters / Band No.		1	2	3	4
Varieties (V)	**Salinity levels (S)**				
Jaya (T)	Control	0.25^{M}	0.49^{M}	0.64^{D}	0.66^{M}
	100 mM (S1)	0.25^{D}	0.49^{L}	0.64^{M}	$0.66^{,}$
	150 mM (S2)	0.25^{D}	0.49^{M}	0.64^{M}	0.66^{D}
	200 mM (S3)	0.25^{M}	0.49^{D}	0.64^{D}	0.66^{L}
Dandi (T)	Control	0.25^{M}	0.49^{M}	0.64^{M}	-
	100 mM (S_2)1	0.25^{D}	0.49^{D}	0.64^{D}	-
	150 mM (S_{32}	0.25^{M}	0.49^{D}	0.64^{D}	0.66^{M}
	200 mM (S_3)	0.25^{M}	0.49^{L}	0.64^{D}	0.66^{D}
CSR-27 (T)	Control	0.25^{L}	0.49^{M}	0.64^{L}	0.66^{M}
	100 mM (S1)	0.25^{L}	0.49^{D}	0.64^{L}	0.66^{D}
	150 mM (S2)	0.25^{D}	0.49^{M}	0.64^{D}	0.66^{D}
	200 mM (S3)	0.25^{M}	0.49^{D}	0.64^{M}	0.66^{M}
GR-3 (S)	Control	0.25^{M}	0.49^{M}	0.64^{M}	0.66^{M}
	100 mM (S1)	0.25^{D}	0.49^{D}	0.64^{M}	0.66^{D}
	150 mM (S2)	0.25^{M}	0.49^{D}	0.64^{M}	0.66^{D}
	200 mM (S3)	0.25^{L}	0.49^{D}	0.64^{D}	0.66^{D}

L = Low, M = Medium, D = Dark

Table 27 : RM values of esterase in rice at 48 h salinity treatment

Parameters / Band No.		1	2	3	4	5
Varieties (V)	**Salinity levels (S)**					
Jaya (T)	Control	0.30^{D}	0.32^{D}	0.44^{M}	0.77^{L}	0.84^{L}
	100 mM (S1)	0.30^{D}	0.32^{D}	0.44^{D}	0.77^{M}	0.84^{L}
	150 mM (S2)	0.30^{D}	0.32^{D}	0.44^{D}	0.77^{D}	0.84^{M}
	200 mM (S3)	0.30^{D}	0.32^{D}	0.44^{L}	0.77^{L}	0.84^{L}
Dandi (T)	Control	0.30^{D}	0.32^{D}	0.44^{D}	0.77^{M}	0.84^{D}
	100 mM (S1)	0.30^{D}	0.32^{D}	0.44^{D}	0.77^{D}	0.84^{D}
	150 mM (S2)	0.30^{L}	0.32^{L}	0.44^{L}	0.77^{M}	0.84^{M}
	200 mM (S3)	0.30^{D}	0.32^{D}	0.44^{M}	0.77^{L}	0.84^{L}
CSR-27 (T)	Control	0.30^{M}	0.32^{M}	0.44^{L}	0.77^{D}	0.84^{L}
	100 mM (S1)	0.30^{M}	0.32^{M}	0.44^{D}	0.77^{D}	0.84^{D}
	150 mM (S2)	0.30^{L}	0.32^{L}	0.44^{D}	0.77^{L}	0.84^{D}
	200 mM (S3)	0.30^{D}	0.32^{D}	0.44^{M}	0.77^{M}	0.84^{M}
GR-3 (S)	Control	0.30^{D}	0.32^{D}	0.44^{M}	0.77^{M}	0.84^{L}
	100 mM (S1)	0.30^{M}	0.32^{M}	0.44^{L}	0.77^{L}	0.84^{L}
	150 mM (S2)	0.30^{D}	0.32^{D}	0.44^{M}	0.77^{L}	0.84^{L}
	200 mM (S3)	0.30^{M}	0.32^{M}	0.44^{L}	0.77^{L}	-

L = Low, M = Medium, D = Dark

3) 72 h salinity treatment

During this treatment clear polymorphism observed in esterase enzyme, 8 isoforms of esterase were identified (Plate XX) with R_m values with in the range of 0.46 to 0.73. Isoform 4, 3, 2, 5, 8 showed clear polymorphism. Isoforms 1 R_m (0.46) was absent at control, at 150 mM salinity level in Jaya and also at 200 mM salinity level in Dandi (Table 28).

Isoform 2 (R_m – 0.49) was absent in control, 150 mM salinity level in Jaya and 100, 150, 200 mM NaCl levels in Dandi. Whereas isoform 4 was completely absent in control among all varieties. CSR-27 did not show isoform of esterase. The intensity was more at 150 mM NaCl level in GR-3.

Isoform 5 (R_m – 0.65) was completely absent in CSR-27 and also at control, 150 mM NaCl level in GR-3.

Isoform 8 (R_m – 0.73) was absent completely in CSR-27 and at 200 mM NaCl level in Jaya, Control, 200 mM level in Dandi and control, 100 mM salinity level in GR-3.

Basu *et al*. (1997) also observed the appearance of one unique esterase band in NaCl adapted callus of rice.

4.2.3 Mineral ion concentration and salinity

Apart from the osmolytes (Garcia *et al*., 1997) certain inorganic ions like Na^+ and K^+ also serve as a potential marker for salinity tolerance (Greenway and Munns, 1990).

Table 28 : RM values of esterase in rice at 72 h salinity treatment

Parameters / Band No.		1	2	3	4	5	6	7	8
Varieties (V)	**Salinity levels (S)**								
Jaya (T)	Control	-	-	-	-	0.65^{L}	0.69^{L}	0.71^{M}	0.73^{L}
	100 mM (S1)	0.46^{M}	-	0.52^{M}	-	0.65^{D}	0.69^{D}	0.71^{D}	0.73^{L}
	150 mM (S2)	-	-	-	-	0.65^{M}	0.69^{M}	0.71^{M}	0.73^{M}
	200 mM (S3)	0.46^{D}	0.49^{M}	0.52^{D}	0.54^{L}	0.65^{M}	0.69^{D}	0.71^{D}	-
Dandi (T)	Control	0.46^{L}	-	0.52^{L}	-	0.65^{L}	0.69^{L}	0.71^{M}	-
	100 mM (S1)	0.46^{M}	0.69^{L}	-	-	0.65^{D}	0.69^{L}	0.71^{M}	0.73^{M}
	150 mM (S2)	0.46^{M}	-	-	-	0.65^{M}	0.69^{L}	0.71^{D}	0.73^{M}
	200 mM (S3)	-	-	-	-	0.65^{M}	-	0.71^{M}	-
CSR-27 (T)	Control	0.46^{D}	-	0.52^{M}	0.54^{M}	-	-	0.71^{L}	-
	100 mM (S1)	0.46^{D}	0.69^{L}	0.52^{D}	-	-	-	0.71^{L}	-
	150 mM (S2)	0.46^{D}	0.69^{M}	0.52^{D}	-	-	-	0.71^{M}	-
	200 mM (S3)	0.46^{M}	-	0.52^{D}	-	-	-	0.71^{L}	-
GR-3 (S)	Control	0.46^{M}	0.69^{D}	0.52^{M}	-	-	0.69^{L}	0.71^{D}	-
	100 mM (S1)	0.46^{M}	0.69^{D}	0.52^{D}	0.54^{L}	0.65^{L}	0.69^{L}	0.71^{M}	0.73^{L}
	150 mM (S2)	0.46^{M}	0.69^{D}	0.52^{M}	0.54^{D}	-	0.69^{D}	0.71^{M}	0.73^{L}
	200 mM (S3)	0.46^{M}	0.69^{M}	0.52^{D}	0.54^{L}	0.65^{L}	0.69^{M}	0.71^{L}	0.73^{L}

L = Light, M = Medium, D = Dark

Table 29 : Pooled analysis of esterase activity in rice varieties

	Esterase activity (Changes in OD min^{-1} g^{-1})			
V_1	0.44			
V_2	0.51			
V_3	0.60			
V_4	0.68			
S.Em.±	0.01			
C.D.	0.02			
T_1	0.50			
T_2	0.67			
T_3	0.62			
T_4	0.44			
S.Em.±	0.02			
C.D.	0.05			
P_1	0.54			
P_2	0.58			
P_3	0.55			
S.Em.±	0.01			
C.D.	0.01			
Interaction T x V	V_1	V_2	V_3	V_4
T_1	0.41	0.44	0.51	0.63
T_2	0.53	0.64	0.72	0.78
T_3	0.48	0.55	0.67	0.77
T_4	0.33	0.41	0.47	0.55
S.Em.±	0.12			
C.D.	0.05			
Interaction P x V	V_1	V_2	V_3	V_4
P_1	0.41	0.50	0.59	0.67
P_2	0.48	0.53	0.63	0.70
P_3	0.42	0.51	0.58	0.67
S.Em.±	0.01			
C.D.	0.04			
Interaction P x T	T_1	T_2	T_3	T_4
P_1	0.48	0.65	0.60	0.45
P_2	0.54	0.73	0.65	0.42
P_3	0.48	0.64	0.61	0.45
S.Em.±	0.01			
C.D.	0.03			
PxTxV	Sig.			
C.V. %	5.68			

4.2.3.1 *Sodium (Na^+)*

Data from the Table 30 showed significant difference in Na^+ among varieties, salinity levels and their interactions in rice seedlings with salinity. Among the four varieties, Jaya recorded the maximum sodium content (355.95 ppm) at 72 h salinity treatment and minimum content of Na^+ was observed in GR-3 (164.05 ppm) 48 h salinity treatment. A similar pattern was also observed in case of sodium content in all the varieties.

Sodium content increased with increasing NaCl level (Fig. 12). The interaction between varieties and salinity levels was also significant among all the varieties (Table 30). Maximum sodium content was noticed in 200 mM sodium chloride level in all the varieties.

4.2.3.2 *Potassium (K^+)*

Potassium content was decreased with salinity among all varieties with 3 different time intervals of salinity (Fig. 13). Among the four varieties Jaya (345.80 ppm) recorded the highest content of K^+ control at 24 h salinity treatment, whereas minimum content of potassium was observed in GR-3 (207.30 ppm) in control at 24 h salinity treatment. In case of 48 h salinity treatment period CSR-27 showed maximum content of K^+ (280.52 ppm) as well as in case of 72 h salinity treatment period. Jaya recorded maximum content of K^+ (269.44 ppm) and minimum content of K^+ was observed in GR-3 in all three salinity treatment periods (Table 31). The interaction between TxV

Table 30 : Changes in sodium ion in seedlings of rice varieties treated with different levels of sodium chloride salinity at different time intervals

Sodium (ppm)

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	24 hour				
V_1	125.57	277.74	378.31	447.06	307.17
V_2	102.05	211.93	330.70	389.55	258.56
V_3	79.23	191.76	240.87	305.41	204.32
V_4	67.96	177.08	211.62	270.13	181.70
Treatment mean	93.70	214.63	290.37	353.04	
	V		S	V x S	
S.Em.	2.27		2.27	4.55	
C.D.	6.30		6.30	8.91	
C.V. %	3.31				
	48 hour				
V_1	134.16	308.36	386.16	463.40	323.03
V_2	123.06	257.96	277.00	292.00	241.16
V_3	101.16	221.46	274.96	307.06	226.16
V_4	81.53	116.70	193.03	264.90	164.05
Treatment mean	107.79	223.63	282.76	340.20	
	V		S	V x S	
S.Em.	2.02		2.02	4.04	
C.D.	5.59		5.59	7.91	
C.V. %	2.93				
	72 hour				
V_1	166.66	335.66	418.70	501.80	355.95
V_2	135.23	248.53	313.46	343.03	260.06
V_3	103.36	291.60	452.60	545.06	348.15
V_4	102.96	147.00	229.00	295.33	193.57
Treatment mean	127.05	255.95	353.44	412.30	
	V		S	V x S	
S.Em.	2.89		2.89	5.78	
C.D.	8.02		8.02	11.34	
C.V. %	3.46				

Table 31 : Changes in potassium ion in seedlings of rice varieties treated with different levels of sodium chloride salinity at different time intervals

Potassium (ppm)

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	24 hour				
V_1	462.06	372.26	317.60	229.86	345.80
V_2	394.63	362.26	287.16	216.83	325.92
V_3	406.50	338.33	271.80	255.40	264.77
V_4	272.66	230.83	182.43	126.80	207.30
Treatment mean	383.80	352.92	264.77	207.30	
	V		S		V x S
S.Em.	3.32		3.32		6.51
C.D.	9.21		9.21		13.03
C.V. %	3.90				
	48 hour				
V_1	408.60	319.60	215.00	166.13	277.33
V_2	304.11	276.60	239.50	188.85	252.26
V_3	353.80	324.68	235.86	207.75	280.52
V_4	252.13	214.50	156.43	113.70	184.69
Treatment mean	330.16	283.84	211.69	169.11	
	V		S		V x S
S.Em.	2.24		2.24		4.48
C.D.	6.22		6.22		8.79
C.V. %	3.12				
	72 hour				
V_1	383.03	328.36	200.80	165.70	269.44
V_2	275.40	246.40	212.26	166.23	225.35
V_3	348.43	308.33	213.23	185.73	263.95
V_4	217.00	197.76	162.93	112.06	172.52
Treatment mean	305.96	270.53	197.34	157.43	
	V		S		V x S
S.Em.	2.09		2.09		4.19
C.D.	5.81		5.09		8.21
C.V. %	3.58				

was also significant. Maximum potassium content was observed in control in all the varieties.

From these results, it was cleared that a contrary relation existing between Na^+ and K^+ content during salinity stress. These results were also in agreement with the findings by Tripathy and Kar (1995) for rice varieties.

From the pooled analysis (Table 32) it was cleared that all the treatment combinations were effective with respect to different treatment periods as well as varieties. Both sodium and potassium contents were significant in all the treatment combinations.

EXPERIMENT – II

EFFECTS OF OSMOPROTECTANTS UPON SODIUM CHLORIDE SALINITY STRESS IN RICE CULTIVARS

4.2.4 Effect of proline on biochemical attributes with salinity in rice

To study the effects of osmoprotectants to counteract the saline treatment an experiment was conducted. Rice seedlings (15 DAG) were treated with two osmoprotectants separately with 1 mM proline and trehalose for 8 h. Treated seedlings were further retreated with NaCl saline solution for 72 h. Changes in biochemical parameters were studied in the seedlings and results for the same have been reported hereunder.

Table 32 : Pooled analysis of sodium and potassium contents in rice varieties

	Sodium (ppm g^{-1})				**Potassium (ppm g^{-1})**			
V_1	110.28				339.87			
V_2	232.15				293.33			
V_3	308.87				224.59			
V_4	368.73				177.92			
S.Em.±	9.85				3.73			
C.D.	34.09				12.91			
T_1	328.64				297.42			
T_2	252.05				264.19			
T_3	259.55				287.49			
T_4	179.80				186.61			
S.Em.±	19.59				8.40			
C.D.	67.81				29.07			
P_1	237.94				295.47			
P_2	237.71				248.58			
P_3	289.38				232.73			
S.Em.±	1.87				1.34			
C.D.	5.26				3.76			
Interaction T x V	V_1	V_2	V_3	V_4	V_1	V_2	V_3	V_4
T_1	142.14	307.26	394.39	470.75	417.90	340.08	244.47	187.23
T_2	120.12	239.48	307.06	341.53	324.72	295.09	246.31	190.64
T_3	94.59	234.94	322.81	385.85	369.58	323.78	240.30	216.30
T_4	84.27	146.93	211.22	276.79	247.27	214.37	167.27	117.52
S.Em.±	20.75				8.72			
C.D.	40.24				25.91			
Interaction P x V	V_1	V_2	V_3	V_4	V_1	V_2	V_3	V_4
P_1	93.71	214.63	290.38	353.04	383.97	325.93	264.75	207.23
P_2	110.67	226.13	282.79	31.84	329.66	283.85	211.70	169.11
P_3	127.06	255.70	353.44	421.31	305.97	270.22	197.31	157.43
S.Em.±	3.75				2.67			
C.D.	10.53				7.52			
Interaction P x T	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
P_1	307.17	258.56	204.32	181.70	345.45	315.23	318.00	203.18
P_2	323.03	237.51	226.17	164.13	277.33	252.27	280.52	184.19
P_3	355.71	260.07	348.16	193.58	269.48	225.08	263.93	172.44
S.Em.±	3.75				2.67			
C.D.	10.53				7.52			
PxTxV	Sig.				Sig.			
C.V. %	5.09				3.58			

4.2.4.1 *Chlorophyll*

One visible symptom of ion accumulation in leaves is a concomitant loss of chlorophyll (Yeo and Flowers, 1983) indicating some form of disruption of the chloroplasts. After treatment with proline no loss of chlorophyll was observed and seedling remained green without wilting and drooping.

A drastic increase in chlorophyll activity was observed in rice upon proline treatment during salinity stress. Proline is a potent osmoprotant act as inorganic nitrogen serve during stress conditions.

The effect was more prominent than the trehalose treatment. Among the four varieties studied, Jaya recorded maximum chlorophyll content (21.60 mg 100 g^{-1}) followed by Dandi (20.96 mg 100 g^{-1}), whereas minimum was observed in GR-3 (13.14 mg 100 g^{-1}). Chlorophyll content was increased towards salinity levels (Fig. 14). The varieties and salinity levels were found to be significant in all the varieties (Table 33).

Among the different salinity levels, 200 mM NaCl level showed maximum chlorophyll content (23.26 mg 100 g^{-1}) however, the minimum chlorophyll content was observed in control (14.05 mg 100 g^{-1}).

When compared to the Experiment I, the chlorophyll content was much more in all the varieties.

Table 33 : Changes in biochemical attributes in rice seedlings upon proline treatment

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	Chlorophyll (mg 100 g^{-1})				
V_1	17.56	18.14	22.56	28.13	21.60
V_2	15.44	19.14	21.84	27.41	20.96
V_3	16.06	17.44	18.18	21.32	18.25
V_4	7.15	14.04	15.21	16.17	13.14
Treatment mean	14.05	17.19	19.45	23.26	
	V		S		V x S
S.Em.	0.12		0.12		0.24
C.D.	0.36		0.36		0.72
C.V. %	2.30				
	Total soluble sugar (g 100 g^{-1})				
V_1	4.67	5.90	7.53	7.24	6.34
V_2	3.42	5.04	5.74	6.68	5.22
V_3	4.37	4.60	4.69	5.53	4.80
V_4	3.35	3.45	3.85	2.36	3.25
Treatment mean	3.95	4.75	5.45	5.45	
	V		S		V x S
S.Em.	0.05		0.05		0.10
C.D.	0.14		0.14		0.28
C.V. %	3.29				
	Proline (mg g^{-1})				
V_1	3.35	4.24	4.51	4.76	4.22
V_2	3.10	3.55	3.97	4.27	3.72
V_3	2.95	3.24	3.32	3.63	3.29
V_4	4.58	4.95	5.10	5.54	5.04
Treatment mean	3.50	4.00	4.23	4.55	
	V		S		V x S
S.Em.	0.05		0.05		0.10
C.D.	0.13		0.13		0.26
C.V. %	3.81				

4.2.4.2 *Total soluble sugars (TSS)*

Data presented in Table 33 suggested a marginal increase in the TSS content in tolerant varieties, but more in sensitive variety GR-3. These results depicted that the effect of Osmoprotectants was more in sensitive varieties than tolerant varieties. Among the four varieties, Jaya recorded maximum TSS content (6.34 g 100 g^{-1}). Whereas minimum TSS was observed in GR-3 (3.25 g 100 g^{-1}). Varieties CSR-27 and Dandi both are at par with each other.

Treatment and varieties as well as interaction effects were significant (Table 33).

4.2.4.3 *Proline*

The results revealed that the content of proline is nearly more than twice than the Experiment I after proline treatment to the rice seedlings under salinity situations.

The results observed were also similar in case of proline with salinity. The increase was more in sensitive variety GR-3 than tolerant varieties *viz.*, Jaya, Dandi, CSR-27. Four varieties studied, GR-3 recorded maximum proline content (5.04 mg g^{-1}) and minimum of proline was noticed in CSR-27 (3.29 mg g^{-1}) (Table 33).

Proline content increased with salinity levels as in the case of Experiment I, 200 mM NaCl level showed highest proline content (4.55 mg g^{-1}) than the control (3.50 mg g^{-1}).

4.2.5 Enzyme activity with proline treatment

4.2.5.1 *Catalase*

As seen in Figure 15 the change in activities of different oxidative stress related enzymes with different levels of salinity of rice seedlings upon Osmoprotectants the activity of catalase was more in Jaya (4.12 unit min^{-1} g^{-1}) followed by GR-3 (3.61 unit min^{-1} g^{-1}). The activity increased with salinity levels among all the varieties (Table 34). The varieties and salinity levels were significant and also interaction between varieties and salinity levels (VxS) significant. Among the salinity levels 200 mM NaCl recorded maximum catalase activity (4.07 unit min^{-1} g^{-1}) and minimum activity was observed in control (2.87 unit min^{-1} g^{-1}).

4.2.5.2 *Peroxidase*

Among the four varieties peroxidase activity was more in Jaya (12.49 unit min^{-1} g^{-1}) followed by CSR-27 (11.21 unit min^{-1} g^{-1}). Whereas minimum activity was observed in GR-3 (6.54 unit min^{-1} g^{-1}). The enzyme activity was increased towards salinity (Table 34).

The varieties salinity levels as well as interaction (VxS) were significant. Among the salinity levels 200 mM showed maximum peroxidase content (14.71 unit min^{-1} g^{-1}) and minimum activity was observed in control (5.53 unit min^{-1} g^{-1}).

Table 34 : Changes in enzyme activities in rice seedlings upon proline treatment

	Control (S_1)	100 mM (S_2)	150 mM (S_3)	200 mM (S_4)	Varietal mean
	Catalase (CAT) (Changes in OD min^{-1} g^{-1})				
V_1	3.38	3.74	4.46	4.91	4.12
V_2	2.88	3.16	3.43	3.64	3.28
V_3	2.14	2.82	3.11	3.46	2.88
V_4	3.09	3.54	3.56	4.27	3.61
Treatment mean	2.87	3.31	3.64	4.07	
	V		S		V x S
S.Em.	0.04		0.04		0.08
C.D.	0.13		0.13		0.26
C.V. %	4.37				
	Peroxidase (POX) (Changes in OD min^{-1} g^{-1})				
V_1	7.50	9.23	14.08	19.16	12.49
V_2	5.88	6.99	8.00	10.93	7.95
V_3	4.69	8.38	13.01	18.77	11.21
V_4	4.06	5.09	7.02	9.97	6.54
Treatment mean	5.53	7.42	10.53	14.71	
	V		S		V x S
S.Em.	0.09		0.09		0.18
C.D.	0.25		0.25		0.50
C.V. %	3.15				
	Super oxidase dismutase (SOD) (Unit min^{-1} g^{-1})				
V_1	20.37	29.49	31.69	38.67	30.06
V_2	18.72	22.56	25.61	29.50	24.10
V_3	28.69	32.79	46.14	47.11	38.68
V_4	27.70	37.26	47.51	49.34	40.45
Treatment mean	23.87	30.53	37.74	41.16	
	V		S		V x S
S.Em.	0.59		0.59		1.18
C.D.	1.71		1.71		3.42
C.V. %	6.16				

4.2.5.3 *Super Oxide Dismutase (SOD)*

SOD activity was more in sensitive variety GR-3 (40.45 unit min^{-1} g^{-1}) followed by CSR-27 (38.68 unit min^{-1} g^{-1}) both are nearly at par with each other.

Data from the Table 34 showing that the SOD activity was increased with salinity level, but the increase was more in sensitive variety GR-3 than tolerant varieties Jaya, CSR-27 and Dandi.

The treatment as well as genotype effects and interaction between varieties and treatments were significant Among salinity levels 200 mM recorded maximum SOD activity (41.16 unit min^{-1} g^{-1}) followed by 150 mM (37.74 unit min^{-1} g^{-1}) and minimum SOD activity was observed in control (23.87 unit min^{-1} g^{-1}).

When compared to Experiment I, the enzyme activity was more with the treatment of proline to the rice seedlings before exposing to salinity.

4.2.6 Effect of trehalose on biochemical attributes of rice with salinity

Trehalose is a non-reducing disaccharide which serve as a potential osmoprotactant during stressful conditions for recovery. There was a significant increase in all biochemical attributes studied than Experiment I.

4.2.6.1 *Chlorophyll*

The similar pattern of results were observed in chlorophyll content with that of proline treatment but the increase was not much more than proline.

Among four varieties, Jaya recorded the maximum chlorophyll content (16.46 mg 100 g^{-1}) followed by CSR-27 (15.13 mg 100 g^{-1}) (Fig. 16) and minimum content of chlorophyll was observed in GR-3 (11.52 mg 100 g^{-1}) which was at par with Dandi (11.77 mg 100 g^{-1}) (Table 35).

The chlorophyll content increased with salinity in all the varieties, the treatment and varieties as well as interaction between VxS was significant.

4.2.6.2 ***Total soluble sugars (TSS)***

When compared to proline treatment total soluble sugar content was rapidly increased by trehalose treatment.

Data from the Table 35 analyzing that the genotype Jaya recorded the maximum TSS content (10.19 g 100 g^{-1}) whereas the minimum content of TSS was observed in GR-3 (7.33 g 100 g^{-1}) (Fig. 16). The increase in TSS was more in tolerant varieties than sensitive varieties.

Among salinity levels, 200 mM recorded the maximum TSS content (9.85 g 100 g^{-1}). But after trehalose treatment, the increase in TSS content was higher in case of sensitive variety GR-3 also than the proline content.

4.2.6.3 ***Proline***

Accumulation of proline was more in case of GR-3 (2.69 mg g^{-1}) whereas minimum content of proline was recorded in CSR-27 (1.11 mg g^{-1}) (Fig. 16). The varieties and treatment effects as well as interaction effects significant (Table 35).

Table 35 : Changes in biochemical attributes in rice seedlings upon trehalose treatment

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	Chlorophyll (mg 100 g^{-1})				
V_1	9.06	13.44	19.16	24.16	16.46
V_2	7.74	10.59	12.27	16.47	11.77
V_3	7.16	10.06	18.08	25.21	15.13
V_4	5.82	10.40	12.99	16.88	11.52
Treatment mean	7.45	11.12	15.63	20.68	
	V		S		V x S
S.Em.	0.19		0.19		0.38
C.D.	0.54		0.54		1.08
C.V. %	4.73				
	Total Soluble Sugar (g 100 g^{-1})				
V_1	9.72	10.39	10.58	10.07	10.19
V_2	9.17	9.46	9.89	10.28	9.70
V_3	8.83	9.02	9.70	10.70	9.56
V_4	6.09	7.10	7.79	8.35	7.33
Treatment mean	8.45	8.99	9.49	9.85	
	V		S		V x S
S.Em.	0.05		0.05		0.10
C.D.	0.15		0.15		0.30
C.V. %	1.98				
	Proline (mg g^{-1})				
V_1	1.14	1.86	2.16	2.59	1.94
V_2	1.65	1.34	1.63	1.88	1.63
V_3	0.91	1.02	1.19	1.33	1.11
V_4	2.20	2.67	2.83	3.03	2.69
Treatment mean	1.48	1.72	1.95	2.21	
	V		S		V x S
S.Em.	0.03		0.03		0.06
C.D.	0.09		0.09		0.18
C.V. %	5.81				

When compared to proline treatment the constant of proline accumulated with trehalose treatment was less.

These results indicated that after the treatment of proline and trehalose even the sensitive varieties may also survive better under salinity conditions.

4.2.7 Enzyme activity with trehalose treatment in rice with salinity

4.2.7.1 *Super Oxidase Dismutase (SOD)*

The activity of SOD was less as compared to proline treatment. Among the varieties Jaya recorded the maximum SOD activity (12.49 unit min^{-1} g^{-1}) and minimum activity was found in GR-3 (6.53 unit min^{-1} g^{-1}) (Fig. 17). The increase in enzyme activity was more in tolerant varieties (Jaya, Dandi and CSR-27) than sensitive variety (GR-3) with trehalose treatment.

Among the salinity level 200mM NaCl was recorded maximum SOD activity (14.69 unit min^{-1} g^{-1}) whereas least activities was observed in control (5.53 unit min^{-1} g^{-1}) (Table 36).

The effect of treatments, varieties as well as interaction between varieties and salinity levels were significant

4.2.7.2 *Catalase*

The activity of catalase was significant in all the varieties. Among the four varieties, GR-3 recorded the maximum activity (4.78 unit min^{-1} g^{-1}) and minimum activities was observed in Dandi (2.73 unit min^{-1} g^{-1}) (Table 36).

Table 36 : Changes in enzyme activities in rice seedlings upon trehalose treatment

	Control (S_1)	**100 mM (S_2)**	**150 mM (S_3)**	**200 mM (S_4)**	**Varietal mean**
	Catalase (CAT) (Changes in OD min^{-1} g^{-1})				
V_1	2.65	2.93	3.01	3.14	2.93
V_2	2.52	2.65	2.82	2.94	2.73
V_3	3.14	3.29	3.44	3.73	3.40
V_4	4.53	4.69	4.88	5.00	4.78
Treatment mean	3.21	3.39	3.54	14.81	
	V	S	V x S		
S.Em.	0.03	0.03	0.06		
C.D.	0.08	0.08	0.16		
C.V. %	2.79				
	Peroxidase (POX) (Changes in OD min^{-1} g^{-1})				
V_1	8.61	10.24	15.60	21.19	13.91
V_2	6.66	8.56	12.21	15.67	10.78
V_3	5.39	7.93	13.74	22.28	12.34
V_4	6.92	8.23	10.32	15.74	10.30
Treatment mean	6.90	8.74	12.97	18.72	
	V	S	V x S		
S.Em.	0.11	0.11	0.22		
C.D.	0.32	0.32	0.64		
C.V. %	3.21				
	Super oxidase dismutase (SOD) (Unit min^{-1} g^{-1})				
V_1	7.50	9.23	14.08	19.16	12.49
V_2	5.88	6.99	8.00	10.87	7.94
V_3	4.69	8.38	13.01	18.77	11.21
V_4	4.06	5.08	7.02	9.97	6.53
Treatment mean	5.53	7.42	10.53	14.69	
	V	S	V x S		
S.Em.	0.09	0.09	0.18		
C.D.	0.25	0.25	0.50		
C.V. %	3.10				

The catalase activity increased with salinity in all the varieties. The increase was more in sensitive variety GR-3 than the tolerant varieties Jaya, Dandi and CSR-27.

Trehalose serves as better osmoprotectant for sensitive cultivar GR-3 in case of catalase activity when compared to proline.

4.2.7.3 *Peroxidase*

Compared to proline treatment, the peroxidase activity was more with trehalose treatment. Among the four varieties studied, Jaya showed maximum peroxidase activity (13.91 unit min^{-1} g^{-1}) whereas minimum activity was observed in GR-3 (10.30 unit min^{-1} g^{-1}) (Table 36). Even sensitive variety GR-3 also showed better peroxidase activity with trehalose treatment.

4.3 ISOENZYME ANALYSIS OF RICE SEEDLINGS WITH PROLINE TREATMENT

4.3.1 Superoxidedismutase

The Plate XXII showed the isoenzyme pattern of SOD. Variation was observed in terms of intensity. Total three isoforms of SOD were noticed after treatment to the salinized seedlings with proline. First and third bands had less intensity where as the second band intensity was more. Rm values are followed in the range of 0.29 to 0.83 (Table 37). After treatment the salinized seedlings with proline even sensitive variety GR-3 also showed maximum intensity of SOD as compare with tolerant varieties.

Table 37 : RM values of super oxidase dismutase with proline treatment in rice

Parameters / Band No.		1	2	3
Varieties (V)	**Salinity levels (S)**			
Jaya (T)	Control	0.29^{L}	0.77^{L}	0.83^{L}
	100 mM (S1)	0.29^{M}	0.77^{M}	0.83^{L}
	150 mM (S2)	0.29^{D}	0.77^{D}	0.83^{M}
	200 mM (S3)	0.29^{D}	0.77^{D}	0.83^{D}
Dandi (T)	Control	0.29^{D}	0.77^{M}	0.83^{M}
	100 mM (S1)	0.29^{M}	0.77^{D}	0.83^{M}
	150 mM (S2)	0.29^{M}	0.77^{D}	0.83^{D}
	200 mM (S3)	0.29^{M}	-	0.83^{D}
CSR-27 (T)	Control	0.29^{D}	0.77^{M}	0.83^{L}
	100 mM (S1)	0.29^{D}	0.77^{M}	0.83^{M}
	150 mM (S2)	0.29^{M}	0.77^{M}	0.83^{L}
	200 mM (S3)	0.29^{M}	0.77^{M}	0.83^{L}
GR-3 (S)	Control	0.29^{D}	0.77^{D}	0.83^{M}
	100 mM (S1)	0.29^{D}	0.77^{D}	0.83^{M}
	150 mM (S2)	0.29^{M}	0.77^{M}	0.83^{M}
	200 mM (S3)	0.29^{L}	0.77^{L}	0.83^{L}

L = Light, M = Medium, D = Dark

4.3.2 Catalase

Plate XXIII showed the only one isoform of catalase of salinized rice seedlings after treatment with proline. The variation was clearly observed among the varieties with different treatment levels. Among the four varieties Jaya showed maximum intensity at 100,150, 200 mM NaCl levels the intensity was more in high concentrated salinity treatments than the control. The intensity was low in the sensitive variety GR-3 but when compared with Experiment I the intensity was increased in GR-3. This shows that after proline treatment even sensitive varieties may also survive better under salinity conditions.

4.3.3 Peroxidase

Plate XXIV showed the isoenzyme pattern of peroxidase, here also there is no clear variation was observed. Only intensity wise variation was seen .Eleven isoforms of peroxidase were noticed after treatment with proline. But there is no clear polymorphism among the varieties .How ever when compared with Experiment I number of isoforms were more during this experiment. It implies that the treatment effect will be their after salinization among the varieties. In control of CSR-27 the 11^{th} band was not seen in control. The Rm values followed in the range of 0.04 to 0.96 (Table 38).

Table 38 : RM values of peroxidase with proline treatment in rice

Parameters/Band No.		1	2	3	4	5	6	7	8	9	10	11
Varieties (V)	Salinity levels (S)											
Jaya (T)	Control	0.04^{M}	0.66^{L}	0.67^{L}	0.68^{L}	0.70^{L}	0.72^{L}	0.73^{L}	0.74^{L}	0.75^{L}	-	0.96^{M}
	100 mM (S1)	0.04^{M}	0.66^{L}	0.67^{L}	0.68^{L}	0.70^{L}	0.72^{L}	0.73^{L}	0.74^{L}	0.75^{L}	0.87^{M}	0.96^{M}
	150 mM (S2)	0.04^{D}	0.66^{M}	0.67^{M}	0.68^{M}	0.70^{M}	0.72^{M}	0.73^{M}	0.74^{M}	0.75^{M}	0.87^{D}	0.96^{D}
	200 mM (S3)	0.04^{D}	0.66^{D}	0.67^{D}	0.68^{D}	0.70^{D}	0.72^{D}	0.73^{D}	0.74^{D}	0.75^{D}	0.87^{D}	0.96^{D}
Dandi (T)	Control	0.04^{M}	0.66^{L}	0.67^{L}	0.68^{L}	0.70^{L}	0.72^{L}	0.73^{L}	0.74^{L}	0.75^{L}	0.87^{L}	0.96^{D}
	100 mM (S1)	0.04^{D}	0.66^{L}	0.67^{L}	0.68^{L}	0.70^{L}	0.72^{L}	0.73^{L}	0.74^{L}	0.75^{L}	0.87^{M}	0.96^{M}
	150 mM (S2)	0.04^{D}	0.66^{L}	0.67^{L}	0.68^{L}	0.70^{L}	0.72^{L}	0.73^{L}	0.74^{L}	0.75^{L}	0.87^{D}	0.96^{D}
	200 mM (S3)	0.04^{D}	0.66^{M}	0.67^{M}	0.68^{M}	0.70^{M}	0.72^{M}	0.73^{M}	0.74^{M}	0.75^{M}	0.87^{D}	0.96^{D}
CSR-27 (T)	Control	0.04^{L}	0.66^{D}	0.67^{D}	0.68^{D}	0.70^{D}	0.72^{D}	0.73^{D}	0.74^{D}	0.75^{D}	0.87^{L}	-
	100 mM (S1)	0.04^{M}	0.66^{L}	0.67^{L}	0.68^{L}	0.70^{L}	0.72^{L}	0.73^{L}	0.74^{L}	0.75^{L}	0.87^{L}	0.96^{D}
	150 mM (S2)	0.04^{D}	0.66^{L}	0.67^{L}	0.68^{L}	0.70^{L}	0.72^{L}	0.73^{L}	0.74^{L}	0.75^{L}	0.87^{M}	0.96^{D}
	200 mM (S3)	0.04^{M}	0.66^{L}	0.67^{L}	0.68^{L}	0.70^{L}	0.72^{L}	0.73^{L}	0.74^{L}	0.75^{L}	0.87^{D}	0.96^{D}
GR-3 (S)	Control	0.04^{M}	0.66^{M}	0.67^{M}	0.68^{M}	0.70^{M}	0.72^{M}	0.73^{M}	0.74^{M}	0.75^{M}	0.87^{D}	0.96^{M}
	100 mM (S1)	0.04^{D}	0.66^{D}	0.67^{D}	0.68^{D}	0.70^{D}	0.72^{D}	0.73^{D}	0.74^{D}	0.75^{D}	0.87^{D}	0.96^{D}
	150 mM (S2)	0.04^{M}	0.66^{M}	0.67^{M}	0.68^{M}	0.70^{M}	0.72^{M}	0.73^{M}	0.74^{M}	0.75^{M}	0.87^{D}	0.96^{M}
	200 mM (S3)	0.04^{M}	0.66^{M}	0.67^{M}	0.68^{M}	0.70^{M}	0.72^{M}	0.73^{M}	0.74^{M}	0.75^{M}	0.87^{M}	0.96^{M}

L = Light, M = Medium, D = Dark

4.4 ISOENZYME ANALYSIS OF RICE SEEDLINGS WITH TREHALOSE TREATMENT

4.4.1 Superoxide dismutase

As seen in Plate XXVI there were presence of two SOD isoforms with the trehalose treatment but when compared with proline treated isoforms of SOD the intensity was very less. The Rm values are with in the range of 0.65 and 0.82 (Table 39) but clear variation was not observed in polymorphism among the cultivars. However, the intensity was more in tolerant varieties only. But as compared with proline treatment even sensitive cultivar GR-3 also showed maximum intensity, here in this case GR-3 was not showed high intensity.

4.4.2 Catalase

As seen in Plate XXVII the presence of catalse isoform pattern was identified. The intensity was less with that of proline treatment. Among the varieties Jaya showed maximum intensity at 150, 200 mM NaCl. The intensity was increased towards salinity levels. During this treatment Sensitive cultivar GR-3 also showed better intensity, it shows the effect of trehalose on sensitive varieties.

Table 39 : RM values of super oxide dismutase with trehalose treatment in rice

Parameters / Band No.		1	2
Varieties (V)	**Salinity levels (S)**		
Jaya (T)	Control	0.65^{L}	0.82^{L}
	100 mM (S1)	0.63^{L}	0.82^{D}
	150 mM (S2)	0.62^{D}	0.81^{D}
	200 mM (S3)	0.61^{M}	0.81^{M}
Dandi (T)	Control	0.59^{M}	0.80^{D}
	100 mM (S1)	0.59^{M}	0.80^{M}
	150 mM (S2)	0.58^{L}	0.79^{M}
	200 mM (S3)	0.58^{L}	0.79^{D}
CSR-27 (T)	Control	0.58^{M}	0.77^{L}
	100 mM (S1)	0.58^{M}	0.77^{M}
	150 mM (S2)	0.61^{M}	0.77^{D}
	200 mM (S3)	0.62^{M}	0.76^{D}
GR-3 (S)	Control	0.62^{D}	0.76^{L}
	100 mM (S1)	0.63^{D}	0.82^{L}
	150 mM (S2)	0.65^{L}	0.82^{M}
	200 mM (S3)	0.65^{L}	0.82^{L}

L = Light, M = Medium, D = Dark

4.4.3 Peroxidase

The isoenzyme pattern of peroxidase was presented in Plate XXVIII. Eight isoforms were observed. Among these first isoform showed variation both in terms of appearance and intensity. In Jaya and CSR-27 it was completely absent except at 200 mM NaCl level. Where as the second isoform was absent in control GR-3.The intensity of isoforms was more than proline treatment.

Table 40 : RM values of peroxidase with trehalose treatment in rice

Parameters/Band No.		1	2	3	4	5	6	7
Varieties (V)	**Salinity levels (S)**							
Jaya (T)	Control	-	0.23^{L}	0.29^{L}	0.92^{D}	0.93^{D}	0.94^{D}	0.96^{D}
	100 mM (S1)	-	0.23^{M}	0.29^{M}	0.92^{L}	0.93^{L}	0.94^{L}	0.96^{L}
	150 mM (S2)	-	0.23^{M}	0.29^{M}	0.92^{M}	0.93^{M}	0.94^{M}	0.96^{M}
	200 mM (S3)	0.23^{D}	0.23^{D}	0.29^{D}	0.92^{D}	0.93^{D}	0.94^{D}	0.96^{D}
Dandi (T)	Control	0.23^{D}	0.23^{D}	0.29^{M}	0.92^{D}	0.93^{D}	0.94^{D}	0.96^{D}
	100 mM (S1)	-	0.23^{L}	0.29^{M}	0.92^{M}	0.93^{M}	0.94^{M}	0.96^{M}
	150 mM (S2)	0.23^{L}	0.23^{M}	0.29^{L}	0.92^{M}	0.93^{M}	0.94^{M}	0.96^{M}
	200 mM (S3)	0.23^{M}	0.23^{D}	0.29^{D}	0.92^{D}	0.93^{D}	0.94^{D}	0.96^{D}
CSR-27 (T)	Control	-	0.23^{M}	0.29^{M}	0.92^{D}	0.93^{D}	0.94^{D}	0.96^{D}
	100 mM (S1)	-	0.23^{M}	0.29^{D}	0.92^{L}	0.93^{L}	0.94^{L}	0.96^{L}
	150 mM (S2)	-	0.23^{M}	0.29^{D}	0.92^{M}	0.93^{M}	0.94^{M}	0.96^{M}
	200 mM (S3)	0.23^{D}	0.23^{D}	0.29^{M}	0.92^{D}	0.93^{D}	0.94^{D}	0.96^{D}
GR-3 (S)	Control	0.23^{D}	-	0.29^{D}	0.92^{D}	0.93^{D}	0.94^{D}	0.96^{D}
	100 mM (S1)	0.23^{M}	0.23^{M}	0.29^{D}	0.92^{M}	0.93^{M}	0.94^{M}	0.96^{M}
	150 mM (S2)	0.23^{M}	0.23^{M}	0.29^{D}	0.92^{D}	0.93^{D}	0.94^{D}	0.96^{D}
	200 mM (S3)	0.23^{D}	0.23^{D}	0.29^{M}	0.92^{D}	0.93^{D}	0.94^{D}	0.96^{D}

L = Light, M = Medium, D = Dark

V. SUMMARY AND CONCLUSION

Salinity is one of the most important abiotic stresses especially for rice, which is mostly grown under irrigated conditions. To elucidate the effects of salinity stress in rice, four different varieties Jaya, Dandi, CSR-27 and GR-3 differing is response to salinity were selected. They were grown in normal pots containing normal soil and FYM upto 15 days. Seedlings were exposed to different levels of sodium chloride for 24, 48 and 72 h. The concentrations of NaCl used were 100, 150 and 200 ml with water as check.

The effect of salinity was studied at germination and at 15 DAG i.e. at seedling growth stage for changes in physiological and biochemical parameters.

Among the various physiological parameters studied, the germination was most crucial stage for salinity stress. CSR-27 the tolerant variety recorded the highest germination percentage, where as susceptible GR-3 recorded the minimum germination percentage. Both Jaya and Dandi were at par.

Seedling growth was also severely affected by salinity. Both the shoot as well as root growth was affected because of excess amount of sodium and chloride in the growth stage. Jaya recorded the highest root length and GR-3 recorded the minimum root length. It was quite opposite to shoot length where GR-3 recorded highest shoot length and Jaya recorded minimum. Both the shoot and root lengths were reduced with increasing salt levels. Dandi, which is

a tolerant to salinity stress recorded highest vigour index, however, tolerant variety CSR-27 recorded minimum vigour index.

Study on biochemical parameters revealed that among the four varieties, GR-3 recorded maximum chlorophyll content at all time intervals except at 24 h salinity treatment period where Jaya recorded maximum chlorophyll content. Chlorophyll content increased upto 150 mM NaCl level thereafter it decreased. The concentration of sodium ions disrupted the chlorophyll structure there by decreasing the content.

Total soluble sugar content also increased with increasing NaCl concentration. Jaya, which is a tolerant variety to salinity recorded maximum total soluble sugar content over the rest of the varieties. TSS content was found to be constantly increase with salinity.

Pooled analyses results also indicated that the chlorophyll and total soluble sugar contents were significant with the treatments levels and also with varieties. Interactions between time periods, varieties and treatment were highly significant both in the case of chlorophyll and total soluble sugar contents.

Protein content was greatly affected by salinity stress, which increased upto 150 mM, there after it decreased. At 24 h treatment period Dandi recorded highest protein content, at 48 h, Jaya and at 72 h and CSR-27 recorded highest protein content. In all three cases, GR-3 recorded minimum protein content.

Proline content continuously increased due to salinity levels and with increasing time intervals. Dandi recorded the highest proline content at 24, 48 h whereas at 72 h GR-3, the susceptible variety recorded maximum proline content.

Salinity stress also influenced the enzymatic activities. Antioxidants are the enzymatic mechanisms for scavenging the reactive oxygen species produced by salt stress. SOD is a major scavenger of O_2^- and its enzymatic action results in the formation of H_2O_2 and O_2. The hydrogen peroxide is then scavenged by catalase and a variety of peroxidases. The SOD activity was maximum in susceptible variety GR-3, this activity increased towards salinity. SOD activity was maximum at 72 h salinity treatment period.

Both catalase and peroxidase activities were maximum in CSR-27 and it increased with salinity levels and time intervals.

Polyphenol oxidase activity showed less variation at 24 h. Jaya recorded maximum PPO activity at 48 h and CSR-27 at 72 h Dandi recorded maximum PPO activity.

Pooled analysis for all the enzymatic activity showed significance levels with effect of varieties, treatment as well as interaction between time periods, varieties and treatments.

Esterase activity was more in Dandi at all incubation periods. The variation was marginal as compared to remaining enzymes. The interaction between varieties, treatments and time periods were non-significant. The

isoenzyme studies of different enzymes like SOD, catalase, peroxidase, esterase revealed the presence or absence of some isoforms with respect to salt stress. The catalase showed presence of single reform with various intensity. Whereas, SOD showed presence of 2 to 3 isoforms with different intensity. The protein profile by SDS PAGE showed presence of nearly 15-25 bands of inducible proteins may be attributed due to salinity stress.

Other than cellular solutes, certain inorganic ions also played an important role in salt adaptation mechanism in rice plants. Mineral ions like sodium, potassium were also highly affected by salinity stress. Jaya recorded highest sodium content in all three time intervals, where as the minimum was noticed in GR-3. Jaya recorded maximum potassium content at all time intervals except at 48 h, where as CSR-27 recorded maximum potassium content.

Osmoprotactants are of either inorganic ions or low molecular weight organic solutes. Both of these played an important role in plant metabolism under stress conditions.

The effects of osmoprotectants on salinized rice seedlings were better understood by different biochemical parameters as well as various antioxidant enzymes. All the biochemical contents were increased after the treatments with osmoprotectant such as proline and trehalose.

Jaya recorded the highest chlorophyll content. This content was very high than the first experiment. It was a clear indication of osmoprotectant efficiency upon salinity.

Not only chlorophyll, but also, osmoprotectant efficiency increased remarkably with proline treatment. Jaya recorded highest TSS content. It was also higher than the earlier experiment. GR-3 recorded highest proline content, which was sensitive to salinity after proline treatment. Even sensitive genotype also with stood salinity upto certain degree and expressed high levels of proline.

The activity of SOD, catalase, peroxidase was not high as observed in first experiment, however sensitive variety GR-3 survived better and secured high enzymatic activity in all three enzymes after treatment with proline.

Trehalose, a non-reducing disaccharide served as a best osmoprotectant next to proline in present investigation. All the biochemical attributes which were analyzed after treatments with trehalose were also high than the earlier experiment but were less in proline treatment. Proline served as a better osmoprotectant to overcome the adverse effects of salinity stress by increasing the levels of different biochemical constituents than trehalose. The concentration of biochemical attributes and the activity of enzyme also vary among the varieties and time intervals.

From the present investigation, it can be concluded that germination percentage, seedling growth, seedling vigor and major biochemical constitutes

like chlorophyll, total soluble sugar, protein, proline and the activity of different enzymes may act as a potential matters for salt tolerance and could be exploited as a stress marker. The antioxidants like SOD, peroxidase and catalase work as a scavenger for deleting the toxic effect produced by salinity stress. Higher level of proline, in all the varieties, proved the role of proline as a osmoregulator. The concentration of Na^+, K^+ also served as a marker for salt tolerance. Isoenzyme and SDS-protein profile are quick methods for identification of salt tolerance.

The selected osmoprotectants, proline and trehalose also performed positively during the salinity stress. Even sensitive genotype GR-3 also showed better performance under salt stress like other tolerant varieties.

The changes in both biochemical parameters as well as enzyme activities varied between genotype and time intervals. The maximum content of biochemical parameters was observed at 72 h salinity treatment period except chlorophyll. But specific stage at which plants could tolerate salinity stress needs to be identified at biochemical as well as molecular levels.

The change in biochemical parameters varied with varieties and the time intervals. However, the changes, not only identified a suitable mechanism for salt tolerance but also a salinity stress as of a multigenic nature. So, it has to be studied in all the directions with all specific growth stages to develop new approaches.

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