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Effect of salinity (NaCl) stress on physiological characteristics of rice (*Oryza sativa L.*) at early seedling stage.

Shakeela B. Solangi[1], Q.I. Chachar[1*], S.D Chachar[2], Afshan B. Solangi[3] and Jameel A. Solangi[4].

[1]Department of Crop Physiology, Sindh Agriculture University Tandojam, [2]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China, [3]Department of Agronomy, Sindh Agriculture University Tandojam, [4]Department of Plant Breeding and Genetics, Sindh Agriculture University Tandojam, Pakistan.

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Abstract: Salinity is a common environmental stress seriously affecting crop growth, production and yield. This experiment was planned to study the effect of salinity stress on physiological characteristics of rice (*Oryza sativa L.*) at early seedling stage. The seeds four rice genotypes (IR88611-B-5, IR83142-B-61-B, IR -72 and FL-478) were grown on plastic boxes [nylon netted frame (5 x 7") fitted in 2.5 L solution capacity] filled with culture solution. The pH of culture solution was maintained at 5.0. These boxes were placed separately in an incubator at 30^{o}C up to ten days. Salinity cause decrease leaf area and potassium (K^{+}) content of rice genotypes; although the genotypes behaved differently. However, membrane injury, chlorophyll content and total sugars of all rice genotypes increased under salinity stresses. A substantial increase in Na^{+} concentration was also observed in all genotypes due to increase in NaCl concentrations. Rice genotypes IR83142-B-61-B and IR -72 had maximum decrease in leaf area under salt stress, whereas, genotypes IR83142-B-61-B and IR -72 had higher proline accumulation. It was concluded that salinity decreased the growth of all tested rice genotypes; Furthermore, genotypes IR88611-B-5 and FL 478 were more tolerant to salt stress than genotypes IR83142-B-61-B and IR-72.

Key words: salinity, seedling growth, rice genotypes chlorophyll, proline, membrane permeability.

Introduction

Rice is one of the most important cereal crops all around the world with exceptional agricultural and economic importance as a staple food for more than 50% population worldwide and Asian farmers produce more than 90% of the total rice (IRRI, 2011).

Salinity is a common environmental stress seriously affecting crop growth, food production and crop yield in many regions, particularly in arid and semi-arid regions **(**Jamil *et al.,* 2010; Osakabe *et al.,* 2011; Hussain *et al.,* 2013). It is estimated that over 800 million hectares of land in the world are affected by both salinity and sodicity globally (Munns, 2005; Kumar *et al.,* 2010 and Tavakkoli *et al.,* 2011). Salt contaminated soils (ECe > 4

*Coressponding Author**:** Q.I. Chachar; e-mail: qdchachar@yahoo.com

dS m^{-1} or 40 mM NaCl or osmotic potential < 0.117 MPa) are defined as saline land, which directly affects plant growth and development in vegetative growth prior to reproductive stage, especially crop species (Chinnusamy *et al.*, 2005; Ashraf *et al.*, 2008; Ashraf, 2009). Some of crop species are susceptible to salt stress (ECe 1.0-1.8 dS m^{-1}), which decline crop growth and productivity about 6-19% *i.e.* rice, corn, bean, eggplant, onion, potato, pepper, sugarcane and cabbage. In general, biochemical, physiological, anatomical and morphological characteristics of plants directly affected by soil salinity is ascribed as (Chinnusamy *et al.*, 2005; Parida and Das, 2005). Various environmental abiotic stresses including high or low temperature, water shortage, high salinity and heavy metals exert drastic antagonistic effects on crop metabolism and thereby plant growth, development and ultimately crop productivity via an osmotic effect on plant water uptake and specific ion toxicities (Munns *et al.*, 2006 and Solangi *et al.*, 2015). The salt stress affected proline and sugars synthesis an accelerated the rate of biosynthesis and higher concentrations of chlorophyll b than chlorophyll a during vegetative growth is observed in many crop plants (Khan *et al*., 2000; Asch *et al*., 2000; Santo, 2004 and Akram *et al*., 2007).

The present research work was aimed to study the effect of salinity (NaCl) stress on seed germination, seedling growth and various physiological parameters including total sugars, ions concentration, chlorophyll content, shoot membrane permeability and proline accumulation in four rice genotypes, namely IR88611-B-5, IR83142-B-61-B, IR-72 and FL-478.

Materials and methods

Seed source:

Seeds of rice genotypes (IR88611-B-5, IR83142-B-61-B, IR-72 and FL-478) were obtained from Nuclear Institute of Agriculture (NIA) Tandojam and (IRRI) type genotypes by Dr. Mohammed Arif, NIBGE, Faisalabad, Pakistan.

This research work was conducted in the research laboratory of the Plant Physiology Division of Nuclear Institute of Agriculture, Tandojam, Pakistan, during the year 2015. The experiment was laid down in Complete Randomize Design (CRD) with three replicates. Four salinity levels (0, 50, 75 and 100 mM NaCl) and four rice genotypes (IR88611-B-5, IR83142-B-61-B, IR-72 and FL-478) were studied in this work at the temperature (30^{o}C).

Healthy seeds were surface sterilized for 20 minutes with 3% sodium hypochlorite (NaOCl) and washed thoroughly with distilled water. The seeds four rice genotypes (IR88611-B-5, IR83142-B-61-B, IR -72 and FL-478) were grown on plastic boxes [nylon netted frame (5 x 7") fitted in 2.5 L solution capacity] filled with culture solution (Yoshida *et al*., 1976). The pH of culture solution was maintained at 5.0. These boxes were placed separately at 30^{o}C in an incubator (Luminine Cube II, ANALIS Model L M-500) up to 10 days. The details of observation recorded are as given below:

Chlorophyll (Chl. a, Chl. b, total Chl.) and carotenoid content

Fresh leaves tissues were cut into small pieces by a scissor and homogenized, then 0.1 gram homogenized tissue sample were taken in a test tube and 10 ml of 80% acetone were added and test tube was wrapped with aluminum foil and incubated at room temperature in the dark overnight. Next day, samples were vortex and wait until the particulates have fallen to the bottom. The extract absorbance was measured at 470.0 nm, 646.8 nm and 663.2 nm using 80% acetone as a blank on a spectrophotometer. Chlorophyll (Chl. *a*, Chl. *b*, Total Chl.) and carotenoid content was determined in (µ mol. g^{-1} fresh weight) using method elaborated by Lichtenthaler (1987).

Calculations:

Chlorophyll *a:* = (12.25* $A_{663.2}$nm - 2.79* $A_{646.8}$nm)* SW

Chlorophyll *b:* = (21.5* $A_{663.2}$nm -5.1 *$A_{646.8}$nm)* SW

Total chlorophyll: = (7.15* $A_{663.2}$nm +18.71* $A_{646.8}$nm)* SW

Carotenoids: = (1000* A_{470} nm -1.82* Chlorophyll *a* -85.02* Chlorophyll *b*)/198

Where:

$A_{648.6}$ = Absorbance at 648.6 nm

$A_{664.2}$ = Absorbance at 664.2 nm

A_{470} = Absorbance at 470 nm

SW = Sample Weight (g^{-1} fresh weight)

Proline content (µ mol. g^{-1} fresh weight)

Free proline content was measured in (µM g^{-1} fresh weight) using method elaborated by Bates *et al. (*1987).

Reagents:

Acid-ninhydrin was prepared by warming 1.25 g ninhydrin in 60 ml glacial acetic acid and 40 ml 6 M phosphoric acid with agitation until dissolved. Kept cool (stored at 4°C), the reagent remains stable for 24 hours. Approximately 0.25g of plant material was grinded and homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered through filter paper (Wattman filter paper# 2). Two ml of filtrate was reacted with 2

ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube, kept in water bath (100°C) for 1 hour and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 20-25 seconds. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance observed at 520 nm using toluene as blank.

Proline content (μg/g shoot fresh weight)

The proline content was determined from a standard curve and calculated on a fresh weight basis as follows:

Proline content = (OD*factor (5.16))*2

Where:

OD = Optical Density / Instrument Reading

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

Sodium and potassium contents were measured in percentage (% g^{-1} fresh weight) using method elaborated by Flowers (1986). For the determination of Na^+ and K^+ in fresh grinded shoot, the plant material was treated with 0.2 mM acetic acid (CH_3COOH) in water bath for 1 hour pre heated at 95^oC. The extracted solution was filtered and suitable dilution was made. Na^+ and K^+ concentration were determined by flame photometer (jenway, Model PFP7).

Leaf area (cm^2)

Leaf area meter (AM-200, ADC Bio Scientific Ltd., England) was used for the measurement of individual plant leaves area by (Khalil *et al.*, 2002).

Membrane permeability (%)

Membrane permeability of the leaves was measured by EC according to Yan *et al.* (1996). Briefly, at the end of experiment the washed leaves were cut into 1 cm pieces from the base part of leaves of 10 plants from each treatment and placed in a test tube containing 10 ml distilled water. The test tubes were kept at 30°C for 3 h and the conductivity of solution was measured by a conductivity meter. The same samples were boiled for 10 min at 100°C and then their conductivity was measured again when the solution was cooled to room temperature. The percentage of electrolyte leakage was calculated as follows:

EC (%) = (C1 /C2) X 100. Where C1 and C2 are the electrolyte conductivities measured before and after boiling, respectively.

Total Soluble sugars (mg g^{-1} dry weight)

Total Soluble sugars were determined in dry leaves according to Riazi *et al.* (1985). One gram chopped dry leaf sample were shaken with 10 ml of 80% ethanol (v/v) overnight in 0.1 ml ethanol extract, 3ml of freshly prepared anthrone was added, heated at 97^0C for 10 minutes, cooled in ice bath and read in spectrophotometer at 625 nm.

Statistical analysis

The data of all parameters were subjected to analysis of variance (ANOVA) to discriminate the superiority of treatment means and least significant difference test at alpha 0.05 for this purpose a Microsoft computer package "Statistics 8.1" was used.

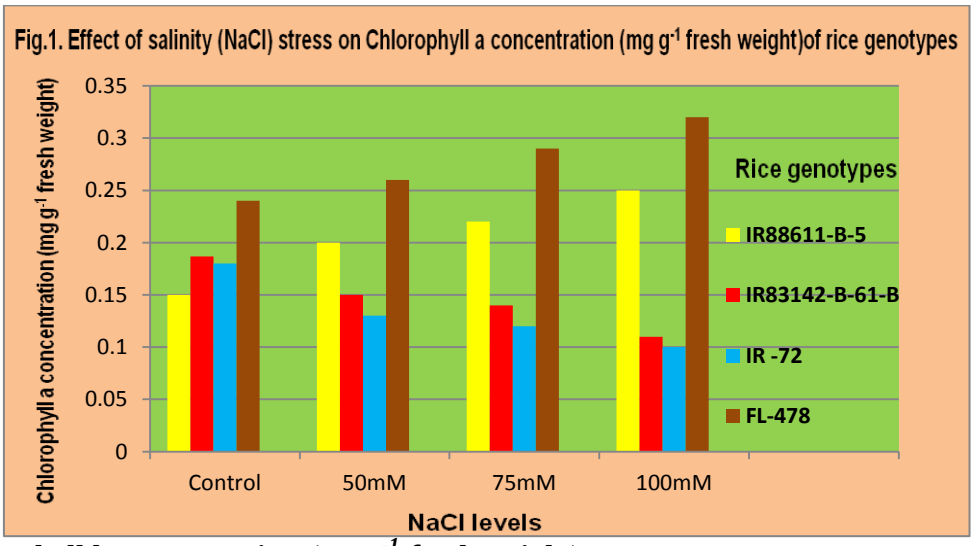
Result and discussions

A laboratory experiment was conducted to study the effect of salinity on physiological characteristics of rice (*Oryza sativa L.*) at germination and seedling stage during the year 2015 at research laboratory of the Plant Physiology Division, Nuclear Institute of Agriculture, Tandojam, Pakistan. Four rice genotypes (IR88611-B-5, IR83142-B-61-B, IR -72 and FL-478) were determined for their effect of different concentration of salinity (0, 50, 75 and 100 mM NaCl) at 30^oC temperature, seed germination, root and shoot length, root and shoot fresh and dry weight, chlorophyll content, proline, ions concentration (Na^+ and K^+), leaf area, shoot membrane permeability and total sugars was recorded after 10 days of incubation.

Chlorophyll a concentration (mg g^{-1} fresh weight)

Chlorophyll *a* concentration under salinity stress presented in Figure 1; all the genotypes responded significantly different with each other. Genotypes FL-478 and IR88611-B-5 maximum chlorophyll *a* concentration was observed (0.32 and 0.25 mg g^{-1} fresh weight) in 100mM NaCl stress. The minimum chlorophyll *a* concentration was recorded (0.24 and 0.15 mg g^{-1} fresh weight) in control respectively. On the other hand, genotypes IR83142-B-61-B and IR-72 maximum chlorophyll *a* concentration was observed (0.18 each mg g^{-1} fresh weight) in control. The minimum chlorophyll *a* concentration was observed (0.11 and 0.10 mg g^{-1} fresh weight) in 100mM NaCl stress respectively.

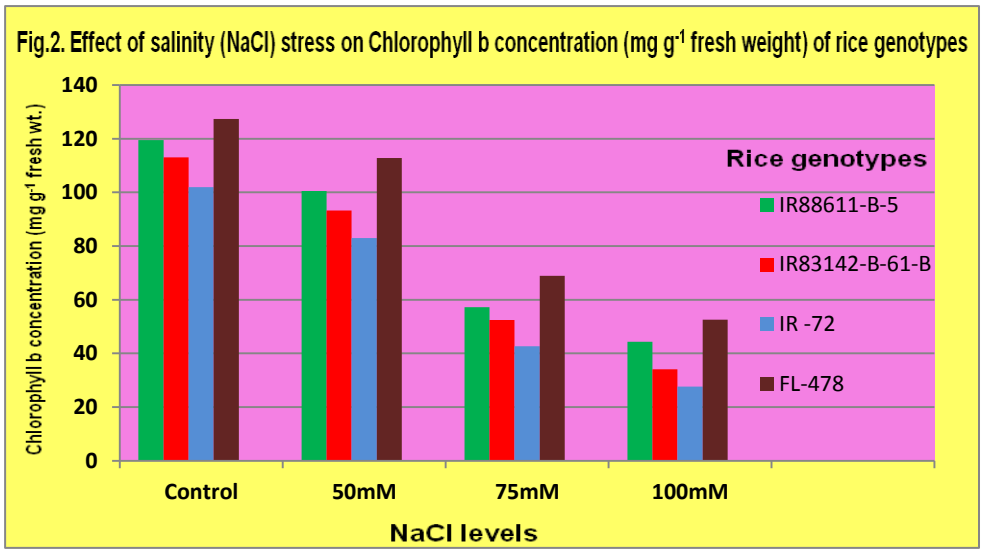
It is shown from the data genotypes FL-478 and IR88611-B-5 chlorophyll *a* concentration increase under increasing salinity levels. Whereas genotypes IR83142-B-61-B and IR-72 chlorophyll *a* concentration decrease under increasing salinity levels. It is paralleled by earlier finding that the photosynthetic pigments in Pokkali (salt-tolerant genotype) can be stabilised better than those in IR29 (salt-sensitive) salt stressed seedlings (200 mM NaCl) (Theerawitaya *et al*., 2012; Boriboonkaset *et al.,* 2012; Zhen-hua *et al.*,2012 and Saeedipour, 2014).



Chlorophyll b concentration (mg g^{-1} fresh weight)

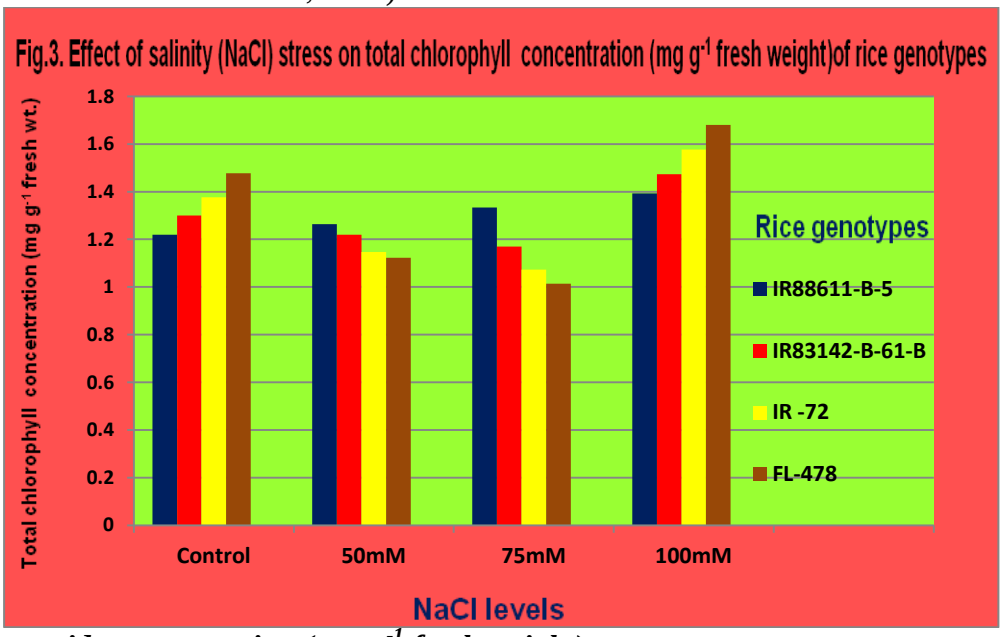
The data of chlorophyll *b* concentration presented Figure 2; all the genotypes under salinity stress responded also significantly different with each other. FL-478 and IR88611-B-5 maximum chlorophyll *b* concentration was recorded (1.363 and 1.223 mg g^{-1} fresh weight) in 100mM NaCl stress. The minimum chlorophyll *b* concentration was recorded (1.1167and 1.0633 mg g^{-1} fresh weight) in control. IR83142-B-61-B and IR-72 genotypes maximum chlorophyll *b* concentration was recorded (1.10 and 1.09 each mg g^{-1} fresh weight) in control. The minimum chlorophyll *b* concentration was observed (0.9767and 0.9133 mg g^{-1} fresh weight) in 100mM NaCl stress respectively.

It is clear that chlorophyll *b* is greater than chlorophyll *a* in all the rice genotypes under salt stress, genotypes indicate same as chlorophyll *a* FL-478 and IR88611-B-5 chlorophyll *b* concentration increase under increasing salinity levels. Whereas, genotypes IR83142-B-61-B and IR-72 chlorophyll *b* concentration decrease under increasing salinity levels. Genotypic variation of pigments under increased salinity levels is also reported by the scientists (Santo, 2004; Akram *et al*., 2007; Theerawitaya *et al*., 2012; Boriboonkaset *et al*., 2012 and Saeedipour 2014).



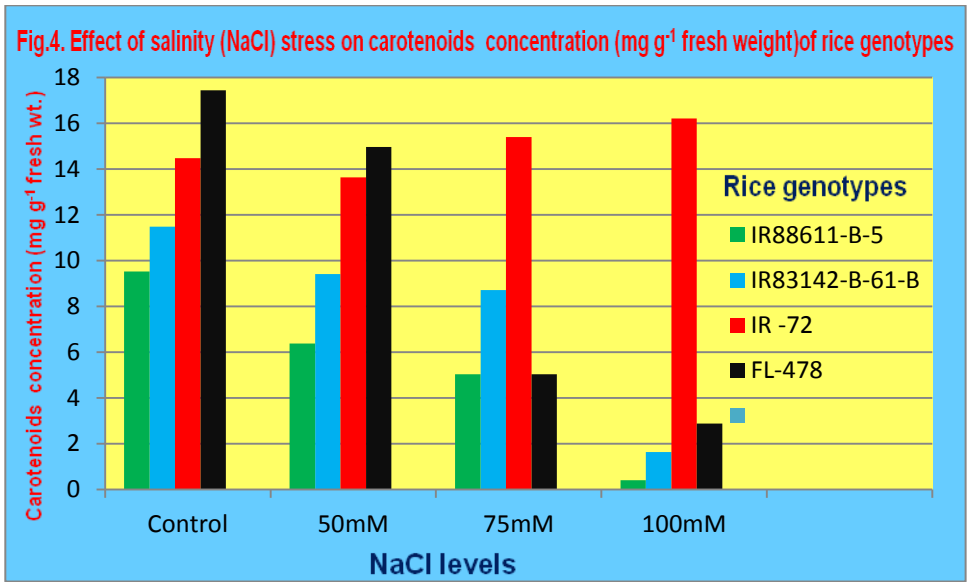
Total chlorophyll concentration (mg g^{-1} fresh weight)

Total chlorophyll concentration of all the rice genotypes responded significantly different with each other under all salinity levels Figure 3. Maximum total chlorophyll concentration was recorded (1.530 mg g^{-1} fresh weight) in 100mM NaCl stress. The total chlorophyll concentration was decrease at 50 and 75mM (1.188 and 1.169 mg g^{-1} fresh weight) than control (1.287 mg g^{-1} fresh weight). Genotypes FL-478 and IR-72 shows maximum total chlorophyll concentration than genotypes IR88611-B-5 and IR83142-B-61-B at 100mM NaCl respectively. Results supported by (Iqbal *et al*., 2006; Ashraf *et al*., 2005; Theerawitaya *et al.,* 2012; Boriboonkaset *et al*., 2012 and Mahmod *et al*., 2014).



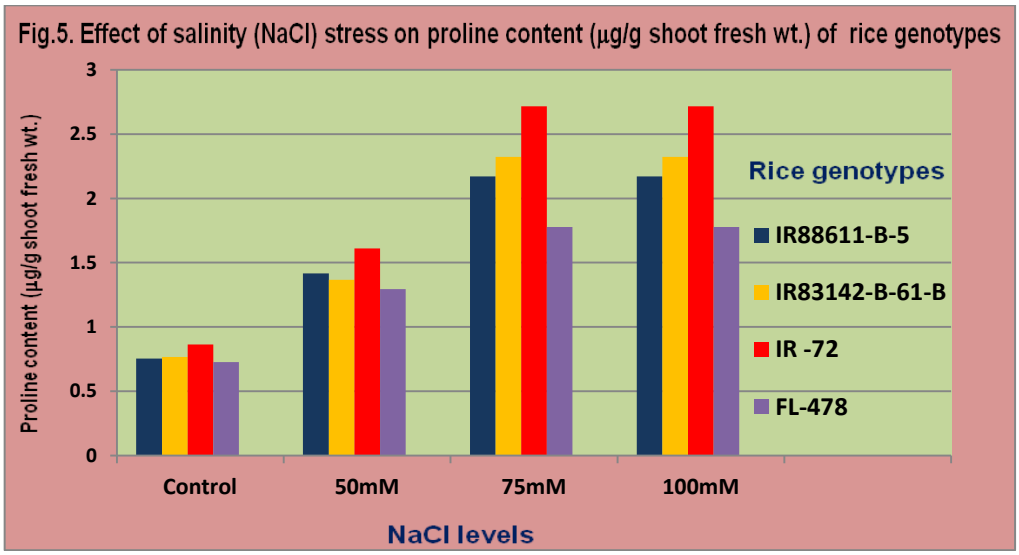
Carotenoids concentration (mg g^{-1} fresh weight)

Salinity stress is effect on carotenoids concentration of rice genotypes observations are presents in Figure 4. Carotenoids concentration of all the genotypes responded decreased significantly with increasing salinity levels except genotype IR-72. Genotypes FL-478, IR83142-B-61-B and IR88611-B-5 maximum carotenoids concentration was recorded (17.43, 11.47 and 9.51 mg g^{-1} fresh weight) in control. The minimum carotenoids concentration was recorded (2.87, 1.63 and 0.40 mg g^{-1} fresh weight) in 100mM NaCl stress. Whereas genotype IR-72 maximum carotenoids concentration was recorded (16.210 mg g^{-1} fresh weight) in 100mM NaCl stress. The minimum carotenoids concentration was recorded (14.470 mg g^{-1} fresh weight) in control respectively. Results supported by (Iqbal *et al*. 2006; Ashraf *et al*. 2005. Theerawitaya *et al.,* 2015; Boriboonkaset *et al*., 2012).



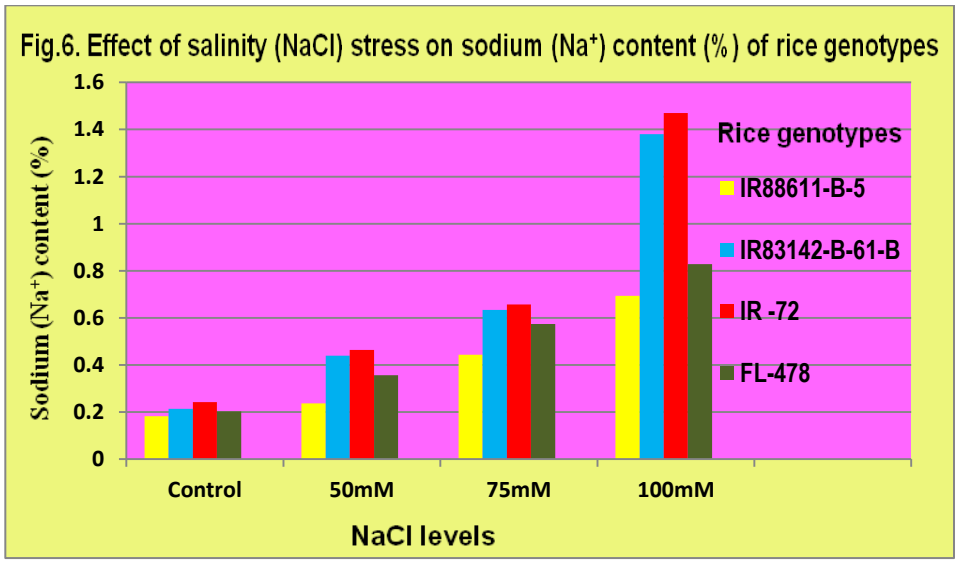
Proline content (µg/g shoot fresh weight)

The observation of the Proline content significantly presented in Figure-5. There was increase in proline with increased salinity levels in all rice genotypes. The Maximum increase of proline was observed (4.130 µg/g fresh weight) at 100mM NaCl stress as compare to control (0.777µg/g fresh wt.), respectively. Genotypic results regarding proline content was observed genotype IR-72 recorded maximum proline (2.725µg/g fresh wt.) than the genotypes FL-478, IR88611-B-5 and IR83142-B-61-B (1.692, 1.970 and 2.187 µg/g fresh wt.), respectively. Proline is a known osmo-protectant, and plays an important role in osmotic balancing, protection of sub-cellular structures, enzymes and in increasing cellular osmolarity (turgor pressure) that provide the turgor necessary for cell expansion under stress conditions (Matysik *et al.* 2002; Sairam and Tyagi 2004). Proline is the key osmolyte, which helps plants to maintain cell turgor and helps to avoid salinity (Farkhondeh *et al.*, 2012). The results are in accordance with the findings of (Zayed *et al.*, 2004; Chutipaijit *et al.*, 2009 Kumar *et al.*, 2009 Danai-Tambhale *et al.*, 2011 and Hakim *et al.*, 2014).



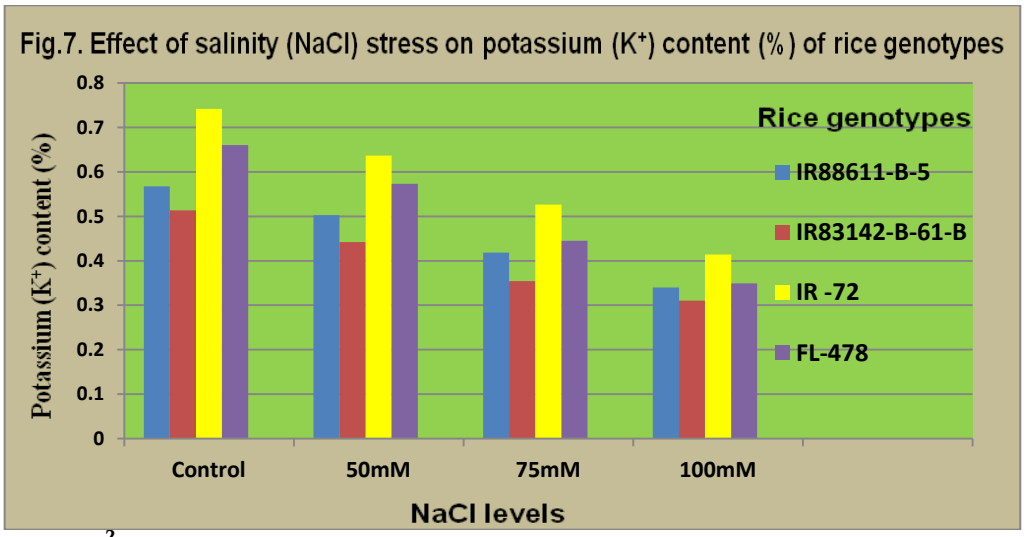
Sodium content (Na^+) (%)

The observation of the shoot sodium content significantly presented in Figure-6. There was increase in sodium content with increased salinity levels in all rice genotypes. The Maximum increase of sodium content was observed (1.09%) at 100mM NaCl stress as compare to control (0.21%), respectively. Genotypic results regarding sodium content was observed genotype IR-72 recorded maximum sodium content (0.70%) than the genotypes IR88611-B-5, FL-478 and IR83142-B-61-B (0.38, 0.49 and 0.66%), respectively. However, the tolerant genotypes IR88611-B-5 and FL-485 had less accumulation of Na^+ as compared to the sensitive genotypes IR-72 and IR83142-B-61-B. Sodium content increased under increasing trend of salinity levels in rice concluded scientists (Djanaguiraman *et al.*, 2006; Momayezi *et al.*, 2009; Mahmood *et al.*, 2009; Ikram-ul-Haq *et al.*, 2010; Nemati *et al.*, 2011 and Theerawitaya *et al.*, 2015).



Potassium content (K^+) (%)

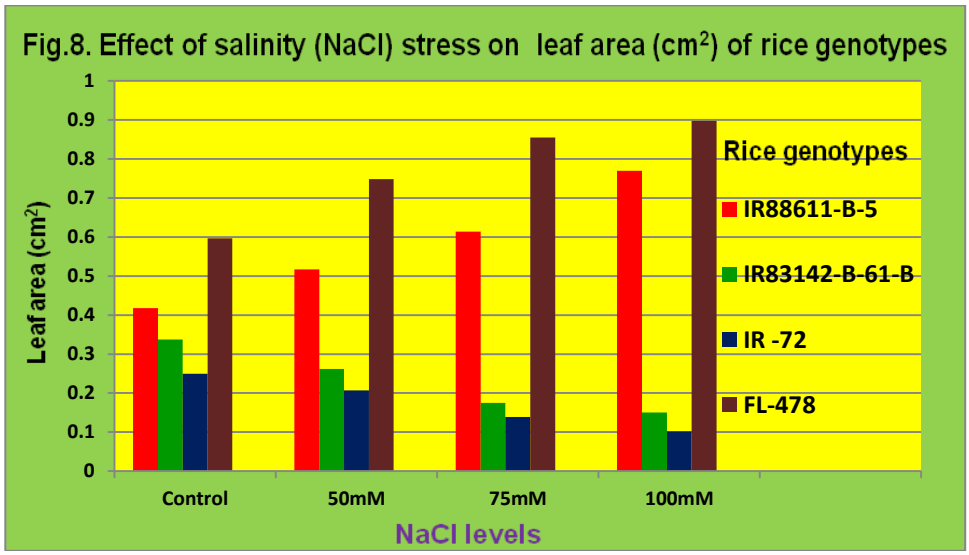
The data regarding the Potassium contents in shoot samples of all the genotypes is presented in figure-7. The data showed to the potassium uptake by rice genotypes decreased with increasing salinity level. The maximum mean values for potassium contents was recorded as (0.62%) in control and the minimum mean value for potassium contents was recorded (0.35%) in 100mM NaCl, respectively. Maximum reduction in potassium uptake was observed at highest NaCl treatment. Genotypic results regarding Potassium contents was observed genotype IR83142-B-61-B recorded maximum reduction at 100mM NaCl than the genotypes FL-478, IR88611-B-5 and IR-72 respectively. The decreasing rate of potassium contents was also reported by researchers (Mahmod *et al.,* 2009; Ikram-ul-Haq *et al.,* 2010; Nemati *et al.,* 2011; Saeedipour 2014 and Bagheri 2014). They reported that potassium contents significantly deceased in response to the increasing NaCl levels.



Leaf area (cm^2)

Leaf area under salinity levels all the genotypes responded significantly varying with each other Figure-8. Genotypes FL-478 and IR88611-B-5 mean value (0.773 and 0.579 cm^2) and the genotypes IR83142-B-61-B and IR-72 was observed mean value (0.231 and 0.174 cm^2), respectively.

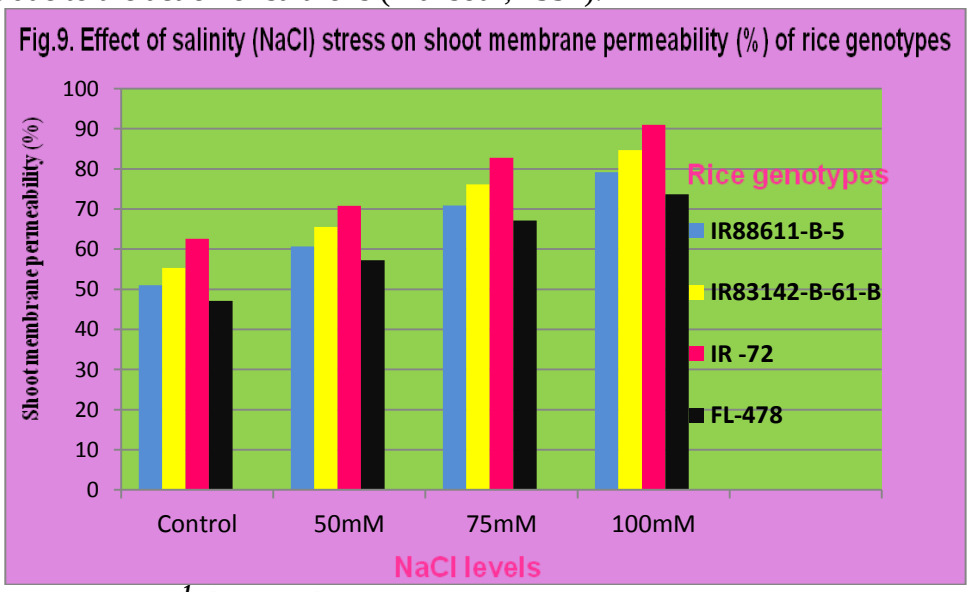
It is shown from the data genotypes FL-478 and IR88611-B-5 leaf area increase under increasing salinity levels. Whereas, genotypes IR83142-B-61-B and IR-72 leaf area decrease under increasing salinity levels. It is paralleled by earlier finding that the leaf area under salt stressed seedlings by the scientists (Ali *et al.*, 2004; Hussain *et al.*, 2013 and Zayed *et al.*, 2014).



Shoot membrane permeability (%)

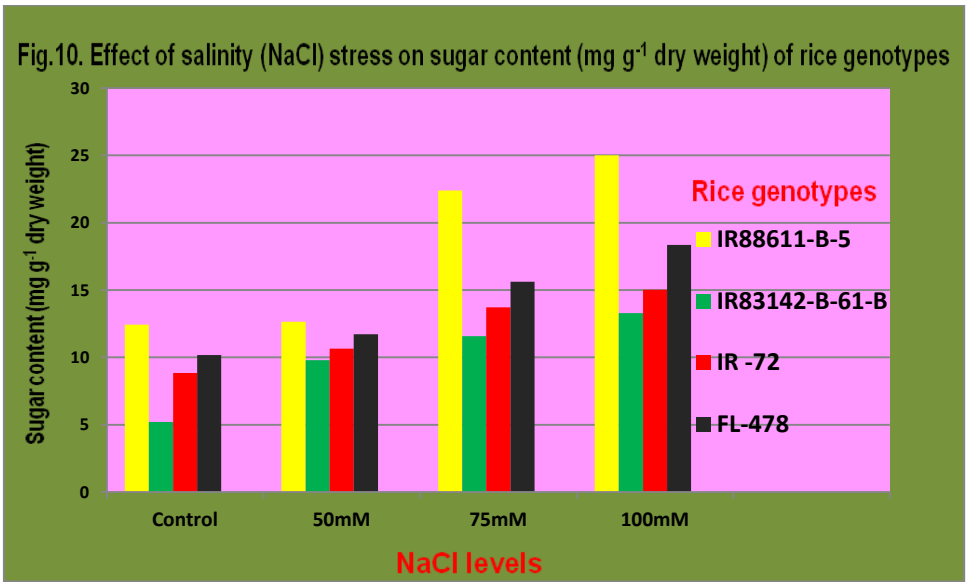
The data regarding shoot membrane permeability of all rice genotypes are presents in Figure-9. Electric conductivity of all the

genotypes responded increased significantly with increasing salinity levels. The minimum mean value was recorded (54.01%) in control. The maximum mean value was recorded (82.10%) in 100mM NaCl stress. Genotypic results regarding shoot membrane permeability genotypes IR83142-B-61-B and IR-72 recorded more electrical conductivity (70.39 and 76.78%) than the genotypes FL-478 and IR88611-B-5(61.26 and 65.43%), respectively. Cellular membranes increasing their ion leakage reported by the scientists (Meloni *et al*., 2003; Menezes-Benavente *et al*., 2004; Hichem *et al*., 2009; Jamil *et al*., 2012;).The effect of salinity on the plasma membrane is prudent due to the action of salt ions (Mansour, 1997).



Sugar content (mg g^{-1} dry weight)

The data of the sugar content significantly presented in Figure-10. There was increase in sugar with increase in salinity levels in all rice genotypes. The Maximum sugar was observed (17.92 mg g^{-1} dry weight) at 100mM NaCl stress as compare to control (9.16 µg/g mg g^{-1} dry weight), respectively. The genotypic results regarding sugar content was observed by genotype IR88611-B-5 recorded maximum sugar (18.12 µg/g mg g^{-1} dry weight) than the genotypes IR83142-B-61-B, IR-72 and FL-478 (9.97, 12.05 and 13.96 mg g^{-1} dry weight), respectively. Total sugars play a major role in osmotic adjustment at the cellular level of crop plants under salt stress reported (Gupta and Kaur, 2005). The results regarding increase sugar to increasing salinity levels with temperature stress are also observed by the scientists (Nemati *et al.*, 2011; Siringam *et al.*, 2011; Danai-Tambhale *et al.*, 2011; Hakim *et al.*, 2014 and Abdelgawad *et al.*, 2014).



Results and discussion

From the results obtained in the present study, we can conclude that overall IR88611-B-5 and FL-478 showed better tolerance to salt stress than IR83142-B-61-B and IR-72, under the effect of salinity levels on physiological response at seedling stage. In addition genotypes IR88611-B-5 and FL-478 showed higher proline, chlorophyll content leaf area and sugars with different salinity levels than IR83142-B-61-B and IR-72 and all these physiological characteristics play an important role in its salt tolerance environment. In this study determined that chlorophyll b greater than chlorophyll a. Chlorophyll *b* concentration with increasing salinity levels which were leading to high photosynthetic ability, there was a increasing trend due to salinity the pigments (chlorophyll a, b total, carotenoids) are directly correlation to membrane injury, leaf area, shoot sodium (Na^+) content and shoot total sugars because salt concentration occurs through transpiration stream, which is highest in the shoot. Furthermore, germination, vegetative growth, biomass and potassium (K^+) content decreased significantly at high salt concentrations (100mM NaCl). However, significant reduction was observed at high salinity levels, symptoms shows from the experiment salinity stress creates toxic effects due to osmotic pressure, inhabit root and shoot growth, leaf rolling, membrane injury and tip burning.

Fig. 11. Seed germination and seedling growth of rice under salt stress at 30^0C temperature

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