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Genotypic responses of rice under salinity and high temperature stresses on seed germination and seedling growth

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Abstract: Seeds of four different rice genotypes (Kharaganja, Shua-92, IR-9 and FL-478) were exposed to increasing concentrations (0, 50, 75 and 100mM) of NaCl and (30 and 38^{0}C) temperature, to investigate the genotypic responses of rice under salinity and high temperature stresses on seed germination and seedling growth. There was a regular decrease in seed germination and seedling growth raised in plastic bowls for ten days with increasing salt concentration and temperature. Highest germination and seedling growth was observed in Kharaganja and FL-478 as compared to Shua-92 and IR-9. It was concluded on the basis of growth characteristics that all the genotypes indicated more sensitive to high temperature stress (38^{o}C) with increasing salinity levels.

Key words: seed germination, seedlings, salinity (NaCl), temperature, rice genotypes

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops among cereals (Eckardt, 2009). It is consumed by 90% in tropical Asia (Parasad*et al*., 2006), and is being used as staple food by world's half population (Muhammad *et al*., 2009). Pakistan, rice is the premium food grain crop and is grown on an area of 2.2 million hectare for domestic consumption and export abroad with foreign exchange return of 33 million US $ (Bashir *et al*., 2010). The yield per hectare has improved growth of 2,437 kg hec^{-1} (Pakistan Bureau of Statistics, 2013-2014). Rice current production rate should increase approximately by 1% per year not only for the world's growing population food demands (Sass *et al.*, 2002) but also due to adverse climatic conditions.

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Production of rice is facing many problems around the world due to various biotic and abiotic stresses (Sabouri *et al.,* 2011). Among abiotic stresses high temperature, drought, water shortage and salinity are the main obstacles for & productivity contributes 50% yield losses (Ren *et al.,* 2010). According to an estimate the crop yield losses due to salinity and high temperature are 20% and 40%, respectively (Athar and Ashraf 2005 and Araus *et al*., 2002). About 20% of earth's land mass and 50 % of irrigated lands are affected due soil salinization and approximately 30% of rice growing area in the world is salt affected (Wang *et al.,* 2012).

In Pakistan where about 80% area is affected by varying degree of salinity causing severe reduction in yield. Irrigated area in Pakistan is approximately 18.92 mha. Hence there is a need to exploit these saline soils by reclamation or via growing salt tolerant plants (Saeed *et al.,* 2012). Reclamation of salinity is complicated and expensive, and not the stable solution of the problem. Identification of salt-tolerant variety is the viable approach to achieve better profit from these lands (Ashraf *et al.,* 2008).

Therefore high temperature stress is another important environmental factor in future scenario for rice crop productivity. Rice with relatively higher tolerance at the vegetative stage is extremely sensitive to high temperature during the reproductive stage, particularly at flowering (Jagadish *et al.,* 2010a, b; Prasad *et al.,* 2006). When rice is exposed to air temperatures higher than 35°C, heat injuries occurs. Global temperature has increased by 0.3–0.6°C since the late 19th century and by 0.2-0.3°C over last 40 years. In the last 140 years, the 1990s was the warmest period (Jones and Briffa, 1992). Kothawale and Rupa (2002) reported a rise of 0.5°C in mean annual temperature over last century.

The arid areas of Pakistan where the evapotranspiration rate is already high and water table is shallow, an increase in air temperature may cause loss of water rapidly and consequently the aridity would increase. If the evapotranspiration rate continues to increase then the water reservoirs in the form of small dams, ponds and tobas etc. would dry more rapidly and water shortage may engrip humans along with plants. Chaudhry *et al*. (2004) described that almost two third area of the country lies in arid zone. Pakistan fulfills its water requirement from winter and summer rainfall along with the melting of snow fall from the glaciers. Due to global warming, variability of summer rainfall has considerably increased and glaciers have started melting at a much faster rate than observed before (Rasul, 2008).The vulnerability of the crop will be increased with a projected global average surface temperature increase of 2.0-4.5°C and the possibility of increased variability about this mean by the end of this century (IPCC, 2007). Thus, this situation is becoming more alarming for rice crop productivity. Increased temperature will affect the physiological processes necessary for crop growth and development and ultimately crop yields are most likely to drop over the present level. Climatic anomalies will play an important role in increasing the uncertainties in crop production. Mackill *et al.* (2010) reported rising temperatures due to accumulation of greenhouse gasses are expected to result in declining rice yields in the tropics. In addition to the direct effect of high temperature in reducing yields, a rise in sea level coupled with more erratic and extreme weather events will result in reduced yields and increase the risks of rice farming.Our study was focused on seed germination and growth responses of rice genotypes under salinity and high temperature stress at seedling stage.

Materials and methods

The research work reported in this manuscript was carried out with an attention to explore the genotypic responses of rice under salinity and high temperature stresses on seed germination and seedling growth. The seeds of rice were obtained from NIA, Tandojam and (IRRI) type genotypes by Dr. Mohammed Arif, NIBGI, Faisal abad.

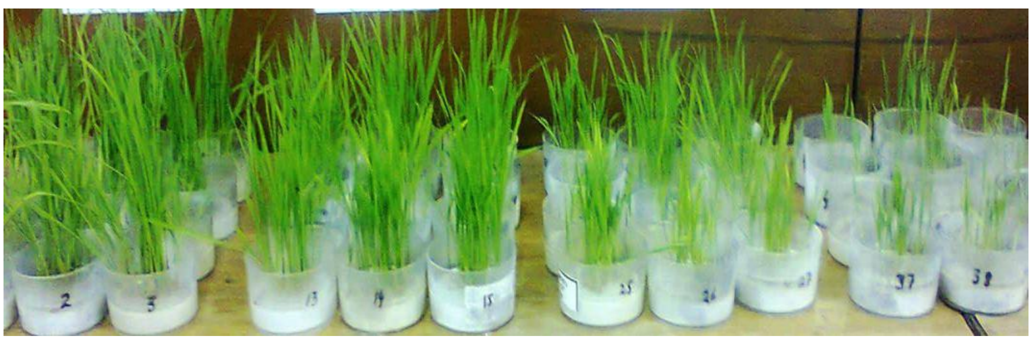
This research work was conducted in the research laboratory of the Plant Physiology Division of Nuclear Institute of Agriculture, Tandojam, Pakistan during the year 2014-2015. The methodology adopted to conduct this research work is described as under:

Water culture experiments were conducted under controlled laboratory conditions with factorial design (Complete Randomize Design), three replicates and four rice (Kharaganja, Shua-92, IR-9 and FL-478) genotypes at different levels of salinity (0, 50, 75 and 100 mM NaCl) and two temperature (30^oC and 38^oC) for studying the effect of salinity and high temperature stresses on seed germination and early seedling growth of rice genotypes. Fifty seeds of each rice genotypes Sterilized (with 3% sodium hypochlorite for 20 min) were placed on plastic bowls filled with culture solution (Yoshida *et al*., 1976). The pH of culture solution was maintained at 5.0. These bowls were placed separately in dark for germination at 30 and 38^oC in incubators up to 10 days. The experimental details are as given below:

The germination percentage, the root and shoot length and their fresh and dry weights were observed after 10 days. The root and shoot length of each treatment were determined in centimeters. At the end of experiment, roots and shoots were cut separately and weighed for their fresh weight. The shoot and root dry weight of the plant was recorded in milligrams after drying in hot air oven (SANYO, Model, MOV-202, Japan) at $65^oC \pm 5^oC$ for 72 hours.

All the data collected was subjected to analysis of variance (ANOVA) to discriminate the superiority of treatment means and least significant difference (LSD) test was applied, following the methods of Gomez and Gomez (1984) to compare the means. For this purpose a Microsoft computer package "Statistics 8.1" was used.

Result and discussions



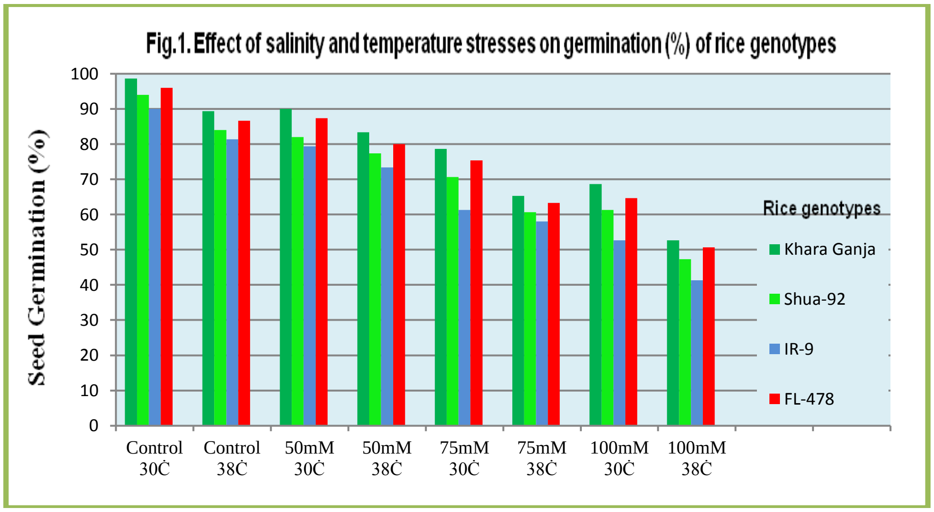
A laboratory experiment was carried out to see the genotypic responses of rice under salinity and high temperature stress on seed germination and seedling growth during the year 2014-2015, Research laboratory of the Plant Physiology Division of Nuclear Institute of Agriculture, Tandojam. Pakistan. Four rice varieties (Khara ganja, Shua-92, IR-9, and FL-478) were investigated for their response to different concentration of salinity (0, 50, 75 and 100 mMNaCl) and temperature (30^oC and 38^oC) Seed germination (%), root and shoot length (cm), root and shoot fresh and dry weight (mg) and root shoot ratio was recorded after 10 days of incubation.

Seed germination (percentage)

The data regarding the seed germination in controlled laboratory condition under various NaCl concentrations and two temperatures is represented in figure 1. The data was subjected to the analysis of variance statistics 8.1.

As it can be seen from the data, the germination percentage depending on salt treatment and high temperature stress all genotypes decreased with increase in salinity levels at 30^{o}C temperature. The maximum seed germination was determined in control (94.66%), followed by (84.66%) in 50mM NaCl, respectively. The lowest seed germination (71.50 and 61.83%) was recorded when NaCl level increased to 75mM and 100mM, respectively. The results regarding the genotypic effect on seed germination indicated that Khara ganja and FL-478 responded significantly better having maximum seed germination (84.00 and 80.83 %) than the genotypes of Shua-92, and IR-9 having (77.00, and 71.`50%), respectively. The results were recorded at 38^{o}C temperature with different salinity levels, the maximum seed germination was determined in control (85.33%), followed by (78.50%) in 50mM NaCl, respectively. The lowest seed germination (61.83 and 48.50%) was determined when NaCl level increased to 75mM and 100mM, respectively. The results regarding the genotypic effects on seed germination indicated that Khara ganja and FL-478 responded significantly better having maximum seed germination (72.66 and 70.16%) than the genotypes of Shua-92, and IR-9 having (67.33, and 64.00%), respectively.

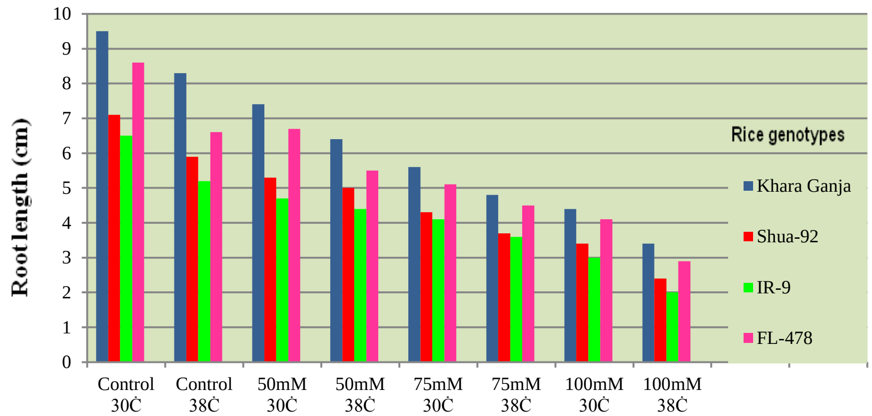
It is clear that all the genotypes indicated most sensitive to germination at high temperature (38^{o}C) stress with increasing salinity levels. The effect of salt treatment and temperature stress on germination of all the genotypes was determined to be statistically significant. Seed germination percentage decreased under the effect of salinity and high temperature stress results ascribed (Mokhberdoran *et al*., 2009; Jamil *et al.*, 2012 and Hakim *et al*., 2010). Reduction in emergence and seedling growth both in hybrids and parents of rice with increasing salinity levels and temperature was reported by (Maiti *et al*., 2009 and Hasamuzzaman *et al.*, 2009).



Root length (cm)

Salt and temperature stresses significantly decreased the root length of all rice genotypes, although to a varying degree (fig. 2). The results determined of root length at 30^0C temperature with increased salinity level of 100 mMNaCl the Khara Ganja genotype showed maximum root length (6.7 cm) as compare by FL-478 and Shua-92 (6.1 and 5.0 cm). The minimum decreased in seedling root length was observed in genotype IR-9 (4.6 cm). At high temperature (38^0C) stress, salinity level of 100 mMNaCl the same genotype Khara Ganja determined better performance having maximum root length (5.7 cm) followed by FL-478 and Shua-92 (4.6 and 4.2 cm) and the least performance was observed on IR-9 (3.8 cm). With increase of salinity and temperature, reduction of root length, was observed by (Roy *et al.,* 2002; Djanaguiraman *et al.*, 2003 and Jamil and Rha 2007). The differences were significant among the genotypes, various NaCl concentrations as well as highly significant among temperatures.

Fig. 2. Effect of salinity and temperature stresses on root length (cm) of rice genotypes



Shoot length (cm)

Shoot length of all the rice genotypes significantly decreased at both stresses temperature and all the salt treatments relative to the control and with increase in salinity (fig. 3). At 30^{0}C temperature, salinity level of 100 mMNaCl the genotypes Khara ganja and FL-478 showed better performance having maximum shoot length (15.5 and 14.0 cm) to followed by genotypes of Shua-92 and IR-9 (13.1 and 11.1 cm), respectively. Same tolerant genotypes decrease shoot length at high temperature stress with increased salinity level of 100 mM NaCl showed better performance having maximum shoot length (10.3 and 9.4 cm) as compare by Shua-92 and IR-9 (8.7 and 5.8 cm), respectively. Increase levels of salinity and temperature results ascribed (Roy *et al.*, 2002; Govinda Raju and Balakrishnan 2002; Djanaguiraman *et al.*, 2003; Jamil and Rha, 2007; Similar findings were reported by Anatai *et al.*, 2009).

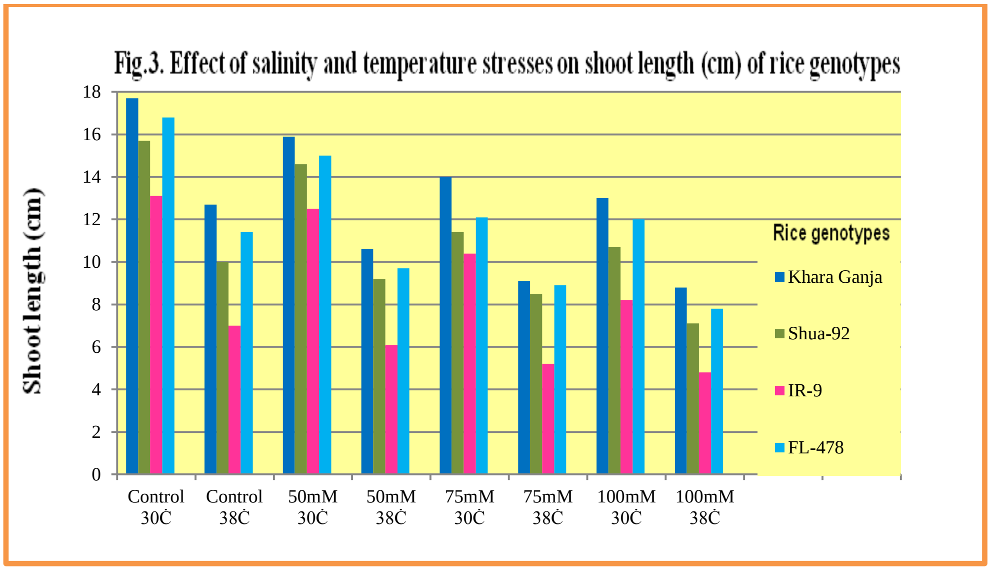
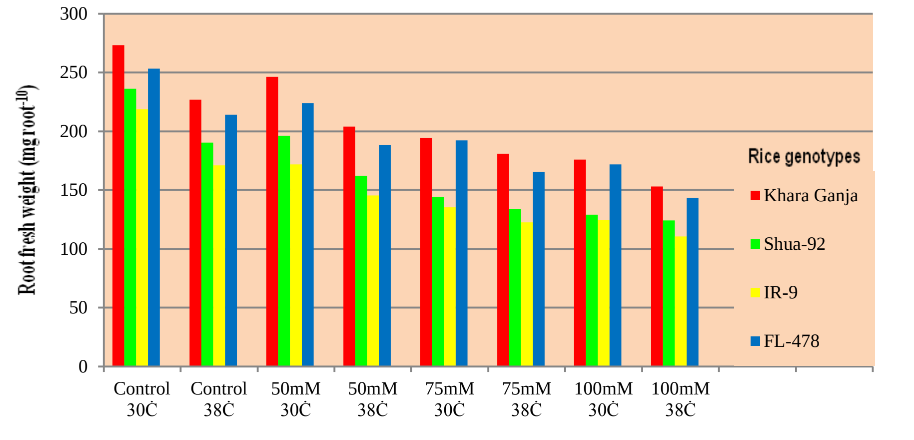


Fig.3. Effect of salinity and temperature stresses on shoot length (cm) of rice genotypes

Root fresh weight (mg root^{-10})

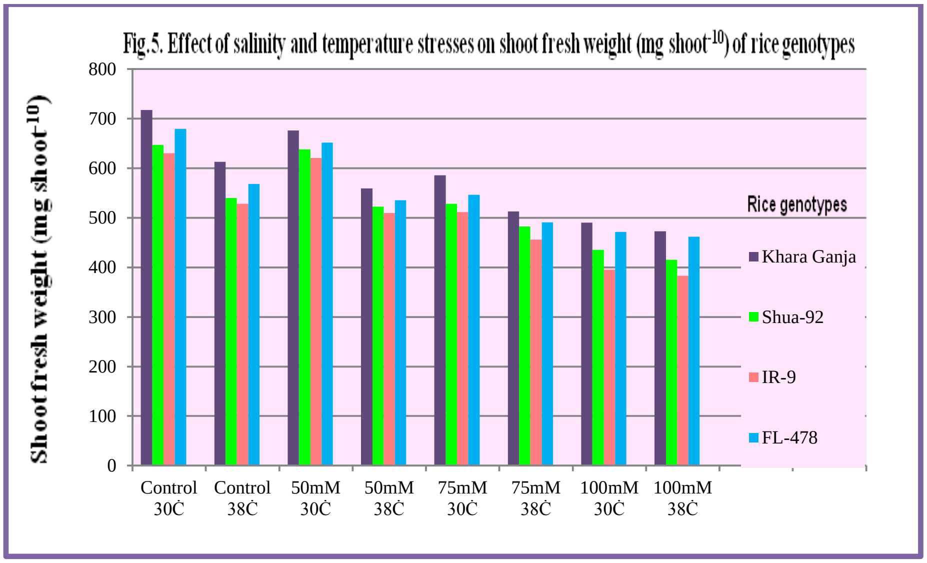
The results pertaining to root fresh weight of different rice genotypes are presented figure 4. At the 30^{0}C temperature root fresh weight of all the genotypes decreased progressively with increase salinity levels up to 75mM NaCl. The lowest root fresh weight was observed at 100mM NaCl salinity level. The results regarding the genotypic effect on root fresh weight indicated that Khara Ganja and FL-478 responded significantly better having maximum root fresh weight (222.42 and 210.33 mg root^{-10}) than Shua -92, and IR-9 (176.29 and 162.72 mg root^{-10}), respectively. At 38^{0}C temperature stress with increased salinity levels same genotypes Khara Ganja and FL-478 having maximum root fresh weight (191.21 and 177.76 mg root^{-10}) than Shua -92, and IR-9 (152.56 and 152.56 mg root^{-10}), respectively. All the genotypes indicated most sensitive to root fresh weight at high temperature (38^{o}C) stress with increasing salinity levels. The effect of salt treatment and temperature stress on root fresh weight of all the genotypes was determined to be statistically significant. The severe inhibitory effect of salts and temperature stress on root fresh weight is reported by (Bandeoglu *et al*., 2004 and Mokhberdoran *et al*., 2009).

Fig.4. Effect of salinity and temperature stresses on root fresh weight (mg root^{-10}) of rice genotypes



Shoot fresh weight (mg shoot^{-10})

The results about shoot fresh weight of different rice genotypes as significantly affected by various NaCl concentrations are shown in figure 5. The results indicated that shoot fresh weight of all the genotypes was decreased with increase NaCl levels at 30^0C temperature. The maximum shoot fresh weight was observed in control (668.55 mg shoot^{-10}), followed by (646.52, 542.82 and mg shoot^{-10}) in 50 and 75mM NaCl, respectively. The lowest shoot fresh weight (448.02 mg shoot^{-10}) was observed at 100mM NaCl level, respectively. The results regarding the genotypic response on shoot fresh weight indicated that Khara Ganja and FL-478 responded significantly better with maximum shoot fresh weight (617.16 and 587.17 mg shoot^{-10}) than Shua-92 and IR-9 (562.14 and 539.42 mg shoot^{-10}), respectively. Same genotypes responded most sensitive to shoot fresh weight at 38^0C temperature with increasing salinity levels, maximum shoot fresh weight was observed in control (562.13 mg shoot^{-10}), followed by (531.71 and 485.30 mg shoot^{-10}) in 50 and 75mM NaCl, respectively. The lowest shoot fresh weight (433.00 mg shoot^{-10}) was observed at 100mM NaCl level, respectively. The results regarding the genotypic response of shoot fresh weight indicated that Khara Ganja and FL-478 responded (539.31 and 513.89 mg shoot^{-10}) than Shua-92 and IR-9 (489.92 and 469.02 mg shoot^{-10}). Results ascribed as (Bandeoglu *et al*., 2004; Kumar *et al*., 2009 and Mokhberdoran *et al*., 2009).



Root dry weight (mg root^{-10})

It was observed from the data (Fig.5) higher root dry weights recorded in genotypes Khara Ganja and FL-478 indicated (23.55 and 19.76 mg root^{-10}) under different concentrations of salinity at 30^{0}C temperature stress. Minimum decrease in root dry weight was observed in genotypes Shua-92 and IR-9 (17.02 and 14.52 mg root^{-10}). Moreover, the same genotypes also sensitive to high temperature stress with increasing salinity. The maximum root dry weight was recorded in Khara Ganja and FL-478 (18.94 and 15.19 mg root^{-10}), followed by (12.83and 10.48 mg root^{-10}) in Shua-92 and IR-9 genotypes, respectively. (Zeng and Shannon 2000; Morales *et al*., 2012; Awala *et al*., 2010 and Kumar *et al*., 2009) reported significant reduction in root biomass of rice genotypes at both stresses greater salinity and high temperature stress.

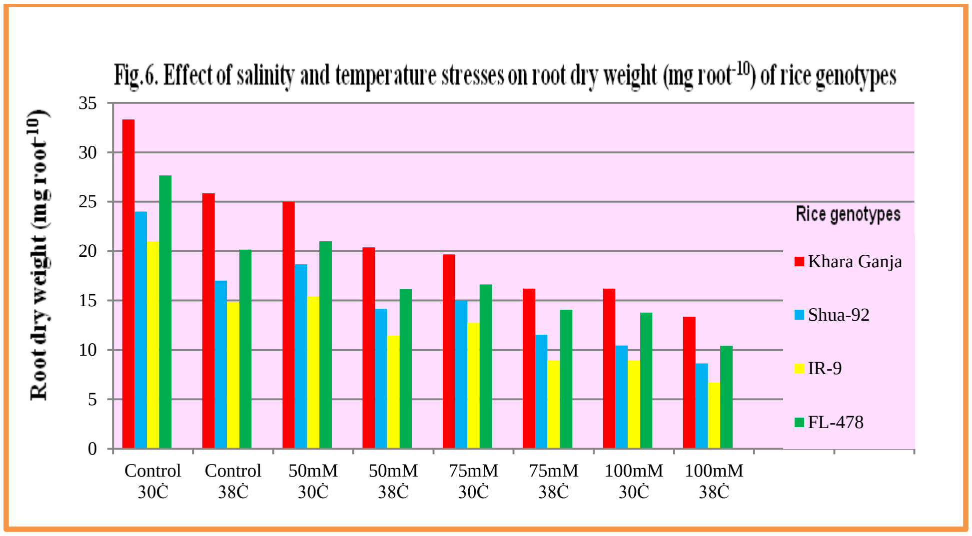
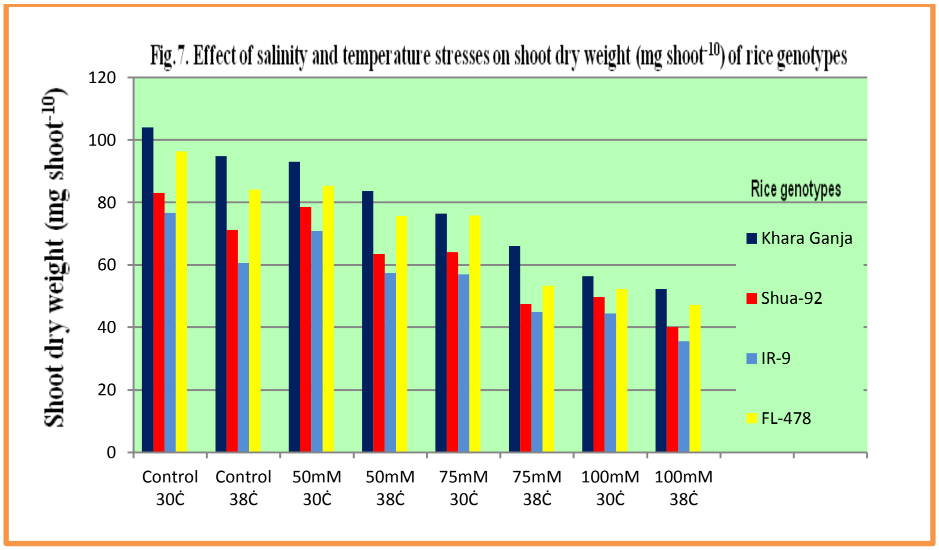


Fig.6. Effect of salinity and temperature stresses on root dry weight (mg root^{-10}) of rice genotypes

Shoot dry weight (mg shoot^{-10})

The data showed that the shoot dry weight was reduced with the increasing salt concentrations (Fig.7). These results indicated that salinity and temperature decrease the growth of rice seedling and led to a decrease in biomass. The maximum shoot dry weight was recorded in control (89.97 mg shoot^{-10}), followed by (81.91 and 68.31 mg shoot^{-10}) at 50 and 75mM NaCl, respectively. The lowest shoot dry weight (50.68 mg shoot^{-10}) was recorded when salinity level increased to 100mM NaCl, respectively. The results regarding the genotypic effect on shoot dry weight determined that Khara Ganja and FL-478 responded significantly better having maximum shoot dry weight (82.46 and 77.44 mg shoot^{-10}) than the genotypes Shua-92 and IR-9 (68.79 and 62.20 mg shoot^{-10}), respectively. All the genotypes highly sensitive to 38^{0}C temperature with increasing salinity levels, Khara Ganja and FL-478 responded (74.18 and 65.08 mg shoot^{-10}) than the genotypes Shua-92 and IR-9 (55.57 and 49.63 mg shoot^{-10}), respectively. Reduction of shoot dry weight reported also (Deng *et al.,* 2011; Mahmood *et al*., 2009 and Mokhberdoran *et al*., 2009).



Conclusion

In conclusion the results indicated that rice genotypes characteristics seed germination, root and shoot length, root and shoot fresh weight and root and shoot dry weight were decreased significantly. All the rice genotypes has been shown to be highly sensitive to higher concentration of salt with high temperature stress, especially 100mM NaCl at 38^{0}C temperature is toxic to seed germination and early seedling growth because of marked effects of osmotic stress and specific ion toxicity resulted inhibition of seed germination, decreased root and shoot growth, rolling of leaves, tip burning and the seedling fresh and dry weight was significantly reduced. The results regarding the genotypic response of seed germination and seedling growth, Khara Ganja determined highest mean values, while IR-9 showed minimum mean values for all the characteristics at different salinity levels and both temperatures.

100mM NaCl 30^{0}C temperature

100mM NaCl 38^{0}C temperature

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