ABSTRACT

Soil salinity is a serious problem for agricultural productivity throughout the world. Rice is an essential food crop and susceptible to salt stress. In the following study, two experiments were performed. The genotypes were screened at germination and seedling stages in the first experiment to select NaCl responsive genotypes. These genotypes were selected based on the gain in radical length, plumule length, radical fresh weight, plumule fresh weight, radical dry weight, plumule dry weight, at germination stage and, at the seedling stage, these were evaluated based on root length, dry root weight, fresh root weight, shoot length, fresh shoot weight, and dry shoot weight. At the germination stage the genotype dular performed better, SRS-66 showed average scores and, the genotype Kihogo did not perform better under NaCl stress while at the seedling stage Basmati-385 perform better, IR-6 and SRS-66 showed average scores and, Jaya did not perform better under NaCl stress. In the second experiment, the selected genotypes were hybridized with two modern cultivars KSK-434 and KSK-282 following the line × tester mating design. The F1 hybrids were evaluated based on the above-mentioned traits at the germination and seedling stages. The obtained data were used to determine the better parent heterosis for the salinity tolerance traits. The parent dular, Basmati-385, KSK-282, and KSK-434 showed higher scores for salinity tolerance traits at germination and seedling stages. The crosses Dular×KSK-282, Basmati-385 × KSK-282 showed higher heterosis for all the salinity tolerance traits. While the cross IR-6 × KSK-434 did not perform better under salt stress conditions.

Keywords: Rice; salinity; screening; heterosis; germination stage; seedling stage.

1. INTRODUCTION

The rapid growth of the population will require excess food crops such as rice, maize, soybeans, and wheat by utilizing the limited land and water resources (Kromdijk and Long, 2016). However, on the global scale soil salinity is a major cause to disturb food security because the soils are affected by salinity due to climatic changes. Salinity has affected an area of nearly 960 Mha of the earth's surface (Wicke et al., 2011). According to the current data, the 2000 km² of land covering 75 countries is regularly influenced by salinity (Reddy et al., 2017). That affects different growth stages of the crop plants. Salt stress induces water deficiency that causes ion toxicity and ultimately resulting in an imbalance in nutrition uptake of the plant. As a result, many reactive oxygen species (hydroxyl, hydrogen peroxide, superoxide, etc.) are produced and accumulate in plant tissues that cause intense osmotic stress and ionic imbalances (Munns et al., 2006). These effects however depend on the salinity contents, ecological conditions, plant type, and the growth stages of the plants. Thus, the effect of salinity stress on plants varies with plant varieties, cultivars, and species, and rice is a susceptible plant to salinity (Torabi, 2014).

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Stunted growth and reduced crop yields are serious problems associated with salinity stress (Ashraf et al., 2009; Ashraf, 2011). Due to the lack of good quality water, farmers are compelled to use brackish groundwater for irrigation purpose that increases the NaCl level in soils. Rice is an important grain crop in Asia that gives food to a large portion of the total population, and it is being affected by the supply of saline water of ground (Ma et al., 2007). Increasing salinity levels is hampering rice production in many areas of the world that are ultimately putting pressure on the global food basket. In Pakistan, one million hectares of the rice-producing regions are affected by salinity, and it causes a 64 percent decrease in the crop yields on these lands (Afzal et al., 2005). Rice is sensitive to salt stress, although a significant genetic variability has been reported in different genotypes of the rice (Flowers and Yeo, 1981). The production of salinity tolerant rice cultivars by traditional and recent molecular methods could assist to resolve the world food security issues (Fischer and Fukai, 2003). Rice germplasm is the source of adaptation loci that can shape climate-smart ideotypes. Selective breeding for these stress tolerance loci has served a lot to agriculture and can further contribute to future food security (Zamir, 2001).

Morphological traits including root and shoot length, root/shoot fresh weight, and dry weight are important for screening rice accessions against salinity stress (Zafar et al., 2015). It was also recorded that the germination potential of seeds differs from one crop to another under salinity conditions and even a substantial difference amongst the different varieties of a crop is observed (Maas and Hoffman, 1977). Germination is an intricate procedure and has a significant influence on the life cycle of plants. As the level of salt increases the seed germination decreases (Saddiqi et al., 2007). Under salinity conditions, during the imbibition process, the decrease in the flow of water into the seeds has occurred which leads to a reduction in the percentage of germination and final seed germination (Hadas, 1977). Since the roots are in direct interaction with the soil and take water from the soil and shoot deliver it to the remaining parts of the plant. For this purpose, the length of plumule and radical give a valuable clue to the salinity stress response of the plants (Jamil, 2006). The radical and plumule length of the seedling grown in a salt stress environment also displays decline suggesting that the salinity affects not only germination but also seedlings production. If at the time of transplantation seedlings are exposed to salt stress it causes reduced root and shoots growth and minimum dry weights.

There are several ways to stabilize rice plants under salinity stress. The exploitation of germplasm resources is the best way to develop salt-tolerant rice plants. Germplasm is the primary component for future farming as it provides the basic material for climate-smart agriculture (Frison et al., 2012; Fu et al., 2015).

With the expanding accessibility of genomic tools and advances in genomic analysis, it is turning out to be progressively evident that gene flow amongst different taxa will produce novel phenotypic diversity, allowing adaptation to new environments. Hybridization can lead to local adaptation by the introgression of novel alleles leading to the creation of new genotypes (Goulet et al., 2017). The use of genotypes stored in seed banks are the sources of genes for resistance to multiple stresses and can help to produce new high yielding and stress tolerant genotypes. To overcome salt stress, salt-tolerant genotypes need to be identified and utilized (Chinnusamy et al., 2005). Potential genotypes of rice are available in seed banks. For this purpose, we tried to find such salt-tolerant genotypes and hybridizing them with modern cultivars, to unlock the hidden potential of the salt tolerance present in these genotypes.

2. MATERIAL AND METHODS

The experiments of screening of 25 genotypes and 10 F1 hybrids under NaCl stress at germination and seedling stages were performed following factorial under CRD with three replication and two treatments (control and 100 mM NaCl stress). The data were subjected to the
analysis of the variance (Steel et al., 1997) to check the variation among genotypes, treatments, and their interaction. Mean data of different traits were used to make biplots for screening salt-tolerant genotypes (Yan et al., 2001). For the evaluation of hybrids, the data were used to determine the better parent heterosis for salinity tolerance traits.

2.1. Plant Material

The rice germplasm was collected from the International Rice Research Institute (IRRI) Philippine and Rice Research Institute (IRRI) Kala Shah Kaku Pakistan. Total 25 accessions were used to find out salt-tolerant genotypes at the germination and seedling stage based on morphological traits (Table 1).

| Sr. No | Genotypes       | Sr. No | Genotypes       |
|--------|-----------------|--------|-----------------|
| 1      | Kaukhyi-ani     | 14     | Ph.577          |
| 2      | Rathuwee        | 15     | SRS-504         |
| 3      | Shaheen Basmati | 16     | PS-2            |
| 4      | Azucena         | 17     | Punjab-Basmati  |
| 5      | Cl 11011        | 18     | Basmati-198     |
| 6      | IR-6            | 19     | Samo trang      |
| 7      | IR-9            | 20     | Basmati-379     |
| 8      | N-22            | 21     | Pokalli         |
| 9      | Basmati-385     | 22     | Kihogo          |
| 10     | SRS-66          | 23     | CS-M3           |
| 11     | Chenab-Basmati  | 24     | Dular           |
| 12     | Basmati-2000    | 25     | Basmati-515     |
| 13     | Jaya            |        |                 |

2.2. Experiment 1: Screening of germplasm at the germination stage

For the genotypic evaluation at the germination stage, a set of 25 rice genotypes was grown between two filter papers placed in Petri plates. Ten seeds of each genotype were immersed in bleach for 10 minutes and then washed five times with distilled water. These genotypes were sown in two sets (control and 100mM NaCl stress) with three replications following the factorial under complete randomized design. In control treatments 4ml distilled water was applied to each Petri plate. In stressed treatments, 4ml of 100mM NaCl solution in distilled water was applied to each Petri plate. This experiment was monitored for three days. At the germination stage, genotypes were evaluated based on radical length (RaL), plumule length (PL), radical fresh weight (RaFW), plumule fresh weight (PFW), radical dry weight (RaDW), and plumule dry weight (PFW). The data were subjected to the analysis of the variance (Steel et al., 1997) to check the variation among genotypes, treatments, and their interaction. Mean data of these traits were used to make biplots for screening salt-tolerant genotypes (Yan et al, 2001).

2.3. Experiment 2: Screening of the germplasm at the seedling stage

The nursery of the genotypes was sown in polythene bags filled with soil for the genotypical evaluation at the seedling stage. At the two-leaf stage, the plants were shifted in hydroponic conditions. Two sets of the treatments (control and 100mM NaCl stress) with three replications were evaluated following the factorial under a completely randomized design. The nutrition of plants in hydroponics was maintained by using Yoshida nutrition media (Yoshida et al., 1976). The pH of the nutrient media was maintained at 6.5 and the media was changed after every three days. After one week of the nursery transplantation, the 100mM NaCl stress was applied to one set of 25 genotypes, and the other set of 25 genotypes remained in nutrient media without NaCl application as the control treatment. The experiment was monitored for two weeks after the stress application. At the seedling stage, the data were recorded for the root length (RL), shoot length (SL), fresh root weight (FRW), fresh shoot weight (FSW), dry root weight (DRW), and dry shoot weight (DSW). The data of these traits were subjected to analysis of variance to check variation among genotypes, treatments, and their interaction, and mean data were used for biplot analysis for screening purposes.

2.4. Experiment 3: Hybridization of selected genotypes

For hybridization, the selected two NaCl tolerant, one moderately tolerant, one susceptible genotype, and two modern cultivars (KSK- 434 and KSK- 282) were sown in the field following the randomized complete block design and hybridized following the line × tester mating design (Kamphorhe, 1957).
For the development of hybrids, the parent plants were sown in the research area of MNS- University of Agriculture Multan. Parent plants were sown in lines, each line has five plants. Line to line and plant to plant distance was maintained at 9 inches.

2.4.1. Evaluation of F₁ hybrids under NaCl stress at germination stage

For the evaluation of hybrids at the germination stage 10 F₁ seeds of each cross were dipped in bleach for 10 minutes for sterilization purpose and wash five times with distilled water. Then sown in two sets (Control and 100mM NaCl stress) with three replications following the factorial under complete randomized design. In control treatments, 4ml distilled water was applied to each Petri plate. In stressed treatments, 4ml of 100mM NaCl solution in distilled water was applied to each Petri plate. This experiment was monitored for three days and data were collected for the above-mentioned traits.

2.4.2. Evaluation of F₁ hybrids under NaCl stress at seedling stage

For the evaluation of hybrids at the seedling stage seeds of each cross were sown in disposable glasses filled with soil. At the two-leaf stage, the seedlings were shifted in hydroponic conditions. Two sets of the treatments (control and 100mM NaCl stress) with three replications were evaluated following the factorial under a completely randomized design. The nutrition of plants in hydroponics was maintained by using Yoshida nutrition media (Yoshida et al., 1976). The pH of the media was maintained at 6.5 and the media was changed after every three days. After one week of the nursery transplantation, the 100mM NaCl stress was applied to one set of hybrids, and the other set of hybrids remained in nutrient media without NaCl application as the control treatment. The experiment was monitored for two weeks after the stress application after that the data were collected for the salinity tolerance traits.

These hybrids were evaluated based on the above-mentioned traits at the germination and seedling stage and the following parameters were recorded, radical length (RaL), plumule length (PlL), radical fresh weight (RaFW), plumule fresh weight (PlFW), radical dry weight (RaDW), plumule dry weight (PlDW), root length (RL), shoot length (SL), fresh root weight (FRW), fresh shoot weight (FSW), dry root weight (DRW) and dry shoot weight (DSW). For the evaluation of hybrids, the collected data were used to determine the better parent heterosis.

Better parent heterosis (BPH) = \( \frac{(F₁ - \text{Better parent})}{\text{Better parent} \times 100} \)

3. RESULTS

3.1. Trait environment biplot analysis for germination stage

At the germination stage, the mean data of the traits were used for the biplot analysis. Based on RaL, PIL, RaFW, PIFW the genotype Dular showed the highest measures under salt stress i.e. 3.33cm, 4.76cm, 0.015g and, 0.015g, respectively. This genotype attained the longest OP and was close to the vector of RaL, PIL, RaFW, PIFW under stress condition as compared to the control condition. Similarly, for RaDW and PIDW under stress condition, the genotype Kaukkyi-ani attained higher scores i.e. 0.003g and 0.002g, respectively. This genotype attained a longer OP on RaDW and PIDW vector. The genotype SRS-66 showed average scores for RaL, PIL, RaFW, PIFW, PIDW, and RaDW under stress conditions i.e. 4.433cm, 3.033cm, 0.017g, 0.009g, 0.001g, 0.003g, respectively. The genotype Kihogo showed the lowest scores.

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Table 2. L × T mating design for rice genotypes

| Tester       | Line | Line   | Line   | Line | Line |
|--------------|------|--------|--------|------|------|
| KSK-434 (MC) | 1    | 2      | 3      | 4    | 5    |
| Jaya (SS)    |      | SRS-66 (MST) | IR-6 (MST) | Dular (ST) | Basmati-385 (ST) |
| KSK-282 (MC) | Jaya (SS) | SRS-66 (MST) | IR-6 (MST) | Dular (ST) | Basmati-385 (ST) |

SS = Salt susceptible, MST = Moderately salt tolerant, ST = Salt tolerant, MC= Modern cultivar.
i.e. 0.33cm, 0.33cm, 0.001g, At the germination stage, RaL, PIL, RaFW, PIFW, RaDW, and PIDW were
0.001g, 0.001g, 0.001g, 0.001g, 0.001g for all the traits i.e. RaL, PIL, RaFW, PIFW, RaDW, and PIDW under stress condition. The biplot is given below for this experiment is given in Figure 1.

**Table 3. Mean square for the screening of rice genotypes at germination stage under NaCl stress**

| Source                  | df | RaL   | PIL   | RaFW  | PIFW  | RaDW  | PIDW  |
|-------------------------|----|-------|-------|-------|-------|-------|-------|
| Genotype                | 24 | 10.502 * | 5.455 * | 0.00013 * | 0.00010 * | 0.0009 NS | 0.0009 NS |
| Replication             | 2  | 11.937 NS | 2.141 NS | 0.0009 NS | 0.0003 NS | 0.0009 NS | 0.0005 NS |
| Treatment               | 1  | 690.36 | 171.735 * | 0.00228 * | 0.00498 * | 0.00283 * | 0.00296 * |
| Genotype*Treatment      | 24 | 3.369 * | 1.287 * | 0.00006 * | 0.00005 * | 0.010 NS | 0.0005 NS |
| Error                   | 98 | 2.685 | 0.533 | 0.00002 | 0.00002 | 0.0008 | 0.0005 |
| Total                   | 149|       |       |       |       |       |       |

*, ** and NS indicate significant differences at p≤ 0.05, p≤ 0.01, and non-significant (p≥ 0.05), respectively. RaL: radical length; PIL: plumule length; RaFW: radical fresh weight; PIFW: plumule fresh weight; RaDW: radical dry weight; PIDW: plumule dry weight

At the germination stage, RaL, PIL, RaFW, PIFW, RaDW, and PIDW were significantly affected under salt stress (p≤ 0.01, Table 3).

3.2. Trait environment biplot analysis for the seedling stage of genotypes

The biplot shows that the RL, SL, FRW, FSW, DRW, DSW in the genotype Basmati-385 has the highest measures of 13.73cm, 50.97cm, 0.44g, and 1.18g, 0.32g, 0.19g, respectively under salt stress condition. This genotype attained the longest OP and was close to the vector of RL, SL, FRW, FSW, DRW, DSW under stress condition as compared to the control condition. The genotypes IR-6 and SRS-66
showed average scores for RL, SL, FRW, FSW, DRW, DSW under stress condition i.e. 8.73 cm, 47.27 cm, 0.90 g, 1.09 g, 0.18 g and 0.38 g, 8.67 cm, 37.93 cm, 0.65 g, 1.02 g, 0.17 g, 0.55 g, respectively. The genotype Jaya showed the lowest scores i.e. 8.67 cm, 37.93 cm, 0.65 g, 1.02 g, 0.17 g, 0.55 g for all the traits RL, SL, FRW, FSW, DRW, DSW respectively under stress condition. The Biplot for these traits is given in Figure 2.

The data of these traits were subjected to analysis of variance to check variation among genotypes, treatments, and their interaction of and principal component analysis for screening purposes. The results indicate that traits including FRW, FSW, and RL were influenced by genotype, treatment, and the interaction between salinity and genotypes. There were also significant differences between genotypes and treatment. The ANOVA table for the above-mentioned traits is given below.

### Table 4. Mean square of absolute values for various seedling traits of 25 rice genotypes grown in control and NaCl stress

| Source              | df  | RL             | SL | FRW   | FSW   | DRW   | DSW    |
|---------------------|-----|----------------|----|-------|-------|-------|--------|
| Genotype            | 24  | 24.030 **      | 985.46 | 0.2056 * | 1.1863 * | 0.02881 * | 686.493 |
| Replication         | 2   | 9.167 NS       | 899.2 NS | 0.031 NS | 0.181 NS | 0.016 NS | 679.5 NS |
| Treatment           | 1   | 81.2544 **     | 202.3 NS | 0.31565 * | 55.6212 * | 0.0194 NS | 668.5 NS |
| Genotype*Treatment  | 24  | 11.4202 *      | 1174.0 NS | 0.09144 * | 0.7373 * | 0.007 NS | 684.8 NS |
| Error               | 98  | 2.8584         | 904.01 | 0.03612 | 0.2394 | 0.00902 | 685.344 |
| Total               | 149 |                |       |        |        |        |        |

*, **, and NS indicate significant differences at p ≤ 0.05, p ≤ 0.01, and non-significant (p ≥ 0.05), respectively. RL: root length; SL: shoot length; FRW: fresh root weight; FSW: fresh shoot weight; DRW: dry root weight; DSW: dry shoot weight.

### Fig 2. The Biplot analysis for the morphological traits at the seedling stage under normal and salinity stress conditions.

FRW: Fresh root weight; FSW: Fresh shoot weight; DSW: Dry shoot weight; DRW: Dry root weight; RL: Root length. The C is indicating the control environment.

At the seedling stage, RL, SL, FRW, FSW, DRW, and DSW were significantly affected under salt stress (p ≤ 0.01, Table 4). 3.3. Determination of better parent heterosis for salinity tolerance traits:

Two NaCl tolerant, two moderately tolerant, and one susceptible genotype, and two modern cultivars hybridized following the line × tester mating design (Kamthorne, 1957). For the evaluation of hybrids, the F1 seeds were sown in Petri plates and hydroponic conditions as
mentioned in the first experiment and evaluated as was the first experiment. The collected data were used to determine the better parent heterosis for salinity tolerance traits. The results of better parent heterosis for salinity tolerance traits are given below in graphs.
Fig 3. Better Parent Heterosis for salinity tolerance traits under 100mM NaCl stress and control condition. Root length: RL; Shoot length: SL; Fresh root weight: FRW; Fresh shoot weight: FSW; Dry root weight: DRW; Dry shoot weight: DSW.

4. DISCUSSION

Soil salinity is the most serious issue in the planting areas. It has a more obstructive impact on crop production worldwide. The soil salinity problem attracts most scientists to develop salt-tolerant lines to resolve the barriers to crop production. Salt stress interferes with the biological absorption of nutrients and water, thus disrupt the required physiological functions necessary for plant growth and development (Munns et al., 2006). Rice is very sensitive to salinity stress. The effects of salt stress have been frequently reported in rice fields. There is a need to broaden the genetic base of present breeding programs so that salt tolerant genotypes could be produced. This is possible by exploiting germplasm. Old (mostly uncharacterized) varieties have great potential as each cultivar may have unique adaptations (Mishra et al., 2016). For this study, 25 rice accessions from the IRRI and RRI were screened at the germination and seedling stage to test the salt stress response. The genotypes were grown to evaluate under salinity stress at germination and seedling stages. The results indicated that at the germination stage, the genotype dular performed better for the above-mentioned traits and was selected as salt-tolerant at the germination stage and the genotype SRS-66 showed average scores for the traits studied thus this genotype was selected as moderately salt-tolerant genotype at the germination stage. The genotype Kihogo did not perform better under salinity stress and was selected as a salt susceptible genotype at the germination stage. At the seedling stage, the genotype Basmati-385 performs so this genotype was selected as tolerant to salinity at the seedling stage and the genotype IR-6 and SRS-66 selected as moderately salt-tolerant genotype at the seedling stage. Genotype Jaya did not perform better under salinity stress and was selected as a salt susceptible genotype at the seedling stage. We found that salt stress reduces plant morphological growth at different developmental stages, similarly, it has already been studied that salt stress is responsible for the deceleration of plant growth and development. It hampers germination and seedling progression (Bera et al., 2006; Hakim et al., 2010). Hybridization is an important strategy to unlock the hidden potential of salt-responsive genotypes. It can broaden the genetic base of rice and give high-yielding salt-tolerant genotypes. Successful hybridization of the potential genotypes produces novel phenotypes. Rice germplasm is the potential source of salinity tolerance and the heterosis breeding has proved the worth of salt-responsive genotypes for transferring a significant proportion of the key traits to their progenies (Gopikannan and Ganesh, 2013).
F1 progenies inherit significant improvement for RL, SL, FRW, FSW, DRW, DSW, in short, hybridization generates such genotypes that can stabilize their biomass and tolerant salt stress (Virmani et al., 1982; Ansari et al., 2003; Sankar et al., 2008). Parallel to these earlier findings, in our study, a significant improvement was observed in F1 populations for the salt-responsive traits. When salt responsive genotypes were crossed with the modern cultivars their progenies performed better for various morphological traits. New phenotypes in F1 were able to acquire more FRW, FSW, DRW, DSW, Rl, and SL.

The results indicated that at the germination stage the F1 hybrids (Dular×KSK-282) and (Basmati-385 × KSK-282) performed better under salinity stress and the hybrid (SRS-66 × KSK-282) showed moderate results for control and salinity stress selected as moderately tolerant to salinity while the hybrid (IR-6 × KSK-434) showed lowest scores for the traits studied and considered as susceptible to salinity. At the seedling stage, the hybrids (Jaya × KSK-434) and (Jaya × KSK-282) perform better under salinity stress. The hybrid (Basmati-385 × KSK-434) was selected as moderately salt tolerant because it showed contrasting scores under salinity stress at the seedling stage. The hybrid (Basmati-385 × KSK-282) showed the lowest scores for salinity tolerance according to the above-mentioned traits and was selected as susceptible under salinity stress. The susceptible genotypes and hybrids exhibited a reduction in trait evaluated at germination and seedling stage under control and salt stress conditions. The reduction occurred in the root, shoot, radical, plumule lengths, and fresh and dry weight of root, shoot, radical, and plumule under saline conditions. Salinity caused a reduction in the shoot length of the susceptible genotypes and hybrids. In short, the rice germplasm stored in germplasm banks keep a large potential of genotypes that can tolerate the salt stress and provide better parent heterosis when hybridized with the modern cultivars.

5. CONCLUSION
In the soil salt concentration is a major abiotic stress factor, which inhibits the growth of plants. The growth stages of rice plants are generally influenced by salinity stress. Rice is considered a salt-sensitive. In general, rice can tolerate a small amount of salt without compromising growth and yield. The rice genotypes stored in the germplasm banks have a great potential to tolerate the salt stress. In this experiment we concluded that rice genotypes dular and Basmati-385 have potential to tolerant the salt stress and capacity to give better parent heterosis for salt tolerant traits when crossed with the modern cultivars.

6. ACKNOWLEDGMENT
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7. CONFLICT OF INTEREST
The authors declare no conflict of interest.

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