Research Article

Assessment of comparative effects of sodium chloride stress on various growth parameters in different varieties of rice (*Oryza sativa* L.)

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Abstract

Rice (*Oryza sativa* L.) belongs to family Poaceae. It is a cereal grain cultivated throughout the world. Rice is one of the largest crop products of Pakistan. In the present study of two varieties of Rice (*Oryza sativa* L.), Swat Sella and Punjab Basmati varieties were studied *in vitro* for the assessment of germination and different growth parameters. These varieties were subjected in hydroponic culture to 100mM, 200mM, and 300mM of NaCl and compared with control. The increase in concentration of NaCl adversely affected the seedling germination and growth parameters. The results showed that Swat Sella rice variety showed greater average number of seeds germination, radicle length and plumule length in 100mM, 200mM, and 300mM of NaCl compared to Punjab variety. However, in controlled medium, it was observed that Punjab variety showed higher fresh weight of seedlings, plumule length, root length of plants, shoot length of plants and dry weight of plants in comparison to Swat variety. It can be concluded that the Swat Sella rice variety is better adapted and can grow better in salinity compared to Punjab variety that grows best in non-saline conditions.

Keywords: Rice (*Oryza sativa* L.); Swat sella; Punjab basmati; NaCl; Hydroponic culture

Introduction

Rice (*Oryza sativa* L.) belongs to family Poaceae which is considered as cereal grain and cultivated throughout the world. Rice plant grows best from the altitude above 25,000 meter above sea level or even at higher altitude. The rice plant is one the largest crop products of Pakistan. It come at second position after the wheat as a staple food and form kharif crop of Pakistan. Rice

is monocotyledonous plant and one of the important export items of Pakistan. Rice holds 2.7% of agriculture and 0.6% of GDP value [1]. Rice is the third mostly cultivated crop after wheat and maize it is mostly cultivated in the mountain and plain areas of Khyber Pakhtunkhwa (KPK). During 2008-2009, 7349 hectares of land was cultivated with rice [2].

The stress is a physiological response to different situations. During the normal condition in which plant grow the metabolism process of the plant help to develop responses to changing environment, that disturb regular, daily and seasonal cycles [3]. Stress on plant is defined as any external biotic (animals, microbes etc.) or abiotic (temperature, water, heat, salinity etc.) feature that restrict photosynthetic rate and limit the biomass production [4]. Under change growth conditions, metabolic processes of the plant adjust itself in such away new state of homeostasis is achieved; this is called as acclimation [5]. Plant stress is measure in term of crop yield, growth, plant death or survival. Productivity of the crops is greatly reduced due to abiotic stresses [6]. The plant can face abiotic stresses that include freezing, immoderate temperature, saline or decrease availability of water, decreased amount of essential nutrient in soil, extreme light, and hindrance to root growth due to hardness of soil [7].

FAO [8] reported one of the biggest challenge toward to the agricultural production is to produce 70% more food crops by 2050 worldwide for extra 2.3 billion people. Salinity is most important stress that ultimately comes in way of increase demand for food crop. More than 20% of cultivated land is affected by salt stress world widely (about 45 hectares). Adaptive evolution of plants is of two types: halophytes (which can grow in saline condition) and glycophytes (which are not salt tolerant and finally die). The world nearly 7% total area seriously affected by salinity [9]. Salinity causes reduction in yield and quantity by affecting plant growth and developmental process [10]. In Pakistan, almost 12.9% of country land is effected badly by salinity as count for 10 million ha area [11].

Like all plants rice also shows variation for salinity tolerance [12, 13]. Plant growth and

development is affected by one of the most important abiotic stresses such as salinity [14, 15]. Rice plants show tolerance toward salinity during germination whereas, the growth stage such as panicle initiation and pollination stages are quite sensitive toward salinity that directly related to yield [16]. High salinity condition causes necrosis and burning of leaf tips due to injection of Na^+ and Cl^- [17].

In Halophytic Grass *Aeluropus lagopoides* due to high salinity the rate of germination decreases, with warmer temperature germination increase however when effected seeds provided with distilled water and warmer temperature seed recover quickly with optimum response [18]. The water stress or salinity effect the plant in many ways i.e. it reduces their ability to take up water, effect the growth rate and it also bring about metabolic changes govern by salinity stresses [19]. Germination is inhibited by increased salinity however; the stimulus of germination toward light depends on type of perennial grasses [20]. Salinity causes sterility in *Oryza sativa* L. that eventually lead to yield losses [21].

Two approaches to produce salt tolerant crops are (i) selection of plant with genetic diversity i.e. through direct selection in saline environment, gene mapping and by setting certain marker (ii) introducing the gene of interest to produce transgenic plant or expression of the gene changed up to degree of salt stress tolerance [22]. Na^+ and Cl^- content increased after salt treatment but K^+ decreases in flag leaves and roots of rice (*Oryza sativa* L.). Phosphorus applied through root inhibit Na^+ and Cl^- accumulation while it increases K^+ , P, Ca^{2+} in leaf and root opposite to effect of salt stress in *Oryza sativa* L. [23]. In rice and wheat accumulation of K^+ and biomass production depend on level of NH_4^+ and K^+ whereas, there interaction changes with the soil type [24]. N, P, K, Ca, and Mg nutrient

present in rice and wheat can be increased by applying chemicals of these nutrients in soil or providing them with plant compost [25]. Under saline condition tolerant genotype show greater leaf area, osmotic potential, glycine-betaine in wheat, chlorophyll and sugar content [26]. Large amount of salinity stress affects rice plant fresh shoot weight, seedling fresh weight and specific shoot length in comparison to that shoot length, root length, seedling length and there dry and fresh weight are less effected [27]. The rice plant should be irrigated with saline water in final growth stages [28]. *Oryza sativa* L. seed breaks dormancy when it is provided with combination of conditions i.e. seed hull removal, heat or chemical treatments then germinated it in optimum temperature [29]. In rice plant growth and yield can be increased by applying Si in suitable concentration at a proper time [30]. Insufficient water for irrigation directly affect morphological, anatomical, physiological and yield of rice plant [31]. Rice plant with salt tolerance genome or genotype could have potential germplasm source in future breeding programs. [32]. Drought effects growth and physiological activities of *Oryza sativa* L. finally, causes decrease in amount of yield further on increasing water stress result in proline accumulation and malondialdehyde [33].

The aims and objective of study was to examine the various parameters of different varieties in *Oryza sativa* L. under salinity stress; effect of NaCl on germination, plumula length, radicle root length, shoot length, leaf chlorophyll content, fresh weight of the plant, dry weight of plant.

Materials and methods

Oryza sativa L. (rice) seeds of two different varieties i.e. Punjab Basmati rice and Swat Sella rice variety were selected. All the research work was conducted in Islamia

College university Peshawar laboratory as well as in botanical garden.

The chemicals were obtained from various sources that include companies such as Riedel-de-haen, Analar (England), Merck (Germany), Sigma-Aldrich (USA) and Scharlau (Spain).

The petri dishes were used for germination of rice seeds before they could be used they were sterilized with distilled water later the thermal sterilization in auto clave at a temperature of 100° C for approximately 2 hours. Later, seeds were germinated on two folds of moistened filter paper. Petri dish was providing with 3 different solution 100mM, 200mM and 300mM of NaCl and one was grown in non-saline water was kept as control. Seeds were kept in an incubator at a temperature of 28° C to 30° C providing it with 90% of humidity. After 10 days germinated seedlings parameters such as length of the plumule, length of radicle and percentage of germination were recorded.

Hydroponic culture technique (Figure 7) was used to grow the seedling further on and to examine the root growth and elongation easily. The seedlings which were grown in non-saline condition are the transferred to the rectangular boxes covered with duct tape in such away root remain in darkness. The plants were provided with essential nutrients via hydroponic culture medium. The hydroponic culture medium consisting to two types of solution, micro nutrients and macronutrients are shown in Table 1. Later, the growth medium was provided with saline condition by adding NaCl to increase the concentration of Na⁺ to about 60mM, for osmotic stress medium was treated with 5%, 10%, and 15% of PEG (polyethylene glycol-4000).

The rice plants were provided with 16 hours of light and almost 8 hours of dark period, the temperature was maintained between 30° C at day and 28° C at night, with about 65% relative humidity. The medium was changed

every 4 days to avoid nutrient deficiency in plants.

Table 1. Growth media for plants (Macronutrients and Micronutrients)

Nutrients	Sources	Preparation of stock sol. (g/l)	MI of stock sol. for medium	Conc. of elements in medium (Mm)
Macronutrients				
N	NH ₄ NO ₃			
P	NaH ₂ PO ₄ .2H ₂ O	91.4	1.25	2.9
K	K ₂ SO ₄	40.3	1.25	0.3
Ca	CaCl ₂	71.4	1.25	1.0
Mg	MgSO ₄ .7H ₂ O	88.6	1.25	1.0
Micronutrients				
Mn	MnCl ₄ .4H ₂ O	1.5	1.25	0.01
Mo	(NH ₄) ₆ .Mo ₇ O ₂₄ .4H ₂ O	0.074	1.25	0.001
B	H ₃ BO ₃	0.934	1.25	0.2
Zn	ZnSO ₄ .7H ₂ O	0.035	1.25	0.0002
Cu	CUSO ₄ .5H ₂ O	0.031	1.25	0.0002
Fe	FeCl ₃ .6H ₂ O	7.7	1.25	0.04
Silica	Na ₂ SiO ₃	0.18	-	-

Growth media for plants

Distilled water is used to prepare stock solutions. In such a way that each of macronutrients were made individually, whereas micronutrients were dissolved separately along with addition of 50ml of conc. H₂SO₄ volume was made up to 1 litre. The micro and macronutrients were added in below proportion. The PH was then retained between 5.6 to 5.7 using NaCl/KCl and HCl in suitable amount [34].

Results

The various parameters of rice plants under different NaCl concentration and comparing it with that of control medium in two varieties were recorded.

Comparing average number of Seeds Germinated, Fresh weight, Radicle and Plumule length in control and different NaCl concentrations

Average number of seeds germinated in Punjab and swat rice varieties in 100mM, 200mM, and 300mM NaCl solutions were recorded. It was observed that in Swat Sella rice variety a greater number of seeds were

germinated among all 100mM, 200mM and 300mM solution of NaCl. However, Punjab Basmati rice variety show at least 2 seeds less than that are germinated in Swat Sella rice variety in all the 3-different solutions of NaCl. In control medium, it was observed that Punjab Basmati rice varieties were germinated in greater number (Figure 1). However, in control medium it was observed that Punjab showed greater number of seeds germination than that of Swat Sella rice variety (Figure 2).

The average weight of seedlings that were grown in control condition and in 100mM, 200mM and 300mM concentration of NaCl were recorded. The data showed that the average weight of Swat Sella rice variety was greater in all 3 solutions of NaCl in comparison to that of Punjab Basmati rice variety (Figure 3). While recording a data, it was observed both the parameter; plumule and radicle length of rice is affected due to salinity. The biomass of rice plant above the ground is almost same below the ground. It was seen that in Swat Sella rice variety the

average plumule length and radicle length is greater than the Punjab Basmati rice variety in 100mM, 200mM and 300mM of NaCl. Whereas, in control medium it was observed

that average length of radicle and plumule in Punjab Basmati rice variety is more than Swat Sella rice variety (Figure 4).

Figure 1. showing rice seeds germination in control and different NaCl concentrations

Figure 2. Comparing average No. of seeds germinated in control and different NaCl concentration.

Figure 3. Comparison of fresh weight of seedling in control and different NaCl concentrations

Figure 4. Comparing average length of radicle and plumule in control and different NaCl concentrations

Fresh weight and dry weight of rice plants in control medium and different concentration of NaCl

The fresh weights of different rice varieties in control medium showed that Swat Sella rice plant have an average weight of 0.7474 grams. Whereas, Punjab Basmati rice plant have an average weight of 0.9488 grams. According to the data, the average weight of fresh biomass in Punjab rice plants are greater as compare to Swat rice variety in control medium. Similarly, when these plants were dried it was seen that Punjab variety contains an average dry weight of 0.2562 grams which was greater than the

average dry weight of Swat variety that was 0.1982 grams (Figure 5).

The fresh weight of Swat variety plants was greater than the Punjab variety plants in 100mM, 200mM, and 300mM concentration of NaCl. However, both varieties show decrease in fresh weight of plants, when subjected to increased amount of NaCl. The dry weight of Swat Sella Rice variety was greater as compare to Punjab Basmati rice variety in 100mM, 200mM and 300mM concentration of NaCl. Dry weight of rice plants decrease with increase in concentration of salinity.

Figure 5. Comparing average fresh weight and dry weight in control and different concentration of NaCl

Comparing average root and shoot length in control and different NaCl concentration

The average root lengths of Punjab Basmati rice variety showed a length of about 11.10 cm which was greater in length from Swat Sella rice variety that was 10.28 cm in controlled medium. The shoot length of Punjab Basmati rice was also greater in length with 18.09 cm whereas, in Swat Sella rice variety, an average length was 16.11 cm in different concentration of NaCl i.e. 100mM, 200mM and 300mM, Swat rice plants showed a greater average length of both root as well as shoot in comparison to Punjab Basmati rice plants. It was observed that with increase in concentration of NaCl (Salinity stress) the average length of both root as well shoot of plants decreases (Figure 6).

In the present study, it was noticed that the average number of seeds germination decreases with increase in the amount of NaCl concentration (Figure 2). This decrease was almost 20% in Punjab Basmati rice variety, Similarly, Swat Sella rice variety show decrease 100mM, 200mM and 300mM. However, the Average number of germinated seeds of swat variety at each 100mM, 200mM and 300mM concentration

was greater than Punjab Basmati rice variety. This result showed that the swat Sella rice variety was more tolerant to salt stress. Whereas, the Punjab Basmati rice variety was more sensitive. This show agreement with those of Kaymakanova [35] who reported that all the plants cultivars show decrease in percentage of germination, seedling growth when treated with NaCl. The decrease in the average number of seed germination is probably because of the greater osmotic potential in the surrounding as compare to the osmotic potential inside the seeds because of which the seeds does not absorb water properly. It was found that under 100mM, 200mM and 300mM of NaCl stress condition the average fresh weight of seedling, average radicle length, average plumule length, average shoot length average root length and fresh weight as well as dry weight of seedling decreases with increase in the concentration of NaCl (salinity) as shown in (Figure 3 and 4). In Swat Sella rice variety, the fresh weight of seedling is greater as compare to Punjab Basmati rice variety. Similarly, average radicle lengths as well as plumula average lengths of seedling of Swat Sella rice variety are greater than Punjab Basmati rice variety.

Figure 6. Comparing average root and shoot length of different rice varieties in control and different concentration of NaCl

Figure 7. Showing plants germination as well as hydroponic cultivation of rice

Discussion

These results showed an agreement with the result of some researchers who specified that salinity stress cases decrease in growth i.e. fresh weight of seedling, radicle length of seedling and plumule length of seedling. Plant growth reduces, when the NaCl concentration is increased [36]. Salinity is most important environment factors that have critical influence on germination and plant establishment i.e. effect imbibition, germination and radicle elongation [37]. The

seedling growth i.e. roots length and shoot length is most sensitive to salt stress [38]. Seeds of most of species only germinate in lowest NaCl concentration [39]. Germination is strongly inhabited at higher salt concentration also the growth of young seedling is reduced, more often at high salt concentration [40]. Due to elevated level of NaCl salinity causes reduction, delay and sometime hinders germination due to osmotic effect or ionic toxicity [41]. The salt stress effect the plant by providing

hindrance for absorption of water due to greater osmotic potential out the seedling and lower osmotic potential inside the seedlings so because of which the lower quantity of water is absorbed by the seedling so decreasing the fresh weight of seedling. The Na^+ also effects the plant growth due to its toxicity but most of all it reacts to plant tissues. It causes osmotic stress which affect the plant by causing important water in plant tissue to be diverted due to which the tissues dry out loses its length and weight.

It was also found that in control medium the comparison of these two-variety showed that in Punjab Basmati rice variety greater amount of fresh weight of seedlings, plumule length, fresh weight of rice plant, root length, shoot length, and dry weight of plant as compare to that Swat variety which show greater length of radicle and seeds germination.

It can be concluded Punjab Basmati have greater ability to grow in non-saline condition. Whereas the Swat Sella variety is more genetically stronger, have a greater ability to tolerate a saline condition and grow best in saline condition Swat rice plant can adopt to this environment easily as compare to the Punjab Basmati rice variety. The different varieties of wheat show different responses to different condition however the changes also vary among same varieties [42].

Conclusion

It was concluded that salinity affects various growth parameters of the rice plants. The effects are more devastating at high concentrations. In a comparative account the Swat Sella rice variety was found to be better adapted to salinity as compared to Punjab variety.

Author's contributions

Designing and performing experiments: S Wali & I Ahmad, designed the experiment: F Tariq, A Ali & SIU Haq, Carried out research work: S Wali & F Tariq, Analysed

the data: I Ahmad & S Wali, Contribution material/tools analysed: I Ahmad & S Wali, Wrote paper: S Wali & F Tariq.

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