Research Article

Effects of sodium chloride on the growth parameters of *Brassica campestris*

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Abstract

The genus *Brassica* is an important agricultural and horticultural crop belongs to family *Brassicaceae*. *Brassica* species and varieties are commonly used for food include broccoli, cauliflower, cabbage, rutabaga, turnip and sometime used as production of canola oil and condiment mustard. Research work was conducted to investigate the effect of different concentrations of sodium chloride on the growth parameters of *Brassica campestris*. For this purpose, an experiment was carried out in triplicate in green house condition at the botanical garden of Islamia College Peshawar. The experimental design consisted of two major groups which were subdivided into each of four sub-groups. Each group pots were arranged in control and experimental soil (to which different concentration of NaCl were added). Group 1 was control (non-saline) and Exp-1, Exp-2 and Exp-3 were the groups to which 62.5 mM, 125 mM, 250 mM sodium chloride were added respectively. Group 2 was control (non-saline) and Exp-1, Exp-2 and Exp-3 have concentrations 375 mM, 500 mM and 625 mM respectively. Overall results showed that high and medium concentrations delay and suppressed the rate of germination, minimize the number of leaves, decrease shoot and root length while low concentration was not affected growth parameters. However, low concentration of NaCl showed no significant decrease in fresh weight, while NaCl medium concentration was decreased the fresh weight of selected plant, significantly as compare to high concentration. It is observed from current research work that *B. campestris* cannot tolerate high concentration of NaCl and damage under a high salt stress condition.

Keywords: *Brassica compestris*; Growth Parameters; NaCl; Salt Stress; Soil

Introduction

Salt stress is the agglomeration of excessive salt contents in the soil which eventually results in the inhibition of crop growth, agriculture productivity, and genetic potential and affects the plants such as ions toxicity, nutritional disorders, alternation of metabolic processes, oxidative stress,

genotoxicity, reduction of the cell division, membrane disorganization. On global scale, salt stress is more dangerous with respect to other toxic substance, 20% cultivated land affected by soil salinity due to impoverished soaking of water and used brackish [1]. About 10-35% of the world's agricultural is effected by salinity in each year. Although

avoid the water management practices, reclaim damaged land [2]. There is a high significant to better crop plants for salinity tolerance, but Brassica has a substantial to growth salt affected region. One of the most perspective is the improvement of salt sufferance of the cultivated species [3]. Salinity change the plant body of the wheat also disturb the metabolism, physiologic al and enzymatic process of the wheat plant [4]. Two basic stresses, osmotic and ionic stresses have been change the plants morphology as well as physiology. Osmotic stress result in inhibition of water uptake, cell expansion while ionic stress develops in excessive sodium cause leaf morality, chlorosis and necrosis [5]. Two basic approaches have been used, first technological approach for reclamation, drainage and irrigation of water, second one biological strategies which focus on development of high qualities of plants which tolerate high level of salts [6]. Growth analysis is one of the most popular treat to study the plant response toward an environmental stress. Genetically and physiologically salt tolerance is difficult among the verities of plants in halophytes and less tolerance plants [7]. Salinity affects photosynthesis by reducing the availability of CO₂, as a result of diffusion limitations and reduces the photosynthetic pigments. Excessive salt content decreasing stomatal and mesophyll conductance to CO₂, which can affect light absorbance [8]. Salinity is one of the major abiotic stresses around the world and causes greater losses to crop plants. The issue of salinity becomes more dangerous when plants are exposed to Cd stress [9]. Dominancy of the Brassica vegetables such as Chinese cabbage, broccoli, cabbage, cauliflower in terms of their nutrient quality for human diet and refined food has increased significant variation was detailed in seed germination between canola cultivars grown under salinity environment. Salinity tolerance is

also reported in species of canola *B. napus* and *B. campestris* [10]. Due to excessive salt contents soils result minimize crop loss, so scientists have searched those plant which have the potential to grow in high concentration of salt [11]. Saline soils expose plants to osmotic stress, due to which reactive oxygen species (ROS) product in large amounts that followed by oxidative damages, e.g., the destruction of proteins, lipids, pigments, and DNA [12]. Furthermore, salinity affects membrane permeability, enzyme kinetics chlorophyll, photosynthesis, stomatal conductance, concentration of ions and metabolite and nutrient uptake through competitive interaction by affecting the ion selectivity of membranes thus resulting reducing plant growth and development [13]. In several regions of the world, salinity stress may occur due to Na⁺ and Cl⁻ which may be greater than those of other salts present in the soil or water [14]. Salinity showed significant reduction at different stage of the species of *Ocimum basilicum*, *petroselinum hortense*, *sweet marjoram* and *thymus maroccanus* [15, 16].

Materials and Methods

Selection of plant

The *Brassica campestris* was selected for the experimental work which is an annual herb grow up to 1 m or higher.

Collection of plant seeds

The seeds of *Brassica campestris* were purchased from the Board Bazar Peshawar, Khyber Pakhtunkhwa, Pakistan. The seeds were cleaned by the same procedure followed by Nisa et al. [17] Nisa et al. [18], Nisa et al. [19], Nisa et al. [20], Nisa et al. [21], Malik et al. [22], Nisa et al. [23], and Gul et al. [24].

Experimental design

Total 24 pots were used in each experiment. These 24 pots were divided into two groups each groups consisted of 12 pots which were arranged on the basis of different concentrations of salt stress used in the experiment. Each experiment was conducted

in triplicate in the greenhouse condition at the botanical garden, Islamia College Peshawar. The experimental design consisted of two major group 1 and group 2, each of them were divided into four minor groups which were arranged in sequences line. The main groups (1, 2) were Control, and experiment 1, experiment 2, experiment 3 were the subgroups.

Seed sowing

240 seeds were used in research work. 120 seeds were sown in each major group. Ten seeds of *B. campestris* were sown in each pot of all groups.

Concentration of salt (NaCl) used in experiment

Different concentrations of sodium chloride stress were used in the experimental groups. No salt (NaCl) was used in control groups (Control 1), while 62.2 mM, 125 mM, 250 mM of NaCl was added in solution form to each one pot of rest of the groups i.e. Exp1, Exp2 and Exp3 respectively. No salt was used in control group (Control 2) while 375 mM, 500 mM and 625 mM of NaCl was added in solution form to each pot of rest of subgroups i.e. Exp 1, Exp 2 and Exp3 respectively.

Procedure for calculation

The procedure followed by Nisa *et al.* [17] Nisa *et al.* [18], Nisa *et al.* [19], Nisa *et al.* [20], Nisa *et al.* [21], Malik *et al.* [22], Nisa *et al.* [23], Gul *et al.* [24] was used for the solution preparation and taking replicates.

1Molar = weight in gram/1 liter of water

1M = 1000 mM solution

So, 1 Molar solution of NaCl = 1000 mM
solution of NaCl = 58.5g NaCl/ 1000 ml

Using the above formula for these three milli Molar solution were taken,

For 62.2 mM=3.65 g NaCl was dissolved in 1000 ml of distilled water, repeated this procedure for three time and added these solutions in each pot of the experimental group 1 (major group 1).

For 125 mM=7.31 g NaCl was dissolved in 1000 ml of distilled water, repeated this procedure for three time and added these solutions in each pot of the experimental group 2 (major group 1).

For 250 mM=14.62 g NaCl was dissolved in 1000 ml of distilled water, repeated this procedure for three time and added these solutions in each pot of the experimental group 3 (major group 1).

For 375 mM=21.93 g NaCl was dissolved in 1000 ml of distilled water, repeated this procedure for three time and added these solutions in each pot of the experimental group 1(major group 2).

For 500 mM=29.25 g NaCl was dissolved in 1000 ml of distilled water, repeated this procedure for three time and added these solutions in each pot of experimental group 2 (major group 2).

For 625 mM=36.56 g NaCl was dissolved in 1000 ml of distilled water, repeated this procedure for three time and added these solutions in each pot of experimental group 3 (major group 2).

Effect of NaCl on the rate of seed germination

The seeds of *Brassica* plant were grown on 27 October, 2019 and give solution of NaCl stress to the experimental groups. After sowing the seeds, the data (rate of seed germination) was recorded with the interval of three or four days. There was a clear difference in the rate of seed germination in control group and those which were treated with sodium chloride stress.

Effect of NaCl on the plant length

The plant length of both control and treated groups (NaCl) were measured in centimeter as taken by Nisa *et al.* (2020, 2016). The plant length was considered from root to apex of the plant.

Effect of NaCl on the number of leaves

The numbers of leaves were counted in the plant grown in control group and those which

were treated with different concentration of sodium chloride stress.

Effect of NaCl on the Fresh Weight

When the *Brassica* plant was reached to maturation stage then were harvested. Measured the fresh weight of *Brassica* plant was determined in gram (g) by using digital balance and recorded the data in control group and experimental groups.

Results

The experiment was conducted in the Botanical Garden Department of Botany Islamia College, Peshawar to find out the effect of sodium chloride on the various growth parameter of *B. campestris*. The experimental period continued for about one month from seed sowing up to the maturity of the plants. During this period data regarding seed germination and growth were recorded. The main growth parameters of the selected plant in relation of the effects of salinity studied in the course of this research work are:

Effect of NaCl on the seed germination

The effect of used six different concentrations of sodium chloride stress on the seed germination. These six different concentrations of sodium chloride stress comprised into two major group no 1 and group No. 2. Each of them three different concentrations given to each one experimental groups. Group 2, (375 mM/ 21.93 g, 500 mM/ 29.25 g and 625 mM/ 36.56g) the effect of used three different concentrations of sodium chloride stress on the rate of seed germination was determined. After a few days the data recorded about germination and seedling growth were exhibited in control group and treated (experimental groups) with different concentrations of sodium chloride stress as shown in the (Fig. 1). Error bar shows that standard deviation which was found statistically significant. Maximum reduction in seed germination was observed at a high concentration of sodium chloride stress on

the soil (Exp 3) followed by (Exp 2) and (Exp 1) while maximum germination was found in untreated group (control group). Overall statistically significant difference was found in the rate of germination in the seeds grown in control and experimental groups using one-way ANOVA (at $P < 0.05$ and P value summary of ****).

The results of group 1 which indicate the effect of three different concentrations, 62.2 mM/ 3.65g, 125 mM/ 7.31g and 250mM/14.62g of sodium chloride is shown in (Fig. 2) on the rate of seed germination. Error bar shows that standard deviation which was found statistically significant. Maximum reduction in seed germination was found at a high concentration of sodium chloride (Exp3) followed by (Exp2) and (Exp1) with respect to non-salinity (control group). Maximum growth of seed germination was observed in control group with respect to experimental groups. Overall statistically significant different was found in rate of seed germination in the soil in control and treated groups (Experimental groups) using one-way ANOVA (at p value <0.05 and p value summary of ***)

Effect of NaCl on number of leave

After maturation of plants were harvested from groups of each pot and counted the number of leaves of each plant. Group 2, showed different data regarding the number of leave in treated (experimental group) and untreated group (control group) as shown in (Fig. 3). Standard deviations were found statistically significant. Maximum reduction in number of leaves was observed at a high concentration of sodium chloride (Exp3) followed by (Exp1) and slightly maximum number of leaves in (Exp2) with respect to other experimental groups. While maximum number of leaves was observed in untreated (control group). Overall statistically significant difference was found in number of leaves in control group and treated (experimental groups) using one-way

ANOVA (at P value <0.05 and P value summary ****).

Group no 1, (Fig. 4) shows statistically significant results. Maximum reduction in number of leaves was found at a high concentrations of sodium chloride (Exp3) followed by (Exp2) and (Exp1) while maximum number of leaves the in saline soil was observed in control group compared to experimental groups. Overall statistically significant difference was observed in control and experimental groups using one-way ANOVA (at P value <0.05 and P value summary **).

Effect of NaCl on plant length

At the time of harvesting, selected ten plants from each pots of each one groups. Group 1, which indicate the effect of three different concentrations of sodium chloride i.e 375 mM/ 21.93 g, 500 mM/ 29.25 g and 625 mM/ 36.56 g is shown in (Fig. 5). Maximum reduction in plant length was observed at a high concentrations of sodium chloride stress: the soil Exp 3 followed by Exp2 and Exp1 while maximum elongation of plant was found in non-saline soil. Overall statistically significant difference was found in untreated (control group) and treated (Experimental groups) using one-way ANOVA (at P value <0.05 and P value summary ****).

The (Fig. 6) shows the effect of three different concentrations of sodium chloride stress (group 1). Maximum reduction in plant elongation was observed at high concentrations of sodium chloride stress in saline soil Exp3, Exp2 and followed by Exp1 while maximum plant elongation was found in control group. Overall statistically significant differences were found in control and experimental groups. One-way ANOVA was used for statistical analysis (P value <0.05 and P value summary *).

Effect of NaCl on fresh weight of plant

Group 2, (the effect of three different concentration of sodium chloride stress i.e

375mM/21.93g, 500mM/29.25g and 625mM/36.56g) is shown in (Fig. 7). Maximum reduction in fresh weight of plant was observed at high concentrations of sodium chloride stress in Exp 3 followed by Exp1 and Exp2 while maximum fresh weight of plant was found in non-saline soil. Overall statistically significant difference was found in non-saline (control) and saline soil (experimental groups) using one-way ANOVA (at P value <0.05 and P value summary ****).

The results indicated by group 1 (the effect of three different concentrations of sodium chloride stress 62.2 mM, 125 mM and 250 mM) are presented in (Fig. 8). Maximum reduction in fresh weight of plant was observed at high concentrations of sodium chloride stress in Exp2 followed by Exp3 while maximum fresh weight of plant was found in Exp1 and non-saline soil (control group). Overall statistically significant difference was found in control and experimental groups 1 using one-way ANOVA (P value <0.05 and P value summary *).

Discussion

The effects of sodium chloride (NaCl) on the rate of seed germination of selected reēsearch plant are presented in (Fig. 1-8). Results showed that increased in concentration of sodium chloride declined the rate of seed germination. However, (b) lower concentration of NaCl has less effect on seed germination. Our results are in agreement with the findings of Midaoui *et al.* [25], who studied the effect of NaCl on sunflower germination (*Helianthus annuus*), the seed of three sunflower genotypes (Oro9, Flamme and Albena) were germinated in petri dishes placed in an incubator maintained at 28°C. Six NaCl concentrations (0, 50, 75, 100, 125 and 175 mM) were used to moisten seeds. Results showed a decline in seed germination of the three genotypes as NaCl concentration was increased in the medium. After six days

of incubation, Significant differences were observed between control and treatments. Similar experiment was also conducted by Chachar *et al.* [26], they studied the effect of NaCl on seed germination and seedling root growth of cotton (*Gossypium hirsutum* L.). In order to provide different degree of salt stress, cotton seeds were exposed to increasing salinity levels, 0 mm, 50 mm, 100mm, 150 mm and 200 mm. It was reported that seed germination was only slightly affected by an increase in salinity (in most cases the differences between treatment were non-significant). So it is evident that from the (Fig. 1) that high level of NaCl solution slightly affect and delayed the rate of germination therefore result findings presented by Zheng *et al.* [27], are in agreement with our findings. Salinity causes the inhibition on the plant growth due to specific ions toxicity. Salinity stress caused a significant increase in Na^+ while decreases in K^+ , as results K^+/Na^+ ratio is high. The excessive K^+/Na^+ ratio was harm full to the growth parameters of wheat plants. Our finding is similar to the results of Amira *et al.* [28]. Results showed that the increased in concentration of NaCl caused the maximum reduction in leaves number while low concentration not significantly cause decrease in number of leaves of selected plant. Sozharajan and Natarajan [29], also reported the effect of salt stress on plant growth and metabolism of bean plant (*Vicia faba* (L.)) No significant effect was observed in the number of leaves with low concentration, while a decrease was noticed, with higher concentrations. Furthermore,

results showed that the increased concentration of sodium chloride caused significant reduction in plant shoot and root elongation. Similar result was conducted by Tuncturk *et al.* [30]. He demonstrated that the length of maize observed from germination of the maize seeds under salinity in laboratory condition, after 14 days observed the data of maize which is significantly reduced the length under salt treatment. Root length also decreased with increase in level of NaCl. The effects of sodium chloride on the fresh weight of *Brassica campestris* showed that increased in concentration of sodium chloride decreased the fresh weight. But to smaller extent NaCl promote the fresh weight of *Brassica* with respect to high concentration of salinity. Similar experiment was conducted by Khan *et al.* [31] who worked on the effect of salinity stress on fresh weight and nutrient composition of some canola (*Brassica napus* L.) cultivars. For the 12 canola genotypes used in the salt treatments, the first marked symptomatic effect of toxic level NaCl for 150 mm dosage was reduction in the green parts weight and inhibition on the plant growth. According to our results, plant fresh weight was influenced significantly by the salt stress ($P < 0.01$) while low and higher concentration not significantly decreased the fresh weight of plant, but medium concentration (125mm) effect the fresh weight. These findings are not matched with the results of Khan *et al.* [31]. However, our findings similar to the results of Khan *et al.* [31] that dry weight of the rice plant are significantly reduce at a high concentration of sodium chloride.

Growth of brassica plant in control and NaCl treated soil

Figure 1. Effect of different concentrations of NaCl on rate of seed germination of *Brassica campestris*

Growth of brassica plant in control and NaCl treated soil

Figure 2. Effect of different concentration of NaCl on rate of germination of *Brassica campestris*

Growth of brassica plant in control and NaCl treated soil

Figure 3. Effect of different concentration of NaCl on number of leave of *Brassica comprestis*

Growth of brassica plant in control and NaCl treated soil

Figure 4. Effect of different concentrations of NaCl on number of leave of *Brassica campestris*
 Figure 5. Effect of different concentration of NaCl on length of *Brassica campestris*

Growth of brassica plant in control and NaCl treated soil

Figure 6. Effect of different concentrations of NaCl on the length of *Brassica campestris*

Growth of brassica plant in control and NaCl treated soil

Figure 7. Effect of different concentration on fresh weight of *Brassica campestris*

Growth of brassica plant in control and NaCl treated soil

Figure 8. Effect of different concentrations of NaCl on fresh weight of *Brassica campestris*

Conclusion

Based on the current research work It is concluded that high concentration of NaCl solution decreased the rate of germination in experimental groups than control group, but in the experimental groups rate of germination was not found significantly different, very high concentration of NaCl solution effect the number of leaves, plant length between experimental groups than low and medium concentration of NaCl as compared to the control group (no stress). NaCl affects the length of shoot and root of a plant, fresh and dry weight of the plant in experimental groups than control group. However low (62.5mM and 125mM) concentration of NaCl was not significantly reduction in the height of plant as compared to high (250mM, 375mM, 500mM and 625mM) concentration and NaCl affects the fresh weight of the plant in experimental groups with respect to control group. It is concluded that high content of sodium chloride in soil or water caused significantly reduction in germination and growth parameters of *Brassica campestris*.

Authors' contributions

Conceived and designed the experiments: MA Sajad & S Ahmad, Performed the experiments: Kainat, Analyzed the data: MA

Sajad & Kainat, Contributed materials/ analysis/ tools: Kainat, H Zafar, S Ahmad, W Haider, Mahnoor & MA Sajad, Wrote the paper: Kainat, ZU Nisa & MA Sajad.

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