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Research Article

SALT INDUCED CHANGES IN GROWTH, PHOTOSYNTHETIC PIGMENTS AND TOTAL CARBOHYDRATE CONTENT IN TWO *BRASSICA JUNCEA* CULTIVARS (L.)

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ABSTRACT

Soil salinity is a serious threat for the production of crops in the major areas of the cultivated land. Two cultivars of *Brassica juncea* L. cv. RH-30 and Laxmi were procured from Indian Agricultural Research Institute, New Delhi, India. Growth was analyzed in the presence of salinity (NaCl) stress having concentrations 50 mM, 100 mM and 150 mM. All treatments of NaCl decreased seed germination, seedling growth, root and shoot length and fresh and dry weight. Salinity stress also affected photosynthetic pigments (chl a,chl b,carotenoids) and total carbohydrate contents. Maximum reduction was observed at 150mM concentration. RH-30 cultivar was found to be more tolerant than Laxmi with response to the parameters studied.

INTRODUCTION

Stress is defined as an external factor that negatively effects growth and development of plants. In developing countries, a large number of factors cause a consequential decline in crop productivity and hence uncertainty to provide adequate food supply to the increasing population. Of them, less availability of agricultural land, exhaustion of fresh water resources and low economic activity in agricultural sector are the most significant elements. Plants are unprotected to several abiotic and biotic stresses, during their period of growth and development that limits their yield and production. Salinity stress has emerged as one of the most serious threat that leads to significant reduction in arid and semi-arid regions across the world (Hasanuzzaman, 2013). Salinity is one of the eminent abiotic stresses in arid and semi-arid regions that diminish the average yield of major crops by more than 50% (Bray *et al.,* 2000). It is the main limiting factor in agricultural productivity in semi-arid and arid regions of the world (Horie, 2004).Profusion accumulations of Na^+ and Cl^+ in plant cells has minimized the absorption of many crucial minerals and responsible for ionic imbalance, specific ion toxicity, osmotic and oxidative stress (Li *et al.*, 2010) that adversely affecting the morphological, physiological and biochemical processes of the plant (Munns, 2005). Saline conditions reduces the internal CO_2 pressure and stomatal opening (Chaves *et al.,*2009) that is responsible for decline in diffusion of gases ultimately affecting the photosynthetic rate (Wani *et al.,*2013).Salinity affects seed germination, seedling growth, root/shoot elongation and dry matter accumulation (Mishra, 1996). Ion cytotoxicity and osmotic stress are the negative impact of salinity on plant growth (Hussain *et al.,* 2008). Under saline conditions, toxicity of ions and osmotic stress leads to generation of oxidative stress (Zhu, 2007). Lipids are major components in membrane which has main aspect in plant cell resistance in proportional to salt stress. Disorganization in membrane lipids and proteins increased under salinity stress (Yordanov *et al.,* 2003). Under condition of salinity stress main changes were observed in lipids metabolism and lipid peroxidation as they increased with higher level of salinity stress (Rahdari *et al.,*2012) which had a relation with plants such as Wheat(Hala *et al.,*2005), Tomato (Neumann, 2001) and Purslane (Yazici,2007). Mustard, a plant within Brassicaceae family, is an economically important oilseed crop extensively grown under broad range of agroclimatic zones all over the world. India contributes nearly 27.8% of total edible oil production being the second largest producer in the world (Shekhawat *et al.,* 2012). Among *Brassica* sp., *Brassica juncea* contributes approx. 85% of total mustard oilseed production (Yadava *et al.,* 2012). However, this production still inadequate to meet the demands of increasing population (Khan *et al.,*2012). It is cultivated mainly in North-West climatic zone

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where soil salinity is the major issue that decreases its production (Sharma *et al.,* 2013). Increasing mustard production under salinity stress has attracted the attention of many investigators (Nazir *et al.,* 2001). Therefore a field experiment was conducted on two cultivars of *Brassica juncea* grown under salinity stress to study the growth and physiological parameters.

MATERIALS & METHODS

Plant material and treatments

The plant material in our study was *Brassica juncea* Cv. RH-30 and Laxmi were selected to assess the effect of salinity stress on growth and physiological changes in relation to phytotoxic effect of salinity stress. The authentic and healthy seeds of *B. juncea* were procured from Indian Agricultural Research Institute, New Delhi, India. Before sowing the seeds were surface sterilized with dilute solution of sodium hypo chlorite to prevent any fungal contamination and then rinsed three times with distilled water. 15 seeds were sown in per pot. The seeds were sown in five sets in an earthen pot containing equal quantities (4kg) of loamy sand soil in Herbal Garden, Maharshi Dayanand University, Rohtak. Salt treatment of NaCl was prepared using sodium chloride salt in concentrations of 50,100 and 150mM in soil, leaving one set as a control. Pots were amended with the recommended dose of fertilizers at the time of sowing. Thinning was done on the 7th day after sowing (DAS). Irrigation was done with tap water as and when required. The samples were taken from four week old seedlings for physiological analysis.

Effect on seed germination and growth rate Germination percentage

Seed germination was recorded daily up to 12 days after the initial day of the experiment. Seeds were considered as germinated when the radical reached a length of 1 mm (Kabir *et al;* 2008) and the germination percentage was calculated as per the following formula:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Root and shoot length

Length of root and shoot was measured with the help of a scale and reading was taken from both treated and controlled plants.

Fresh and dry weight

Plants were removed along with the soil and dipped in a bucket, filled with tap water, to remove the adhering soil particle, ensuring the safety of the roots. The plants were blotted and weighed to record their fresh mass and then placed in an oven, run at 80 ºC, for 24 hrs. The samples were weighed again after allowing them to cool to room temperature to record their dry weight.

Soil pH

The pH of the soil was determined by dissolving soil in water and soil mixture to set until the soil settles to the bottom and the water is clear. Insert the litmus paper into the water. If the litmus paper turns red, the soil is acidic; if it turns blue, the soil is alkaline.

To estimate the photosynthetic pigments

Amount of leaf sample used for extraction depended upon the availability and other requirements which ranged from 100-500 mg. The chlorophylls and carotenoids were extracted by the method of (Arnon, 1949). For extraction, 250 mg of plant sample was homogenized with about 10 ml of ice cold 80% acetone (AR Grade). A pinch of $CaCO_3$ was added to avoid the destruction of chlorophylls and other pigments. Extraction has to be carried out under dim light to avoid photoxidation of the pigments. It was centrifuged in a Remi centrifuge at low speed (5000 rpm) for about 20 min. Ice cold pastle and mortar were used for grinding the samples. After centrifugation cell wall debris were settled down and was discarded. Only supernatant was taken out and raised to a specific volume of 10 ml with ice cold 80% acetone. Absorbance was recorded soon after extraction was over with the help of UV-Vis spectrophotometer (Specord-205 Analytik Jena, Germany) at wavelengths of 663, 645, 510 and 480 nm. The amount of total chlorophylls and carotenoids were estimated. Pigments were calculated in terms of mg/g plant tissue on fresh weight.

Chlorophyll a = 12.7(A663) - 2.69(A645×V/1000×10 (mg g^{-1} fr. wt.)
Chlorophyll b =22.9(A645) - 4.68(A663) ×V/1000×10 (mg g^{-1} fr. wt.)
Total Chlorophyll = Chlorophyll a + Chlorophyll b (mg g^{-1} fr. wt.)
Caretonoids = 7.6(A480) - 1.49(A510) ×V/1000×10 (mg g^{-1} fr. wt.)

Where
A = Absorbance of specific wavelength.
V = Final volume of chlorophyll and caretonoids in 80% acetone.
W = Fresh weight of tissue extract.

Total soluble carbohydrates

Total soluble carbohydrates were determined using anthrone reagent by the method (Yemm and Willis, 1954). 100 mg fresh samples of leaves were homogenized separately in 80% ethanol using acid washed sand as an abrasive. The homogenate was refluxed volume made to 80% ethanol. The extract so obtained was used for estimation of Total Soluble Carbohydrates.

Anthrone reagent: - Anthron reagent was prepared by dissolving 0.4 g anthrone in 100 ml concentrated H_2SO_4.

An aliquot from the above extract measuring 0.2 ml was evaporated to dryness in a test tube in a boiling water bath. On cooling the residue left in the tube was dissolved in 1 ml of distilled water and mixed with 4.0 ml of anthrone reagent. The mixture was heated in a water bath for 10 minutes. After cooling, absorbance was recorded at 620 nm using spectrophotometer.

RESULTS

Effect of NaCl on seed germination

Effect of different conc. of NaCl (50,100,150 mg) on seed germination was evaluated in two different cultivars of *Brassica juncea.* The effect of NaCl on seed germination and seedling growth in two varieties of *Brassica* is presented in Fig.

1 Result showed that plants were more sensitive at high concentrations and the maximum reduction was found in Laxmi variety at 150mM concentration of NaCl. Figure 1 clearly shows that plants exhibited a marked decrease in percentage of germination. With increase in salinity stress germination rate was also declined.

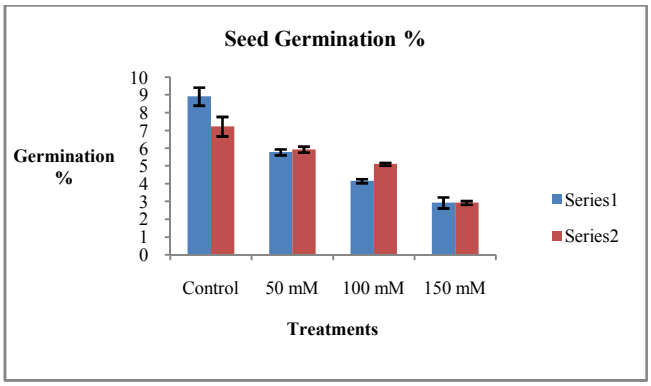

Figure 1 Effect of different concentrations of NaCl (50 mM, 100 mM and 150 mM) on germination percentage of two *Brassica juncea* cultivars at 30 DAS.Bars represents the standard errors

Effect of NaCl on root and shoot length

Decrease in root and shoot length with increasing concentrations of NaCl. Shoot length was found to be more affected than root length and again plants were more affected by NaCl in both the two varieties. RH-30 variety was more affected as compared to Laxmi variety as the concentration of NaCl was increased.

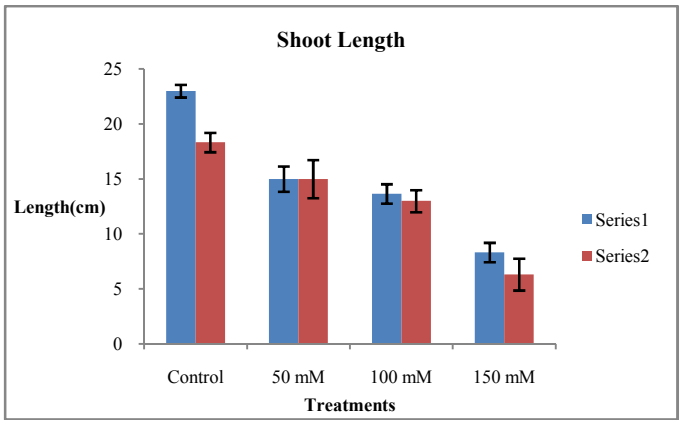

Figure 2 Effect of different concentrations of NaCl (50 mM, 100 mM and 150 mM) on shoot length of two *Brassica juncea* cultivars at 30 DAS. Bars represent the standard errors.

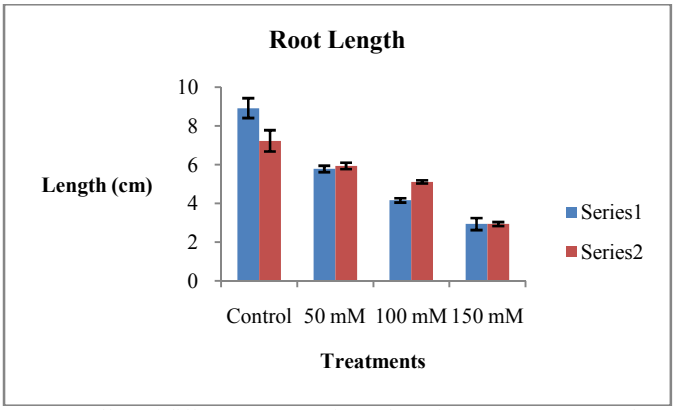

Figure 3 Effect of different concentrations of NaCl (50 mM, 100 mM and 150 mM) on root length of two *Brassica juncea* cultivars at 30 DAS. Bars represent the standard errors.

Effect of NaCl on fresh and dry weight

An increase in different concentrations of NaCl decreased the fresh and dry weight of the plants. As we increase the concentration of NaCl, weight was decreased. Maximum effect was shown at 150mM NaCl concentration as clearly shows that plants exhibited a marked decrease in biomass due to toxic effects of NaCl salt.

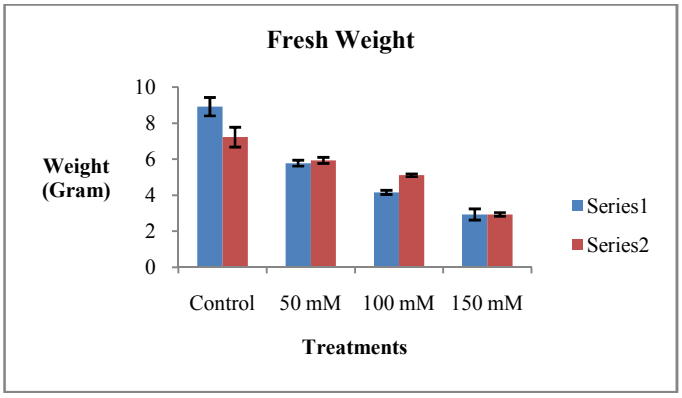

Figure 4 Effect of different concentrations of NaCl (50 mM, 100 mM and 150 mM) on fresh weight of two *Brassica juncea* cultivars at 30 DAS. Bars represent the standard errors.

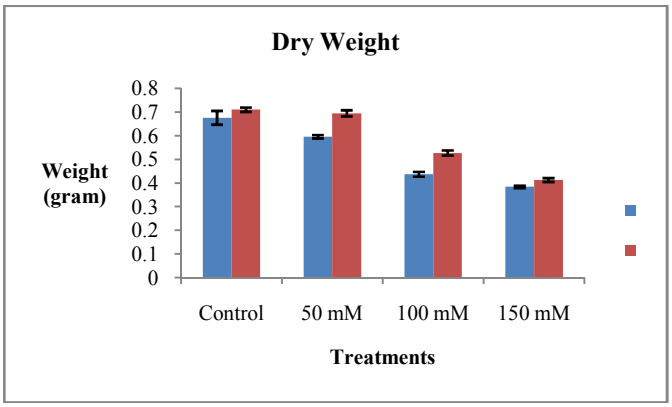

Figure 5 Effect of different concentrations of NaCl (50 mM, 100 mM and 150 mM) on dry weight of two *Brassica juncea* cultivars at 30 DAS. Bars represent the standard errors

Effect of NaCl on Soil pH

With increase in NaCl concentration, pH of the soil decreases which reduces the growth of brassica.

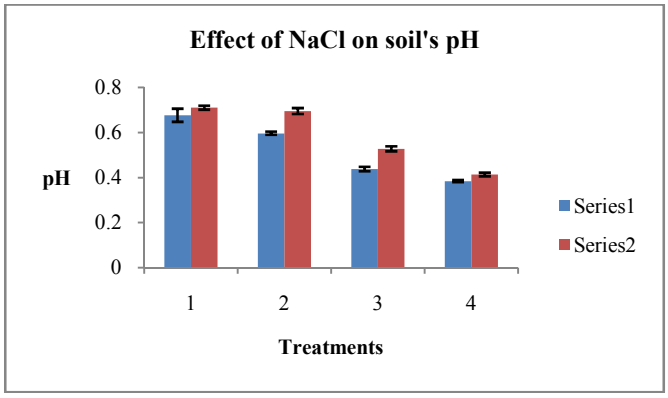

Figure 6 Effect of different concentrations of NaCl (50 mM, 100 mM and 150 mM) on soil's pH of two Brassica juncea cultivars at 30 DAS. Bars represent the standard errors

Effect of NaCl on Photosynthetic Pigments

Different concentrations of NaCl affected the chlorophyll amount of leaves. The reduction of chlorophyll *a* and *b* contents of leaves was detected with enhanced NaCl accumulation in leaves. Light-induced chlorophyll accumulation was inhibited by increasing the concentration of NaCl. Under salt stress, the chlorophyll *a* content of leaves was more affected than the chlorophyll *b* content. Total chlorophyll content of the leaves decreased significantly with increased the concentration of NaCl .The 150mM NaCl concentration caused the maximum decrease of Chlorophyll amounts as compared to all others. The highest carotenoid content was measured in control plants and it decreased with increasing salt concentration.

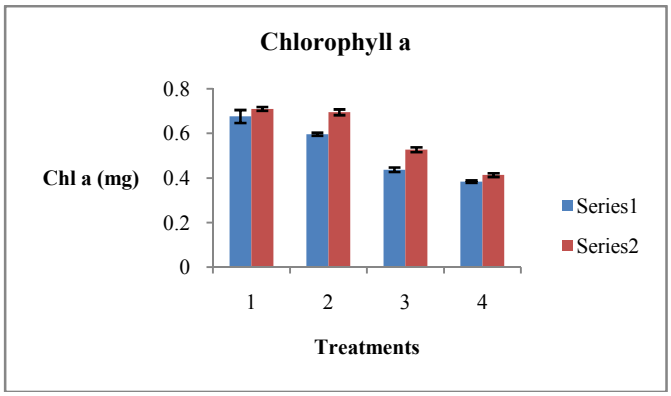

Figure 7 Effect of different concentrations of NaCl (50 mM, 100 mM and 150 mM) on Chlorophyll a of two *Brassica juncea* cultivars at 30 DAS. Bars represent the standard errors

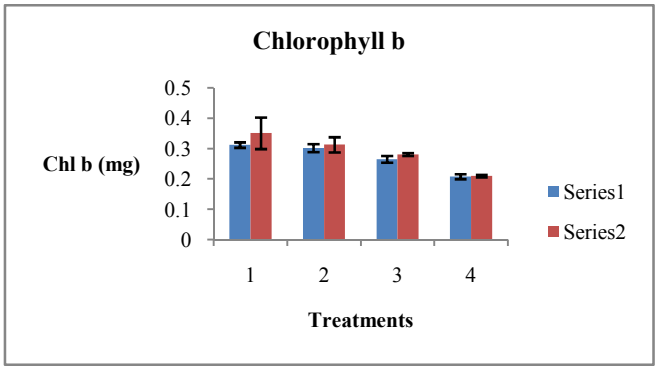

Figure 8 Effect of different concentrations of NaCl (50 mM, 100 mM and 150 mM) on chlorophyll b of two *Brassica juncea* cultivars at 30 DAS. Bars represent the standard errors

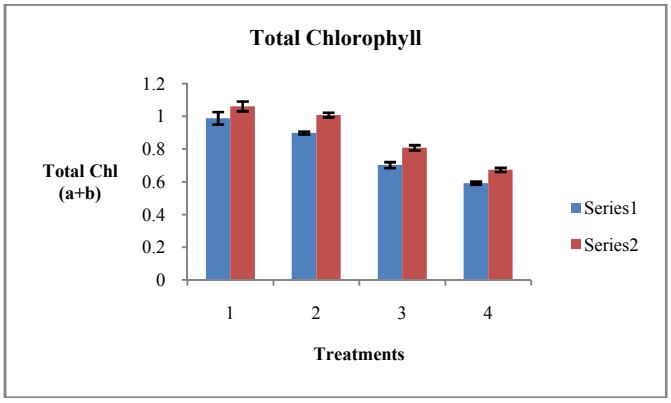

Figure 9 Effect of different concentrations of NaCl (50 mM, 100 mM and 150 mM) on total chlorophyll of two *Brassica juncea* cultivars at 30 DAS. Bars represent the standard errors

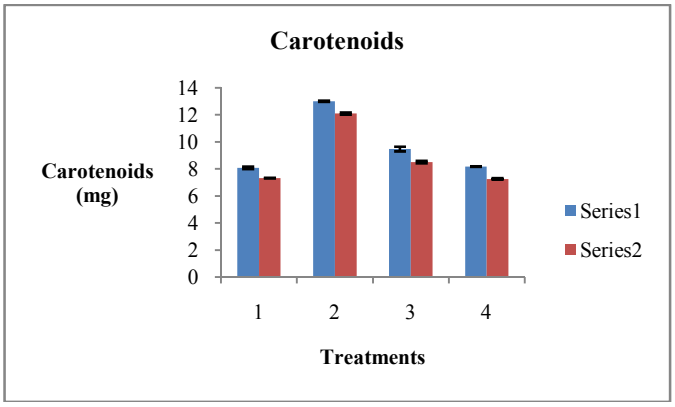

Figure 10 Effect of different concentrations of NaCl (50 mM, 100 mM and 150 mM) on carotenoid content of two *Brassica juncea* cultivars at 30 DAS. Bars represent the standard errors

Effect of NaCl concen. on Carbohydrate content

NaCl treatment affects the carbohydrate amount of leaves. The decrease in carbohydrate contents of leaves was detected with enhanced NaCl accumulation in leaves. Total Carbohydrate content of the leaves decreased significantly with increasing NaCl concentration. The NaCl concentration of 150mM caused the maximum decrease of carbohydrate amounts.

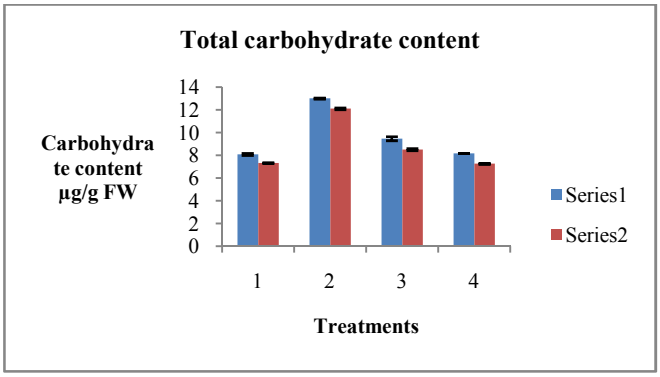

Figure 11 Effect of different concentrations of NaCl (50 mM, 100 mM and 150 mM) on total carbohydrate content of two *Brassica juncea* cultivars at 30 DAS. Bars represent the standard errors

DISCUSSION

The extent of plant injury by elevated concentration is specific and strongly depends on the environmental condition. In the present study we took the two varieties of *Brassica* RH-30 and Laxmi. These two varieties were grown under NaCl stress. The NaCl affects various parameters of *Brassica* at different concentrations of NaCl. When we increase the concentration of NaCl it inhibits seed germination and seedling growth. Lower concentration of NaCl showed less effect on seed germination whereas higher concentration of NaCl showed deleterious effects on seed germination.

Figure 1 indicated that seed germination % decreases with increase in NaCl concentration. Maximum reduction of seed germination observed in 150mM NaCl concentration. As salinity stress adversely affects the germination parameters. Based on experimental study we found that Laxmi is more affected by salt stress as compared to RH-30 variety.

Figure 2 indicated that as the toxic effect of incerease NaCl conc. the growth of shoot decline. Shoot length was found to be more affected than root length. Shoot length showed a

significant decrease in Laxmi when compared to RH-30. Table no.4 indicated that fresh weight/dry weight also decreases with increase in NaCl concentration, showing the harmful effects of salinity on growth parameters. Laxmi showed more decrease in fresh weight/dry weight ratio as compared to RH-30. Figure no.9 total Chlorophyll content in leaves of *Brassica* seedling decreased significantly with increase in NaCl concentration maximum reduction in chlorophyll content was noted in 150 mg Kg^{-1} NaCl treated seed. In the present study chlorophyll content decrease with increase in NaCl concentration which might be due deterioration of chlorophyll pigment that eventually lead to decrease in photosynthetic efficiency in plants and ultimate reduction in plant growth. It showed that toxic effect of NaCl was reflected by reduction in growth of parameters and photosynthetic pigments. Result has shown that chlorophyll content decreases more in case of Laxmi than RH-30.

Figure 11 showed that carbohydrate content of *Brassica* increase at 50 mg/kg NaCl concentration. After that it reduces with inc. in NaCl concentration. Maximum reduction in carbohydrate content was noted in 150mM NaCl treated seeds in *Brassica* variety Laxmi. Carbohydrate content increases more in RH-30 as compared to Laxmi variety. Reduction in biomass and yield components might be due to direct toxic effects of NaCl on biochemistry and physiological processes in the *Brassica* plant.

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