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Influence of Seed Priming with $ZnSO_4$ and $CuSO_4$ on Germination and Seedling Growth of *Brassica rapa* under Nacl Stress

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Abstract: Abiotic stress is an important environmental problem limiting crop growth and productivity. Micronutrients have ability to overcome stress up to some extent. This study was carried out to investigate the effects of priming with micronutrients (Zn and Cu) on *Brassica rapa* grown under NaCl stress. Seeds were primed with 100 and 200 ppm $ZnSO_4$ and $CuSO_4$ and then treated with different concentrations (0 mM, 60 mM, 90 mM, 120 mM) of NaCl. Effect of NaCl, $ZnSO_4$ and $CuSO_4$ were observed on germination, seedling growth separately. It was observed that germination and different seedling growth parameters viz. shoot length, root length, fresh and dry biomass were decreased under different concentrations of NaCl (without treatment of $ZnSO_4$ and $CuSO_4$). It was also observed that treatment with different doses of $ZnSO_4$ and $CuSO_4$ exhibited increase in germination and seedling growth in both salinity levels as well as in non-saline conditions.

Key words: *Brassica rapa* · Micronutrients · Salinity · Seedling · Germination

INTRODUCTION

Brassica is a genus of plants in the mustard family (Brassicaceae). *Brassica rapa* sub sp. rapa) is a root vegetable commonly grown in temperate climates. The purpose of priming is increasing germination percent, decreasing mean of germination time and improving growth and vigor of seedling at very wide favor and unfavored environmental conditions. This method is successful in small seed plants and the most medicinal plants that have great economic value with quick and uniform emergence requirement (Cutforth *et al.*) [1].

Salt is a natural element of soils and water. The ions responsible for salinization are: Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-. As the Na^+ (sodium) predominates, soils can become sodic. Salinity stress delays this process even though there may be no difference in the percentage of germinated seeds from one treatment to another (Maas and Poss, ,) [2]. According to Romero *et al.* [3] increase of salt in the root medium can lead to a decrease in leaf water potential and affect many plant processes. The relation between salinity and mineral nutrition of crops are very complex (Grattan and Grieve) [4]. Salinity with an adequate supply of calcium reduces shoot growth, particularly leaf area, more than root growth (Läuchli and Epstein) [5]. Reduction in shoot growth due to salinity is commonly expressed by a reduced leaf area and stunted shoots (Läuchli and Epstein) [5].

MATERIALS AND METHODS

The experiments were carried out to investigate the effect of seed priming with $CuSO_4$ and $ZnSO_4$ under different NaCl levels in Plant Physiology Laboratory and Botanical Garden of Abdul Wali Khan University, Mardan, Pakistan.

Experimental Design:

Source of Seeds: Seeds of *Brassica rapa* were collected from Agricultural Research Institute, Tarnab Farm, Peshawar.

Seeds were surface sterilized with 70% ethanol for 30 seconds and then washed with distilled water.

Germination response was studied on different levels of NaCl salinity Control, 60 mM (1.4 dS/m), 90 mM (2.1 dS/m) and 120 mM (3.2 dS/m), $ZnSO_4$ (100 and 200 ppm) and $CuSO_4$ (100 and 200 ppm) replicating each concentration three times. 60 petri dishes were prepared

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lined with filter paper and moistened with 3ml water/solution. All plates were kept in incubator at 27°C for germination which was started after 36 hrs (seeds were considered to be germinated with the emergence of the radicle). The germinated seeds were counted after every 24 hrs. Continuous increase was observed in germination percentage and after 7 days of seed germination, germination percentage, shoot and root lengths, fresh and dry biomass of seedlings were recorded.

RESULTS AND DISSCUSSION

Soil salinity affects the plant growth by several physiological and biochemical means. In the present study effects of salinity and seed priming was investigated on germination growth, vegetative and biochemical parameters of *Brassica rapa* plant. In the literature, dealing with seed viability, there are a number of reports that hydration-dehydration treatments as well as treatments of seeds with chemicals can favorably influence the viability status of seeds (Kanp and Bhattacharjee, ,; Sengupta *et al.*; Pati and Bhattacharjee,, Mondal *et al.*) [6-9]. In our study enhancement of seed germination percentage and the other parameters i.e. growth parameters, biochemical, electrolyte leakage, leaf water loss and relative water content showed the beneficial effect of seeds pretreatment with the selected micronutrients grown under normal and stressed condition.

NaCl showed non-significant reduction in germination percentage in *Brassica rapa* seeds, except 60mM NaCl which do not show any response to salinity in this parameter. Similar results were reported by Sheoan and Garo [10] that germination percentage reduced among different pea cultivars when salinity concentrations increased from 0 to 20 mmohs cm^{-1}. There are many reports which are in agreement with the present findings indicating that salinity stress severely reduces seed germination and early seedling growth in safflower Kaya *et al.,* Khodadad *et al*. [11-12] and other species such as triticale Atak *et al.* [13] wheat CR Hampson *et al*. [14] and chickpea B Murillo-Amador *et al*. [15].

Seeds primed with $ZnSO_4$ (100ppm) showed non-significant reduction in non saline as well as in saline medium, except 60mM which do not show any response in this parameter as compared to control.

Seeds primed with 200ppm $ZnSO_4$ showed significant ($P<0.05$) reduction in germination percentage in different concentrations of NaCl as compared to control. A comparison between these two concentrations showed that 200ppm exhibited high germination percentage as compared to 100ppm. The decrease in seed germination of cluster bean due to zinc treatment is in conformity with our findings (Mahalakshmi,; Mahmood) [16-17].

When seeds primed with $CuSO_4$ (100ppm and 200ppm) both treatments did not show any response in different concentrations of NaCl as well as in control plants. Arshad [18] investigated effects of different seed priming on chickpea and reported that priming with solutions of copper sulfate 1% for 4 h will enhance germination by 17.1% as compared to untreated seeds. Dey Sumanta *et al*.[19] also observed similar effects in two photosensitive varieties of rice.

Different concentrations of NaCl showed non-significant reduction in seedling shoot length of *Brassica rapa* plant. Present results are in accordance with observation of Zaiter and Mahfouz [20] who noted a decrease in shoot length of common and tepary beens under hydroponic solution and sandculture. Seeds primed with 100ppm $ZnSO_4$ showed significant ($P<0.05$) reduction in this parameter while seeds primed with 200 ppm $ZnSO_4$ also showed significant ($P<0.05$) increase in this parameter as compared to respective control. A comparison between these two concentrations 100 and 200ppm showed that 200ppm showed more promotion as compared to 100ppm and control group. These results are in agreement with the results obtained by Nematollahi *et al*.[21] on cumin and also Amjad *et al*.[22] who indicated that halo-priming and osmo-priming results an increase in the stem length of hot pepper seedling. These results are in agreement with the results obtained by Nematollahi *et al*.[21] on cumin and also Amjad *et al*.[22] who indicated that halo-priming and osmo-priming results an increase in the stem length of hot pepper seedling.

Seeds primed with 100ppm $CuSO_4$ showed significant ($P<0.05$) reduction in this parameter, while seeds primed with $CuSO_4$ (200ppm) also showed significant ($P<0.05$) increase in seedling shoot length as compared to their respective control. Comparison between these two concentrations 100 and 200ppm and control group showed that 200ppm exhibited more promotion as compared to 100ppm and control group. These results are also observed by Palma *et al*. [23] in pea plant.

Different concentrations of NaCl showed Non-significant reduction in seedling root length of *Brassica rapa* plant. The findings of present study positively correlate with the findings of Harris *et al* .[24] on chickpea and wheat plants. Gulzar *et al*. [25] reported a decrease in the growth of *Urochondra setulosa* (Trin.)

Table 1: Effect of seed priming with different doses of $ZnSO_4$ and $CuSO_4$ on Germination percentage of *Brassica rapa* germinated under different levels of NaCl.

Treatment	Control	100ppm $ZnSO_4$	200ppm $ZnSO_4$	100ppm $CuSO_4$	200ppm $CuSO_4$
Control					
Mean	100a	96.666a	100a	100.000	100.000
SE	+0.000	+3.333	+0.000	+0.000	+0.000
60mM NaCl					
Mean	100a	96.666a	90b	100.000	100.000
SE	+0.000	+3.333	+0.000	+0.000	+0.00
	(+0)	(+0)	(+10)	(+0)	(+0)
90mM NaCl					
Mean	90a	90a	90b	100.000	100.000
SE	+10.000	+5.773	+0.000	+0.000	+0.000
	(-10)	(-6.896)	(-10)	(+0)	(+0)
120mM NaCl					
Mean	93.333a	76.666a	93.333b	100.000	100.000
SE	+6.666	+14.529	+3.333	+0.000	+0.000
	(-6.666)	(-20.896)	(-6.667)	(0)	(+0)
$LSD_{0.05}$	19.597	26.627	5.430	0.000	0.000

Mean followed by different letters in the same columns differ significantly at 95% probability level, Values in parenthesis indicates % promotion (+) and reduction (-) over control.

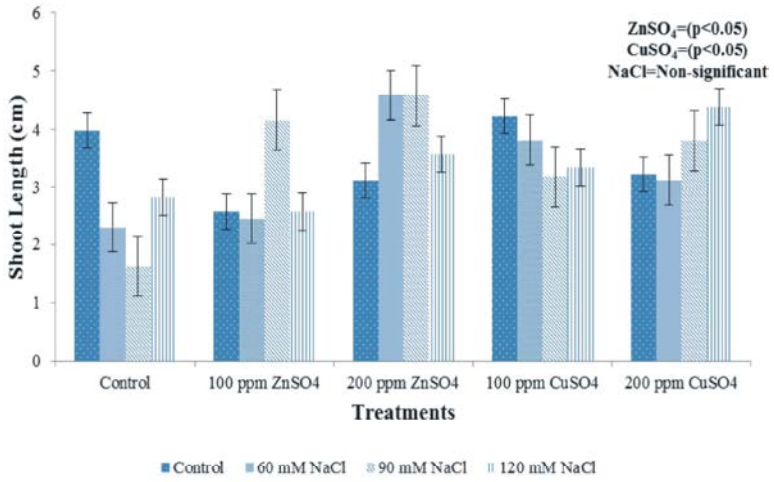

Fig. 1: Effect of seed priming with different doses of $ZnSO_4$ and $CuSO_4$ on seedling shoot length of *Brassica rapa* grown under different levels of NaCl.

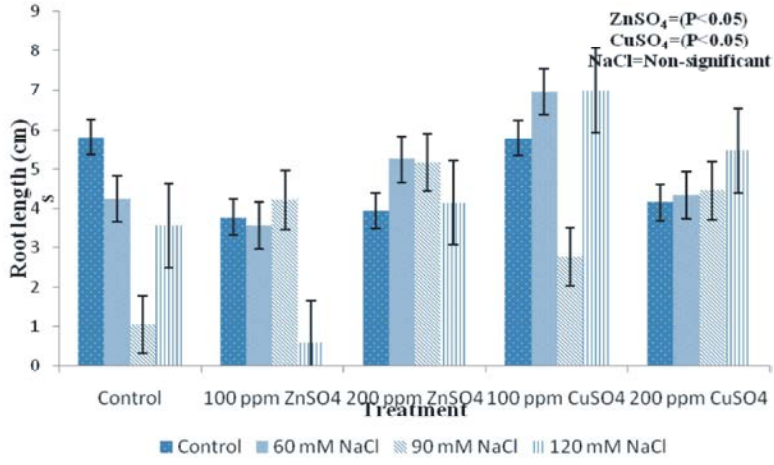

Fig. 2: Effect of seed priming with different doses of $ZnSO_4$ and $CuSO_4$ on seedling root length of *Brassica rapa* grown under different levels of NaCl

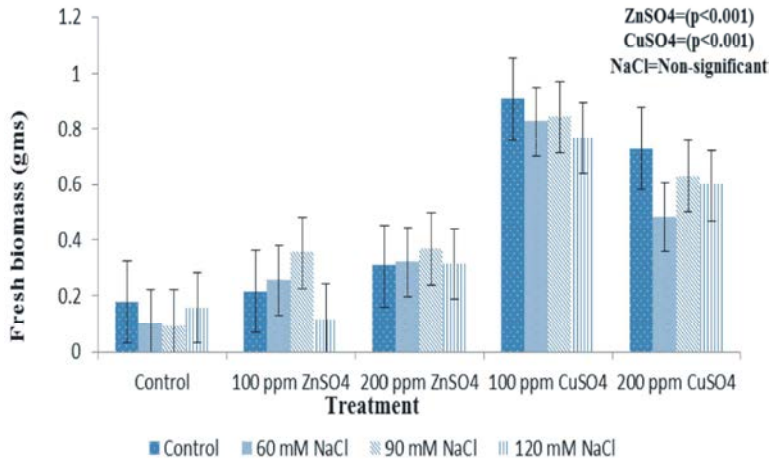

Fig. 3: Effect of seed priming with different doses of $ZnSO_4$ and $CuSO_4$ on seedling fresh biomass of *Brassica rapa* grown under different levels of NaCl.

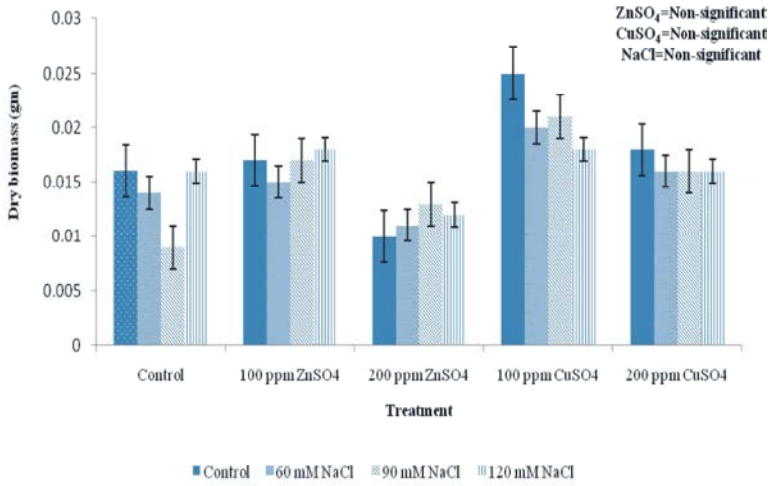

Fig. 4: Effect of seed priming with different doses of $ZnSO_4$ and $CuSO_4$ on seedling dry biomass of *Brassica rapa* grown under different levels of NaCl.

under the influence of salinity and a significant ($P < 0.05$) inhibition of root length, shoot length, number of tillers and number of leaves at NaCl salinity level above 200mM. High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant (Werner *et al*.[26] may be another reason for this decrease. Neumann [27] indicated that salinity can rapidly inhibit root growth and hence capacity of water uptake and essential mineral nutrition from soil. Our results were also similar with the findings of Hussain and Rehman [28] who found that the roots of seedlings were more sensitive than the shoots.

Different concentrations of NaCl showed non-significant reduction in seedling fresh biomass of *Brassica rapa* plant. Our results are in agreement with the previous report of Sharma *et al* .[29]. In general, seedling shoot fresh weight (SFW) was more affected by salt than seedling root fresh weight (RFW). This is in accordance with previous research reports in wheat and triticale genotypes (Shalaby *et al.)* and sugar beet, cabbage, amaranth and pak-choi (Jamil *et al*.) [30-31].

Seeds primed with 100ppm $ZnSO_4$ showed significant ($P<0.001$) reduction in this parameter in saline medium while seeds primed with 200ppm $ZnSO_4$ showed significant ($P<0.001$) increase in saline media as compared to 200ppm and control. A comparison between these two concentrations 100 and 200ppm and control group showed that 100ppm showed more reduction as compared to 200ppm. Similar effect was observed by Nada [32] in *Paulonia Tomentosa (*Thunb*).*

When seeds primed with (100 and 200ppm) $CuSO_4$ showed significant ($P<0.001$) reduction in this parameter as compared to their respective control. Comparison between these two concentrations of $CuSO_4$ (100 and 200ppm) and control group showed that 100ppm exhibited better result as compared to 200ppm and control group. The fresh and dry biomass of wheat slightly decreased with copper concentration (Singh *et al.)* [33].

Different concentrations of NaCl showed non-significant decrease in seedling dry biomass of *Brassica rapa* plant and agrees with previous findings in rice (Shannon *et al.)*, *Phaseolus* species (Bayuelo-Jimenez *et al.)*, wheat (Afzal *et al.), Catharanthus roseus* (Jaleel *et al.*) [34-37].

Seeds primed with 100ppm and 200ppm $ZnSO_4$ showed Non-significant promotion in this parameter as compared to their respective control. Comparison between these two concentrations 100ppm and 200ppm showed that 100ppm exhibited better results as compared to 200ppm and control group in this parameter. Already increase in plant biomass was recorded in tomato (Ejaz et al.), sunflower (Khan *et al.*), cucumber (Sudhan and Shakila), rice,corn, dry bean and soybean with Zn application (Fageria *et al*.) [38-41].

When seeds primed with 100ppm and 200ppm $CuSO_4$ showed Non-significant reduction in this parameter as compared to their respective control. Comparison between these two concentrations. Similar observations in crops had been observed by Hailing *et al*. [42]. The toxicity of some metals may be so severe that plant growth is reduced before large quantities of the element can be translocated (Haghiri) [43].

CONCLUSION

It was concluded that salinity showed inhibitory effects on seed germination, seedling emergence of *Brassica rapa* plant. These adverse effects were overcome by application of micronutrients (copper and zinc) through seed priming with $ZnSO_4$ and $CuSO_4$ and all the above mentioned parameters showed improvement.

RECOMMENDATIONS

It is recommended that seeds should be primed with micronutrients (e.g copper and zinc) before sowing to create tolerance upto some extent for NaCl stress. 200 ppm of $ZnSO_4$ and 100 ppm of $CuSO_4$ exhibited enhancement effects on germination, seedling growth in *Brassica rapa*.

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