Research Article

Influence of seed priming with CuSO₄ and ZnSO₄ on germination and seedling growth of oat under NaCl stress

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Abstract

Salinity is a grave problem across the world which retards growth and productivity of plants. Seed priming is a technique which enhances growth and yield of crops by overcoming salt stress. Present study was conducted to examine the effect of grains priming of *Avena sativa* L. with different micronutrients (Cu & Zn) grown under NaCl stress. In this study grains of *Avena* were primed with two different concentration levels (100 & 200ppm) of CuSO₄ & ZnSO₄ solutions. After priming, these grains were treated with different levels of NaCl (60mM, 90mM & 120mM) and these results are compared with control groups. The effect of CuSO₄, ZnSO₄ and NaCl was observe on different physiological growth parameter including germination speed, germination percentage, root and shoot length, root and shoot fresh and dry biomass and biomoisture contents of root and shoot were observed. It was observed that grains without priming exhibited reduced growth under NaCl stress. Whereas priming of grains with CuSO₄ and ZnSO₄ showed improved growth in all growth traits as compared to non-primed seeds. It was evaluated that grains primed with ZnSO₄ at 200ppm showed enhanced germination speed. Whereas grains treated with CuSO₄ at 200ppm showed decreased germination speed. It was also demonstrated that priming of grains with both micronutrients (CuSO₄ & ZnSO₄) at 200ppm concentration exhibited higher shoot length as compared to Grains without priming grown in NaCl. It was concluded that seed priming technique can overcome the effects of salinity to some extent.

Keywords: *Avena sativa* L.; CuSO₄; NaCl; Salinity; Seed priming; germination speed and percentage; ZnSO₄

Introduction

Grasses (Family Poaceae) consists of approximately 11,500 species and about 786 genera and are considered as the fifth largest family of flowering plants in the World [1]. This family covers about 40% of the land area and hence dominated the

terrestrial ecosystem [2]. Oat is an important cereal crop which is used primarily for seeds, for human utilization as well as for fodder of livestock [3]. They are rich in soluble fibre, balanced proteins, vitamins as well as minerals which are valuable for human health [4, 5]. The

harmful effects of high levels of minerals like Na^+ and Cl^+ ions on plant is termed as Salt stress [6]. High concentrations of soluble salts are the characteristics of saline soils [7]. Among various environmental stresses one of the most important is the salt stress that influence the growth period of the whole plant and restrict the growth and productivity of crops by decreasing the osmotic potential and by impairing the absorption of certain nutrients. The absorption of essential ions such as potassium, calcium, ammonium and certain nitrate ions reduces as the level of sodium and chloride ions increases. Moreover increasing sodium and chloride ions blocks the structure of membrane and decreases the absorption of certain enzymes [8]. Increased salinity levels cause considerable decrease in different growth parameters including root and shoot dry weight, leaf length and leaf area [9]. Soil salinity is one of the major effective barriers that reduces the crop productivity [10]. In Pakistan, total irrigated area is 16.795 million ha. Slightly saline soil covers an area about 10%, 4% soil is moderately saline and 7% area is strongly saline, 6% is miscellaneous and 73% is termed as non-saline [11]. Salinity is the important abiotic stress, imposes negative effects on growth and productivity of crops [12]. The crop yield reduces upto 50% due to salinity in arid and semi-arid areas [13]. Stunted growth, decreased chlorophyll content and increased levels of reactive oxygen species are the most important symptoms of salt-stressed plants [14]. Around 9.5 billion hectares of the soil across the world are saline. This figure excludes large areas of secondarily salinized soil which is present in the currently cultivated land. Additionally, freshwater resources are getting scarce day by day. Under the present conditions it is essential to find plants which have economic value which can grow under saline conditions [15]. Seed priming is basically a pre-sowing approach for affecting the seedling development by balancing pre-germination metabolic

processes before the development of radical and usually improves the rate of germination and plant performance [16]. Seed priming is one of the easiest and simplest technique to create tolerance in crops against salinity [17]. Before germination, seed priming lead to hydration in seeds but roots do not emerge [18]. Moreover, under salt and drought stresses hydropriming enhanced germination and seedling growth [19]. Micronutrients are essential for human health and plant growth. Through seed treatments the application of micronutrients increases the yield and micronutrient grain content in most cases [20]. Under salt stress, micronutrient deficiencies are common due to high PH. Micronutrients improves plant production rate, leaf area and grain yield as a result of improvement in the enzymatic systems of plants [21]. Zn is an essential nutrient that is necessary for normal plant growth. It is directly involved in the biosynthesis of growth substances such as auxin. Cu is one of the most important nutrient that is essential for carbohydrate and nitrogen metabolism [22].

Materials and methods

Experiments were carried out to investigate the effects of seed priming of *Avena sativa* L. with CuSO_4 and ZnSO_4 under different levels of NaCl concentrations following the method but with little modifications as described by [23].

Seeds priming and experimental design

Seeds of *Avena sativa* L. were surface sterilized with 70% ethanol for 30 seconds and then washed with distilled water. Seeds were then primed with two concentration levels of CuSO_4 and ZnSO_4 i.e, 100 and 200ppm for 1hour and then dried. Germination period was studied in 4 control groups with different experiments like seed without priming grown in fresh water, primed seed grown in distilled water, only sterilized seed without priming grown in distilled water, treatment of seeds without priming with different NaCl concentrations and experimental group in which seeds were primed with CuSO_4 and ZnSO_4 then

grown in different concentrations of NaCl salinity (60mM, 90mM, 120mM). Repeating each experiment 3 times 64 petri plates were prepared. In each plate 7 seeds were placed which were lined with filter paper and moisten with 10-12 ml soln. All plates were kept at 10-13°C for germination being the optimum temperature. Germination started after 36 hrs (with the emergence of radical seeds were considered to be germinated).

Recording of data

Germination of seeds were counted after every 24 hrs and after 8 days of germination, germination percentage and germination velocity were recorded but root and shoot length, fresh and dry biomass of seedlings were determined after 15 days of sowing. Root, shoot length were measured by using the simple ruler. Fresh weight was

obtained using the electronics sartorius balance TE214S and after 24 hrs dry weight was determined for each case and percentage moisture content was obtained.

Statistical analysis

The data was statistically analysed using t-test.

Results and discussion

Germination speed

Germination speed is badly affected by salinity. In the present studies, it was investigated that in control groups, the slowest germination speed was recorded at 120mM NaCl concentration as (804.25) in comparison to 60mM NaCl as (690.41) and 90mM NaCl as (775.22). And fastest germination speed was observed in seeds grown in fresh water as (508.87) and distilled water as (510.72) (Fig. 1).

Figure 1. Effects on germination speed of *Avena sativa* L. under different control groups

Seeds primed with CuSO₄ 100ppm showed higher germination speed as (602.57) in comparison to that of 200ppm as (635.73) which also showed that CuSO₄ delayed germination in seeds of *Avena sativa* L. In case of priming with CuSO₄, 100ppm had (0.02) and 200ppm had (0.04) P(T<=t) values. Both treatments of CuSO₄ showed

significant effects in this regard. In case of seeds priming with ZnSO₄, 100ppm of ZnSO₄ showed slow speed of germination as (540.96) than that of 200ppm as (531.66). Statistical analysis revealed that priming of ZnSO₄, at 100ppm (0.009) and at 200ppm (0.018) P(T<=t) showed significant effect (Fig. 2).

Figure 2. Effects of seeds priming with different concentrations of CuSO₄ and ZnSO₄ on germination speed of *Avena sativa* L. under different levels of NaCl

Germination percentage%

Seeds grown in distilled water showed highest germination percentage (94.44 %) as compared to seeds grown in fresh water as (88.88 %) as shown in fig 2. whereas

seeds grown in different concentrations of NaCl i.e, 60, 90 and 120mM without priming exhibited lowest germination percentage as (50 %), (44.44 %) and (38.89 %) respectively (Fig. 3)

Figure 3. Effects on germination percentage of *Avena sativa* L. under different control groups

Priming of *Avena* seeds with CuSO₄ at two different concentrations demonstrated that 100ppm reduced germination percentage (68.05 %) as compared to that of 200ppm with highest value (80.55 %). In case of seeds priming with CuSO₄, 100ppm had (0.007) and 200ppm had (0.015) $P(T \leq t)$

values. Both doses of CuSO₄ showed significant effects in this regard. A comparison between two concentrations of ZnSO₄ seeds priming showed that 100ppm showed lower germination percentage (74.99 %) than that of 200ppm as (80.55 %). Seeds primed with ZnSO₄ at both 100

and 200ppm concentrations demonstrated (0.009) and (0.018) $P(T \leq t)$ values respectively which showed that 100 &

200ppm exhibited significant effects on this growth parameter (Fig 4).

Figure 4. Effects of seed priming with different concentrations of CuSO₄ and ZnSO₄ on germination percentage of *Avena sativa* L. under different levels of NaCl

Biomosture content or %age O.D weight of shoot

It was clear from fig 3 that highest %age O.D weight of shoot was obtained in distilled water as (80.1%) while the %age O.D weight of shoot in fresh water was observed as (75.5%). In case of seeds

grown in salinity without priming higher %age O.D weight of shoot was obtained in the treatment of 90mM NaCl as (70.1%) whereas %age O.D weight of shoot in 60mM and 120mM NaCl was as (69%) and (65%) respectively (Fig. 5).

Figure 5. Effects on % O.D weight of shoot of *Avena sativa* L. under different control groups

Treatment of CuSO₄ at 100ppm contributed to more %age O.D weight of shoot

(81.05%) in comparison to that of 200ppm as (72.62%). In case of priming with

CuSO₄, 100ppm had (0.09) and 200ppm had (0.18) $P(T \leq t)$ values. Data represented that both treatments of CuSO₄ showed non-significant effects in this regard. Results revealed that treatment of 100ppm ZnSO₄ showed lower %age of O.D

weight of shoots (78.1%) than that of 200ppm as (79.9%). Seeds primed with 100 and 200ppm of ZnSO₄ showed non-significant $P(T \leq t)$ values as (0.307) and (0.614) respectively (Fig. 6).

Figure 6. Effects of seed priming with different concentrations of CuSO₄ and ZnSO₄ on %O.D weights of shoot of *Avena sativa* L. under different levels of NaCl

Biomoiature content or %age O.D weight of root

Fig 4 demonstrated that higher %age O.D weight of root was found in the treatment of fresh water (88%) whereas %age O.D weight of root in distilled water was as

(80%). Lowest %age O.D weight of root was observed in treatment of 120mM (60%). While 60mM and 90mM NaCl showed %age O.D weight as (69%) and (65%) respectively (Fig. 7).

Figure 7. Effects on %O.D weights of root of *Avena sativa* L. under different control groups

Priming with CuSO₄ at 100ppm exhibited much higher %age of O.D wt. of roots as

(81.1%) than that exhibited at 200ppm as (63.1%). In case of priming of *Avena* seeds

with CuSO_4 , 100ppm had (0.01) and 200ppm had (0.03) $P(T \leq t)$ values which showed significant effects at both treatment levels on this growth physiological parameter. A comparison between two concentrations of ZnSO_4 revealed that 100ppm of ZnSO_4 showed higher %age

O.D weight of roots as (67.1%) than that of 200ppm with (65.3%). Both treatments of ZnSO_4 at 100 and 200ppm exhibited (0.009) and (0.018) $P(T \leq t)$ values and showed significant effects on this growth trait (Fig. 8).

Figure 8. Effects of seed priming with different concentrations of CuSO_4 and ZnSO_4 on %O.D weight of roots of *Avena sativa* L. under different levels of NaCl

Seedlings fresh biomass of root (g)

Fig 5 clearly demonstrated that higher fresh biomass of seedlings root was found in seeds grown in distilled water (0.1105 g) as compared to fresh water (0.0853 g). While 60mM NaCl has fresh weight of root as

(0.0765 g) and 90mM exhibited fresh weight of root as (0.0524 g) which was lower than 60mM NaCl treatment without priming. And 120mM NaCl showed great decline in fresh weight of root as (0.0398 g) (Fig. 9).

Figure 9. Effects on seedling fresh biomass of root of *Avena sativa* L. under different control groups

The root fresh weight of germinated seeds or seedlings primed with CuSO_4 at 100ppm was as (2.775 g) and was much more reduced as compared to that of 200ppm as (4.057 g) almost double in quantity. In case of priming with CuSO_4 , 100 and 200ppm concentrations showed (0.081) and (0.162) $P(T \leq t)$ values which had non-significant effects on this physiological parameter. A

comparison between two concentrations of ZnSO_4 used for *Avena sativa* L. seeds priming indicated that 100ppm exhibited much reduced root fresh weight as (0.0322 g) as compared to that of 200ppm as (0.0788 g). In case of ZnSO_4 , at 100 and 200ppm concentrations (0.110) and (0.220) $P(T \leq t)$ values were recorded as non-significant in this growth trait (Fig. 10).

Figure 10. Effects of seed priming with different concentrations of CuSO_4 and ZnSO_4 on fresh biomass of root of *Avena sativa* L. under different levels of NaCl

Seedling dry biomass of root (g)

Fig. 6 demonstrated that seedling root dry biomass of *Avena sativa* L. was lower in fresh water as (0.0514g) with respect to distilled water recorded as (0.0765g). The

root dry biomass in different concentrations of NaCl i.e. 60mM, 90mM and 120mM was recorded as (0.0492g), (0.0298g) and (0.0265g) respectively (Fig. 11).

Figure 11. Effects on dry biomass of root of *Avena sativa* L. under different control groups

CuSO_4 at 100ppm concentration exhibited reduced roots dry weight of seedlings as

(0.0334 g) in comparison to that of 200ppm with (0.0408 g) dry weight. . In case of

priming with CuSO₄, both 100 and 200ppm concentrations had (0.08) and (0.16) P(T<=t) values and both exhibited non-significant effects on this growth trait. 100ppm of ZnSO₄ showed lower dry weight

of root as (0.0285 g) than that of 200ppm as (0.0295 g). 100 and 200ppm of ZnSO₄ exhibited (0.471) and (0.942) P(T<=t) values, show non-significant effects on root dry weight (Fig. 12).

Figure 12. Effects of seed priming with different concentrations of CuSO₄ and ZnSO₄ on dry biomass of root of *Avena sativa* L. under different levels of NaCl

Seedlings fresh biomass of shoot (g)

The fig 7 clearly described that seeds grown in fresh water (1.0567 g) attained high fresh biomass of shoot. It can be seen that shoot fresh biomass of 60mM as (0.4285 g),

90mM as (0.3213 g) and 120mM as (0.2931 g) NaCl showed reduction in this growth trait as compared to fresh water (1.0567 g) and distilled water (0.8675 g) (Fig. 13).

Figure 13. Effects on fresh biomass of shoot of *Avena sativa* L. under different control groups

CuSO₄ at 100ppm exhibited reduced fresh weight of shoot as (0.489 g) as compared to that of 200ppm with greater biomass as

(0.646 g) which demonstrated that higher concentration of CuSO₄ enhanced this growth parameter in seeds of *Avena sativa*

L. In case of priming with CuSO_4 , 100ppm had (0.29) and at 200ppm $P(T \leq t)$ value was as (0.58) and both treatments of CuSO_4 showed non-significant effects for this growth parameter. Seeds primed with 100ppm of ZnSO_4 showed reduced fresh

weight of shoot (0.418 g) than that of 200ppm as (0.632 g). In ZnSO_4 for 100ppm data the $P(T \leq t)$ value was (0.09) and for 200ppm it was (0.19), both being non-significant as far as this physiological parameter is concerned (Fig. 14).

Figure 14. Effects of seed priming with different concentrations of CuSO_4 and ZnSO_4 on fresh biomass of shoot of *Avena sativa* L. under different levels of NaCl

Seedling dry biomass of shoot (g)

The dry biomass of shoot in fresh water (0.8827 g) was observed that was found to be higher as (0.8827 g) than in distilled water as (0.5971 g). Among various concentrations of NaCl it was observed that

60mM, 90mM and 120mM exhibited dry weight of shoot as (0.2739 g), (0.1861 g) and (0.0985 g) respectively. The lowest dry weight was found in 120mM NaCl as demonstrated in Fig 15.

Figure 15. Effects on dry biomass of shoot of *Avena sativa* L. under different control groups

CuSO₄ at 100ppm exhibited reduction in dry shoot biomass of shoot as (0.275 g) when compared to that of 200ppm as (0.5008 g) and sufficiently increased this physiological parameter regarding dry biomass of shoot of *Avena sativa* L. In case of seeds priming with CuSO₄, 100ppm showed non-significant value as (0.056) while 200ppm also exhibited non-significant values recorded as (0.113).

Results indicated that priming of seeds at 100ppm of ZnSO₄ declined in dry biomass of shoot as (0.221 g) than that of 200ppm as (0.357 g). In case of ZnSO₄ *Avena* seeds priming at 100 and 200ppm concentrations, (0.071) and (0.142) P(T<=t) values were recorded. Results showed that priming at 100ppm and 200ppm of ZnSO₄ showed non-significant effect in this parameter (Fig. 16).

Figure 16. Effects of seed priming with different concentrations of CuSO₄ and ZnSO₄ on seedling shoot dry biomass of *Avena sativa* L. grown under different levels of NaCl

Seedlings root length (cm)

It was clear from the fig 9 that seeds grown in distilled water (6.276 cm) without priming have higher root lengths with respect to fresh water (5.855 cm). It was observed that root length in 60mM (4.123

cm) and 90mM (3.213 cm) levels of NaCl have very minute differences but root length in 120mM (2.143 cm) level of NaCl showed great decline in this growth trait (Fig. 17).

Figure 17. Effects on root length of *Avena sativa* L. under different control groups

CuSO₄ 100ppm showed reduced roots length up to (2.775 cm) in comparison to that of 200ppm being (4.057 cm). In case of priming with CuSO₄, 100ppm had (0.081) and 200ppm as (0.162) P(T<=t) values. Both treatments of CuSO₄ showed non-significant effects. 100ppm of ZnSO₄ concentration exhibited (4.745 cm) and that

of 200 (5.460 cm) increased roots length. In case of priming with ZnSO₄, for 100 and 200ppm (0.126) and (0.253) P(T<=t) values were recorded respectively. Results showed that priming with both concentrations of ZnSO₄ showed non-significant effects on root length growth parameter (Fig. 18).

Figure 18. Effects of seed priming with different concentrations of CuSO₄ and ZnSO₄ on root length of *Avena sativa* L. under different levels of NaCl

Seedlings shoot length (cm)

Seeds grown in fresh water (12.767 cm) showed greater shoot lengths than in distilled water as (12.676 cm). Whereas treatments of 60mM and 90mM NaCl

showed shoot lengths as (6.437 cm) and (5.725 cm) respectively. Results indicated that shoot length was much reduced in treatment of 120mM NaCl (4.105 cm) (Fig. 19).

Figure 19. Effects on shoot length of *Avena sativa* L. under different control groups

In case of priming of seeds with CuSO₄, 100ppm reduced shoot length as (6.77 cm) in comparison to that of 200ppm as (7.99

cm) which indicated that CuSO₄ showed enhancement in this parameter of *Avena sativa* L. seedlings. In case of priming of

Avena seeds with CuSO₄, 100 and 200ppm had (0.0009) and (0.0019) P(T<=t) values respectively. Both treatments of CuSO₄ showed significant effects on shoot length. Results indicated that priming of *Avena sativa* L. seeds with 100ppm of ZnSO₄ showed lower shoot length as (7.51 cm) as

compared to that of 200ppm as (9.084 cm). In case of ZnSO₄ at 100 and 200ppm concentrations, P(T<=t) values recorded were as (0.145) and as (0.291) respectively. Both these two treatments showed non-significant effects on this growth parameter (Fig. 20).

Figure 20. Effects of seed priming with different concentrations of CuSO₄ and ZnSO₄ on shoot length of *Avena sativa* L. under different levels of NaCl

The goal of this research work was to evaluate the effects of seeds priming of *Avena sativa* L. under salt stress. Results revealed that oat seedlings demonstrated decreased growth under salt stressed conditions. The results indicated that the germination of oat seedlings was negatively influenced by NaCl stress under different concentrations (60mM, 90mM and 120mM). Seeds germination percentage and seedlings emergence were delayed by higher salinity levels as described by [24] and our results are in agreement with these findings. The same results were reported in mustard by [25]. These results are also in conformity with our findings regarding salt stress.

The inhibition of germination induced by salts could be associated with specific ion toxicity or osmotic stress as reported by [26]. Increased salinity level of the medium caused great reduction in germination percentage as reported by [27, 28] and these results are similar with our findings.

From our results it was also demonstrated that high salinity levels caused severe reduction in seedlings shoots and roots length and fresh and dry weights. Shoot and root lengths are the important growth parameters that are readily affected by salt stress due to the reason that roots take in water due to direct contact with soil and then shoots empower its supply in whole plant. Due to this reason shoot and root length administer critical indications of a plant's response to salt stress as reported by [29]. Our results showed reduction in fresh and dry biomass as salt stress increases which was strongly supported by [30, 31] in which they described the reason that salinity influences the metabolic processes, by decreasing water potential. Reduction in seedling growth under salinity is due to increase in sodium chloride toxicity and aggregation of sodium ions in the photosynthetic tissues as demonstrated by [32] and our results are in agreement with these findings. Present results showed that seeds primed with 200ppm of ZnSO₄

showed considerable increase in seedlings shoot length of *Avena sativa* L. as compared to their respective controls. Same results were demonstrated on Cumin plant by [33] and our results are in accordance with these findings. Osmo-priming and halo-priming caused in increased stem length of hot pepper seedlings as demonstrated by [34] and our results are in agreement with these findings. Priming in maize with 1% ZnSO₄ (for 16h) extensively increased the crop growth, grain yield and Zn content as reported by [35]. Similar results were obtained in rice where seed priming with Zn caused enhanced growth and grain yield which was found more suitable than any other soil application [36]. Our results are also in conformity with these findings. Seeds priming with Zn considerably increased the yield and related traits in common bean (*Phaseolus vulgaris* L.) as reported in findings by [37]. Similar results were also reported in barley (*Hordeum vulgare* L.) where the improved germination and seedlings development was observed due to seeds priming with Zn as reported by [38]. Our findings are in conformity with these findings. Explained that In another research it was demonstrated that the improved growth after nutrient primings of seeds may be due to the reason that in newly developed radicals and coleoptiles during seed germination the Zn content was found to be much higher (upto 200 mg kg⁻¹) thus proving the involvement of Zn in Physiological processes during early seed germination as reported by [39]. Copper and Zinc plays an important role in cellular metabolism because both of them are being involved in many proteins [40]. So all these findings support our findings for improved growth after seed priming with different micronutrients.

CuSO₄ treatments on *Avena sativa* L. seeds had no significant effects on germination speed in our present studies. Our results clearly demonstrated that seeds primed in 100ppm CuSO₄ showed reduction in seedlings shoot length as compared to CuSO₄ at 200ppm and their respective

controls. Results revealed that CuSO₄ had no significant effects on germination speed. Priming of wheat seeds with CuEDTA (0.04 to 0.16 kg Cu ha⁻¹) considerably improved grain yields but had no significant effects on seedlings emergence as reported by [41]. These results were in conformity with another experiments on oats in which seeds treated with Cu (0.001% solution of CuSO₄) had no considerable effects on seedling germination although caused increase in yield of 16.5% with respect to controls as described by [42]. A comparison was made between 100 and 200ppm of CuSO₄ and controls which indicated that 200ppm promoted more growth. Similar results were obtained in pea plant as reported by [43]. These results were in accordance with our's results and thus support our findings.

Conclusion and recommendations

From the research work it was concluded that salinity caused reduction in seedlings germination and emergence and also affected the other growth physiological parameters of *Avena sativa* L. Our results revealed that seeds priming was proved to be helpful in overcoming the effects of salinity on seeds germination and growth. The negative effects of salinity were reduced by using the micronutrients (Cu and Zn) through seeds priming with CuSO₄ and ZnSO₄ at two concentration levels (100 & 200ppm). It was concluded that by the application of these micronutrients, all the growth parameters including seed germination, root and shoot length and fresh and dry biomass showed improvement in saline as well as in non-saline conditions. It is suggested that to produce tolerance in plants against NaCl stress, seeds should be primed with micronutrients before sowing. Moreover, priming techniques can enhance the germination rate, growth and ultimately yield of some important crops. Furthermore, it is environment friendly technique that's why it may be used in future for beneficial results.

Authors' contributions

Conceived, designed the experiments and supervised the research work: AM Khan, Performed the experiments and wrote the article: S Iqbal, Helped in experiments and data

analysis: I Dilshad, K Moatter & T Ahmed,
 Proof read the article: SA Gilani.

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