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# The determination of gibberellic acid effects on seed germination of *Echinacea purpurea* (L.) Moench under salt stress

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**Abstract**

The research was conducted to determine the effect of gibberellic acid pre-treatments on germination of *Echinacea purpurea* seeds under salt stress. In the study, three different gibberellic acid concentrations (0, 100, 200 and 300 ppm) were pre-treated. Seeds planted in petri dishes were left to germinate after salt applications at 0, 50 and 100 mM (NaCl) concentrations. Responses of *echinacea* to priming and salt stress treatments were observed on the bases of some growth and viability (radicle and hypocotyl length, radicle fresh and dry weight, hypocotyl fresh and dry weight, germination power, germination speed, mean germination time, germination and sensitivity index) parameters.

According to the result of the research; when the salt concentrations increase, the germination and growth parameters of *echinacea* seeds were inhibited. It was conducted determined that increasing doses of gibberellic acid pre-treatments had significant and positive effects on the germination and growth parameters of *echinacea* seeds under salt stress conditions. In physiological enhancement of echinacea seeds, the best results were obtained from the 300 ppm $GA_3$+0 mM (control) salt combination.

**Keywords:** Gibberellic acid, Salt, Echinacea (*Echinacea purpurea* L.), Germination

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## Introduction

*Echinacea* which is a medicinal and aromatic plant, has been used for traditional medicine because of its pharmacological properties in many countries. The first reports of the use of the plants belonging to *Echinacea* genus date to the beginning of the twentieth century, in the Ethnobotanical studies made by WHO, documented uses as a primitive antibioticits use against snakebites to heal wounds (Anonymus, 1999). Although medicinal plants have been used for thousands of years in every culture of the world, serious scientific researches supporting their therapeutic value have begun around 1960 (Lopez and Shepard, 2007). *Echinacea* is an indigenous herbal plant genus of North America and occupies an important place among medicinal plants due to its immunostimulatory properties against respiratory ailments. The genus *Echinacea* is one of the prominent genera of the plants that are utilized in medicinal preparations and drugs (Ahmad et al., 2017). Echinacea plant which is perennials has 9 species. Being commonly used in traditional medicine, E. purpurea, E. pallida and E. angustifolia have been the most investigated of these species (Ivanova et al., 2014). Echinacea which belongs to the family *Asteraceae (Compositae*) is a plant commonly found in the world. *Echinacea purpurea* (L.) Moench has been traditionally also used for the treatment of toothache, snake bite, bowel pain, skin disorders, chronic arthritis, seizure and cancer (Grimm and Muller, 1999). Salinity is considered to be one of the environmental stresses that reduce the growth and efficiency of most glycophytic plants worldwide. Induces both osmotic and ionic stresses leading to deterioration of many physiological and biochemical processes including salinity, water relations, ionic homeostasis, gas exchange and mineral nutrition (Parida and Das, 2005; Munns and Tester, 2008). Gibberellins are involved in the stimulation of enzymes involved in seed germination.

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Gibberellic acid ($GA_3$) is one of the hormones proposed to control primary dormancy by inducing germination. Cavusoglu et al. (2007) reported that $GA_3$ was the most effective salt stress reducing agent among plant growth regulators used in the research.

This study was carried out to detect the effects of salt doses with pre-applications in various levels of Gibberellic acid ($GA_3$) on germination of *Echinacea purpurea* L. seeds.

**Materials and Methods**

In order to evaluate the effect of seed priming on the germination and seedlings' growth traits of *Echinacea purpurea* L. under different salinity stress levels, a factorial experiment was conducted in completely randomized design (CRD) with four replications, at the laboratory of Seed Science and Techonology in the Department of Field Crops, Faculty of Agriculture, Van Yuzuncu Yil University, Turkey in 2018. Three salt levels (0, 50, 100 NaCl mM) and four gibberellic acid ($GA_3$) levels (0, 100, 200 and 300 ppm) were used in the experiments. Seeds were rinsed with water and distilled water, then their surface sterilized in 20% sodium hypochlorite (vol/vol) for 5 minutes, followed by 70% alcohol (vol/vol) treatment for 1 minutes, after then, were thoroughly rinsed with sterile deionized water before priming.

The seed lots were imbibed within different doses gibberellic acid ($GA_3$) in priming treatments. Treated seed lots with $GA_3$ were kept in darkness in an incubator at 25 ± 0.5 °C for 24 h. Seeds, removed from the solutions ($GA_3$) were air dried for 24 hours at 25 °C to reach the original moisture content (12-13 %) and then proceed for germination test as suggested by ISTA. Unprimed seeds were used as control. Treated seeds were placed in 9 cm diameter sterile petri dishes with filter paper and 20 seeds were placed in each petri dish and 5ml of salt solution was added dish to all petri except for control treatments, to the control treatments was given distilled water (5 ml), then petri dishes were placed in a totally dark incubator which stable at temperature (25±0.5ºC). As a final step, after 14 days all parameters were observed, measured and recorded. The root and shoot vigors were calculated as the sum of total root length (cm) and shoot length (cm) of all the seedlings of a replicate divided by the number of seedlings. Fresh root and shoot were then placed in a hot air oven (70 $^0$C for 24 hours) to dry (Anonymus, 2012). Root and shoot dry masses were weighed germination rate, germination power, germination index, mean germination time and sensitivity index were calculated with the following formulas. It was considered that number of germinated seeds on 7$^{th}$ day as "germination rate" and number of germinated seeds on 14$^{th}$ day as "germination power". Germination rate (GR) was calculated using the Equation 1 (Akinci and Caliskan, 2010).

GR = Total seeds germinated after day 14/Total number of planted seeds (Equation 1)

Germination index (GI) was calculated using the Equation 2 (Wang et al., 2004).

GI=Σ(Gi/Tt) (Equation 2)

GI: Germination index; Gi: i. Days germinated seed rate; Tt: count day

Mean germination time (MGT) was calculated using the Equation 3 (Ellis and Roberts, 1980).

MGT=Σ(fx)/Σf (Equation 3)

f: Number of seeds germinated x: germination day

Sensivity index (SI) was calculated using the Equation 4 (Foolad and Lin, 1997).

SI= MGT in the salt application/ MGT in the control application (Equation 4)

Data were analyzed statistically by using analysis of variance with COSTAT (version 6.3) software. The variance analysis of data was performed (ANOVA) and the multiple comparison of the means was made according to the Duncan test (Duzgunes et al., 1987).

**Results and Discussion**

According to the research results, salinity and seed priming showed a significant effect on all parameters. Only, the effect of gibberellic acid on the radicula length wasn't found insignificant. Furthermore, salinity stress x seed priming interaction that it was was found important with regard to parameters such as dry radicula weight (mg) and sensitivity index (%). Salinity stress x seed priming Interaction in terms of dry radicula weight seem seeds primed with $GA_3$ unlike untreated seeds had the highest germination value at all salinity levels. While the highest sensitivity index was obtained as 1.54 % from seeds primed with 100 ppm $GA_3$, in 100 mM salt stress conditions, the highest dry radicula weight was reported as 5.70 mg from seeds primed with 300 ppm $GA_3$ in non-stress (0 mM NaCl) conditions.

Highest germination power, germination rate and germination index were determined as 92.50, 66.25 and 14.64 % from 0 mM (control) salt treatments, also the lowest values were as obtained as 72.08, 13.75 and 8.56 % from 100 mM NaCl applications. In addition, the effect of gibberellic acid on the germination rate was found significant and the best mean (47.77 %) was determined from 300 ppm $GA_3$, lowest germination rate (33.88 %) is due to control of $GA_3$. But, among the first three doses of gibberellic acid there isn't any a different as statistically. Based on statistical analysis, salinity significantly decreased mean germination time of seeds primed with 200 and 300 ppm $GA_3$ applications. In salt doses of 50 mM and 100 mM, the applications of gibberellic acid in concentrations of 100, 200 and 300 ppm caused to the increase in germination power or rate, respectively. In the result of this research, mean germination time (6.37 day) has shortened with control (0 mM) salt application. The longest average germination time recorded as 9.57 day from 100 mM NaCl treatments. As for the sensitivity index, increasing salt applications have also increased these values. Sensitivity index value is 1.46 % in 100 mM salt dose and 0.46 % in 0 mM salt dose. In this study, we observed that germination percentage delayed and decreased with increasing NaCl concentration and drastically reduced at 100 mM NaCl. The results showed that echinacea is highly sensitive to salinity in the germination stage. In carried previous work by Yuonesi and Moradi (2015), reported that is consistent with the hypothesis that under salinity stress, priming can prepare a suitable metabolic reaction in seeds and can improve seed germination performance and seedling establishment. These results agree with Singh and Jakar (2018), in *Vigna mungo* and Fardus et al. (2018) in wheat, they suggested that salinity may influence germination by decreasing the water uptake, and germination percentage declined with increase of salinity level. However, osmotic stresses reduce germination percentage (Cornelia and Bandici 2008; Maghsoudi and Arvin 2010) although, the seeds pre-treated with $GA_3$ solutions exhibited higher germination percentage. Sabra et al. (2012) reported that photosynthetic activity in echinacea was affected by salinity and Zollinger et al. (2007) also found a similar reduction in photosynthetic rate and stomatal conductance in *E. purpurea* irrigated with increasing concentrations of salinity. In addition, Sanam et al. (2014), different levels of salinity significantly reduced germination rate, germination vigor and index. The results also showed that by increasing salinity levels, the percentage of germination and normal seedlings significantly decreased and the mean time to germination increased, compared to the control treatment. But the seeds treated with $GA_3$ showed higher viability and better performance under salinity stress condition. Ali et al. (2012) and Zadeh et al. (2015) reported that application of $GA_3$ enhanced growth parameters (shoot length, shoot fresh weight, shoot dry weight, root lenght, root fresh weight, root dry weight, leaf area etc.) under saline condition. These results are consistent with those of Jamil and Rha (2007) in sugar beet, Erdemli and Kaya (2015) in sunflower, Yıldız et al. (2017) in sweet william, Cavusoglu et al. (2007) in radish seed, Singh and Jakar (2018), in *Vigna mungo* and Zadeh et al. (2015) in echinacea. Promotion in seed germination with $GA_3$ may be due to increased uptake of oxygen and a-amylase activity.

Yalcın (2010) and Yildiz et al. (2017) recorded that the positive and significant effects of $GA_3$ applications on germination power were found. Jamil and Rha (2007) reported that water uptake of primed seeds also increased significantly with increasing concentration of $GA_3$ as compared to control. Erdemli and Kaya (2015), recorded that it was concluded that seed treatment with $GA_3$ can be beneficial for decreasing the effect of abiotic stress conditions on germination. It was seen that as gibberellic acid dose increases germination parameters were improve when compared to control.

Table 1. Effect of gibberellic acid treatments on germination of echinacea seeds under salt stress

| Applications | | Germination power (%) | Germination rate (%) | Mean germination time (day) | Germination index (%) | Sensitivity index (%) |
|---|---|---|---|---|---|---|
| Salt Doses | GA3 | | | | | |
| Control (T0) | GA0 (control) | 90.00 | 68.33 | 6.50 | 15.51 | - |
| | GA100 | 91.66 | 66.66 | 6.62 | 13.39 | 1.01de |
| | GA200 | 95.33 | 60.00 | 6.54 | 14.27 | 1.00 de |
| | GA300 | 95.00 | 70.00 | 5.81 | 15.41 | 0.89 e |
| T0 Means | | 92.5 a | 66.25 a | 6.37 c | 14.64 a | 0.72 c |
| 50 mM (T5) | GA0 (control) | 88.33 | 26.66 | 8.29 | 11.78 | 1.41 ab |
| | GA100 | 83.33 | 40.00 | 8.05 | 12.48 | 1.23 c |
| | GA200 | 86.66 | 41.66 | 7.28 | 12.10 | 1.15 cd |
| | GA300 | 88.33 | 50.00 | 7.51 | 13.55 | 1.15 cd |
| T5 Means | | 85.41 a | 39.58 b | 7.78 b | 12.48 b | 1.23 b |
| 100 mM (T10) | GA0 (control) | 70.00 | 6.66 | 9.99 | 7.60 | 1.53 a |
| | GA100 | 76.66 | 10.00 | 10.03 | 9.93 | 1.54 a |
| | GA200 | 76.66 | 15.00 | 9.84 | 7.81 | 1.50 a |
| | GA300 | 70.00 | 23.33 | 8.43 | 8.91 | 1.29 bc |
| T10 Means | | 72.08 b | 13.75 c | 9.57 a | 8.56 c | 1.46 a |
| $GA_3$ Doses Means | GA0 (control) | 82.77 | 33.88 b | 8.26 a | 11.63 ab | 0.98 c |
| | GA100 | 83.88 | 38.88 b | 8.23 a | 11.94 ab | 1.26 a |
| | GA200 | 82.22 | 38.88 b | 7.89 ab | 11.39 b | 1.20 ab |
| | GA300 | 84.44 | 47.77 a | 7.25 b | 12.62 a | 1.11 b |
| CV (%) | | 10.28 | 19.39 | 8.37 | 9.76 | 11.20 |

*There is no significant difference between the means indicated with same letter (5%).

Table 2. Effect of gibberellic acid treatments on some characteristics of echinacea seedlings

| Applications | | Radicula lenght (cm) | Plumula lenght (cm) | Fresh radicula weight (mg) | Fresh plumula weight (mg) | Dry radicula weight (mg) | Dry plumula weight (mg) |
|---|---|---|---|---|---|---|---|
| Salt Doses | GA3 | | | | | | |
| Control (T0) | GA0 (control) | 1.23 | 2.65 | 57.66 | 374.7 | 5.20 ab | 29.26 |
| | GA100 | 1.71 | 3.01 | 59.43 | 388.66 | 5.03 a-d | 32.0 |
| | GA200 | 1.67 | 3.13 | 64.66 | 417.6 | 5.13a-c | 33.66 |
| | GA300 | 1.63 | 3.43 | 63.90 | 441.23 | 5.70 a | 34.76 |
| T0 Means | | 1.56 a | 3.05 a | 61.41 a | 405.5 a | 5.26 a | 32.42 a |
| 50 mM (T5) | GA0 (control) | 1.03 | 1.66 | 54.53 | 354.63 | 4.33 c-f | 24.20 |
| | GA100 | 0.78 | 2.36 | 57.26 | 357.06 | 4.23 d-f | 26.33 |
| | GA200 | 1.10 | 2.46 | 63.86 | 397.23 | 4.63 b-e | 24.96 |
| | GA300 | 1.28 | 2.53 | 60.23 | 432.96 | 4.03 ef | 30.30 |
| T5 Means | | 1.05 b | 2.25 b | 58.97 a | 385.47a | 4.30 b | 26.45 b |
| 100 mM (T10) | GA0 (control) | 0.50 | 0.91 | 33.33 | 165.5 | 1.63 g | 21.76 |
| | GA100 | 0.46 | 1.15 | 39.20 | 188.80 | 3.80 f | 21.66 |
| | GA200 | 0.70 | 1.10 | 40.66 | 195.7 | 3.66 f | 23.10 |
| | GA300 | 0.72 | 1.48 | 40.93 | 224.76 | 3.73 f | 23.70 |
| T10 Means | | 0.59 c | 1.16 c | 38.53 b | 193.69 b | 3.20 c | 22.55 c |
| $GA_3$ Doses Means | GA0 (control) | 0.92 b | 1.74 c | 48.51 c | 298.27 b | 3.72 b | 25.07 b |
| | GA100 | 0.98 ab | 2.17 b | 51.96 bc | 311.51 ab | 4.35 a | 26.66 ab |
| | GA200 | 1.15 ab | 2.23 b | 56.40 a | 336.84 ab | 4.47 a | 27.24 ab |
| | GA300 | 1.21 a | 2.48 a | 55.02 ab | 366.32 a | 4.48 a | 29.58 a |
| CV (%) | | 9.12 | 11.51 | 7.98 | 7.98 | 8.01 | 8.20 |

*There is no significant difference between the means indicated with same letter (5%).

The effect germination inhibiting in seeds of salt has been revealed in many studies. It has been reported that it was caused to the inhibition of water intake of high salt concentration on the decrease of germination rate, toxicity of salt and the can not be activated due to salt stress of required enzymes during germination (Mansour, 1994; Essa, 2002; Sadeghian and Yavari 2004). However, Seyedi et al. (2012) reported that, with increasing salinity stress germination characteristics such as germination percentage, germination rate and seedling fresh weight decreased. All of the early growth parameters have decreased as parallel with salt application.

Priming of seeds with different materials particularly $GA_3$ was useful for alleviating salt stress effects and improving germination and seedling establishment under salt stress. Under salinity condition, primed seeds possessed more germination and emergence than control. According to the results of the study; the highest values for investigated characters such as radicula and seedling lenght, fresh radicula and seedling weight and dry radicula and seedling weight were determined as 1.56 cm, 3.05 cm, 61.41 mg, 405.5 mg, 5.26 mg and 32.42 mg from control applications (0 mM NaCl), respectively. Also, the most negative results were determined as 0.59 cm, 1.16 cm, 38.53 mg, 193.69 mg, 3.20 mg and 22.55 mg from 100 mM NaCl applications, respectively. In terms of gibberellic acid applications the most positive results for the characters such as radicula and seedling lenght, fresh radicula and seedling weight and dry radicula and seedling weight were obtained from 300 ppm $GA_3$ applications as 1.21 cm, 2.48 cm, 56.40 mg, 366.32 mg, 4.48 mg and 29.58 mg, respectively In this experiment, we observed that, growth parameters reduced with increasing salinity (Table 2) which was similar with the report of Sabra et. al. (2012) in echinacea species, Nazarian (2016) in rapeseed, Salahuddin et al. (2017), in mungbeen, Fardus et. al. (2018) in wheat and Singh and Jakhar (2018), in *Vigna mungo*.

## Conclusions

In the trial conducted to evaluate some germination and growth characteristics (radicle length, shoot length, radicle age and dry weight, shoot age and dry weight, germination strength, germination rate, average germination time, germination and sensitivity index) of echinacea was concluded that germination and growth parameters of echinacea seeds were inhibited as the salt concentration increases. Echinacea seeds exposed to the salt stress and gibberellic acid concentrations were found to have a significant positive effect on germination and growth properties of echinacea.The best results were obtained from the combination of 300 ppm $GA_3$ and 0 mM (control) salt in the improving as physiological of echinacea seeds. As a result, it was determined that GA3 treatments eliminated the germination-inhibiting effects caused by salt stress and shortened the average germination time.

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