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Research Article

Quantifying seed germination responses of *Echinops* and *Centaurea*, to salinity and drought stresses

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Abstract

Seed germination may significantly interrupt by water stress due to drought and salinity condition. Salinity can cause osmotic pressure and induce drought stress. Water deficit stress affect normal seed germination and reduce seedling vigor. The objective of this investigation was to determine the effect of drought and salt stresses on germination characteristics of *Echinops ritro and Centaurea virgata*. Seeds were germinated with the concentrations of sodium chloride (0, 50, 100, 150 and 200 mmol) or in polyethylene glycol PEG6000 (0, -0.2, -0.4, -0.6, -0.8, -1 and -1.2 MPa). The highest values of germination parameters were obtained with no osmotic potential or salinity stress. At treatment by PEG, the germination was severely decreased at -0.6 MPa. While, no germination occurred at- 0.8 MPa by PEG. Results revealed that under 118 Mmol salinity, the seed germination of *Centaurea virgata* declined to 43% which was as close as half of its total seed germination. However, 50% reduction in seed germination of *Echinops ritro* was observed at 193 mmol salinity. Results indicated *Echinops ritro* and *Centaurea virgata* germination was sensitive to both the stresses. However, seedling growth was more sensitive to PEG than NaCl.

Keywords: germination; logistic; water deficit; weed

Introduction

Salinity and drought stress are the most widespread abiotic stresses which threatens successful agricultural productions (Forni *et al.*, 2017). Salinity caused ion imbalances in the soil water which in higher concentration resulted in hyper osmotic stress and reduced the available water to plants (Zhu, 2001). Drought stress not only interferes normal physiological process in plant tissues but also may be followed by reactive oxygen damages to cell and active organs, known as oxidative stress (Nath *et al.*, 2017). Plants use various strategies to overcome abiotic stress such as salinity and drought stress. However, the most sensitive part of the plant life cycle to such stresses is seed germination. Understanding the possible tolerance limit of abiotic stress at the seed germination stage will help researchers to increase biodiversity at the highly infected areas and minimize the risks of desertification and biodiversity loss. Among weedy species, *Echinops ritro* and *Centaurea virgata* are finding great interest to weed and seed sector of Iranian agricultural research as they are rapidly invading the agronomic lands of Iran.

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The present study was, therefore, undertaken in order to compare the effects of salinity and drought stresses induced by NaCl and polyethylene glycol (PEG 6000) on germination processes in two weedy species *Echinops ritro* and *Centaurea virgata* quantifying their level of salt and drought resistance.

Materials and Methods

Seed collection site

This study was carried out at Seed technology laboratory of the Department of Plant Production and Genetics, College of Agriculture, Khuzestan Agricultural Sciences and Natural Resources University, Iran. Seeds of the two weed species, *Echinops ritro* and *Centaurea virgata* were collected from herbicide-free fields of research farm during 2017.

Salinity and drought stress treatments

Salinity treatments (50, 100, 150, 200 and 250 mmol) were prepared by solving the right amount of NaCl (Merc Inc. 99% purity) in distilled water. Drought stress was simulated by limiting available water to seeds using polyethylene glycol 6000 (PEG). Drought stress treatments were -0.2, -0.4, -0.6, -0.8, -1 and -1.2 MPa that were prepared by solving PEG 6000 in the distilled water. The required PEG to induced osmotic potentials (drought) were calculated using (Michel and Kaufmann, 1973) equation.

Germination conditions and evaluations

Seeds of both weeds were germinated inside the controlled germinators based on the procedure recommended by (ISTA, 2013). Seeds were placed at the 90 mm Petri dishes containing 5 ml of test solutions. Seeds germinated at distilled water considered as the controls of the experiment. During for 14 days the germinated seeds were counted. A seed was considered to have germinated when the radicle was visible around 2 mm in length.

Methods of germination expression

Seed germination indices were calculated for all the experimental treatments.

The final germination percentage of each treatment was calculated based on equation 1.

Equation 1: FGP= Final number of germinated seeds *100

Mean germination time (MGT) was calculated using equation 2 (Orchard, 1977).

Equation 2: $$MGT = \frac{\sum nidi}{\sum ni}$$ Where

ni= number of germinated seeds, di= Days after imbibition

Seedling vigour was calculated using equation 3.

Equation 3: Seedling vigour index = seedling length * germination percentage

Statistical analysis

Each weed seeds were subjected to abiotic stress tolerance evaluations using factorial experiment based on the randomized design with four replications. Due to large seed size of *Echinops ritro*, it was not possible to put more than 25 seeds in each petri dishes, thus, in this experiment for both plants, each replication was replicated twice. Then pooled data from each of two replicas (25 seeds) were used to form each of replicates (50 seeds).

Three parameter sigmoidal and logistic models were fitted on the germination data to estimate time required to completion of 50 % of total seeds germination at each of the experimental treatments. Using equation 4.

Equation 4: Sigmoid 3 parameter: $Y=\frac{a}{1+e^{-(\frac{x-x0}{b})}}$

Where a is Y or upper asymptote, b was slope, x0 Critical point or the x that reached 50% of Y and y0 is lower asymptote.

Data analysis was performed using sigma plot 14 and Minitab version 16.

Results

Cumulative seed germination of both *Echinops ritro* and *Centaurea virgata* seeds were significantly affected by salinity and drought stresses ($P < 0.001$ in all cases). Seed germination of *Echinops ritro* mostly reached 80-100% in salinity stress treatments till 200 Mmol which there was 40% declined in a number of germinated seeds (Figure 1). The time course of seed germination was a sigmoidal with rapid germination rate in salinity levels below 200 Mmol compared with *Centaurea virgata*.

For *Centaurea virgata* seeds, the maximum seed germination was less than 90% in control treatment (85%) while in *Echinops ritro* due to pappus removal from the seed coat, the seed germination was reached 100% (Figure 1). The maximum seed germinating of *C. virgata* was observed in control treatment. Under salinity conditions higher than 100 Mmol, cumulative seed germination of *C. virgata* was drastically reduced (Figure 1). Both plants were able to germinate in 250 Mmol Salinity while there was no significant difference between their germination percentages (Figure 1).

The time required to reach 50% of total seed germination in each weed was estimated using three parameters sigmoid model. The time required to reach 50% of total seed germination of *Echinops ritro* under 250 Mmol salinity was 87.42 h while this was only 11.49 h in control treatment which was eight-time greater. For *Centaurea virgata*, time to 50% of total seed germination did not considerable varies as the salinity stress level increased. However, there was a deistical reduction in cumulative seed germination from 83% (control) to 15% (250 Mmol salinity). This might raise from the fact that the seed coat is critical for the behaviour of seed germination to environmental factors. The imbibition kinetics of seed germination is also affected by seed coverings. Thus, the difference in response of these weeds to salinity might by related to their different seed coat structure.

Seedling vigor index of *Echinops ritro* was much higher than *Centaurea virgata* which was due to the longer, bigger root and shoot system of the seedling. The salinity concentration which led to 50% reduction in seedling vigor of *Echinops ritro* and *Centaurea virgata* was 118.66 and 81.62 Mmol, respectively. This showing that *Echinops ritro* loss 50% of seedling vigor at 37 Mmol higher salinity than *Centaurea virgata* (Table 2).

Table 1. The effect of salinity stress on germination of two weeds *Echinops ritro* and *Centaurea virgata*

	Echinops *ritro*				*Centaurea virgata*			
NaCl concentration	G_{max}	b	Ec_{50}	$R^{sqr(Adj)}$	G_{max}	b	Ec_{50}	$R^{sqr(Adj)}$
control	99.52(0.35)	0.87(0.16)	11.49(0.11)	0.99	83.75(1.59)	25.17(2.15)	73.68(2.49)	0.94
50Mmol	99.58(0.47)	1.61(0.14)	11.97(0.13)	0.98	69.15(1.48)	25.01(2.43)	70.78(2.79)	0.93
100Mmol	95.91(0.97)	4.29(0.52)	16.00(0.55)	0.94	43.69(1.87)	21.68(4.50)	63.26(5.07)	0.75
150Mmol	87.24(1.71)	14.23(1.59)	47.07)1.89)	0.92	34.23(1.59)	16.11(4.14)	62.12(4.69)	0.72
200Mmol	40.67(1.52)	32.10(4.39)	95.24(5.72)	0.87	23.17(0.50)	18.92(2.18)	72.78(2.46)	0.93
250Mmol	17.53(0.85)	30.08(5.74)	87.42(7.16)	0.77	15.77(0.56)	16.47(3.40)	76.51(3.81)	0.84

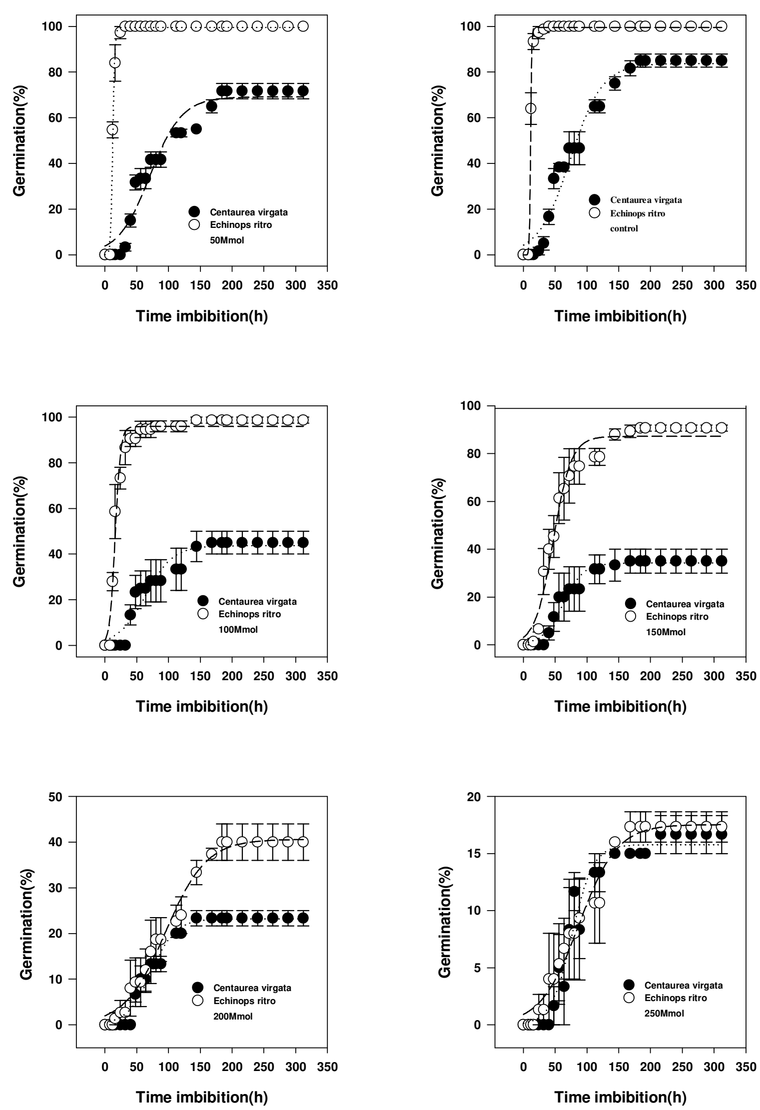

Figure 1. Seed germination rate of two weed species affected by salinity stress treatments

Table 2. Estimated of parameters logistic on final germination and vigor index of two weeds *Echinops ritro* and *Centaurea virgata* in response to salinity levels

	Final germination				Vigor index			
NaCl conc.	Gmax	b	Ec50	Rsqr (Adj)	Vmax	b	Ec50	Rsqr (Adj)
Echinops ritro	99.49 (1.44)	7.43 (0.63)	193.03 (2.46)	0.98	832.49 (31.36)	4.27 (0.64)	118.66 (5.31)	0.96
Centaurea virgata	85.52 (3.26)	1.79 (0.20)	115.09 (8.432)	0.95	297.05 (11.80)	2.84 (0.33)	81.62 (5.01)	0.96

The three parameters logistic model was fitted on data obtained from final seed germination and vigor index of both weeds under different salinity levels (Figure 2). Results of logistic function estimated the concentrations of salinity stress which led to 50% germination reduction. At the 193.03 Mmol and 115.09 Mmol salinity stress seed germination was 50% decline for *Echinops ritro* and *Centaurea virgata* respectively.

According to the estimated EC 50% parameter, it was shown that the *Echinops ritro* is more tolerate to the same salinity concentrations than *Centaurea virgata* which was approximately 78 Mmol. Results of logistic function showed that although there was a reduction in germination percentage of *Echinops ritro* at concentrations above 150 Mmol was started by they already declined it's vigor index at the concentration of higher than 50 Mmol. Interestingly, seed germination seed germination and seedling vigor of *Centaurea virgata* both start to decline at salinity concentrations higher than 50 Mmol (Figure 2). Seed germination of *Echinops ritro* at salinity concentration of 200 Mmol was equal with seed germination of *Centaurea virgata* at a salinity of 100 Mmol.

There was no seed germination of both weeds at -1.2 MPa. The *Echinops ritro* was capable to tolerate drought stress till -0.4 MPa without any reduction of seed germination while for *Centaurea virgata* germination reduction due to drought stress was initiated from -0.2 MPa. Our results revealed that difference of seed germination between both weeds we not statistically significant at drought stress with -0.8 MPa but interestingly when drought stress increased to -1 MPa, then *Centaurea virgata* (24%) exhibited higher drought tolerance four times of *Echinops ritro* (6%) (Figure 3). As presents in Figure 4, seed germination and seedling vigor of *Echinops ritro* were drastically reduced at drought stress higher than -0.6 MPa. Results showed 50% of the reduction in seed germination potential of *Echinops ritro* and *Centaure virgata* at a concentration of -0.78 and -0.74 MPa, respectively. Seedling vigor, however, was more sensitive to drought stress and germination rate. Seedling vigor of *Echinops ritro* and *Centaure virgata* declined to 50% at concentrations of -0.36 and -0.41, respectively (Table 2). These results suggested that *Centaure virgata* slightly provide more drought tolerance to *Echinops ritro*.

Our results showed that *Echinops ritro* seeds germinated better in NaCl than drought treatments. Lower germination percentage obtained from PEG compared with NaCl at equivalent water suggest that adverse effects of PEG on germination were due to osmotic effects rather than specific ion accumulation. These results agree with Murillo-Amador *et al.* (2002) for cowpea, Demir and Van De Venter (1999) for watermelon for which they reported that drought or salinity may influence germination by decreasing the water uptake. Under salt stress, Na^+ and Cl^- may be taken up by the seed and toxic effect of NaCl might appear.

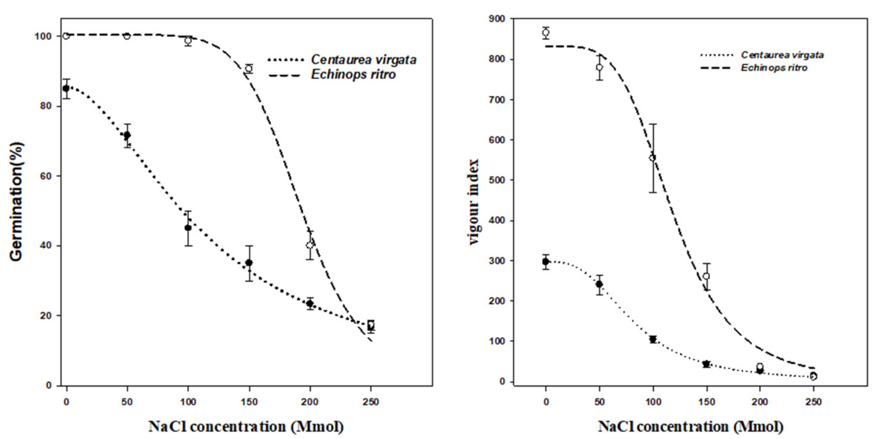

Figure 2. Fitted nonlinear regression model on the data obtained from seed germination and seedling vigor of *Echinops ritro* and *Centaurea virgata* under salinity concentrations

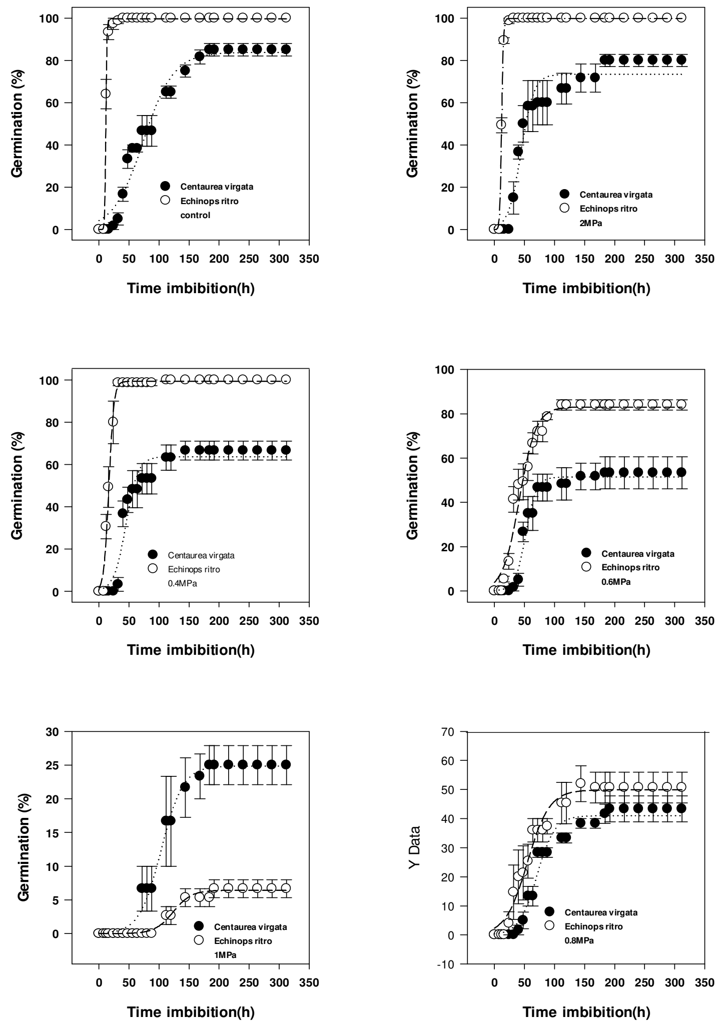

Figure 3. Seed germination rate of two weed species affected by drought stress treatments

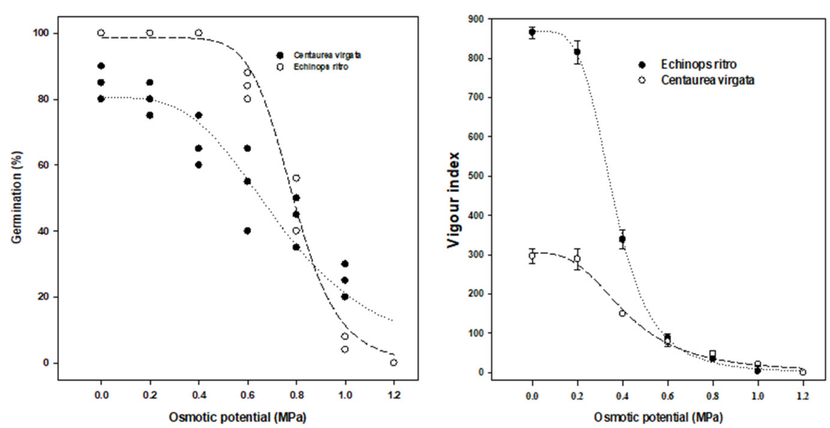

Figure 4. Fitted nonlinear regression model on the data obtained from seed germination and seedling vigor of *Echinops ritro* and *Centaurea virgata* under drought stress

Discussion

Soil salinity and drought stresses are wide spread challenges all around the globe. They are the main reasons for limiting crop production and yield losses (Parihar *et al.*, 2015; Wang *et al.*, 2017). The plant response to salinity stress are a complex network of gene expression and physiological alterations (Gupta *et al.*, 2014a, 2014b). Our main purpose to user sigmoid and logistic models was to provide better illustrations for NaCl and drought injuries to seedling vigor by showing that the low concentrations of salt have little effect on growth, but at higher concentrations seedling vigor rapidly declined as a sigmoidal function of the NaCl and drought concentrations (Figure 2 and 4). These findings are in line with the reports of (Willenborg *et al.*, 2005; Bybordi and Tabatabaei, 2009; Claeys *et al.*, 2014). Results revealed that under 118 Mmol salinity, the seed germination of *Centaurea virgata* declined to 43% which was as close as half of its total seed germination. However, 50% reduction in seed germination of *Echinops ritro* was observed at 193 Mmol salinity (Table 2). This is the indication of better salt resistant mechanism in *Echinops ritro* and we expect to germinate seed of this weed at high saline soils than the *Centaurea virgata*. We are suggesting that the sensitivity of *Centaurea virgata* to Na+ or Cl- is the main reason for the lower salt tolerance. It was observed that both weed species losses about 50% of their germination potential at -0.8 MPa (Table 3). Therefore, as previously described, salinity may influence the plant physiological process through either induction of osmotic stress or ion imbalance and toxicity or both (Munns and Tester, 2008; Parihar *et al.*, 2015). Thus, it is concluded that the ion imbalance is the main cause of lower salinity tolerance in *Centaurea virgata*. In addition, it was shown that the salinity stress led to disturbance of hormone balance in seeds which may reduce utilization of seed reservoirs (Albacete *et al.*, 2014).

The response of seeds to drought stress includes a series of cellular, physiological, molecular and biochemical processes (Shinozaki *et al.*, 2007). It was previously reported that drought stress can cause serious damage to seed germination (Kaya *et al.*, 2006; Lin *et al.*, 2017). However, quantifying seed germination response of various plant species may still be helpful to understand and predict the time of appearance or emergence at the farms facing water deficit stress. In this regard, our results showed that, at winter fields of Iran, especially the ones facing serious drought challenge, both *Echinops ritro* and *Centaurea virgata* may interfere with crops increase intra species due to their considerable drought stress tolerance.

Conclusions

The salinity threshold for *Centaurea virgata* to loss 50% of its seed germination potential was 118 Mmol salinity while 50% reduction in seed germination of *Echinops ritro* was observed at 193 Mmol salinity. Therefore, at saline lands, it is expected to seed more *E. ritro* seedlings than *C. virgata*. Both weeds germinated better in saline condition than drought treatments. Lower germination percentage obtained from PEG compared with NaCl at equivalent water suggest that adverse effects of PEG on germination were due to osmotic effects rather than specific ion accumulation.

Authors' Contributions

Ahmaz Zare: Conceptualization (weed species; drought stress); Data curation; Formal analysis; Writing. Seyed Amir Moosavi: Conceptualization (seed germination; salinity stress); Methodology; Data curation; review and edition. Both authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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