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Effect of drought and salinity tensions on germination and seedling growth of Common Yarrow *(Achillea millefolium* L.) in laboratory conditions

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ABSTRCT

A major problem of arid and semi-arid regions is environmental stresses like salinity and drought. To study the effect of Artichoke germination under different levels of salinity and drought, two separate experiments were performed. The experimental treatments included different salinity levels of NaCl (0, 50, 100, 150 mM) and five drought levels by PEG (0, 10%, 15%, 20% and 25%) with three replications that used based on completely randomized design. Results showed that drought and salinity stresses reduced significantly germination rate, germination percent, and shoot length. In both conditions, there was insignificant difference between treatments for dry weight of root and shoot. There was insignificant difference between treatments for root length in salinity condition, but in drought condition, there was significant difference for this trait. Moisture range optimum for germination was determined from non-stress to 20% PEG concentration (in this study). It seemed that among traits, shoot length was more sensitive than others to salinity and drought stresses. It was observed that Common Yarrow fairly tolerated salinity better than drought conditions. It can be concluded that Common Yarrow can tolerate salinity up to 150 Mm, but in drought conditions it cannot germinate in 25% PEG.

Key words: Common Yarrow, Drought, Germination, Salinity

1- INTRODUCTION

Achillea millefolium, known commonly as yarrow or common yarrow, is a flowering plant in the family Asteraceae. It is native to temperate regions of the Northern

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Hemisphere in Asia, Europe, and North America. Common yarrow is frequently found in the mildly disturbed soil of grasslands and open forests. Active growth occurs in the spring. It has also been used as herbal tea to relieve gastrointestinal inflammations (Innocenti *et al.*, 2007). *Achillea millefolium* is cultivated as an ornamental plant by many plant nurseries. It is planted in gardens and natural landscaping settings of diverse climates and styles. The plant is a frequent component of butterfly gardens. The plant prefers well-drained soil in full sun, but can be grown in less ideal conditions. The plant flowers from June to September (Batanouny, 1999; Boulos, 2000). Yarrow is an herbaceous perennial, and is best suited to cottage rather than a formal garden (Halevy, 1999). The plant also has a long history as a powerful 'healing herb' used topically for wounds, cuts and abrasions (Batanouny, 1999). It has medicinal and cosmetic uses (Rohloff et al., 2000), and extensively grown in drought-prone environments due to its numerous leaf and several stems developed from the horizontal rootstock (Bartram, 1995). Plants are usually exposed to different environmental stresses which limit their growth and productivity as well as cause considerable loss to worldwide agricultural production [Shao et al., 2008]. One of the most important factors affecting plant growth and the production of secondary metabolites is the salt stress [Nikolova et al., 2005]. Production of green buds and seeds is strongly affected by drought and salinity stress, resulting in poor plant establishment in dry and saline soils (Afzal et al., 2005; Soltani et al., 2008). Germination is one of the most salt-sensitive plant growth stages severely inhibited with increasing salinity (Sosa et al., 2005; Zare et al., 2006). This negative response of seed germination under salt stress was reported by many authors on *Ocimum basilicum*, *Eruca sativa*, *Petroselinum hortense*, chamomile, sweet marjoram, and *Thymus maroccanus* [Miceli et al., 2003; Ramin, 2005; Ali RM et al., 2007. Belaqziz et al.,2009]. Sodium chloride has been used to simulate osmotic stress effects in Petri dish (*in vitro*) for plants to maintain uniform water potential throughout the experimental period (Kulkarni and Deshpande, 2007; Young et al., 1983). Drought stress is another most important environmental factor in reduction of growth, development and production of plants. It can be said that it is one of the most devastating environmental stresses. Iran, with an annual rainfall of 240 mm, is classified as one of those dry regions (Jajarmi, 2009). It is necessary to cultivate tolerated plants in these regions. According to Hayat and Ali (2004), Moisture stress is a limiting factor for crop growth in arid and semi-arid regions due to low and uncertainty precipitation. Enhancement of seed germination rate might be result in better seedling establishment especially under salinity and drought stresses condition (He et al., 2002). Research carried out on *Seidlitzia rosmarinus* (Hadi et al., 2007) and *Salsola* sp. (Zahtabian et al., 2005), suggested that increased level of NaCl and polyethylene glycol provides unsuitable medium for seeds germinations so that the more salinity and drought stress levels, the less germination rate. Salehi Sardoei (2013) observed that germination of *Lycopersicon esculentum* L. var *Cal-ji* decreased with increasing in salinity, significantly. Therefore, concentrations of 200 to 250 Mm caused to reducing germination up to 29% and 17%, respectively.

The present study was therefore mainly conducted to evaluate the effect of salinity and drought based on PEG and NaCl on seed germination and seedling growth of *Achillea millefolium* L.

2- MATERIALS AND METHODS

Common Yarrow (*Achillea millefolium* L.) seeds were obtained from the Department of Natural Resources, Agriculture Research Center of Hamedan. Two separate experiments were carried out in a research lab of scientific – Applied University of Jihad- Agriculture of Hamedan, Iran. The first experiment was consisted of five levels of drought potential (0, 10%, 15%, 20% and 25%) which was prepared using the chemical polyethylene glycol 6000 (PEG-6000). The second experiment also included four levels of salinity (0, 50, 100 and 150 mM) which NaCl was used to establish the levels of salinity; also, distilled water was used for stress surface of zero establish in both experiments. Studies have shown that these materials are suitable for laboratory experiments on salinity and drought (Michel and Kaufman, 1973). Both experiments were conducted in completely randomized designs with three replications. Before experiments, seeds were disinfected with 2% sodium hypochlorite (bleaching liquid) for 2 min. Then, they were washed with distilled water and sterilized with Benomyl 0.2% (for 2 min). Gama sterilized Petri Dishes were used for these tests. Sterilized Whatman filter papers were placed on the bottom of Petri Dishes. Fifty seeds were selected and placed into Petri Dishes on Whatman papers. Then, for drought and salinity treatments, 5 ml polyethylene glycol solution and 5 ml sodium chloride solution were added to each Petri Dish, respectively. Petri Dishes and seeds treated in 24 ± 1° C. Petridishes are monitored on a daily basis and afterwards germinated seeds (according to exit the root shell to the size of 2 mm from seed Testa) were measured and recorded daily in each Petri Dish (Emmerich and Hardegree, 1991; Fernandez and Johnston, 1995). After 10 days of the experiment, seed germination percentage was calculated, and then root length and shoot length of seeds were measured with a ruler. Root and shoot dry weight was measured (after maintaining in the oven at 70 ° C for 48 h) by a digital scale (0.0001 g). Germination percentage and germination rate were calculated using following formula.

$G = (n/N)*100$ (Jefferson and Penachchio, 2003)
$RG = (Ni /Di)$ (Jefferson and Penachchio, 2003)

G: germination percentage, n: number of germinated seeds, N: total number of seed in each Petri Dish, RG: rate of germination (seed /day), Ni: germinated seeds in each numeration, Di: day of each numeration. Statistical analysis of data from two experiments was performed to compare the concentrations independently and to compare stress type in equal concentrations and in the same situations by SAS statistical software (SAS Inst. Cary, NC, USA, 2004). Comparing all averages by Duncan's multiple test range was conducted in level 5%. All graphs were plotted with Excel software.

3- RESULTS AND DISCUSSION

Salinity:

Results showed that there were significant differences between treatments based on rate of germination, germination percent, shoot length and root length ($p<0.01$). Seed germination decreased. The highest germination percent was related to control treatment (96.00%) and the lowest related to concentration of 150 mM (56.67%) (Fig. 1: 1c). Also Seyyed Sharifi (2007) in an experiment with study on four salinity levels (0, 25, 50 and 100 Mm) NaCl on *Silybum marianum* germination showed that salinity stress has significant effect on germination percentage, germination uniformity and germination percentage average in salinity potential 25 and 50 Mm NaCl decreased in compared to control (distilled water) respectively 17.20 and 43.00 percent. the salinity in the seed germination stage damage the membrane of the cell, particularly the cytoplasmic membrane that results in increasing permeability of the membrane due to the replacement of Ca^{2+} by Na^{+} that resulted to increasing in K^{+} losses (Takel, 2000). Also, Salehi Sardoei (2013) found that with in increasing in salinity from 50 to 150 Mm, germination of *Lycopersicon esculentum* L. var *Cal-ji* reduced about 18.21%. Also, germination rate decreased with increasing in salinity that the highest germination rate related to control treatment (13.2 $p.d^{-1}$) and the lowest related to concentration of 150 mM (5.2 $p.d^{-1}$) (Table 1). With increasing in concentrations, root length of seedlings decreased. The longest root length obtained in control treatment (6.8 mm) and the shortest obtained in concentration of 150 mM (2.1 mm) (Fig. 1: 1a). Also, shoot length reduced with increasing in salinity, like other parameters, but it showed more sensitivity to salinity, therefore, in concentrations of 100 and 150 mM, shoot length reduced notably (Fig. 1: 1a). The highest shoot length related to control (9.53 mm) and the lowest related to 150 mM (5.40 mm) (Table 1). Salinity impressed seed germination through osmotic pressure and therefore reduction of water absorption by seeds and also from the toxic effects of Na^{+} and Cl^{-} (Rehman *et al.*, 1996). In this study, reduction of different germination parameters can be related to reduction of speed and rate of water absorption. In addition to the osmotic effect which reduces the uptake due to the specific toxicity effect of ions, the loss of germination percentage is caused by the interruption of nutrients uptake which has been confirmed by Safarnejad et al. (1996), Penuelas et al. (1997) and Shalhevet (1993), too. It has been shown that the raising of salinity increased the uptake of Na, K and P and decreased the uptake of N which can be the reason for the loss of germination percentage (Tarzi, 1995). Salinity may inactivate the germination-affecting enzymes, especially by increasing the uptake of K^{+} which brings about a secondary peak which as a result, inhibits the activation and/or synthesis of germination-affecting enzymes and the uptake of Ca^{2+} increases in a confrontation with Na^{+}. Nabizadeh (2002) stated that the adverse effect of salinity on plants can be caused by the loss of osmotic potential of root medium, specific ion toxicity and the lack of nutritional ions.

Drought:

With increasing in drought stress, there was significant difference between treatments in all evaluated traits (germination rate, germination percent, shoot length, root length, root dry weight and shoot dry weight) ($p<0.01$) (Table 1). With increasing of PEG concentration, root length of Common Yarrow seedlings was decreased. The longest root length related to the control (6.80 mm) and the shortest related to concentration of 25% PEG (no germination). Also, shoot length reduced like other characteristics, but it showed more sensitivity to drought. The longest and the shortest shoot length related to control (9.53 mm) and concentration of 25% PEG (zero), respectively (Fig. 1: 1b) (Table 1). Root length decreasing reported with increasing in water stresses and one of the causes of shoot length reduction in drought stress conditions has been found reduction or non-transfer of nutrients from seed storage tissues to the embryo by Takel (2000). Generally, germinated seeds in environments under stress conditions have shorter shoot and root (Katergi *et al.*, 1994). Results showed that germination of Common Yarrow reduced. The highest percentage of germination related to the control treatment (93.33%) and the lowest related to concentration of 25% PEG (no germination) (Fig. 1: 1d). It has been reported similar results in Uniyal and Nautiyal studies (1998). Germination rate decreased with increasing in drought. The highest germination rate was 13.19 $P.d^{-1}$ that related to control treatment and the lowest was zero (25% PEG). Barzegar and Rahmani (2004) reported that there was a significant difference between values of percentage averages and germination speed of Hyssop seed (*Hyssopus officinalis*) under affection of drought stress levels (0, -1, -3, -6 and -9 bar), and with increasing stress intensity, the values of these traits got lower. Their results are consistent with our results. If water absorption disturbed or delayed, it could delay in seed metabolic processes. It is resulted that time for radicle outing increased and therefore, germination rate is decreased (Marchner, 1995). Reduction of germination process due to drought stress can be related to a decrease in water absorption by seeds. Reduction of root length with increasing in water potential has been reported by Takel (2000). One of the factors that caused shoot length decreased in during drought conditions is reduction or no transfer of nutrients from seed storage tissues to embryo. In addition, decreasing in water absorption by seed under drought stress causes to decrease in hormones secretion and enzymes that result to impair seedling growth (root and shoot) (Asghari, 2002). Falleri (1994) reported that germination rate of *Pinus Pinaster* seeds in drought stress conditions reduced more than germination percent. In general, the effect of drought stress on germination rate and germination percent is more than other traits.

4- CONCLUSION

From the result of this study, it can be concluded that Common Yarrow can tolerate salinity up to 150 Mm, but in drought conditions it cannot germinate in 25% PEG. The result clearly demonstrated that the effectiveness of salinity on the root length was more than shoot length and similar results were observed in drought conditions. Also, germination rate decreased with increasing in salinity and drought that the highest germination rate related to control in both condition and the lowest related to concentration of 150 mM NaCl and 25% PEG, respectively for salinity and drought.

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Table 1: Effects of PEG and NaCl on germination characteristics and seedling growth of Common Yarrow (*Achillea millifolium*)

Treatments	Germination percent (%)	Germination Rate (p.d^{-1})	Root length (mm)	Shoot length (mm)	Root dry Weight (mg/plant)	Shoot dry Weight (mg/plant)
NaCl						
Distilled water (control)	93.33 a	13.2 a	6.80 a	9.53 a	0.3000 a	0.4667 a
50 mM	96.00 a	12.7 a	2.80 b	9.27 a	0.1000 a	0.3333 a
100 mM	74.00 b	8.1 b	2.40 b	6.13 b	0.1000 a	0.4667 a
150 mM	56.67 c	5.2 c	2.10 b	5.40 b	0.1000 a	0.3133 a
P value	**	**	**	**	ns	ns
CV	9.16	12.27	21.16	10.87	23.67	21.70
PEG						
Distilled water (control)	93.33 a	13.19 a	6.80 a	9.53 a	0.3000 a	0.4667 a
10%	94.00 a	10.53 b	5.00 cd	8.87 a	0.1667 ab	0.6000 a
15%	74.00 a	7.73 c	2.13 ab	5.87 b	0.1667 ab	0.5333 a
20 %	20.00 b	1.89 d	3.77 bc	3.00 c	0.1000 ab	0.4000 a
25%	0.00 b	0.00 d	0.00 d	0.00 d	0.0000 b	0.0000 b
P value	**	**	**	**	**	**
CV	14.64	21.13	20.47	20.28	22.37	24.11

ns, * and **: Not significant, significant at 5% and 1% probability levels, respectively.
Amounts of P value are means of square in ANOVA.
Similar letters in each column (between two horizontal lines) indicate no significant difference at 5% probability levels (Duncan test).

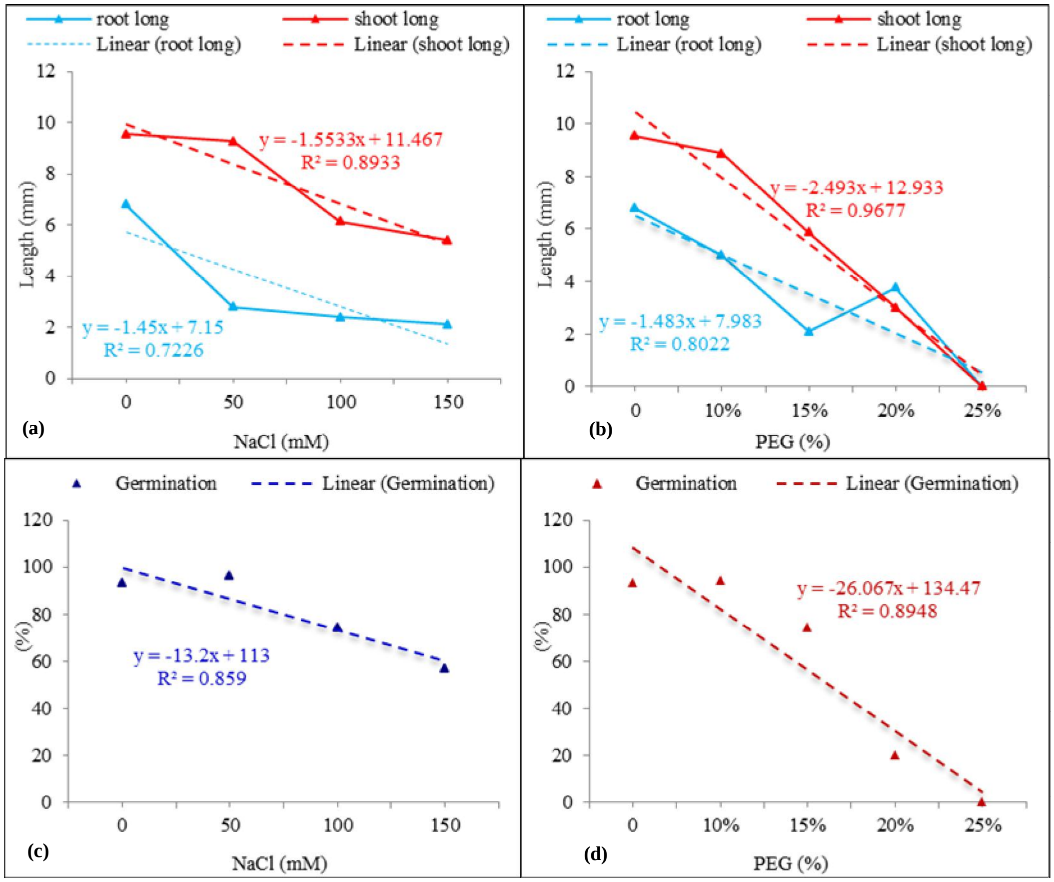

Fig 1: Effect of NaCl on (a) root and shoot length, (c) germination and PEG on (b) root and shoot length, (d) germination.