Research Journal of Applied Sciences, Engineering and Technology 4(21): 4524-4529, 2012
ISSN: 2040-7467

Submitted: May 15, 2012 Accepted: June 08, 2012 Published: November 01, 2012

Effects of Salt and Drought Stresses on Germination and Seedling Growth of Swallow Wort (*Cynanchum acutum* L.)

[1]Farid Golzardi, [1]Saeed Vazan, [2]Hossien Moosavinia and [1]Ghasem Tohidloo
[1]Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, Iran
[2]Shahid Chamran University of Ahwaz, Ahwaz, Iran

Abstract: In order to study the effects of drought and salinity stresses on germination indices in swallow wort (*Cynanchum acutum* L.), an experiment was conducted as factorial form, using a completely randomized design arrangement, with four replications. In this study, 6 levels of PEG 6000 including 0 (distilled water), -0.2, -0.4, -0.6, -0.8 and -1 MPa and 6 levels of NaCl consisting 0, 100, 200, 300, 400 and 500 mM were applied in 2011. Results indicated significant differences among the treatments ($p<0.01$) in all the traits. Control treatment was better for radicle and hypocotyle length as well as seedling fresh weight and seed germination than the other treatments considerably. Results showed that the drought and salinity stresses effect essence on germination and early seedling growth was different. Osmotic potential up to -0.6 Mpa and salinity stress up to 300 mM had little effect on seed germination. Seed germination was less than 13% at -1 Mpa osmotic potential and less than 9% at 500 mM salinity.

Keywords: Hypocotyle length, NaCl, osmotic potential, PEG, radical

INTRODUCTION

To protect natural habitats from the detrimental effects of invasive plant species, there is an urgent need to develop effective and sustainable management programs. The control of invasive species generally requires an integrated approach to limit their growth and reproduction, because a single strategy oftenmight not be effective and sustainable (Van Wilgen *et al.*, 2000). Invasive species pose one of the greatest threats to native plant populations and diversity, second only to habitat loss Wilcove *et al*. (1998). The ability to predict seed germination and emergence in response to environmental conditions is essential in order to allow better timing of mechanical, biological and other management strategies (Ghorbani *et al*., 1999).

Swallowwort is problematic in disturbed habitats, including recently abandoned agricultural fields and roadsides, but they also invade rocky outcrops and old fields (DiTommaso *et al*., 2005). Swallowwort (*Cynanchum acutum* L.) is an herbaceous perennial vine in the Asclepiadaceae family, originating in southern Europe (Albania, Romania, France, Greece, Spain, Portugal and Russia) (Davis, 1984; Tewksbury *et al*., 2002). It is widespread in Mediterranean regions from Spain, east to Iraq (Shu, 1995) and found extensively in cultivated fields, orchards, fence rows and roadsides (Mozaffarian, 1998). In Iran, swallowwort was first found in 1988 in Moghan province and is now widespread across northern Iran. Complete eradication of swallowwort is unlikely, because of its enormous seed production, dispersal by wind and water and the ability to propagate vegetatively (Pahlevani *et al*., 2008).

Agriculture has been being affected by environmental stresses such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress which reduce crop yield fifty percent, approximately and water stress that is caused by salinity and drought is a prevalent problem in the world. However, plants are affected by drought and salinity similarly (Khayatnezhad *et al*., 2010). Salinity and drought stresses are physiologically related, because both induce osmotic stress and most of the metabolic responses of the affected plants are similar to some extent (Kumar *et al*., 2011; Farsiani and Ghobadi, 2009). Salinity is one of the most important restrictions on crop production in the world, particularly in arid and semi-arid regions (Dadkhah and Grrifiths, 2006).

Salinity through production of exterior osmotic potential which cease water uptake and Na^+ and Cl^- ions toxic effects influences on seed germination (Turhan and Ayaz, 2004). The effect of saline stress on seed germination is to make easier toxicions ingestion which alters particular seed enzymatic or hormonal activities (Sayar *et al*., 2010). Seed germination has been reported to be the most critical stage influencing crop establishment in areas under saline conditions (SitiAishah *et al*., 2010).

Corresponding Author: Farid Golzardi, Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, Iran

The presence of water has a considerable role in crucial processes such as enzymatic reactions, metabolites transportation and solubilization as well as a factor in proteins, lipids and carbohydrates hydrolytic breakdown in seeds (Bialecka and Keoczynski, 2010). Seed priming methods are safe, effective and easily adopted by farmers (Dursun and Ekinci, 2010) such as seed soaking in solutions of Polyethylene Glycol (PEG) was expressed as sowing seeds in an osmotic solution that permits seed to absorb water for germination, but inhibits radicle extension via seed coat (Janmohammadi *et al*., 2008; Giri and Schillinger, 2003). The reaction of seed to priming is associated with some factors including duration of priming, seed maturity, species and environmental conditions (Armin *et al*., 2010). It was indicated that priming induces synthesis of DNA in tomato radicle cells, pepper and maize radicle cells (Rouhi *et al*., 2011). Duman (2006) observed no positive effect of seed priming on lettuce plumule and radicle growth in both laboratory and greenhouse. A practical method which has been applied to identify water stress as a restraining factor for seed germination is Polyethylene Glycol (PEG) (Mantovani and Iglesias, 2010) in Petri dishes because of high molecular weight and it can't pass through plant cell walls Kumar *et al*. (2011).

This research was conducted to study the effects of drought and salt stresses induced by PEG and NaCl on germination and early seedling growth of swallowwort (*Cynanchum acutum* L.).

MATERIALS AND METHODS

In order to study of effects of salinity and drought stresses on germination and early seedling growth of swallow wort (*Cynanchum acutum* L.), an experiment was conducted as factorial form, using a completely randomized design arrangement, with four replications. In this study, 6 levels of PEG 6000 including 0 (distilled water), -0.2, -0.4, -0.6, -0.8 and -1 MPa and six levels of NaCl consisting 0, 100, 200, 300, 400 and 500 mM were applied in 2011.

Swallowwort pods were collected from Karaj of Iran in 2010. The seeds were removed from the pods and the silky white hair (coma) removed from the seeds and kept at room temperature for the period of study. Immediately prior to each experiment, seed viability was tested using tetrazolium (Moor, 1985).

During experiments, the seeds were surface-sterilized by being soaked in 1% solution of carbandazime fungicide for 5 min, washed with several flushes of distilled water and dried at room temperature. Fifty seeds were placed on filter paper (Whatman No. 2) in 11-cm-diam Petri dishes. The filter paper was moistened with 7 ml of test solution. To maintain adequate moisture in Petri dishes, 4 mL distilled water was added when needed. The seeds were considered germinated when the radicle visibly protruded through the seed coat. Germination, radicle length and hypocotyle length were recorded after 7 d incubation.

Aqueous solutions with osmotic potential of 0 (distilled water), -0.2, -0.4, -0.6, -0.8 and -1 MPa were prepared by using Polyethylene Glycol (PEG) 6000 in deionized water. The following equation (Agrawal and Dadlani, 1992; Burlyn and Kaufmann, 1973) was used for calculation of water potential from known concentrations of PEG 6000:

$$\text{Water potential} = -(1.18 * 10^{-2)} C - (1.18*10^{-4}) C^2 + (2.67*10^{-4}) CT + (8.39 * 10^{-7}) C^2T \quad (1)$$

where, C is concentration of PEG (g/kg distilled water) and T is temperature (C). Two layers of filter paper were placed in the bottom of the 11-cm-diam Petri dishes. The Petri dishes were placed in a 12 h light incubator at 3°C. Seed germination percentage and radicle and hypocotyl lengths of the seedlings were determined up to 7 d.

Salt effects on seed germination were studied using solutions of 0, 100, 200, 300, 400 and 500 mM NaCl. Petri dishes containing filter paper and seeds were placed in an incubator with 12 h of light at 3°C and 12 h of darkness at 25°C. Seed germination percentage, radicle length and hypocotyl length of the seedlings after 7 d, were determined.

Prior to analyses the data were tested for normality of distribution with the Shapiro-Wilk test. Germination data were arcsine-transformed to ensure homogeneity of variance. Data were subjected to Analysis of Variance (ANOVA) procedures (SAS 9.1) and LSD test was applied at 5% probability level to compare the differences among treatment means.

RESULTS AND DISCUSSION

Salinity is a major environmental stress factor that affects seed germination in coastal salt marshes (Khan, 2002) where salinity ranges from 0.8 to 2.4%. Saline sodic and non-sodic soils have high salt content of sodium, calcium and magnesium that could reach 8% (Waisel, 1972) in these soils while the more important anions are chloride, sulfate and bicarbonates (Bewley and Black, 1994).

Analysis of data presented in Table 1 showed that salt and osmotic stresses had significant effect ($p<0.01$) on Radicle length and hypocotyle lengths. According to

Table 1: Analysis of variance (mean of square) of measured traits of swallow wort under drought and salinity stress

S. O.V	Radicle length	Hypocotyle lengths	Germination	Seedling fresh weight
Drought stress	2146.4**	123.87**	1.250**	1741.46**
Salinity stress	1938.9**	148.72**	1.21**	1534.98**
Drought * salinity	35.4**	1.20**	0.03**	12.70^{ns}
Error	47.00	0.80	0.009	4.50
C.V	11.13	10.91	8.240	7.85

**: Significant at p≤0.01 level; ns: not significant

Table 2: Mean comparison of drought and salinity stress on measured traits of swallow wort using LSD method

Treatment	Radicle length (mm)	Hypocotyle length (mm)	Germination (%)	Seedling fresh weight (gr)
Drought stress (MPa)				
0	3.84^{a}	1.97^{a}	83.25^{a}	3.76^{a}
-0.2	3.29^{ab}	1.42^{b}	68.75^{ab}	3.02^{b}
-0.4	2.88^{ab}	1.16^{bc}	45.5^{b}	2.61^{bc}
-0.6	2.12^{b}	0.97^{bc}	39.75^{b}	1.97^{c}
-0.8	1.47^{bc}	0.68^{c}	21.5^{bc}	1.35^{d}
-1.0	0.56^{c}	0.28^{d}	12.25^{c}	0.32^{e}
Salinity stress (mM)				
0	3.79^{a}	1.88^{a}	85.5^{a}	3.81^{a}
100	2.56^{b}	1.25^{b}	63.25^{b}	2.18^{b}
200	1.65^{c}	0.76^{c}	32.25^{c}	1.45^{bc}
300	1.47^{c}	0.69^{c}	28.75^{c}	1.12^{bc}
400	1.14^{c}	0.43^{cd}	9.5^{d}	0.76^{c}
500	0.89^{c}	0.21^{d}	8.75^{d}	0.32^{c}

Values in a column bearing different superscript are significantly different at 0.01 level using LSD methods

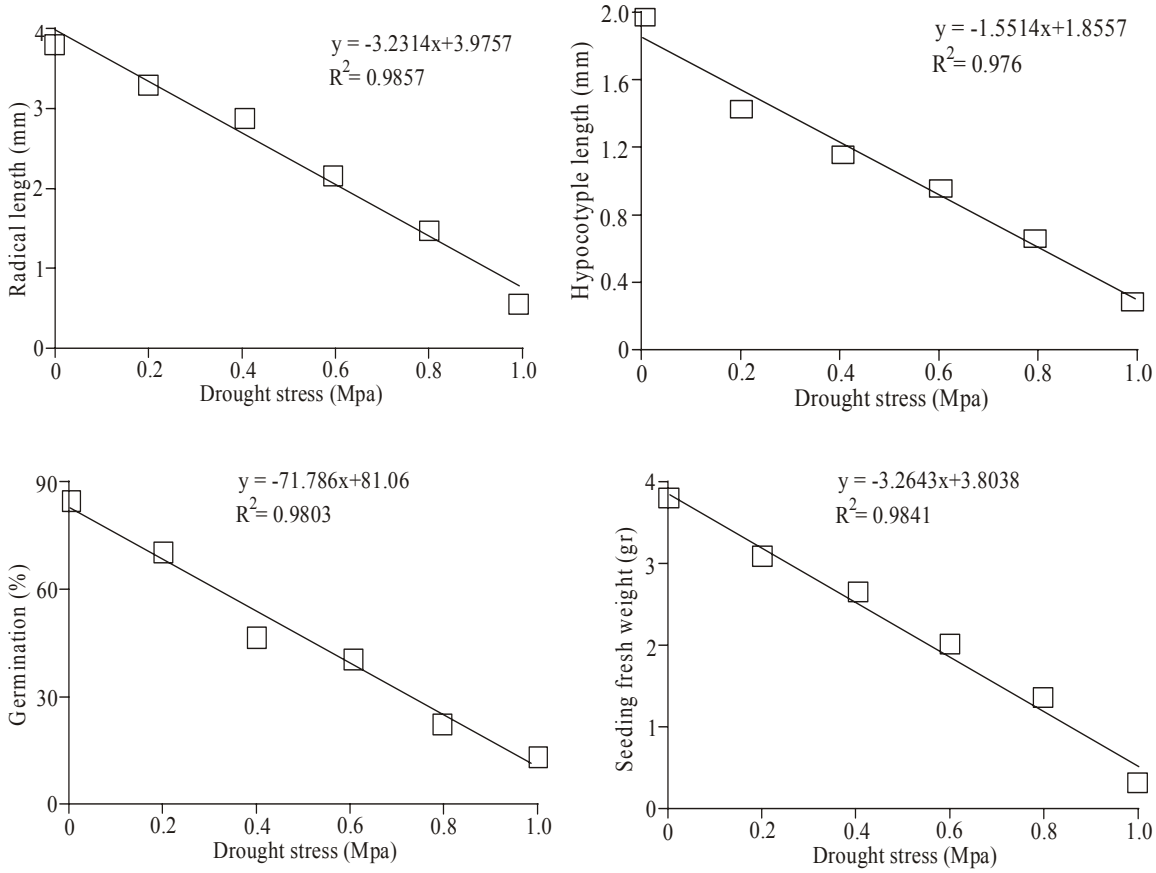

Fig. 1: Radicle length, hypocotyle length, seed germination and seedling fresh weight of swallow wort exposed to various drought levels. A linear trend line and regression equation is shown. Each point represents the mean of observations

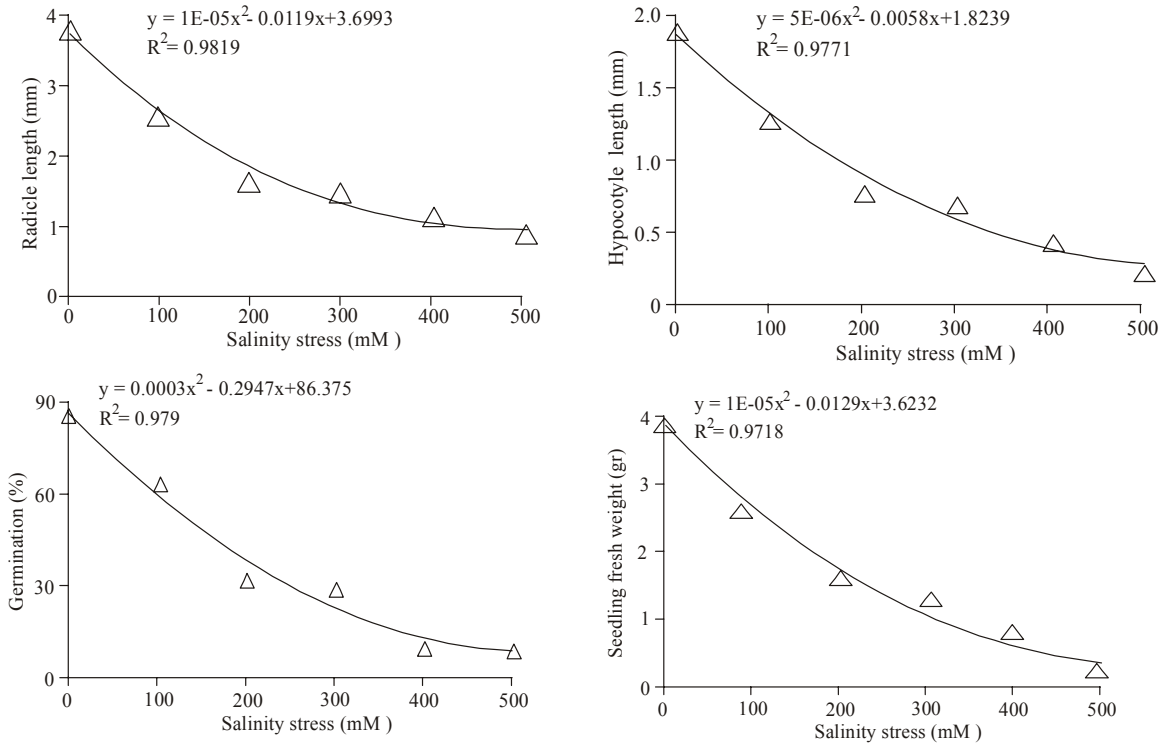

Fig. 2: Radicle length, hypocotyle length, seed germination and seedling fresh weight of swallow wort exposed to various NaCl concentrations. A second order polynomial trend line and related regression equations are shown. Each point represents the mean of observations

results of mean comparison control treatment of salinity levels and control treatment, -0.2 and -0.4 MPa of drought levels has the highest amounts of Radicle length and hypocotyle lengths (Table 2, Fig. 1 and 2). Khayatnezhad *et al*. (2010) report that the effects of salinity and drought levels on Radicle length and hypocotyle lengths were significant. Delachiave and De Pinho (2003) stated that too much amount of salt in cell wall led to decrease in hypocotyle length. Macar *et al.* (2009) found that drought stress induced by PEG prevented and radical extention in Chickpea. Rahimi *et al*. (2006) in their study made clear that NaCl and PEG were inhibitory to Radicle and hypocotyle elongation in plantago species because of a decrease in water potential gradient between seeds and their surroundings.

Results of analysis of variance showed significant difference of factor on Germination percent at 0.01% level (Table 1). Mean comparisons by LSD method displayed that salinity stresses at control treatment and drought stresses at control treatment and -0.2 MPa levels had the highest amount of germination percentage (Table 2, Fig. 1 and 2). But Yari *et al.* (2010) observed the highest germination rate in seeds which soaked in distilled water (control). According to Òeèajeva and Ievinsh (2007) in *Chenopodium glaucum* and Khodarahmpour (2001) in corn seed germination percentage reduction is associated with decrease in water uptake by seeds at imbibitions and turgescence stages.

Analyze of variance showed that salt and osmotic stresses had significant effect ($p<0.01$) on seedling fresh weight (Table 1). Results of mean comparison discovered that control treatment of salinity level had the highest value of seedling fresh weight (Table 2, Fig. 2). With attention to Table 2 and Fig. 1 it was concluded that decreasing osmotic potential and increasing salinity levels reduced seedling fresh weight. Heshmat *et al*. (2011) in cowpea and Armin *et al*. (2010) in watermelon reported that cowpea seedling growth was ceased by both NaCl and PEG. Rouhi *et al*. (2010) claimed that there was no significant effect of priming on seedling fresh weight. Taghipour and Salehi (2008) in their study noticed that seedling fresh and fresh weights of Iranian barley were decreased by the salt stress.

CONCLUSION

Timing of swallowwort seed germination is an important factor in determining the success or failure of

a seedling. The effects of soil moisture and salinity on relative time of emergence have implications for weed management. Results of this study showed that seed germination, seedling fresh weight, radicle length and hypocotyle length of swallowwort decreased sharply with increasing NaCl concentration and linearly with increasing osmotic potential. However swallowwort seeds were able to germinate at a wide range of water potentials and salinity. Osmotic potential up to -0.6 Mpa and salinity stress up to 300 mM had little effect on seed germination. Seed germination was less than 13% at -1 Mpa osmotic potential and less than 9% at 500 mM salinity. In order to achieve promising control of swallowwort, one should consider its biology during the early part of the growing season. Swallowwort's response to water availability suggests that soil should be worked shallowly at crop seeding in order to reduce transferring of seeds from lower to upper soil layers in infested areas. Swallowwort has the attributes to be a serious weed problem in conventional and nonconventional cropping systems of Iran. Based on the present study, this weed has the potential for invading a wide range of new areas with a broad range of environmental conditions.

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