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Effects of drought and salt stress on seed germination of three leguminous species

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The seeds of *Medicago sativa* (L.), *Astragalus adsurgens* (Pall.) and *Coronilla varia* (L.) were evaluated at germination for tolerance to salt (NaCl) and drought conditions induced by polyethylene glycol (PEG) in an experiment of orthogonal design. The results reveal that the germination percentages of *M. sativa* and *A. adsurgens* were much higher than that of *C. varia*. The radicle and hypocotyl lengths of *M. sativa* were significantly longer than that of *A. adsurgens* and *C. varia* (P<0.05). The effects of PEG (PEG 6000) on the radicle length showed a trend similar to that of NaCl where the radicle length decreased significantly; additionally, the hypocotyl length was inhibited by 10 and 15% PEG and 50 and 100 mM NaCl. The fresh weights of the three species decreased as a result of the combined effects of PEG and NaCl, yet no difference was observed on the dry weights of the early seedlings in comparison with the control and the seed germination of *M. sativa* was not inhibited by 5% PEG + 50 mM NaCl. This study indicates that the seed germination of the three species was inhibited by PEG and NaCl but there was no inhibitory effect on *M. sativa* at a low concentration of PEG and NaCl.

Key words: Drought and salt stress, germination, leguminous species.

INTRODUCTION

Biological stress is an adverse force or a condition that inhibits the normal functioning and well-being of a biological system, such as plants (Jones et al., 1989), and the environment exerts great effects on plant growth and productivity. Worldwide agricultural productivity is subject to increasing environmental constraints (Bartels and Sunkar, 2005) in the form of abiotic and biotic stresses that adversely influence plants (Hussain et al., 1990). In fact, abiotic stresses are the principal cause of crop failure, decreasing average yields for major crops by more than 50% (Buchanan et al., 2000) and threatening the sustainability of the agricultural industry (Mahajan and Tuteja, 2005). Drought and salinity are two major abiotic determinants (Wang et al., 2009b) due to the high magnitude of their impact and wide occurrence (Bartels and Sunkar, 2005). Severe drought and high salinity could promote the desertification and salinization of land, processes which are rapidly increasing on a global scale: More than 10% of arable land has become desertified or salinised, and greater than 50% of the average yields of major crops have been reduced (Boyer, 1982). Therefore, drought and salt stress are of utmost concern.Seeds are the main way through which plants propagate, and a seed contains all of the genetic material of the plant. As seed germination is the beginning of the life cycle of plants, seedling emergence is critical for the establishment of plant populations (Khan and Gulzar, 2003).

Accordingly, an understanding of the effects on seed germination is vital to an understanding of the effects of drought and saline conditions.Drought stress is one of the main obstacles to seed germination. Germination is

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adversely affected by unfavourable moisture conditions due to lack of rainfall (Mwale et al., 2003) and irrigation. At sowing, inadequate soil moisture results in irregular seed germination and unsynchronised seedling emergence, affecting the establishment of a stand, with negative effects on the yield (Mwale et al., 2003; Okcu et al., 2005).

Salinity is another major constraint to seed germination. Soil salinity affects germination by either an osmotic stress or ion toxic effect (Bewley and Black, 1982): Salinity may create an external osmotic potential limiting water absorption by the seeds, or sodium and chloride ions may accumulate in the germinating seed, resulting in a toxic effect. Under drought and salt stress, the mobilisation of stored re-serves is prevented or reduced in seeds (Bouaziz and Hicks, 1990), and the structural organisation and synthesis of proteins are restricted in germinating embryos (Ramagopal, 1990). Consequently, the germi- nation of seeds is inhibited by both drought and salt stress (Abid et al., 2011; Li et al., 2011; He et al., 2011).

There have been numerous reports on the effects of salt and/or drought stress (Erdal et al., 2011; Fethi et al., 2011; Bajehbai, 2011; Saeedipour, 2011; Abbasi et al., 2010). The germination of dragon spruce (*Picea asperata* Mast.) seeds pretreated with gibberellin (GA) in response to water stress has been investigated; with decreasing water potential, the germination percentage and germination index had decreased gradually and was especially prominent under -0.6 MPa (Yang et al., 2010).

Similarly, a study in black gram (*Phaseolus mungo*) has shown that the germination percentage and all of the seedling growth parameters showed inhibition with an increase in osmotic potentials generated by polyethylene glycol (PEG) 6000 (Pratap and Sharma, 2010). In another report, four lentil (*Lens culinaris* M.) genotypes were treated with salt stress (0, 50, 100, 150 or 200 mM NaCl), and it has been reported that the increasing NaCl concentration had reduced the germination percentage, the growth parameters and the relative water content (Sidari et al., 2007). The seed germination of *Panicum turgidum* has been significantly reduced and slowed at high concentrations of both NaCl and KCl and was completely inhibited at 300 and 400 mM (El-Keblawy, 2004). Both NaCl and KCl has been reported to reduce the final germination percentage and germination rate of two arid-land varieties of wheat (*Triticum aestivum* L.); furthermore, the salt had increased the production of abnormal seedlings (Al-Ansari, 2003). Seed germination and the growth of young seedlings of sugar beet (*Beta vulgaris* L.) have been reported to be inhibited by NaCl treatment (Wang et al., 2011; Ghoulam and Fares, 2001). Additionally, the germination and emergence rates have been delayed and the seedling growth has been reduced by both NaCl and PEG-6000 solutions in two durum wheat cultivars (*Triticum durum* Desf.), although, it was reported that NaCl had demonstrated a smaller effect in terms of germination and seedling growth (Sayar et al., 2010). Similarly, both NaCl and PEG-6000 have been shown to inhibit the germination and seedling growth in two *Phaseolus mungo* varieties, where the effect of NaCl was also shown to be less than PEG-6000 (Garg, 2010). The concentration limits for germination in *Dianthus chinensis* L. have been reported to be 150 mM for NaCl and -1.66 MPa for PEG 6000; again, it was reported that NaCl had exerted less effect than PEG on the final germination percentage (He et al., 2009).

Medicago sativa (L.), *Astragalus adsurgens* (Pall.) and *Coronilla varia* (L.) are important perennial leguminous forage species and green manure crops. *M. sativa* is a very palatable and productive herbaceous legume with worldwide distribution that is high yielding and high in quality. *M. sativa* is grown primarily for hay, but it can be ensiled or used as pasture, either alone or in combination with grasses (typically, orchardgrass or smooth bromegrass). *M. sativa* has a strong drought tolerance, and it can be cultivated in marginal lands because of its deep root system that obtains scarce moisture from the soil (Moran et al., 1994). However, *M. sativa* is more sensitive to salt stress during the seed germination stage than the mature plant stage. Another plant that is nutrient rich and can be used for fresh forage, silage, hay modulation, hay meal and formula feed, *A. adsurgens* is also an excellent forage for grass crop rotation, as the stubble has a lot of organic matter and nitrogen, thus, improving soil fertility. *A. adsurgens*, a type of psammophyte, is also tolerant to drought stress and is resistant to windy and sandy conditions. *C. varia* is a spreading, long-lived, winter- hardy and drought-tolerant herbaceous legume with angular stems that grow to a height of 60 to 90 cm during the blooming period, after which the plant forms a dense mat approximately 30 cm deep. Its principal use is for erosion control, soil building and ground cover (Richardson and Diseker, 1965), especially for highway embankments. Stands of *C. varia* improve with age and gradually outcompete other weeds. *C. varia* is also used for its ornamental value on steep banks and hillsides. The plant can be established from seeds or crowns (Wheeler and Hill, 1957) and can grow in 0.5% soil salinity.

An orthogonal array design is often applied in biological field experiments because it can reveal the parameters that interact efficiently (Stenlund et al., 2009) and also determine the effect of a factor. Orthogonal refers to balanced and separable, and, as such, when the effect of a factor is calculated, the influence of other factors is removed (Hedayat et al., 1999). Thus, different effects can be observed independently.

Although, there have been many previous reports on the stress treatments of seeds, these studies have focused mainly on drought stress or salt stress alone, and there is no significant amount of information available in the literature about the combined effects of drought

Table 1. Assignment of control factors and levels in the experimental design using an orthogonal matrix ($L_9[3^4]$).

Factor	Species	PEG solution (w/v) (%)	NaCl solution (mM)
Level 1	*M. sativa*	5	50
Level 2	*A. adsurgens*	10	100
Level 3	*C. varia*	15	150

and salt stress. Therefore, to better understand the coupling effects of drought and salt stress on the germination of *M. sativa*, *A. adsurgens* and *C. varia*, we employed an orthogonal design to evaluate the tolerance of these three species to salt and drought conditions at the germination stage.

MATERIALS AND METHODS

This study was carried out at the Department of Grassland Science, Northwest A&F University, Shaanxi Province, China. The seeds of *M. sativa*, *A. adsurgens* and *C. varia* from Jindao Seeds Inc. were used as the experimental materials.

Experimental design

The experiment was carried out in an orthogonal design: an $L_9(3^4)$ orthogonal array was adopted to assign three factors and one vacancy (Hedayat et al., 1999). Each factor was placed into three levels. The first factor was species (*M. sativa*, *A. adsurgens* or *C. varia*), the second was the PEG concentration (w/v: 5, 10 or 15%), and the third was the NaCl concentration at 50, 100 or 150 mM. Additionally, three control treatments were conducted, including the three species of *M. sativa*, *A. adsurgens* and *C. varia* at 0% (w/v) PEG solution and 0 mM NaCl solution. The assignment of the three factors and their levels are shown in Table 1. The $L_9(3^4)$ matrix with factors and their levels is shown in Table 2. The vacant column was used to account for the statistical error of the orthogonal method (Hedayat et al., 1999).

Germination tests

Drought stress was induced by polyethylene glycol (PEG 6000) treatments, and the salt stress strength was expressed as the concentrations of the sodium chloride solutions. The test solutions were prepared according to Table 2. Distilled water served as a control.

Seeds of uniform shape of *M. sativa*, *A. adsurgens* and *C. varia* were selected, surface sterilised in an aqueous solution of 75% ethanol for 5 min to prevent fungal attack and rinsed in several changes of sterile distilled water. The seeds were germinated in 9-cm sterile Petri dishes lined with two sterile Whatman No.1 filter papers with 5 ml of distilled water or the respective test solutions (Rejili et al. 2010); there were 50 seeds per Petri dish and three replicates in each treatment. Germination tests were conducted under conditions of a 12-h light/dark cycle at 25°C. Distilled water was added into the Petri dishes each day to maintain the concentration of the test solutions. A seed was considered to have germinated when the radicle was 2 mm long (Kaya et al., 2006; Kim et al., 2006). The germination percentage was determined by counting the number of germinated seeds every 24 h. The radicle and hypocotyl length and the early seedling fresh and dry weights were measured on the 14th day.

Statistical analysis

Statistical analyses were performed using SAS software (version 8.2, USA). Trends were considered significant when the mean values of the compared sets were different at $P<0.05$.

RESULTS

Effects on germination percentage

The germination percentage responses of the three leguminous species to a range of PEG and salinity treatments are shown in Figure 1. Germination was reduced under the combination of the two stresses with varying responses for each species. The germination percentages of *M. sativa* and *A. adsurgens* were higher (57.55 and 39.56%, respectively) than *C. varia* (14%).

As compared to the non-stressed seeds, no significant difference on the germination percentage was observed in *M. sativa* when the seeds were treated with 5% PEG + 50 mM NaCl. However, the seed germination of *M. sativa* decreased with an increase in the PEG and NaCl concentrations, where the 15% PEG + 150 mM NaCl treatment was lower (13.33%) than control (92.67%). Statistical analyses showed significant effects of PEG and NaCl ($P<0.01$) on the seed germination percentages of *A. adsurgens* and *C. varia* (Table 2) in comparison with the control.

Effects on radicle and hypocotyl length

The differences of the radicle length determined among the species were significant ($P<0.05$) (Figure 2), and the hypocotyl length of *A. adsurgens* and *C. varia* differed significantly from *M. sativa* ($P<0.05$). The radicle and hypocotyl lengths of *M. sativa* were longer than that of *A. adsurgens* and *C. varia*. Furthermore, the radicle length was severely influenced by PEG and NaCl, and their effects showed a similar trend. The radicles from seedling under non-stress conditions were the longest, whereas those under the highest PEG or NaCl concentration were

Table 2. Assignment of factors and levels in the experiment using a $L_9(3^4)$ matrix and the responses to stress (germination percentage, fresh weight and dry weight).

Treatment	*1 (Species)	2 (PEG)	3 (NaCl)	4 (Vacancy)	Germination percentage (%)	Fresh weight (mg/5 seedlings)	Dry weight (mg/5 seedlings)
1	1 (*M. sativa*)	1(5%)	1(50)	1	89.33 ± 6.11^{Aa}	121.9 ± 28.54^{ABb}	8.8 ± 1.0^{Aab}
2	1	2(10%)	2(100)	2	70.00 ± 14.42^{BCbc}	77.2 ± 3.8^{CDEFd}	8.5 ± 0.9^{ABab}
3	1	3(15%)	3(150)	3	13.33 ± 5.03^{EFg}	49.7 ± 4.9^{EFGef}	6.0 ± 0.1^{BCcd}
4	2 (*A. adsurgens*)	1	2	3	50.67 ± 14.19^{CDde}	71.3 ± 17.7^{DEFGde}	$4.0\pm0.7C^{Def}$
5	2	2	3	1	34.67 ± 2.31^{Def}	45.5 ± 10.6^{FGf}	2.8 ± 0.2^{Df}
6	2	3	1	2	33.33 ± 5.03^{Def}	38.2 ± 2.3^{Gf}	4.9 ± 0.6^{CDde}
7	3 (*C. varia*)	1	3	2	10.67 ± 9.02^{Fgh}	$48.2\pm0.9E^{FGef}$	9.4 ± 2.4^{Aab}
8	3	2	1	3	28.67 ± 9.87^{Ef}	90.0 ± 25.8^{CDcd}	9.0 ± 0.5^{Aab}
9	3	3	2	1	2.67 ± 1.15^{Fh}	0.0 ± 0.0^{Hg}	0.0 ± 0.0^{Eg}
Control 1	*M. sativa*	0	0	-	92.67 ± 2.31^{Aa}	105.5 ± 11.8^{BCbc}	7.8 ± 1.2^{ABbc}
Control 2	*A. adsurgens*	0	0	-	82.67 ± 6.11^{ABab}	80.9 ± 7.6^{CDEd}	3.5 ± 0.9^{CDef}
Control 3	*C. varia*	0	0	-	57.33 ± 11.55^{CDcd}	146.2 ± 0.7^{Aa}	9.9 ± 1.8^{Aa}

*Values are the means ± SE. Different lowercase letters in the same column represent a significant difference at P=0.05, and different capital letters in the same column represent a significant difference at P=0.01. *Factor.

the shortest. Increasing the PEG concentration resulted in a decrease in the hypocotyl length, but no significant difference between 5% PEG and the control was observed. The hypocotyl length was noticeably inhibited by 100 and 150 mM NaCl but not by 50 mM.

Effects on the fresh and dry weights of early seedlings

Increasing the stress strength of PEG and NaCl caused a remarkable decrease in the early seedling fresh weight of *M. sativa*, but the early seedlings under the 5% PEG + 50 mM NaCl treatment did not show a significant difference for the fresh weight in comparison with the non-stressed seedlings (Table 2). The fresh weight of *A. adsurgens* early seedlings was much lower than the control when the seeds were treated with 10% PEG + 150 mM NaCl or 15% PEG + 50 mM NaCl, and the early seedling fresh weight of *C. varia* was drastically reduced in response to PEG and NaCl stress. Conversely, no difference was observed in the dry weight of early seedlings, in comparison with the control, in the three species.

DISCUSSION

Seed germination and early seedling growth are affected by both genetic and environmental factors, and different species have evolved different mechanisms to adapt to adverse conditions. As a result, the seed germination of varying species can be different under a similar environment. The research presented here indicated differences in the germination percentage and radicle and hypocotyl lengths of the three species studied. The germination percentage of *C. varia* was much lower than that of *M.sativa* and *A. adsurgens* (Figure 1). However, it has been reported that the final germination percentage of *Melilotus officinalis* was much higher than that of *M. sativa* and *A. adsurgens* at 300 mM NaCl (Wang et al., 2009c), and the germination rate in six alfalfa cultivars was also differentially affected by treatments with 200 mM NaCl and 35% PEG (Wang et al., 2009b). Vicente et al. (2009) have observed varying responses to saline solution of the seeds of three plant species (*Arthrocnemum macrostachyum*, *Juncus acutus* and *Schoenus nigricans*) and different germination recovery of the seeds after submersion in hypersaline solution of different salt types. We observed differences among *M. sativa*, *A. adsurgens* and *C. varia* that were significant for the radicle length (P<0.05), and the radicle and hypocotyl lengths of *M. sativa* were higher than that of *A. adsurgens* and *C. varia* (Figure 2). However, no differences in the

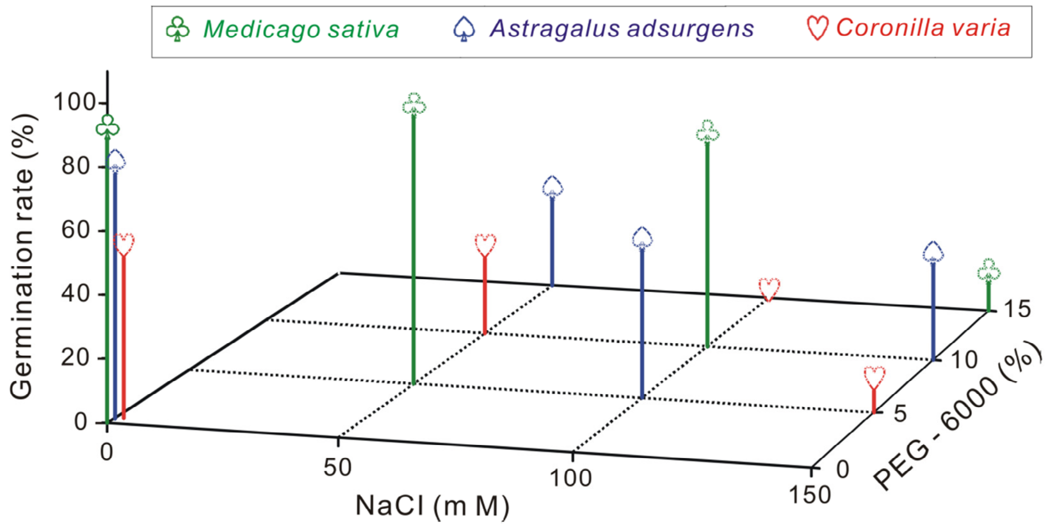

Figure 1. Combined effects of PEG and NaCl on the germination of *M. sativa*, *A. adsurgens* and *C. varia*. The bars at 0, showed the controls of the three experimental plants, respectively.

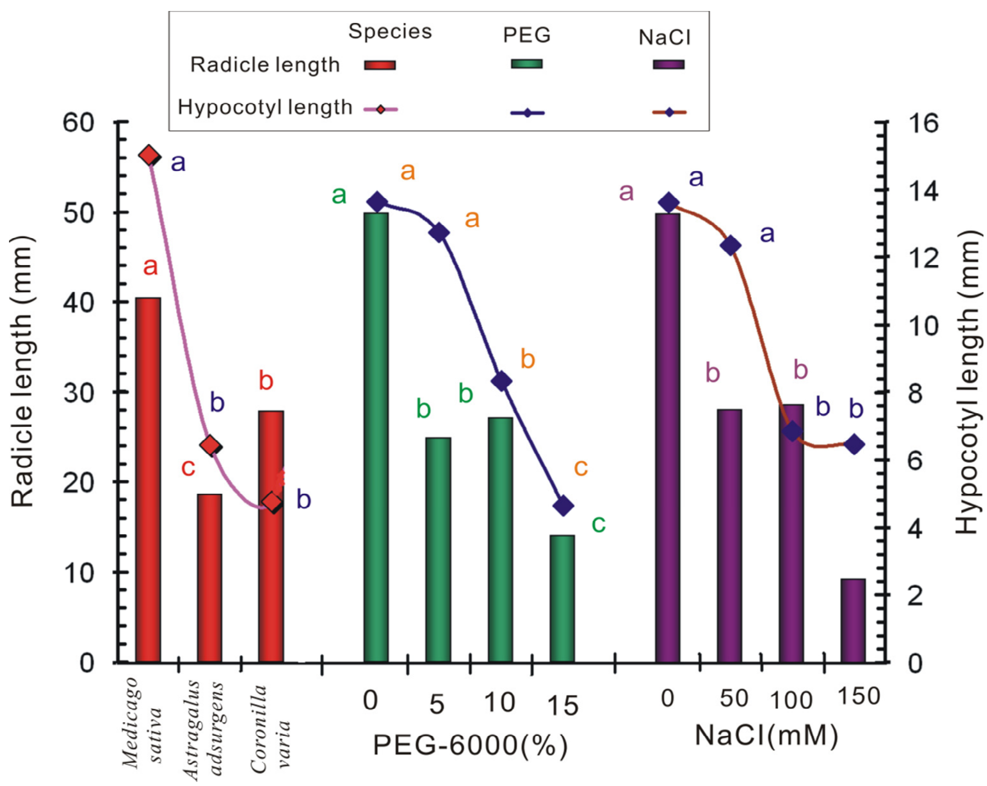

Figure 2. Radicle and hypocotyl length under PEG and NaCl treatments in *M. sativa*, *A. adsurgens* and *C. varia*.

fresh and dry weights of the three species were observed.

PEG is frequently used to simulate drought stress (Chen et al., 2010; Farahani et al., 2010; He et al., 2009; Khajeh-Hosseini et al., 2003; Tohidloo and Kruse, 2009; Zhu et al., 2006) as an inert osmoticum in germination tests (Dodd and Donovan, 1999) and is a non-penetrating solute (Almansouri et al., 2001), which results in osmotic stress that inhibits seed germination through the prevention of water uptake. However, it has been reported that the inhibitory effect of PEG on germination may not be solely related to water imbibition (Almansouri et al., 2001). In the present study, radicle length was inhibited significantly by PEG (P<0.05), and it was the shortest under the highest PEG concentration (15%); moreover, increasing the PEG concentration resulted in a decrease in the hypocotyl length. Wang et al. (2009b) have observed that the fresh weight and the length of the roots and shoots of two alfalfa cultivars (Xinmu No.1 and Northstar) were significantly inhibited by 35% PEG treatment. For a potential medicinal plant, *Matricaria chamomilla*, both the seed germination rate and seedling growth have been found to be reduced with the PEG-mediated increasing osmotic potential of the growth medium (Afzali et al., 2006).

The adverse effects of NaCl on seed germination are osmotic stress or/and ion toxicity (Huang and Redmann, 1995; Kaya et al., 2006; Rehman et al., 1997; Zhou and Xiao, 2010). Rejili et al. (2010) have found that the decreased germi-nation percentage in a Oued Dkouk population was due to the osmotic effect of salt because the NaCl concentrations used did not injure the embryo, and the ungerminated seeds were able to germinate when the salt stress wasremoved, a finding that was in agreement with that of Rehman et al. (1997). Wang et al. (2009a) have also observed that seeds were not affected by ion toxicity, as evidenced by a high germination recovery percentage and pink embryos in a viability test of ungerminated seeds in 2, 3, 5-triphenyl-2H-tetrazolium chloride (TTC) solution. In contrast, the observed germination decreases using Msarref seeds which was attributed to the toxic effect of salt, as no germination was observed when the salt stress was removed (Rejili et al., 2010). Dimorphic seeds of the halophytes, *Atriplex prostrate* and *Atriplex patulawere*, treated with various iso-osmotic solutions of NaCl and polyethylene glycol (PEG) have suggested that the influence of NaCl is a combination of an osmotic effect and a specific ion effect (Katembe et al., 1998). In the present study, the radicle length was significantly reduced by NaCl ($P<0.05$), and the shortest length measured was under 150 mM NaCl. The hypocotyl length was markedly inhibited by 100 and 150 mM NaCl but not 50 mM. Wang et al. (2009b) have found that the fresh weights and lengths of the roots and shoots of two alfalfa cultivars (Xinmu No.1 and Northstar) were significantly inhibited by 200 mM NaCl treatment, and an increase in the NaCl concentration has been found to progressively inhibit the seed germination of *Kalidium capsicum* (Chenopodiaceae), with a critical salinity tolerance for seed germination of 198 mM NaCl and an ultimate salinity tolerance of 278 mM NaCl (Wang et al., 2009a).

Water moves from high water potential to low water potential (Mahajan and Tuteja, 2005), and sodium and chloride ions can penetrate the germinating seeds and decrease the internal osmotic potential, thus, allowing water uptake to occur and alleviating osmotic stress. Although, the detrimental effects of NaCl may be linked to the long-term effects of accumulated toxic ions (Almansouri et al., 2001), some studies have demonstrated that NaCl has a less inhibitory (or detrimental) effect on seed germination than an iso-osmotic concentration of PEG (Garg, 2010; Sadeghian and Yavari, 2004; Sayar et al., 2010; Sousa et al., 2008). Moosavi et al. (2009) have treated seeds of cultivated species of Amaranth with NaCl and PEG and found that at lower osmotic potentials, the germination percentage, germination rate, root and shoot length were higher under NaCl than PEG at the same water potentials, and the seeds were able to germinate under all the concentrations of NaCl, but no seed germination was observed at -1.2 MPa of PEG. Similar results have been reported by Demir and Mavi, (2008). In contrast, Katembe et al. (1998) have found that higher concentrations of NaCl (-1.0 MPa) were more inhibitory to the imbibition, germination and seedling root elongation of *Atriplex prostrate* and *A. patulawere* than iso-osmotic PEG solutions. Seeds of *Zizyphus joazeiro* have been treated with different solutions of NaCl and PEG 6000, and the results have suggested that salt stress provided a further reduction in the germination and speed of germination than the water stress (De Lima and Torres, 2009). In addition, a germination test has demon- strated that *Gliricidia sepium* seeds show less tolerance to drought stress than to salt stress (De Farias et al., 2009). Therefore, the effects of PEG and NaCl on seed germination may be related to the species. Clearly, the effects of PEG and NaCl stress on germination require further research.

In the present study, the seed germination of *M. sativa*, *A. adsurgens* and *C. varia* was detrimentally influenced by the combined effects of PEG and NaCl. The germination percentage and radicle length of *A. adsurgens* and *C. varia* were much lower than under non-stress conditions, and the fresh weight of *C. varia* was significantly reduced ($P<0.01$). However, as compared to the control, the radicle length of *M. sativa* increased significantly ($P<0.05$) when the seeds were treated with 5% PEG + 50 mM NaCl, and no clear difference was observed in the germination percentage, hypocotyl length or fresh and dry weights. These results suggest that the seed germination of *M. sativa* was not inhibited by a low concentration of PEG and NaCl.

In conclusion, we revealed that drought and salt stress, in general inhibited the germination of *M. sativa*, *A. adsurgens* and *C. varia* seeds and decreased the germination percentage, radicle and hypocotyl lengths and fresh weight of the three species by the combined effects of PEG and NaCl. Specifically, the seed germination of *M. sativa* was not inhibited by 5% PEG + 50 mM NaCl, and no significant difference was observed in the dry weight of early seedlings in comparison with non-stressed seedlings of the three species.

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