Vietnam Journal of Agricultural Sciences

ISSN 2588-1299

VJAS 2018; 1(2): 134-141
https://doi.org/10.31817/vjas.2018.1.2.03

Effects of Osmotic Stress Induced by PEG and NaCl on the Germination and Early Growth of Mung Bean

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Abstract

This study was performed to evaluate the effects of osmotic stress (measured by different water potentials) induced by Polyethylene Glycol 6000 (PEG) and NaCl solutions at the germination stage of five mung bean varieties (DX11, DX208, DX14, DX17, and DX22). Five water potentials: 0 (control), -0.15, -0.49, -1.03, and -1.76 Mpa were used as treatments in this study. The germination rates, root and shoot lengths, root and shoot fresh weights, and dry weights of the plants were measured. The results showed that the germination rate of the mung bean varieties decreased with increased NaC1 or PEG concentrations. The germination rates of the mung bean varieties in the PEG treatments were higher than those in the NaCl treatments. In addition, low water potentials induced by NaCl (-1.76 Mpa and -1.03 Mpa) inhibited germination and seeding growth of all the mung bean varieties. The growth parameters of the mung bean seedlings, such as root and shoot lengths, fresh weights of roots and shoots, and plant dry weights, were reduced under low water potentials treated with either PEG or NaCl. However, more severe damage in seedling growth was observed in the NaCl induced treatments. Amongst five mung bean varieties used in this study, DX17 was more drought and salt tolerant than the other mung bean varieties.

Keywords

Germination, mung bean, NaCl, PEG (polyethylene glycol)

Introduction

Seed germination is considered the most critical stage in seedling establishment for determining successful crop production (Almansouri *et al.*, 2001; Finch Savage and Bassel, 2016). Crop establishment depends on an interaction between the seedbed environment and seed quality (Khajeh Hosseini *et al.*, 2003). Many

Received: March 19, 2018
Accepted: September 7, 2018

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factors adversely affect seed germination including seed sensitivity to drought stress (Wilson *et al.*, 1985; Sadeghian and Yavari, 2004) and salt tolerance (Almas *et al.*, 2013). Water and salinity stresses are two of the most common environmental factors that regulate plant growth and limit plant production. Salinity may also affect the germination of seeds by creating an external osmotic potential that prevents water uptake or by the toxic effects of sodium and chloride ions on the germinating seeds (Khajeh-Hosseini *et al.*, 2003).

Water availability and movement into the seeds are very important to promote germination, initiate root growth, and initiate shoot elongation (Bewley and Black, 1994). Only highly negative water potentials, especially during early germination, may influence seed water absorption, making germination not possible (Bansal *et al.*, 1980). Under water stresses, the germination of seeds is affected by the external osmotic potential that prevents water uptake due to the toxic effects of Na^+ and Cl^- ions both during imbibition and seedling establishment (Murillo-Amador *et al.*, 2002). The relation of various seedling growth parameters to yield components and yield under drought and saline conditions are very important for the development of salt tolerant cultivars for production under drought and saline conditions.

Mung bean [*Vigna radiata* (L.) Wilczek] is an important leguminous crop and is being used in annual crop rotations on increasingly larger areas of depleted soils in many regions of Vietnam. Different developmental stages of this crop are sensitive to drought and salinity stress. In order to select mung bean genotypes that can endure salt and drought stresses, the objective of this study was to evaluate the effects of PEG-6000 and NaCl induced treatments during germination on five mung bean varieties.

Materials and Methods

Plant materials and growth conditions

This study was conducted in the laboratories of the Biology Department, Faculty of Biotecnology at Vietnam National University of Agriculture. Five mung bean cultivars (*Vigna radiata*) were used in this study (Table 1). The seeds of all the cultivars were germinated in petri dishes on two layers of filter paper containing solutions of polyethylene glycol (PEG) 6000 or sodium chloride (NaCl) with osmotic potentials of -0.15, -0.49, -1.03, or -1.76 MPa (Table 2) at 25°C in a tissue culture room (Nayer and Reza, 2008). In order to keep the filter paper moist, 3 mL of the PEG or NaCl solutions was added to the Petri dishes every 12 h.

Germination test and seedling growth measurements

The germination test was conducted with five replications per treatment, in which 15 seeds

Table 1. The name and origin of the mung bean varieties used in this experiment

Variety name	Origin
DX11	Selected by the Legume Research and Development Center, from Thailand CN36 lines
DX208	Selected by the Southern Seed Corporation
DX14	Selected by the Legume Research and Development Center, from Korea Germplant in 2004
DX17	Selected by the Legume Research and Development Center, from DX7 x PAEC3
DX22	Selected by the Legume Research and Development Center

Table 2. Details of the sodium chloride and polyethylene glycol 6000 amounts used to induce different water potentials

Osmotic potential (Mpa)	PEG-6000 (g L^{-1} of distilled water)	NaCl (g L^{-1} of distilled water)
0	0	0.00
-0.15	10	1.76
-0.49	20	5.74
-1.03	30	12.07
-1.76	40	20.88

seeds counted as a replication. Prior to the germination test, the mung bean seeds were surface sterilized by immersing them in 1% $HgCl_2$ for 2 min and rinsing repeatedly with distilled water. Germination was recorded every day for 6 days. Seeds were considered to have germinated when both the plumule and radicle had extended more than 2 mm. The final germination rate, shoot lengths, root lengths, and shoot and root weights (fresh and dry) were recorded on the 6^{th} day. The fresh shoots and roots were dried in an oven (MOV-212F, Sanyo Electric Co., Ltd., Osaka, Japan) at 80°C for 72 h for the dry weight measurements. Only 25 normal seedlings in each treatment were randomly selected for the seedling growth parameter measurements.

Statistical analysis

Mean values were taken from the measurements of five replicates on a total of 25 seedlings (five seedlings in one replication). The standard deviations of the means were calculated. Analyses were completed using Microsoft Excel version 2013.

Results

Effects of osmotic stress induced by PEG and NaCl on final germination rates of mung bean varieties

The results showed that the final germination rates were inversely proportional to the NaCl concentrations. Compared to the control, a higher reduction in mung bean germination rates was recorded in the NaCl treatments than in the PEG treatments. In the PEG treatments, the DX11, DX17, and DX22 varieties had final germination rates of 100% at all the osmotic potentials induced by PEG. There were no significant differences between the germination rates of the DX208 and DX14 varieties at 0, -0.15, -0.49, -1.03, or -1.76 Mpa when induced by PEG, but the final germination rates of the DX208 and DX14 varieties were lower than the rates of the other mung bean varieties in the -1.76 Mpa treatment. In the NaCl treatments, the two lowest water potentials, -1.76 Mpa and -1.03 Mpa, inhibited germination and inhibited seeding growth of all the mung bean varieties, respectively. The low osmotic potential (-1.03 Mpa) treatment induced by NaCl significantly decreased the germination rate of the five mung bean varieties. Compared to the other mung bean varieties, a higher germination rate was recorded in DX17 in both the PEG and NaCl treatments.

Effects of osmotic stress induced by PEG and NaCl on root and shoot lengths of mung bean varieties

Variations of responses to water deficits caused by both PEG and NaCl were recorded across the five mung bean varieties (Figures 1 and 2). The highest values of root and shoot lengths were observed in the control treatment. However, the root and shoot lengths decreased with increased PEG and NaCl concentrations.

Table 1. Effects of different osmotic potentials induced by PEG and NaCl on final germination rates of mung bean varieties (%)

Factors	Osmotic potentials (Mpa)	Mung bean varieties				
		DX11	DX208	DX14	DX17	DX22
Control	0	100.00	100.00	100.00	100.00	100.00
PEG	-0.15	100.00	100.00	100.00	100.00	100.00
	-0.49	100.00	100.00	100.00	100.00	100.00
	-1.03	100.00	100.00	100.00	100.00	100.00
	-1.76	100.00	97.55	97.55	100.00	100.00
NaCl	-0.15	100.00	100.00	100.00	100.00	100.00
	-0.49	100.00	100.00	100.00	100.00	100.00
	-1.03	78.33	53.33	67.78	97.55	85.55
	-1.76	0.00	0.00	0.00	0.00	0.00

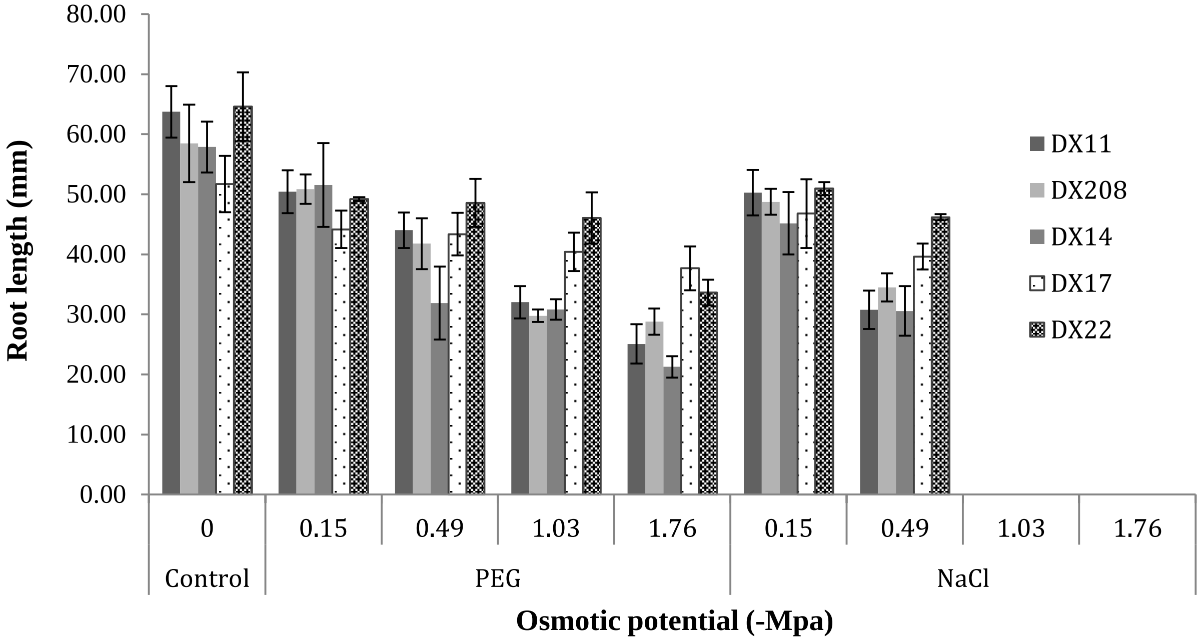

Figure 1. Effects of different osmotic potentials induced by PEG and NaCl on root lengths of mung bean varieties. Vertical bars represent ± SD, n = 25.

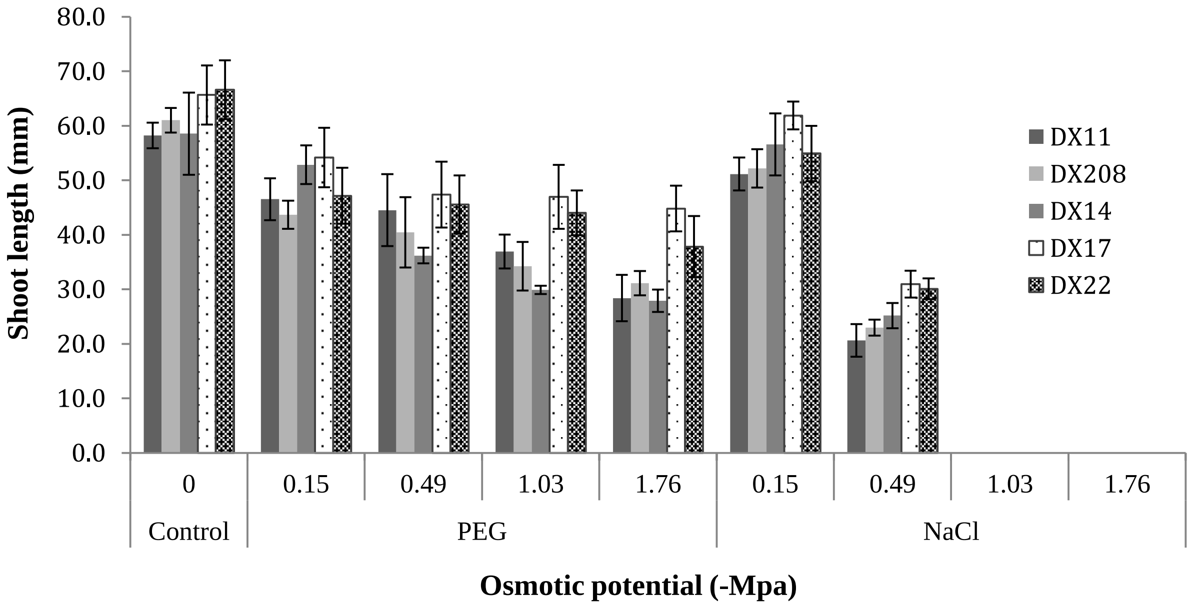

Figure 2. Effects of different osmotic potentials induced by PEG and NaCl on shoot lengths of mung bean varieties. Vertical bars represent ± SD, n = 25.

High concentrations of PEG or NaCl also led to significant declines in root and shoot lengths of mung bean seedlings in the early growth stages. In addition, the low water potential treatment (-1.03 Mpa) induced by NaCl inhibited seedling growth of all the mung bean varieties. Within the -0.15 Mpa NaCl treatment, all the mung bean varieties were observed with significantly longer shoot lengths compared to those in the PEG treatment. However, in the -0.49 Mpa PEG treatment, all the mung bean varieties were observed with significantly longer shoot lengths compared to those in the NaCl treatment. No significant differences in root lengths were found at -0.15 Mpa when induced by PEG or NaCl, but significant effects were observed at -0.49 Mpa when induced by both PEG and NaCl in all the mung bean varieties.

Effects of osmotic stress induced by PEG and NaCl on root and shoot fresh weights of mung bean varieties

Results from this study also revealed that root and shoot fresh weights decreased with increased concentrations of both PEG and NaCl (Figures 3 and 4). In the PEG treatments, while significant decreases in shoot and root fresh weights were observed in almost all the mung bean varieties, no significant decreases were recorded in DX17 as the osmotic potential decreased from -0.15 to -1.76 Mpa. In the NaCl treatments, the decline in osmotic potential from -0.15 to -0.49 Mpa resulted in significant decreases in root and shoot fresh weights in all

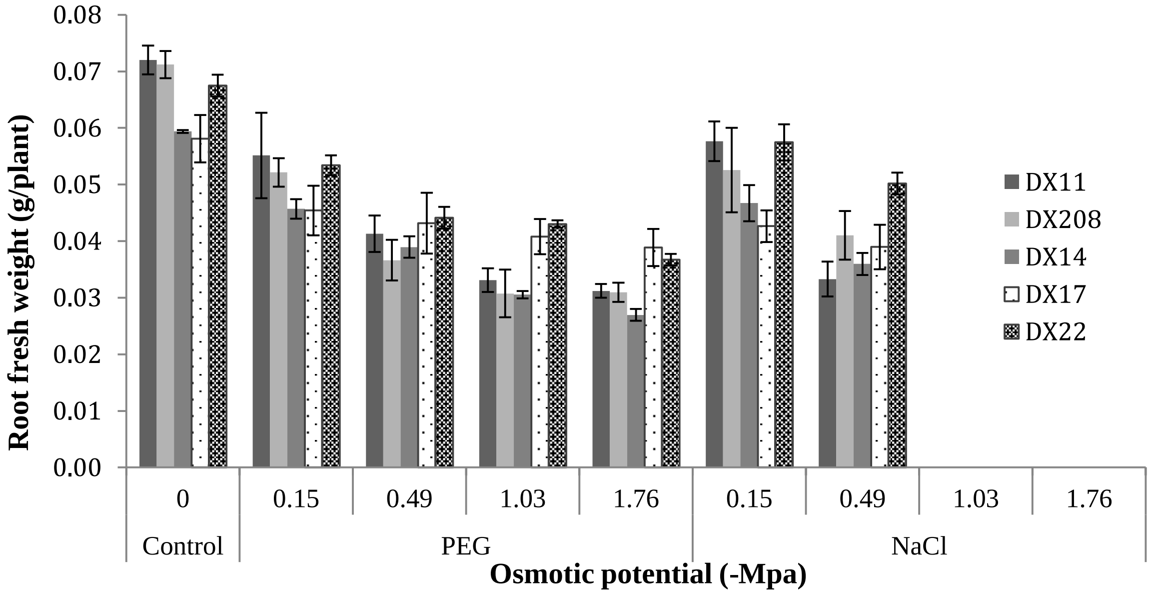

Figure 3. Effects of different osmotic potentials induced by PEG and NaCl on root fresh weights of mung bean varieties. Vertical bars represent ± SD, n = 25.

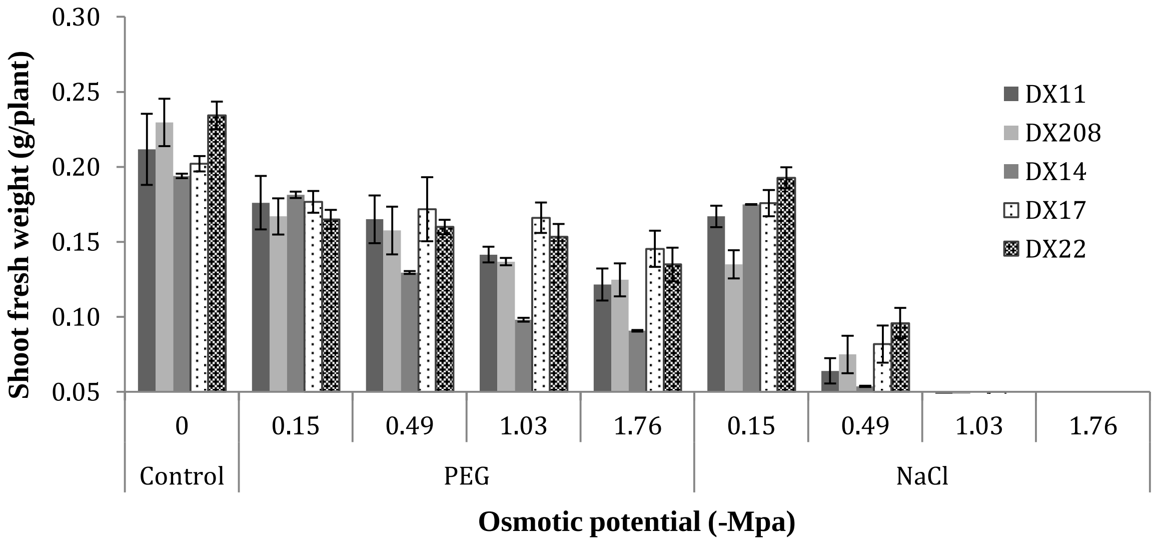

Figure 4. Effects of different osmotic potentials induced by PEG and NaCl on shoot fresh weights of mung bean varieties. Vertical bars represent ± SD, n = 25.

the mung bean varieties except the root fresh weight of DX17. The effects of water deficits in the NaCl treatments were more noticeable compared to those in the PEG treatments. When comparing the five mung bean varieties, DX17 was more drought and salt tolerant than the other mung bean varieties.

Effects of osmotic stress induced by PEG and NaCl on plant dry weights of mung bean varieties

Variations in the responses to different levels of osmotic potentials was recorded among the mung bean cultivars in both the NaCl and PEG treatments (Figure 5). Low water potentials induced by both NaCl and PEG resulted in significantly lower plant dry weights in all the mung bean varieties compared to those in the control. However, reducing the water potential from -1.03 to -1.76 Mpa in the PEG treatments did not lead to any significant reductions in plant dry weights across all the mung bean varieties. The highest values of plant dry weights were recorded in DX17 and DX22 at the water potentials of -0.15, -0.49, -1.03, and -1.76 Mpa induced by PEG and at -0.49 Mpa induced by NaCl.

Discussion

Reduced water potentials induced by both NaCl and PEG decreased germination and seedling growth of all the mung bean varieties in this study. Similar responses have been reported in rice (Alam *et al.*, 2002), pepper (Demir and Mavi, 2008), lentil (Musculo *et al.*, 2014), and mugwort (*Artemisia vulgaris* L.) (Almas *et al.*, 2013). These results revealed that the consequences of the decreased water potential gradients between the seeds and the surrounding media which adversely affected germination and subsequent seedling growth. In addition, Alam *et al.* (2002) showed that elevated concentrations of NaCl and PEG prevented water uptake into seeds, thereby inhibiting germination. In this study, NaCl was observed to be more inhibitory to seed germination of the mung bean varieties compared to the PEG treatments. This result agreed with the germination results of Roundy

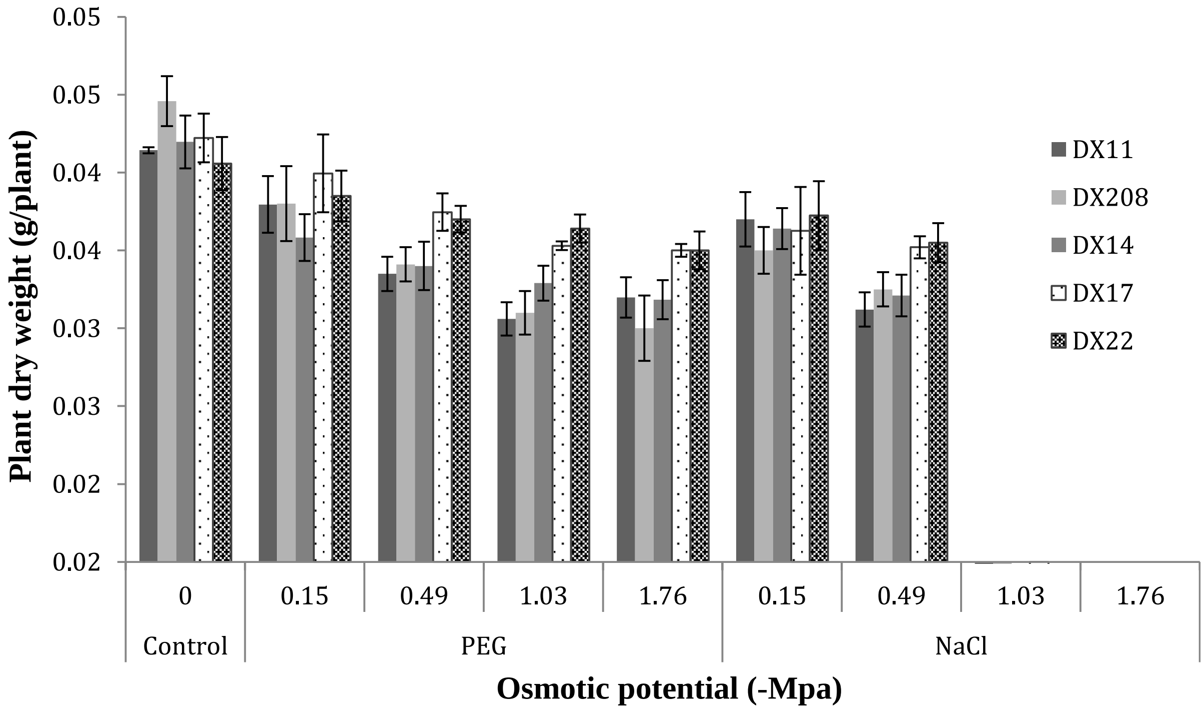

Figure 5. Effects of different osmotic potentials induced by PEG and NaCl on plant dry weights of mung bean varieties, Vertical bars represent ± SD, n = 25.

et al. (1985) who studied wheat grass and wild rye (Katembe *et al.*, 1998). A low water potential (-1.76 MPa) caused by NaCl appeared to be lethal for all mung bean cultivars. This showed that mung bean seeds can remain viable for a considerable period under drought stress but not salinity stress (Hampson and Simpson, 1990) and can be explained by the toxic effects caused by Na^+ and Cl^-. Though NaCl is believed to readily cross the cell membrane and trigger seed hydration, high concentrations of Na^+ and Cl^- in the cell membrane, cytoplast, and cell nuclei can cause damage to seed metabolism (Alam *et al.*, 2002).

Subsequent seedling growth was progressively decreased as the water potential decreased in both the NaCl and PEG treatments. Reductions in root fresh weights, shoot fresh weights, and plant dry weights as consequences of low water potentials in the NaCl treatments were more noticeable compared to those in the PEG treatments. These results were consistent with the report of Roundy *et al.* (1985) and Katembe *et al.* (1998). The more noticeable effects of NaCl on seedling growth can be explained by looking at the role of Ca^{2+} at the cell level. As Ca^{2+} plays a central role in maintaining cell membrane permeability, high Na^+ concentrations can displace Ca^{2+} in the cell membrane and thus, cause more severe membrane leakage compared to PEG (Hampson and Simpson, 1990). Differences in germination and seedling growth among the mung bean varieties in response to low water potentials were also recorded in the study. These differences among cultivars may be due to differences in critical water potentials or hydration levels leading to germination inhibition and prevention (Alam *et al.*, 2002). However, in comparing the five mung bean varieties, higher germination rates and seedling growth parameters were recorded in DX17 than in the other mung bean varieties in both the PEG and NaCl treatments.

Conclusions

In conclusion, reduced water potentials caused by both NaCl and PEG decreased germination and seedling growth parameters such as root and shoot lengths, fresh weights of roots and shoots, and plant dry weights of the five mung bean varieties. However, higher reductions in mung bean germination rates were recorded in the NaCl treatments than in the PEG treatments. Compared to the other mung bean varieties, DX17 was more tolerant to drought and salt stress than the other mung bean varieties.

Acknowledgments

Instrumental analyses and chemicals were supported by the laboratory of the Industrial and Medicinal Plants Department and the laboratory of the Biology Department of the Faculty of Biotechnology, Vietnam National University of Agriculture.

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