Investigation of Salinity Tolerance in Dodonaea viscosa L.

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Abstract

Dodonaea viscosa Linn is an important medicinal plant that is sensitive to saline soil. In order to well understand the salinity effects on germination and seedling growth of this species, we selected nine different salinity treatments including CacL₂, NaH₂Po₄ and KNo₃ + KH₂Po₆ each one 100 mM, 200 mM and 300 mM. Seeds of *D. viscosa*, before the germination in controlled growth chamber, were treated with boiling water and H₂SO₄. Biomass and germination parameters were measured after 56 days. Results showed that the dry weight of seedling was inhibited by high concentrations of NaH₂Po₄. The dry and fresh weight of root at level of 100mM KNo₃+KH₂Po₄ was higher than that of other treatments. Stem length increased at level of 100mM KNo₃+KH₂Po₄. The fresh weight of seedling severely increased at level of 100 mM KNo₃+KH₂Po₄. The seedlings succulence was enhanced to 87 % at level of 100 mM KNo₃+KH₂Po₄. Faster germination was recorded in 100 mM KNo₃+KH₂Po₄. Final germination index and seed stamina were higher than that of other treatments at level of 100 mM KNo₃+KH₂Po₄. Overall, biomass and germination parameters of *D. viscosa* in unsalinized control were significantly more than that of other salinity treatments except for total dry weight.

Keywords: Dodonaea viscosa Linn . Salinity level . Germination . Seedling biomass

INTRODUCTION

Salt stress leads to unfavorable functional changes and damage to plant tissues [1, 2]. Saline soils contain soluble salts in quantities that affect plant growth at various stages [3, 4]. Some scientists found that seedling respiration is decreased as salinity level is increased [5]. Katembe et al. [6] reported that high concentration of NaCl inhibits imbibitions, germination and seedling root elongation. Moreover, El-keblawy and Rawai [7] proved that *Prosopis juliflora* germination rate was significantly greater at 40°C than at 15°C and 25°C in lower salinities and the reverse was true at higher salinities. Germination in light was significantly greater than in the dark at lower salinity levels and high temperature.

Dodonaea viscosa Linn is used in folk medicine to treat various ailments [8, 9]. This species is a shrub belonging to family Sapindaceae. The centre of origin of Dodonaea viscosa is believed to be Australia, but it occurs throughout the tropics and subtropics, widely distributed in temperate regions of Australia, Africa, Mexico, New Zealand, India, Virgin Islands, Florida, Arizona, South America and elsewhere [10]. The common names of Dodonaea viscosa are hopbush, hopseed, hopwood, soapwood and candlewood.

It was demonstrated that the germination of seeds is strongly influenced by the nature of the ions in the salt solutions. This interaction causes severe damage to germination, especially in the high salinity [11, 12]. Meloni et al. [13] have discovered that the root and shoot growth of *Prosopis alba* decreased at

high level of NaCl. Previously, many investigators proved that at high salinities a delay in final germination often occurred, because salts in the seed bed may cause osmotic or specific toxicity [14, 15]. Salt stress inhibits seed germination [16]. Moreover, Chen et al. [17] results illustrated that both the vegetative growth and the reproductive growth of cotton were inhibited by the high soil salinity. Kurban et al. [18] found that total plant dry weight increased at low salinity but decreased at high salinity in Alhagi pseudalhagi seedlings. Many social and economic problems are caused by salinity that affects the growth, productivity and distribution of plants [19, 20]. It has been reported that salinity has negative effect on germination rate, root length, shoot length, fresh root weight and fresh shoot weight [21, 22]. Nevertheless, some species can grow on saline habitats due to their tolerance to moderate salinity at germination and seedling stage [23].

The dry weight of *Acacia ampliceps* and Acacia holosericea decreased under NaCl stress conditions [24]. In another study, the shoot growth of *P. euphratica* was not so reduced even on the medium containing 100 mM NaCl. However, growth of the other poplars reduced with 10 mM NaCl [25]. Sidari et al. [26] indicated that the germination of Pinus pinea seeds is influenced by the concentrations and even more by the nature and interactions of the ions present in the solutions. The degree to which salinity affects germination and whether salt tolerance varies in different species is still a subject of research. So, the objective of present study was to determine salt tolerance in *D. viscosa* Linn species at two early growth stages.

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MATERIAL AND METHOD

Dormancy Breaking and Salinity Treatments

Laboratory experiments of liquid culture were conducted in forestry department of natural resources faculty, sari, Iran. The seeds of *Dodonaea viscosa* were collected in May 2006 from south of Iran. Seeds were scarified with boiling water for 5 min and H₂SO₄ 98% for 45 min and then washed thoroughly with teepul and distilled water. The use of the sulfuric acid pretreatment on *Dodonaea viscosa* seeds was also observed in past studies [27, 28]. Seeds were planted in sterilized glass plates on two layers of filter paper and cotton moistened with 20cc solution of different salts as well as control in L₂ medium. Nine different salinity treatments including three salt types of CacL₂, NaH₂Po₄ and KNo₃ + KH₂Po₄ each one with three salinity levels of 100 mM, 200 mM and 300 mM were used in this study. The plates were maintained in growth chamber at 23°C and completed light condition.

Seed Germination

Seed germination in each glass plate was observed weekly and germinated seeds were counted. Final germination (FG) percentage (Eq. 1) and Mean Daily Germination (MDG) (Eq. 2) was calculated.

$$FG = \frac{n}{N} \times 100$$
 [29]

$$MDG = \frac{FG}{D} \tag{2}$$

Where n is the number of germinated seeds, N is the total number of seeds, FG is the final germination percentage, D is the number of days to final germination percentage. To assess the germination rate (GR), the mean germination time was calculated as follows (Eq. 3):

$$GR = \sum_{i=1}^{n} \frac{n_i}{t_i} \tag{3}$$

Where n_i is the number of germinated seeds in day t_i . Germination index (GI) was calculated according to following equation (Eq. 4):

$$GI = \frac{\sum t_i n_i}{N} \tag{4}$$

Seedling Growth (Biomass)

Seedling succulence (SU) (Eq. 5) and seed stamina (S) (Eq. 6) was calculated according to following equations:

$$SU = \frac{FW - DW}{FW} \times 100$$
 [31]

$$S = \frac{L \times FG}{100} \tag{6}$$

Where FW is the seedling fresh weight and DW is the seedling dry weight and L is the mean of seedling length (mm). Experiment was concluded after 56 days and various growth indices such as root and stem length, stem fresh weight, root fresh weight, stem dry weight and root dry weight were measured. Dry weight was determined after drying the seedlings in an oven at 80°C for 24 hours. Seedlings weight was measured by digital balance with accuracy of mg.

Statistical Analyses

Completely randomized design was used with five replications and 10 seeds in each replicate. Data were analyzed using GLM procedure in SAS program. The statistical significance of the differences among means was evaluated using Student Newman Kouls (SNK) test at probability level of 1%. Diagrams were designed by Excel software.

RESULTS AND DISCUSSION

Seed Germination

The present study clearly demonstrated that *Dodonaea viscosa* Linn germination parameters were influenced not only by the salt type but also by the salt concentrations. Nevertheless, the interaction between salt concentrations and salt type on these parameters wasn't statistically significant (Table 1). There was a significant difference between the unsalinized control treatment and various concentrations of salts for the final germination. Germination index, mean daily germination and germination rate of *Dodonaea viscosa* decreased with increasing salinity (P<0.01, Table 2). The findings agree with those observed by Sosa et al. [12], who demonstrated that increasing the salinity could decrease seed germination. Similarly, West and Francois [14] reported that Cowpea seeds could overcome the salinity in low concentration of salt.

Salts in the seed bed may cause osmotic or specific toxicity effects on germinating seeds that may result in reduced or retarded germination. The damaging effects of salt on plants are caused not only by osmotic forces, but also by toxic levels of ions. This is in agreement with our recent report of *Dodonaea viscosa* tolerant to salinity. Present study indicated that germination parameters of *Dodonaea viscosa* in unsalinized control were significantly more than that of salinity treatments. Besides, seeds could not germinate in 200 and 300 mM CacL2 as well as 100, 200 and 300 mM NaH2Po4+ KNo3+KH2Po4.

Responses of germination parameters to different treatments of salinity were similar. No seeds germinated in salinity levels of 200 mM and 300 mM of CacL₂ (Table 4). Faster germination was recorded at level of 100 mM KNo₃+KH₂Po₄. Final germination, germination index and seed stamina of *Dodonaea viscosa* at level of 100 mM KNo₃+KH₂Po₄ were higher than that of other treatments (Table 5). Ions that contribute to soil salinity include Cl⁻, HCO⁻³, Na⁺, Ca⁺², Mg⁺², and rarely NO⁻³ and K⁺. Salt tolerant plants (Plants less affected by salinity) are better able to adjust internally to the osmotic effects of high salt concentration than salt-sensitive plants. Salt-tolerant plants are more able to absorb water from saline soil [32]. This could be a reason behind the successful germination of this species in saline soil.

Seedling Growth (Biomass)

Dodonaea viscosa Linn biomass was influenced not only by the salt type but also by the salt concentrations (Table 1). There

Table 1. Analysis of variance showing the effect of the salt type, different concentrations of salinity and their interaction on germination and biomass of *Dodonaea viscosa* Linn.

Source of variation	Salt type			Salt concentration			Salt type × Salt concentration		
	SS	MS	F	SS	MS	F	SS	MS	F
Biomass production									
Stem length (SL)	28.9	14.4	6.4***	108	54.0	24***	0.99	0.49	0.22^{ns}
Root length (RL)	11.6	5.8	12***	30.2	15.1	31***	0.58	0.29	$0.59^{\rm ns}$
Total length (TL)	74.8	37.4	13***	251	125	44***	1.80	0.90	0.32^{ns}
Fresh weight of stem (FWS)	0.06	0.3	1.1 ^{ns}	0.44	0.2	8.1***	0.03	0.01	$0.05^{\rm ns}$
Fresh weight of root (FWR)	0.02	0.01	6.8***	0.38	0.02	13***	0.09	0.05	3.27 ^{ns}
Dry weight of stem (DWS)	0.03	0.02	0.8 ns	0.07	0.03	1.6 ns	0.03	0.02	0.83^{ns}
Dry weight of root (DWR)	0.001	0.01	5.4 ns	0.01	0.01	17***	0.02	0.01	2.57ns
Total fresh weight (TFW)	0.14	0.07	2.6 ns	0.73	0.4	13***	0.08	0.04	0.15^{ns}
Total dry weight (TDW)	0.04	0.02	0.8 ns	0.09	0.1	2.0^{ns}	0.03	0.01	0.70^{ns}
Succulence (SU)	1556	77.9	0.1 ns	543	2719	4.9*	689	344	$0.62^{\rm ns}$
Germination									
Germination index (GI)	784.8	392	5.1*	147	738	9.5***	123	61.8	$0.80^{\rm ns}$
Final germination (FG)	1078	538	1.5 ns	723	3618	9.9***	1697	848	2.34^{ns}
Mean daily germination (MDG)	0.34	0.17	1.5 ns	1.31	1.1	9.9***	0.54	0.27	2.34^{ns}
Germination rate (GR)	2.47	1.23	4.9*	3.69	1.8	7.3***	0.18	0.09	$0.35^{\rm ns}$
Seed stamina (S)	4282	2141	17***	125	6269	48***	690	345	2.67ns

^{*,**, ***:} Significant in probability level of 5, 1 and 0.1%, respectively; ns: not significant SS: Sum of squares, MS: Mean Square, F: this value is calculated by dividing MS source to MS error in SAS software.

Table 2. Influence of salinity levels on germination and biomass production of *Dodonaea viscosa* Linn

Salinity (mM)	SL (cm)	RL (cm)	FWS (g)	FWR (g)	DWS (g)	DWR (g)	TDW (g)	SU (%)	GI	MDG	GR	S
100	4.38 b	2.32 b	0.29 b	0.08^{b}	0.0991a	0.016^{b}	0.115 ^a	76ª	29.1 ^b	1.22ab	1.4 ^b	46 b
200	1.23 °	0.57°	$0.06^{\rm b}$	0.03^{bc}	0.0080^{a}	$0.005^{\rm c}$	0.013a	81ª	18.2 ^{bc}	0.97 b	0.8^{bc}	12°
300	0.02°	0.30^{b}	0.001^{b}	$0.007^{\rm c}$	0.0007^{a}	$0.002^{\rm c}$	0.003^{a}	44ª	7.1°	0.42°	0.3°	0.8 °
0	8.97°	4.04 a	0.57 a	0.19^{a}	0.0600^{a}	0.026^{a}	0.086^{a}	88ª	54.4ª	1.74ª	3.7ª	127ª

Means followed by different lower-case letters within columns are significantly different (P<0.01)

Table 3. Influence of salt type on germination and biomass production of *Dodonaea viscosa* Linn

Salt type	SL (cm)	RL (cm)	FWS (g)	FWR (g)	DWS (g)	DWR (g)	TDW (g)	SU (%)	GI	MDG	GR	S
CacL ₂	1.8 b	0.7^{b}	0.2 b	0.02^{b}	0.04^{a}	0.01^{b}	0.04^{a}	76ª	28^{b}	1.1 ^b	1.4 ^b	17 ^b
NaH ₂ Po ₄	2.0^{b}	1.4 b	$0.1^{\rm b}$	0.03^{b}	0.07^{a}	0.01^{b}	0.08^{a}	64ª	13°	0.8^{b}	0.5^{b}	17 в
$KNo_3 + KH_2Po_4$	3.0 b	1.4 b	0.2^{b}	0.08^{b}	0.02^{a}	0.01^{b}	0.03^{a}	75ª	28^{b}	1.1 ^b	1.4 ^b	37 в
Control	9.0°	4.0 a	0.6 a	0.19^{a}	0.06^{a}	0.03 a	0.09^{a}	88ª	54ª	1.7ª	3.7^{a}	126ª

Means followed by different lower-case letters within columns are significantly different (P<0.01)

were differences among seedling lengths in response to salinity. Fresh weight of seedling decreased at 300mM Salinity. Fresh weight of root was markedly reduced by salinity (P<0.01, Table 2). The same trend was observed when the seedling biomass was subjected to different salt types. In contrast to controls, seedling biomass decreased by salt stress (P<0.01, Table 3).

In this study, 100 mM NaH₂Po₄ severely increased length of the root. The dry and fresh weight of stem was higher than that of other treatments at level of 100 mM NaH₂Po₄. The inhibition effect of salinity on dry weight of seedling was

observed in treatment of 300 mM KNo₃+KH₂Po₄. In 100mM KNo₃+KH₂Po₄ treatment, dry and fresh weight of root was also greater than in other treatments. The length of stem at 100 mM KNo₃+KH₂Po₄ was higher than in other treatments. Fresh weight of seedling was accelerated by 100 mM KNo₃+KH₂Po₄ after 56 days of treatment. Results suggest that seedlings had a higher succulence under salt stress of 100 mM KNo₃+KH₂Po₄, as compared to CacL₂ and NaH₂Po₄ (Table 4).

Our experimental results showed that the length, fresh weight and final germination of *D. viscosa* decreased with the

Table 4. Biomass features of <i>D. viscosa</i> seedlings treated with	n different types of salt together with increasing concentrations of
salinity (Mean ± Standard deviation)	

Salt type	Salinity (mM)	SL (cm)	RL (cm)	FWS (g)	FWR (g)	TFW (g)	DWS (g)	DWR (g)	TDW (g)	SU (%)
$CacL_2$	100	1.80±0.20	0.8±0.1	0.211±0.060	0.020±0.0010	0.191±0.0224	0.031±0.0010	0.007±0.0009	0.042±0.0066	75±14
	100	5.20±0.40	3.3±0.3	0.333±0.010	0.060±0.0020	0.390±0.0562	0.202±0.0100	0.010±0.0011	0.222±0.0192	67±15
NaH ₂ Po4	200	0.80±0.10	0.6±0.1	0.040±0.001	0.030±0.0010	0.073±0.0093	0.007±0.0006	0.006±0.0003	0.013±0.0013	78±12
	300	0.03±0.01	0.3±0.1	0.001±0.000	0.008±0.0001	0.012±0.0012	0.001±0.0001	0.002±0.0001	0.003±0.0002	47±6
	100	5.50±0.80	2.6±0.6	0.321±0.040	0.150±0.0118	0.490±0.0232	0.040±0.0030	0.021±0.0012	0.063±0.0096	87±18
KNo ₃ +KH ₂ Po ₄	200	1.80±0.50	0.5±0.1	0.090±0.001	0.030±0.0052	0.121±0.0715	0.009±0.0016	0.004±0.0005	0.013±0.0019	85±19
	300	0.00±0.00	0.3±0.1	0.000±0.000	0.004±0.0009	0.004±0.0006	0.000±0.0000	0.001±0.0001	0.001±0.0001	37±6
Control	0	9.00±0.03	4.0±0.7	0.600±0.010	0.200±0.0500	0.762±0.0913	0.060±0.0100	0.022±0.0050	0.086±0.0096	88±9

Table 5. Germination features of *D. viscosa* seedlings treated with different types of salt together with increasing concentrations of salinity (Mean \pm Standard deviation)

Salt type	Salinity	FG (%)	GI	MDG	GR	S
$\mathrm{CacL}_{\scriptscriptstyle 2}$	100	60±19	28±3.9	1.07±0.82	1.41±0.85	17.312±2.40
	100	52±12	20±5.4	0.94 ± 0.11	1.03 ± 0.05	43.514±8.90
NaH ₂ Po ₄	200	55±10	12±3.2	0.98 ± 0.14	0.44 ± 0.07	7.910±1.120
	300	22±5	5±0.9	0.40 ± 0.08	0.16 ± 0.01	0.823 ± 0.150
	100	90±8	39±7.1	0.61±0.09	1.85±0.02	71.50±11.10
KNo ₃ +KH ₂ Po ₄	200	53±9	25±5.6	0.95±0.16	1.32±0.04	17.112±2.20
	300	25±4	11±2.3	0.45 ± 0.07	0.59 ± 0.02	0.891 ± 0.110
Control	0	97±5	54±9.8	1.74±0.14	3.75±0.32	126.7±23.20

increase in concentration of the salts. Similar kind of observation was detected by Jamil et al. [21]. They reported that salt stress reduced the *Dodonaea viscosa* seedling biomass (root and stem length, fresh and dry root and shoot weight). In another study, it has been reported a significant reduction in root and shoot growth of *Prosopis alba* with an increase in NaCl concentration [13]. Moreover, Jing et al. [33] showed that with an increase in salinity, the biomass of H. rhamnoides seedlings clearly decreased. In our study, the reduction in *Dodonaea viscosa* L. biomass may be a consequence of several physiological responses, including modification of ion balance, water status, mineral nutrition, stomatal behavior and photosynthetic efficiency [33].

The ability of plants to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions, and maintain ion homeostasis [34]. Reduction of plant growth by salinity may be due to the inhibitory effect of ions. The reduction in root and stem development may be due to toxic effects of the CacL₂, NaH₂Po₄, KNo₃ and KH₂Po₄ [21]. Salt does affect the cell membrane of imbibing seeds and that some, but not all, salts can penetrate the membrane. Thus, there may be intracellular, as well as membrane, responses to the presence of salt. It seems

likely to us that the inhibitory effect of salt on seed germination will ultimately be traceable to the effect of salt on a number of cellular processes [19, 20].

The results indicated that the total dry weight under the 100 mM NaH₂Po₄ and the total fresh weight under the 100 mM KNo₂+KH₂Po₄ were the highest. Seedlings succulence under the treatment of 100 mM KNo₂+KH₂Po₄ was higher than those under the other salinity treatments (Table 4). Salt affects plant growth mainly through toxicity from excessive uptake of salt substances such as sodium, reduced water uptake, known as water stress and reduction in uptake of essential nutrients particularly potassium [35]. This leads to sharp decrease in the cytosolic K⁺/Na⁺ ratio and causes salt sensitivity [36]. Similar results were recorded by Kulkarni and Nautiyal [20]. Wang et al. [37] reported that P. euphratica leaves exhibited a higher capacity to exclude salt in a longer period of increasing salinity, thus limited salt-induced lipid peroxide and MP, which contributed to membrane integrity maintenance and salt tolerance of P. euphratica. The effect of NaCl on Paxillus involutus were investigated by Zhang et al. [38]. Results indicated that the growth of Paxillus strains was enhanced by 100 mmol·L⁻¹ NaCl but severely inhibited at the concentration of 500 mmol· L^{-1} .

CONCLUSIONS

In summary, our results indicated that the different response of stems and roots might be due to varied level of salinity. Furthermore, this study may be helpful in minimizing the impact of high salt stresses, which limit germination and biomass production of plants. Seedlings were more resistant against the destructive effects of salt in salinity level of 100 mM. In the present study, total dry weight increased in seedlings in 100 mM NaH₂Po₄ compared with that of the control and this may partially explain the positive growth in 100mM NaH₂Po₄ in *Dodonaea viscosa* Linn.

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