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Full Length Research Paper

Response of three *Calligonum* species to salinity at germination and seedling stages

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In order to study the effects of salinity stresses on seed germination characteristics and seedling growth of *Calligonum*, three species of *Calligonum,* namely, *C. comosum*, *C. polygonoides* and *C. persicum* were evaluated. Three separate experiments was carried out; Experiment 1: salinity in four levels of 0, 6, 12 and 18 dS/m NaCl, were applied and the traits; germination percent, germination rate, length and dry weight of plumule and radicle and Na^+ concentration in seedling tissues were measured. In Experiment 2, non-germinated seeds from Experiment 1 were transferred into distilled water and then seed germination recovery was studied. In Experiment 3: two levels of salinity and three *Calligonum* species were conducted with three replications in pots and time course of survival percentage were recorded. Results showed that salinity reduced all the above mentioned traits. With increasing salinity the Na^+ concentration in *C. persicum* increased more than the other two species. The *C. polygonoides* species had the highest germination recovery index. The results of probit analysis showed that *C. polygonoides* has the highest Lt50 and salinity tolerance. Cluster analysis on the basis of studied traits and Ward's method revealed that there is a significant level of genetic diversity between the three species.

Key words: *Calligonum*, salt tolerance, LT50, probit analysis.

INTRODUCTION

The *Calligonum* species are dominant perennial shrubs in active sand dunes and stabilize sand fields in most desert areas (Mao and Pan, 1986; Tao, 2000). They can exist in mobile sand dunes in condition of hard drought and suitable for re-vegetation of deserts (Zang, 1992; Tao, 2000). These species have great potentials to produce forage and traditional medicine as well as perform services like halting desert encroachment and stabilizing sand dune (Tao, 2000).

A number of *Calligonum* species such as *C. polygonoides* are economically important resources in the arid regions (Bhandari, 1995; Bewal et al., 2009). Flowers are used to bake bread or cooked with clarified butter and its roots and stems are used as fuel (Bewal et al., 2008).

The *C. comosum* species is also a desert plant, which grows in sandy desert of Saudi Arabia and has been used as fuel in winter as well as in the treatment of stomach ailments (Liu et al., 2001). Its vegetative parts have high levels of crude protein, potassium and calcium content which makes it favored by camels in deserts (Munton, 1988). The *Calligonum* species have extended in desert areas of Khozestan, Sistan and Baluchestan, Semnan, Kerman and Yazd provinces in Iran.

Seed germination and seedling stabilization are the most critical stages for survival of plant under extreme condition (Kitajima and Fenner, 2000) and this is particularly true in saline deserts such as deserts in northwestern of China where rainfall is low and unpredictable (Qu et al., 2008).

Different temperatures, salinity, dormancy breaking and pre-sowing treatments have been studied to evaluate their effect on germination of *Calligonum* species (Taia et al., 2007; Bahrani and Kazempour, 2007; Ren and Tao, 2004) .Positive effects of scarification and acid treatment

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on germination have been reported for *Calligonum* species (Ren and Tao, 2004). Maximum germination percentage has been obtained at 20°C and 0 mmol NaCl for *C. comosum* and a significant correlation was observed between seed germination and salt concentration (Taia et al., 2007).

In an experiment *Calligonum* species germinated easily between 18 to 22°C (Ren et al., 2005). Seed germination was not affected by salinity up to 5 and 6 dS/m for *Calligonum* and *Hammada* species; however the germination percentage decreased with an increase in salinity. Radicle growth decreased more than plumule growth with an increase in salinity in both species (Bahrani and Kazempour, 2007). Germination of a desert shrub, *Kalidium capsicum* was inhibited by 0.2 and 0.6 M NaCl completely. Non-germinated seeds germinated after they were transferred from NaCl solutions to distilled water; however germination percentage in this condition was not greater than control (the recovery of seed germination was not significant). On the other hand, radicle elongation recovery and survival of seedlings decreased significantly with the increase in pretreatment salinity (Qu et al., 2008). There are scarce information in literature regarding salinity effects on percentage, rate and recovery of seed germination and specially seedling survival of *Calligonum* species. For natural or manmade desert vegetation, the number of emerged seedlings and seedling survival in saline soils are very important. Any strategy, which increases seed water imbibitions, germination velocity and survival time at seedling stage in stress condition, is beneficial for shrubs establishment and desert vegetation (Ren et al., 2005). The aim of this research was to study salinity effects on seed germination characteristics and seedling survival of three *Calligonum* species use for stabilizing sand dunes and desert re-vegetation in deserts of Iran.

MATERIALS AND METHODS

The seeds of *Calligonum* were collected from the Natural Resources and Forestry Organization of Yazd province of central Iran at late May 2009. The dried and homogene-sized seeds were selected for each species. Three separate experiments were conducted:

Experiment 1

To evaluate salinity stress effects on seed germination characteristics and seedling growth, a factorial experiment was conducted in a completely randomized design with three replications in sterilized petri dishes as medium for germination. Salinity factor in four levels including 0, 6, 12 and 18 dS/m induced by NaCl, were applied. Three species including *C.comosum*, *C. polygonoides* and *C. persicum* were used. To do the breaking dormancy, the seeds were soaked in sulfuric acid (96%) for 30 min (Tao and Ren, 2004) and then washed with distilled water for 45 min. The acid treated seeds were washed by sodium hypochlorite 10% containing 0.2% benomyl to prevent fungal infection. Twenty seeds were placed on two layers of Whatman filter paper in each petri dish and then 15 ml of NaCl solutions with 6 or 12 or 18 dS/m EC were added to each petri dish accordingly. The petri dishes were then transferred to germinator at 20°C. Germinated seeds were daily recorded for 19 successive days. Seeds with 2 mm plumule and radicle lengths were considered as germinated ones. Lengths of plumule and radicle were measured at the end of experiment. The dry weights of plumule and radicle were measured in each experimental unit after oven dried at 75°C for 48 h. All tissues of plumule and radicle were burned at 550 to 600°C and then used to determine tissue Na^+ ion concentration by dry ash method (Kingsbury et al., 1984). The Na^+ concentration was measured with a flam photometer. Germination percentage and germination rate were calculated as follow, respectively;

Ger (%) = 100 n/N.
n: number of germinated seeds,
N: total number of seeds in one Petri dish;

$GR= x_1/y_1 + (x_2-x_1)/y_2 +\ldots+ (x_n-x_{n-1})/y_n$. (Maguire, 1962).

$x_1 \ldots. x_n$: percentage of germinated seeds from the first day to nth day,
y_n: days from sowing to last day.

Experiment 2

The non-germinated seeds of each salinity treatment (6, 12 and 18 dS/m) from Experiment 1, were transferred into new petri dishes containing distilled water and after 8 days percentages of germination were measured in each petri dish. Recovery of germination was calculated according to Vicente et al. (2004).

The data were analyzed in a factorial experiment in complete randomized design with three replications.

Experiment 3

Seedlings survival of three *Calligonum* species were evaluated in a factorial experiment with 5 replications in greenhouse at 25 to 30/15 to 20°C day/night temperature. Salinity factor with two levels were at 0 and 12 dS/m EC. Plants were grown in pots with 2.5 kg soil comprising a mixture of clay and sand (1:3 respectively). The final number of grown plants was 13 to 15 per pot. Ten days after germination and establishment, salinity treatments were applied. The amount of necessary NaCl was calculated based on Richard (1954) and added to the irrigation water. To maintain salinity level during the growth period irrigation was accomplished with a determined amount of distilled water (presumed no leaching) to soil field capacity (FC). After salinity application, the number of survived plant in each pot was recorded from zero to 21 days with 7days interval and survival percentage was calculated as follow:

Sur (%) = (number of alive plant at present stage/ number of alive plant at previous stage) × 100.

All statistical analyses were conducted with MINITAB (version14), MSTATC and Excel.

RESULTS AND DISCUSSION

Experiment 1

Germination characteristics

Analysis of variance showed that species and salinity levels had significant effects on all studied traits and there were significant interactions between salinity and

Table 1. Analysis of variance results for survival at 7, 14 and 21 days after applying salt stress and other traits under study.

S.O.V	MS										
	Survival			Ger (%)	GR	PL	RL	PW	RW	TW	Rec
	7 days	14 days	21 days								
Salinity	0.0420^{ns}	1.088**	2.220**	552**	0.285**	486**	201**	0.052**	0.012**	0.113**	690^{ns}
Species	0.0483*	0.022^{ns}	0.077*	344**	0.243**	359**	224**	0.072**	0.007**	0.123**	1240*
Salinity*species	0.0979*	0.0543*	0.040^{ns}	97^{ns}	0.049*	88*	88**	0.013*	0.003*	0.028**	1221*
Error	0.01216	0.0139	0.0137	39	0.016	24	15.95	0.0037	0.0003	0.005	280

Ger (%): Germination percent, GR: germination rate, PL: plumule length, RL: root length, PW: plumule dry weight, RW: root dry weight, TW: total dry weight, Rec: recovery.

species for all but germination percentage (Table 1). Germination percentage was significantly and negatively correlated with EC (Table 3).This is in agreement with findings of Taja et al. (2007) and Bahrani and Kazempour (2007). However, in *C. persicum* germination stopped at 6 dS/m, then started at 12 dS/m and stopped again at 18 dS/m. This observation is in agreement with those of Taja et al. (2007) which found similar results in one *Comosum* population in Saudi Arabia. In this study, *C. comosum* and *C. polygonoides* had the highest and *C. persicum had the lowest germination percentage. The C.comosum showed 5% germination at 18 dS/m EC* suggesting its more tolerant to salinity at germination stage than the other species (Table 2). Species with higher germination rates are able to establish more rapidly and use favorable conditions (Ren and Tao, 2004).

Salinity significantly reduced plumule and radicle lengths and there was a significant negative correlation between salinity and these traits (Table 3). Generally, plumule and radicle lengths significantly reduced after 6 dS/m for all species; however plumule length decreased more rapidly than radicle length. Munns and Termaat (1996) found similar results but Bahrani and Kazempour observed that in *C. intertextum* radicle length was more injured than plumule length by salinity. Radicle lengths at 6, 12 and 18 dS/m were not significantly different (Table 2). Interaction between species and salinity in the case of plumule and radicle lengths indicates different responses of species to salinity. Plumule length in *C. comosum* was significantly reduced by salinities more than 6 dS/m relative to control but this reduction was not observed for other species. The *C. comosum* and *C. persicum* had the highest and lowest radicle and plumule lengths, respectively. In all species, salinity effects on radicle and plumule weight and total dry mass showed almost similar trends to radicle and plumule lengths. A very high correlation was also obtained among plumule and radicle length and seedling dry weight.

C. comosum showed the highest plumule, radicle and total dry weight with no significant difference with *C. polygonoides.* In germination experiment, a positive trend was obtained between the sodium concentration of seedlings and the accordance EC. For *C. persicum*, increase in Na^+ concentration was not significantly different than in *C. comosum* and *C. polygonoides*. For the *C. comosum* and *C. polygonoides* sodium concentration correlated significantly and negatively ($p < 0.01$) with all germination and early seedling growth traits. This suggests that these two species have higher salinity tolerance than *C. persicum.* One possible reason for higher tolerance in *C. comosum* and *C. polygonoides* is the lower accumulation of sodium (20.7 and 19.5 mg per gram dry weight, respectively) in these species compared to *C. persicum* (85 mg/g dry weight). Munns et al. (2006) discussing salinity effects on plant physiology, explained that salinity can suppress plant growth by reducing root growth which is a consequence of reduced soil water potential and toxicity of certain ions. Metabolic toxicity of Na^+ is aroused from its capability to compete with potassium to bind to special (critical) cellular receptors. More than 50 enzymes are activated by K^+ and not by sodium (Tester and Davenport, 2003). Some avoidance mechanisms for salinity stress in plants are minimizing sodium import, sodium exclusion from tissues or its compartmentation to vacuole (Munns et al., 2006). Therefore, species in which Na is excluded from roots may be more tolerant to salinity (Munns et al., 2002).

Experiment 2

After transferring non-germinated seeds of salinity treatments to a control medium with no salt (EC = 0), more germination percentage were observed in all species with the highest number in *C. polygonoides* (56%) (Table 4). The effect of salinity was not significant on the number of recovered plants (Table 1). There was also no significant correlation between EC and the recovery (Table 3). However the interaction between salinity and species was significant (Table 1) showing that the amount of recovery is varied among the species with change in EC. In *C. comosum*, the recovery increased with increasing EC from 6 to 18, so the highest recovery was obtained in EC = 18 (70%) while in the other two species recovery values did not changed by EC. This observation is on the contrary to those of Ungar and Jamalkhan (1996) which found in *Haloxylon recurvom*.

This experiment suggested that in order to increase

Table 2. Mean comparision of traits under study for three *Calligonum* species.

Trait	Species	EC				Mean
		0	6	12	18	
Ger%	*C. comosum*	92.6^{a}	21.6^{ab}	10^{bc}	0^{d}	30.9^{a}
	C. polygonoides	95.6^{a}	10^{bc}	5^{c}	5^{c}	28.8^{a}
	C. persicum	93^{a}	0^{d}	5^{c}	0^{d}	24.5^{b}
	mean	26.11^{a}	8.889^{b}	6.667^{bc}	1.66^{7c}	.
GR	*C. comosum*	0.8^{a}	0.308^{bc}	0.296^{bc}	0^{c}	0.351^{a}
	C. polygonoides	0.57^{ab}	0.023^{c}	0.05^{c}	0.037^{c}	0.17^{b}
	C. persicum	0.2^{c}	0^{c}	0.048^{c}	0^{c}	0.062^{b}
	mean	0.53^{a}	0.111^{b}	0.131^{b}	0.012^{b}	.
$PL_{(mm)}$	*C. comosum*	27.25^{a}	16.73^{ab}	8.93^{bc}	0^{c}	13.229^{a}
	C. polygonoides	28.05^{a}	4.2^{c}	3.567	0.73^{c}	9.138^{b}
	C. persicum	7.17^{bc}	0^{c}	1^{c}	0^{c}	2.043^{c}
	mean	20.82^{a}	6.978^{b}	4.5^{bc}	0.244^{c}	.
$RL_{(mm)}$	*C. comosum*	22.75^{a}	7.967^{b}	2.66^{b}	0^{c}	8.346^{a}
	C. polygonoides	6.95^{b}	5.433^{b}	3.76^{b}	0.63^{b}	4.196^{b}
	C. persicum	1.87^{b}	0^{c}	0.33^{b}	0.^{c}	0.550^{c}
	mean	10.52^{a}	4.467^{b}	2.256^{b}	0.231^{b}	.
$PW_{(mg)}$	*C. comosum*	40^{a}	20^{b}	12^{bc}	0^{c}	18.1^{a}
	C .polygonoides	18^{b}	4.6^{bc}	6.3^{bc}	1^{c}	7.5^{b}
	C. persicum	9b^{c}	0^{c}	0.7^{c}	0^{c}	2.4^{c}
	mean	22^{a}	8.2^{b}	6.4^{bc}	0.3^{c}	.
$RW_{(mg)}$	*C. comosum*	16^{a}	3.7^{b}	1.5^{b}	0^{b}	5.3^{a}
	C. polygonoides	15^{a}	0.7^{b}	1.2^{b}	0.5^{b}	4.4^{a}
	C. persicum	1^{b}	0^{b}	0.4^{b}	0^{b}	0.4^{b}
	mean	11^{a}	1.5^{b}	3^{b}	0.2^{b}	.
$TW_{(mg)}$	*C. comosum*	51^{a}	23.6^{bc}	13.6^{cd}	0^{d}	22.1^{a}
	C. polygonoides	32^{b}	5.3^{cd}	7.5^{cd}	1.1^{d}	11.5^{b}
	C. persicum	09^{cd}	0^{d}	1.1^{d}	0^{d}	2.5^{c}
	mean	31^{a}	9.7^{b}	7.4^{bc}	0.4^{c}	
$Na^{+}{}_{mg/gr.dw}$	*C. comosum*	2.46^{b}	20.95^{b}	38.73^{b}	*	20.71^{b}
	C. polygonoides	4.10^{b}	21.69^{b}	32.69^{b}	*	19.50^{b}
	C. persicum	6.77^{b}	76.7^{a}	171.7^{a}	*	85.05^{a}
	mean	4.44^{c}	39.78^{b}	81.04^{a}		

(Ger (%): Germination percent, GR: germination rate(percentage per day), PL: plumule length and RL: root length(millimeter), PW: plumule dry weight, RW: root dry weight and TW: total dry weight(milligram), Na^{+}: sodium concentration (milligram per gram dry weight))*: There was not enough plant material to measure.

germination percentage in seedling production programs, one can use soaking seeds in water with EC = 18 for *comosum* and EC 6 to 12 for the other two species. It was also concluded that seeds are able to survive in soils with salinities over the tolerance limit and germinate after reduction in salinity level in wet periods. This finding is in accordance with Ajmalkhan and Unger (1996). Similar results have been found by Unger (1995) in some annual halophytes too.

Experiment 3

Analysis of variance for seedling survival trait in each

Table 3. Correlation between the traits under study.

Trait	EC	Ger (%)	GR	PL	RL	PW	RW	TW	Rec	Na^+
EC	1									
Ger	-0.679**	1								
GR	-0.595**	0.886**	1							
PL	-0.611**	0.875**	0.938**	1						
RL	-0.504**	0.817**	0.756**	0.749**	1					
PW	-0.582**	0.874**	0.937**	0.944**	0.786**	1				
RW	-0.519**	0.800**	0.814**	0.802**	0.852**	0.807**	1			
TW	-0.588**	0.889**	0.940**	0.942**	0.837**	0.985**	0.896**	1		
Rec	0.293	-0.640**	-0.495**	-0.566**	-0.440*	-0.557**	-0.505**	-0.554**	1	
Na^+	0.719*	-0.930**	-0.808**	-0.946**	-0.864**	-0.770**	-0.991**	-0.862**	-0.4	1

Ger (%): Germination percent, GR: germination rate, PL: plumule length, RL: root length, PW: plumule dry weight, RW: root dry weight, TW: total dry weight, Rec: recovery.

Table 4. Means comparsion for seed germination recovery after transfered from ECs 6 , 12 and 18 dSm^{-1} to distilled distilled water.

Species	EC			Mean (%)
	6	12	18	
C. comosum	7.77^{d}	29.21^{cd}	70.00^{a}	35.66^{b}
C. polygonoides	53.00^{ab}	58.90^{ab}	56.14^{ab}	56.01^{a}
C. persicum	40.00^{bc}	40.29^{bc}	28.33^{cd}	36.20^{b}
Mean%	33.59^{a}	42.8^{a}	51.49^{a}	

Table 5. Survival difference between salinity stress and control for each species and their mean comparison in different recording times (0 , 7 ,14 and 21 days).

Days after applying stress	Survival means for species at different times, after applying salt stress			Difference from control			LSD0.05	LSD0.01
	C. comosum	*C. polygonoides*	*C. persicum*	*C. comosum*	*C. polygonoides*	*C. persicum*		
0	1	1	1					
7	0.9714^{a}	0.8^{b}	0.9^{ab}	-0.0035	-0.2000*	-0.1	0.1471	0.2021
14	0.5335^{a}	0.5138^{a}	0.5416^{a}	-0.3120**	-0.4611**	-0.3611**	0.1576	0.2165
21	0.2089^{a}	0.3020^{a}	0^{b}	-0.5867**	-0.6479**	-0.8331**	0.1548	0.2148

recording time (Table 1) showed that 7 and 21, but not 14 days after salinity application survival was significantly different in all three species (Table 5, Figure 1) but rate or level of survival in all three species at 14 and 21 days was significantly different ($p < 0.01$) compared to the control. Survival in *C. persicum* 21 days after salinity application reduced to zero but the other two species did not show any differences (Table 5) suggesting that the *C. persicum* is more susceptible than the other species.

Probit analysis was performed to linearize sigmoidal curve of seedling mortality (Figure 2) during the proposed time (Finny, 1971). The time (days) for 50% mortality (Lt = 50) was also determined for each species (Table 6). Results showed that the *C. polygonoides* seedlings are more tolerant to salinity than the other two species (Lt50 = 24 days and b = 0.07). The lower values of b (the line slope showing the level of salinity sensitivity) indicate lower sensitivity and higher tolerance. Cluster analysis revealed that (based on all traits related to salinity tolerance) in 0.42 similarities both *C. polygonoides* and *C. Comosum* were in one group indicating high genetic similarity between these two species. However, based on 1.36% similarity all three species were in same group suggesting high genetic diversity among *Calligonum* species in salinity tolerance (Figure 4).

Conclusion

Comparison of three species responses to salinity stress

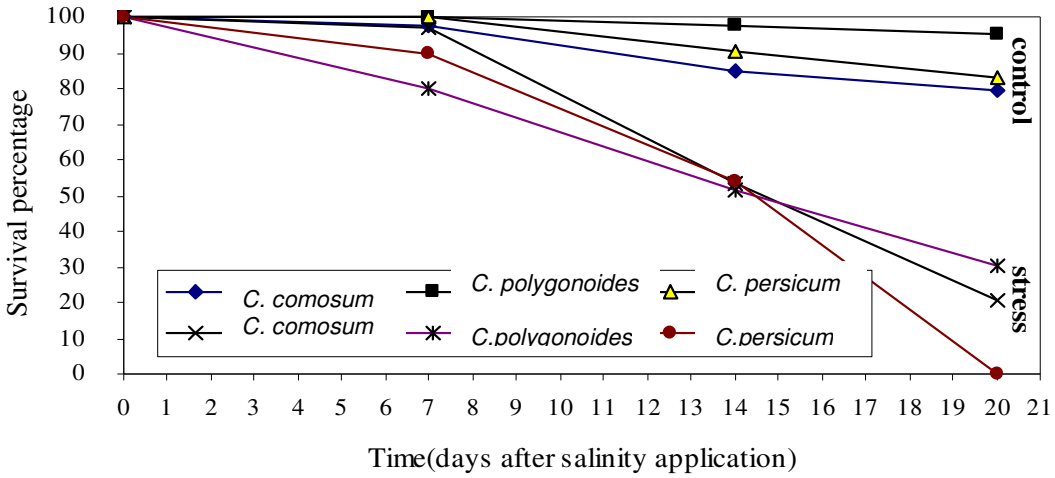

Figure 1. Survival graphs for three species in salinity stress and control condition over time.

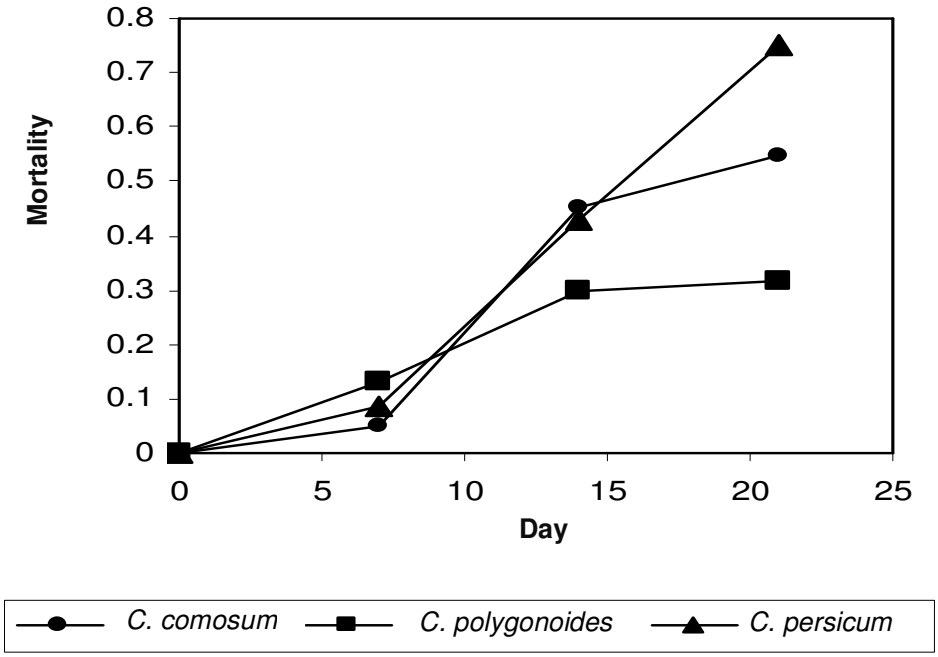

Figure 2. Mortality over time(days) after applying salt stress for diferent species.

Table 6. Probit analysis for determination time to 50% mortality (LT50)in three species.

Species	LT50(day) ± SE	B ± SE	Prob	Goodness-of-fit (χ^2)
C. comosum	17.5 ± 1.25	0.13 ± 0.02	0.000	5.5^{ns}
C. polygonoides	24 ± 2.4	0.07 ± 0.02	0.002	0.147^{ns}
C. persicum	15.3 ± 1.26	0.16 ± 0.03	0.000	0.218^{ns}

b: Regression coefficient ,SE:standard error, Prob:significance level for b, χ^2 :Chi Square ns: non-significant at 0.05 probability.

showed that *C. polygonoides* and *C. comosum* had higher germination percentage and root dry mass. The other seedling characteristics including seedling dry weight, radicle and plumule length were the highest in *C. comosum*. Sodium concentrations in seedlings of *C. polygonoides* and *C. comosum* were much lower than *C.*

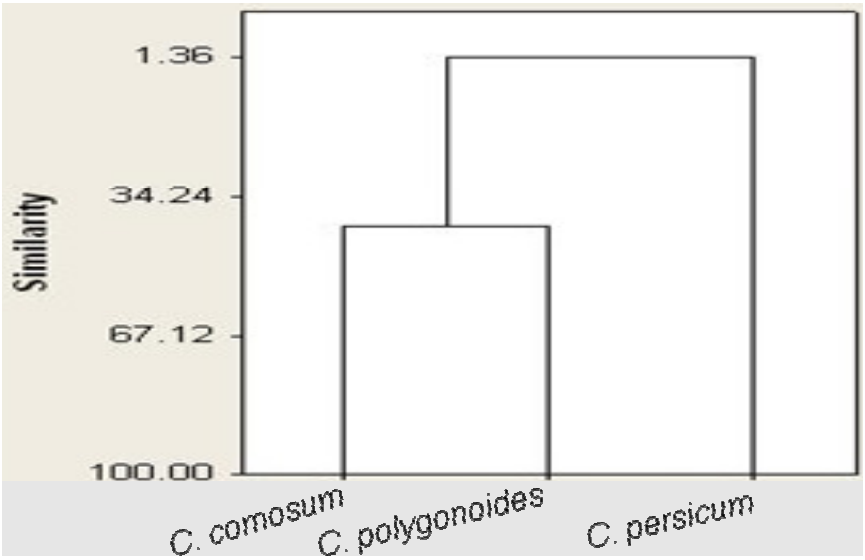

Figure 3. Dendrogram with ward linkage method.

persicum which may be a possible reason of higher tolerance to salinity during germination in these two species. Considering results of seedlings' survival assay and probit analysis the *C. polygonoides*, having the highest Lt50 and lowest regression slope, is the most tolerant species to salinity stress during the germination and early growth. This species had also the highest recovery. Finally, cluster analysis and grouping of species based on means of all measured traits revealed that all three species were in one group (Figure 3), which indicates a high genetic variation among species in salinity tolerance in *Calligonum*.

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