



Effects of salinity and drought on early seedling growth and survival of *Artemisia herba-alba*

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Received: 1st January, 2015

Accepted: 31st May, 2015

Abstract

Seedlings of *Artemisia herba-alba* grown in glasshouse were watered with differing salinities (0, 150, 250 or 350 mM NaCl) and watering frequencies of 3, 7, 14 or 21 days for a period of 6 weeks. At the end of the study, plant survival, dry matter yield, biomass allocation (shoot and root), leaf area, relative growth rate (RGR), net assimilation rate (NAR), specific leaf area (SLA) and leaf area ratio (LAR) were recorded. When watered with 0 mM NaCl, *Artemisia herba-alba* plants had similar ($P > 0.9$) final dry matter weight and 100% survival regardless of watering frequency. However, plants did respond to watering frequency by allocating less biomass proportionately to below ground reserves as watering frequency decreased ($P < 0.05$). A threshold for survival of *Artemisia herba-alba* to cumulative salt concentrations above 20 g salt per kg soil was recorded. Once salinity concentrations passed the threshold, survival decreased dramatically from 80% at 30 g salt per kg soil to 60% at 70 g salt per kg soil. As well, within each level of watering frequency, as salt concentrations increased, seedling biomass decreased. *Artemisia herba-alba*'s drought tolerance makes it an ideal candidate on low salinity sites with low to moderate soil moistures.

Keywords: Biological diversity, Desertification, Mediterranean basin, Rangeland, Salinization

Introduction

Drought and salinity are major factors that reduce crop yields worldwide. Salt and drought often co-exist to create some of the most inhospitable environments for plant growth. Salinity may affect growth by decreasing the ease with which the plant absorbs water, and/or facilitating the entry of ions in amounts high enough to be toxic, and/or reducing the absorption of nutrients through ion imbalances (Romo and Eddleman, 1985). While salinity

and drought are two independent environmental factors, they often not only co-exist but exasperate one another; therefore the uncoupling of salinity versus drought effects on plant growth is nearly impossible. Typically, researchers study salinity and drought independently through the use of known concentrations of salt solutions applied at regular intervals, similar to the current study.

Within Syria, approximately 10.2 million hectares, or about 60% of the land base, falls below the 200 mm isohyet and are designated as *Badia* or *šsteppe* (Ghassali *et al.*, 2011; Louhaichi *et al.*, 2012b). Within the *Badia*, *Artemisia herba-alba* is the dominant planthence, commonly referred as artemisia steppe. The artemisia steppe overlaps the very low precipitation zone of the Syrian deserts (100 mm/yr) and the barley/wheat livestock zones (150-300 mm/yr). As such, the artemisia steppe is highly susceptible to being lost through desertification from over grazing or through the expansion of rain fed cereal crops (Bounejmate *et al.*, 2004).

The value of *Artemisia herba-alba* in the restoration of the artemisia steppe regions cannot be underestimated. *Artemisia herba-alba* is widely used for fuel and heavily exploited within grazing systems. Its palatability increases from moderate in spring to good in autumn (Al-Oudat *et al.*, 2005). Changes in palatability are likely linked to seasonally dimorphic leaves, which is just one of many features that allow the species to thrive under the drought and saline conditions of its native range. It is able to tolerate soil water potentials from -5.5 MPa (Ourcival *et al.*, 1994) to -10.0 MPa (Schulze *et al.*, 1980), and saline conditions as high as 200 mM NaCl (Mariko *et al.*, 1992). Under stress *Artemisia herba-alba* is able to amputate portions of above ground biomass in order to maintain a smaller functional core plant (Ourcival *et al.*, 1994). It also appears that water transport within the plant is some

what sectorial as each branch is physiologically independent, as a result water potentials within a single plant can vary from shoot to shoot (Evanari *et al.*, 1976; Ourcival *et al.*, 1994). The objective of this study was to evaluate the combined effects of salinity and drought on *Artemisia herba-alba* growth responses in an effort to determine suitable site conditions for restoring *Artemisia herba-alba* plant communities.

Materials and Methods

Site and experimental design: The experiment was conducted within an environmentally controlled glasshouse at the International Center for Agricultural Research in the Dry Areas (ICARDA), located 30 km south of Aleppo, Syria (36° 01 N, 36° 56 E). Seeds of *Artemisia herba-alba* were collected from ICARDA's research station in February 2010, cleaned and dry stored. In October 2010, a total of 200 seeds were sown in plastic bags (10 × 25 cm), and filled with 1:1:1 clay, manure and sand resulting in soil with 8.4 pH (alkaline); 0.29% organic matter content and 0.22 mS/cm EC. In March 2010, eighty plants of similar size were randomly selected and transplanted into plastic pots (25 × 14 cm). At the time of transplanting, each pot was watered once with distilled water and after 10 days, salt-drought treatments were imposed. The experimental design was completely randomized design (CRD) with five salinity levels and four irrigation intervals in 5 replications. The pots were filled to field capacity (1050 mL) with watering solution of 0, 150, 250 and 350 mM NaCl and applied every 3rd, 7th, 14th and 21st day for a period of 6 weeks. The pots were kept inside the naturally lit and temperature-controlled glasshouses. During watering no solution dripped out from bottom of the pots. Cumulative salt added to the pots through irrigation ranged from 6 g salt per kg soil at 150 mM NaCl × 21 day watering frequency to 70 g salt per kg soil at 350 mM NaCl × 3 day watering frequency.

Growth and survival parameters: Seedling survival was recorded at the end of six week trial period. All surviving plants were uprooted, washed and the tissue was separated into above ground (stem) and below ground parts (roots) for morphological analyses at the end of the trial. Above and below ground tissue was oven dried for 7 days at 70°C. For each plant, the weight of the stems and roots were recorded.

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Physiological characters: Data was calculated on; relative growth rate (RGR), net assimilation rate (NAR), specific leaf area (SLA) and leaf area ratio (LAR). Using following equation:

$$RGR = (\ln(W_2) - \ln(W_1)) / T_m$$

$$NAR = (W_2 - W_1) [\ln(L_2) - \ln(L_1)] / (L_2 - L_1) / T_m$$

$$LAR = (L_2/W_2 + L_1/W_1) / 2$$

Where, W_i and L_i are the plant dry weight and leaf area at time T_i , respectively (i ; 1 and 2), and T_m is the growth period in days (42 days in the present study). Five plants were harvested at t_{-1} , when the treatments were started and the five plants of each treatment were harvested at t_2 , when the treatments were terminated.

Specific leaf area (SLA) was calculated as:

$$SLA = L_2 / LW_2$$

Where, LW_2 is the leaf dry weight at time t_2 . Dry matter partitioning, which is the ratio of the dry weight of each plant part (leaf, stem and root) to the total dry weight, was calculated as leaf weight ratio (LWR), stem weight ratio (SWR) and root weight ratio (RWR) on the basis of data obtained at t_2 .

Leaf area was measured by the *vegMeasure* software program developed at Oregon State University for measuring plant cover in rangelands to make automated cover measurements (Louhaichi *et al.*, 2001; Louhaichi *et al.*, 2012a). Photos of each plant were taken weekly using a high resolution camera. Images were acquired on the same day, with the same light and time conditions, as well as the same camera settings. The position and the height of camera during the first photo shoot was determined based on the canopy vegetation cover conditions, so that the camera's lens faced exactly perpendicular to the ground (Louhaichi *et al.*, 2010). The images were processed through *vegMeasure*.

Statistical analyses: The analysis of variance was carried out using completely randomized design error structure and combined over the irrigation levels to account for the variation due to irrigation, salinity and their interaction. Irrigation frequency may be seen as having effects confounded with the tables; therefore, our

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interest does not lie in irrigation frequency. However, we believe the interaction with salinity would primarily be due to irrigation frequency rather than from the table position; therefore, the interaction will be interpreted accordingly. Since salinity and irrigation frequencies are quantitative, the response means were modeled as follows: if salinity $\times$ irrigation frequency were significant ($P < 0.05$), the means were modeled in terms of linear and quadratic effects of salinity and irrigation frequencies, and their interaction between linear components. The model was further reduced by dropping the insignificant terms, but retaining the lower order if higher order terms were significant, in favor of higher percent variance accounted for (adjusted R^2). All analyses were undertaken using Genstat software (Payne, 2013).

Results and Discussion

In this study, drought and salinity stress were imposed independently and short periods of water stress applied did not cause any major detrimental effect on the survival and growth of *Artemisia herba-alba* seedlings.

Watering frequencies: Independent from the effect of salinity, watering frequencies (3, 7, 14 or 21 days) had no significant effect ($P > 0.05$) on plant growth, survival and most of the physiological parameters. Regardless of the watering frequencies, all *Artemisia herba-alba* seedlings survived the duration of the study. Watering frequencies had no significant affect ($P > 0.05$) on any parameters, except the net assimilation rate (NAR; $\text{g cm}^2 \text{day}^{-1}$). The NAR of plants decreased linearly from 0.0006 to 0.00038 $\text{g cm}^2 \text{day}^{-1}$ as the frequency of drought prolonged from 3 to 21 days (Fig. 1). Drought affected the NAR positively, but this effect was profoundly when the watering interval was longer than 7 days. The lone significant physiological response of the seedlings to drought was the reduction of NAR which typically occurs in plants that are subjected to drought. Similar effects of prolonged drought on NAR have been reported in a number of studies with various plant species (Polley *et al.*, 2002; Khalil *et al.*, 2011).

In this study, the irrigation regime was set to imitate the precipitation for the Badia rangelands in the Middle East. The lowest watering level of 150 mL every 21 days is near the average precipitation and soil moisture conditions of the Syrian Badia. The watering of 150 mL 3 kg^{-1} soil translates to 50 g kg^{-1} , over twice the average soil moisture of the field conditions reported in this study. However, global climate change is likely to result in substantial declines in precipitation across the Middle

East (Evans, 2009) where *Artemisia herba-alba* can cope with deteriorating moisture conditions. Evans (2009) predicts an increase in the length of the dry season of approximately two months for most of Syria, lying below the 200 mm/year isohyet. The dry season is a time when animals must rely on supplemental feed for sustenance; thus compounding the economic pressures associated with sheep raising and increasing the tendency to overgraze during the grazing period.

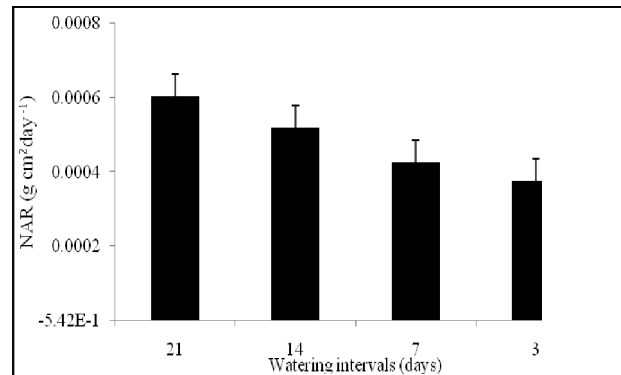


Fig 1. Effect of watering intervals on net assimilation rate ($\text{g cm}^2 \text{day}^{-1}$) of *Artemisia herba-alba*. Bar represents SE.

In this study, the irrigation regime was set to imitate the precipitation for the Badia rangelands in the Middle East. The lowest watering level of 150 mL every 21 days is near the average precipitation and soil moisture conditions of the Syrian Badia. The watering of 150 mL 3 kg^{-1} soil translates to 50 g kg^{-1} , over twice the average soil moisture of the field conditions reported in this study. However, global climate change is likely to result in substantial declines in precipitation across the Middle East (Evans, 2009) where *Artemisia herba-alba* can cope with deteriorating moisture conditions. Evans (2009) predicts an increase in the length of the dry season of approximately two months for most of Syria, lying below the 200 mm/year isohyet. The dry season is a time when animals must rely on supplemental feed for sustenance; thus compounding the economic pressures associated with sheep raising and increasing the tendency to overgraze during the grazing period.

Salinity levels: A clear threshold for the survival of *Artemisia herba-alba* was recorded for cumulative salt concentrations above approximately 20 g salt per kg soil (Fig. 2). Regardless of watering frequency, all *Artemisia herba-alba* plants survived cumulative salt concentrations below 20 g salt per kg soil. Once cumulative salt concentrations exceeded 20 g salt per kg soil, plants began to die. Survival decreased to 80% at 30 g salt per kg soil and 60% at 70 g salt per kg soil.

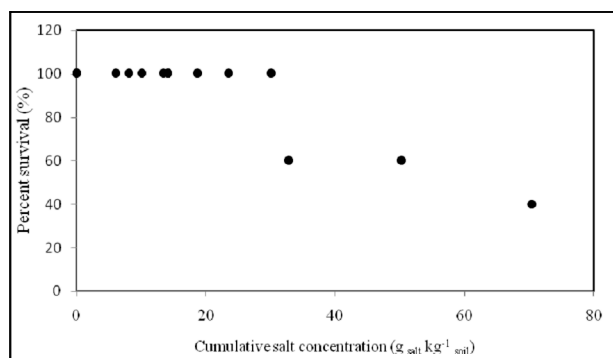


Fig 2. Survival rate (%) of *Artemisia herba-alba* seedling as affected by cumulative salt concentration (g salt kg⁻¹ soil)

A clear effect of salinity on the physiological parameters and growth of *Artemisia herba-alba* was recorded. As the salinity levels increased, the dry weight of leaves, stems and roots declined ($P<0.05$) significantly (Fig. 3).

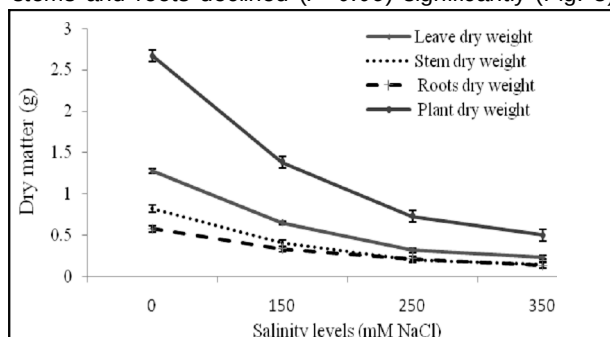


Fig 3. Effect of salinity levels (mM NaCl) on dry weight (g) of leave stem and roots of *Artemisia herba-alba*. Bars represent SE.

When plants were subjected to the highest level of salinity (350 mM NaCl) the dry weight of the plant, leaves, stems and roots decreased to 81, 82, 83 and 77% respectively less compared to the 0 mM NaCl treatment. A highly significant negative effect ($P<0.001$) of salinity on stem height and root length was observed (Fig. 4).

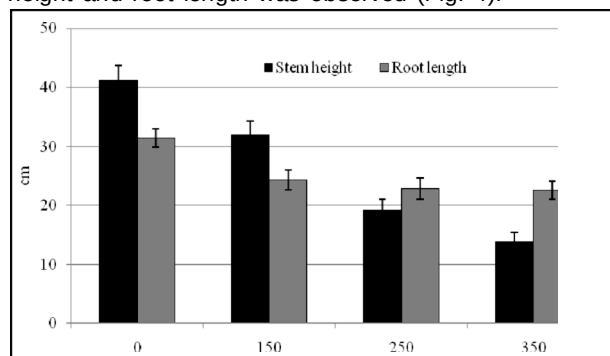


Fig 4. Effect of salinity (mM NaCl) levels on stem height (cm) and root length (cm) of *Artemisia herba-alba*. Bars represent SE.

Stem height decreased linearly from 41.2 cm at 0 mM level salinity to 13.9 cm at 350 mM NaCl. The root length decreased from 31.4 cm to 24.4 cm as the level of salinity increased from 0 mM NaCl to 150 mM NaCl. However further increase in the salinity level (up to 350 mM NaCl) did not cause any significant changes ($P<0.001$) in the root length of *Artemisia herba-alba* plants. Salt stress induced a dramatic decrease ($P<0.001$) in NAR (g cm² day⁻¹), specific leaf area (SLA; cm² g⁻¹) and leaf area ratio (LAR; cm² g⁻¹) (Table 1).

Table 1. Effect of the salinity levels on net assimilation rate, specific leaf area and leaf area ratio of *Artemisia herba-alba*

Salinity levels (mM NaCl)	Net as simulation rate (NAR) (g cm ⁻² day ⁻¹)	Specific leaf area (SLA) (cm ² g ⁻¹)	Leaf area ratio (LAR) (cm ² g ⁻¹)
0	0.001055	62.9	48.21
150	0.000659	56.4	46.23
250	0.000256	46.6	43.05
350	0.000047	41.5	41.08
Mean	0.000504	51.8	44.6
P	<0.001	<0.001	<0.001
LSD	0.0002	9.56	1.901

Salinity levels of 150, 250 and 350 mM NaCl decreased the NAR by 38, 76 and 96%. However, reduction in specific leaf area (10, 26 and 34%) and leaf area ratio (4, 11 and 15%) as a response to increasing salinity was less profound as compared to NAR.

Interactions between watering frequencies and salinity levels: Analysis of variance revealed significant interactions between watering frequencies and salinity levels for leaf area (cm²), plant dry weight (g) and relative growth rate (RGR; g day⁻¹) (Table 2). Hence, as salinity levels increased, *Artemisia herba-alba* leaf area (cm²) significantly declined ($P=00.4$) for each level of watering frequency (Table 2). Salinity levels of 150, 250 and 350 mM NaCl decreased the leaf area by 54, 81 and 88%; the RGR by 39, 77 and 98%; and the NAR by 38, 76 and 96%. However, the reduction in specific leaf area (10, 26 and 34%) and leaf area ratio (4, 11 and 15%) as a response to increasing salinity was less dramatic.

The interaction between 21 days watering frequency × 0 mM NaCl salinity level and 14 days watering frequency × 150 mM NaCl salinity level resulted in significant leaf area decrease. However, there was no significant effect of the watering rates on leaf area when the salinity levels

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Table 2. Effect of the salinity levels × watering frequencies on leaf area, plant dry weight and relative growth rate of *Artemisia herba-alba*

Item	Watering frequencies (day)	Salinity levels (mM NaCl)				Mean
		0	150	250	350	
Leaf area (cm ²)	3	85.7	44.9	13.9	4.3	37.2
	7	83.2	41.8	15.7	6.3	36.7
	14	85.5	23.0	12.5	10.2	32.8
	21	65.7	37.2	18.6	17.1	34.6
	Mean	80.0	36.7	15.2	9.5	
	P	0.004				
	LSD	13.52				
Plant dry weight (g)	3	2.63	1.55	0.60	0.37	1.29
	7	2.53	1.53	0.75	0.36	1.29
	14	3.01	0.97	0.74	0.58	1.33
	21	2.50	1.46	0.80	0.70	1.37
	Mean	2.67	1.38	0.72	0.50	
	P	0.011				
	LSD	0.40				
Relative growth rate (g day ⁻¹)	3	0.042	0.042	0.002	-0.005	0.017
	7	0.041	0.041	0.011	-0.011	0.018
	14	0.045	0.045	0.012	0.003	0.019
	21	0.040	0.040	0.013	0.010	0.023
	Mean	0.042	0.042	0.0097	0.0007	
	P	0.016				
	LSD	0.01				

were 250 and 350 mM NaCl (Table 2). Likewise, the plant dry weight and RGR (g day⁻¹) declined ($P < 0.05$) as watering frequency decreased, but did not significantly changed with increasing watering intervals when salinity level increased to 250 and 350 mM NaCl. Furthermore, there was a significant decrease ($P < 0.05$) in the dry weight for the 14 day treatment when the salinity level was 0 and 150 mM NaCl. Similarly, RGR was significantly decreased for the 14 day treatment when the salinity level was 150 mM NaCl.

Unlike the minor effect of irrigation frequency, salt stress induced a dramatic decrease in *Artemisia herba-alba* leaf area, which subsequently caused a significant decline in relative growth rate (RGR; g day⁻¹), net assimilation rate (NAR; g cm² day⁻¹), specific leaf area and leaf area ratio (LAR; cm² g⁻¹). As an indication of salt stress, a significant reduction in the net assimilation rate and stomatal conductance for various plants was also reported (Flexas *et al.*, 2004; Koyro, 2006). *Artemisia herba-alba* is considered to be low salt tolerant (Mayzlish-Gati and Steinberger, 2007) plant species. Similarly, the results on the seedling survival and growth performance obtained in this study indicated that *Artemisia herba-alba* has a potential in low - moderate salt affected dry

areas. Our results also confirm the finding of Mariko *et al.* (1992) who reported that *Artemisia herba-alba* can only tolerate the salinity level upto 200 mM NaCl. This is also highlighted in a study reported by Friedman *et al.* (1977) who recorded that *Artemisia herba-alba* abundance on north facing slopes was linked to sites with maximum sodium concentrations of approximately 0.9 mg sodium per g dry soil, and maximum chloride concentrations of approximately 4.0 mg chloride per g dry soil. In the current study, there existed a threshold of approximately 20 g salt per kg soil (or 20 mg salt per g soil) after which the species was affected by salt, regardless of the drought treatment. However, it should be noted that while plants survived salt rates near 20 g salt per kg soil, growth was marginal and plant quality was poor. Thus, the field observations of Friedman *et al.* (1977) are likely closer to limitations one could expect to find for healthy specimens of *Artemisia herba-alba*. While unaltered rangelands in Syria are not typically affected by salinity (Ilaiwi, 2001), in other regions of the Mediterranean Basin, in particular Iraq, salinity does affect vast tracts of land (Schnepf, 2004). Additionally, the conversion of steppe to rain fed agriculture has resulted in increased levels of salinity on marginal lands in Syria. Since the middle of the 1980s rainfed agriculture expand

within the Syrian steppe from 36,000 ha in 1982 to 552,000 ha in 1992 (Ilaiwi, 2001). As a result the conversion of steppe to rain fed agriculture was made illegal in 1995. The fragile nature of these Aridisol soils leaves them highly susceptible to wind erosion, where approximately 25% of the Syrian steppe is affected by wind erosion (Ilaiwi, 2001). The detrimental effects of rainfed agriculture has left hundreds of thousands of hectares in need of restoration, ideally back to rangeland condition. *Artemisia herba-alba*'s good palatability, combined with drought tolerance makes it an ideal candidate on low-moderate salinity sites with low to moderate soil moistures. Salinization and alterations to the soil structure, compounded by global climate change, may preclude the use of *Artemisia herba-alba* on the most challenging sites.

Conclusion

In the Mediterranean Basin, land managers are becoming increasingly aware of the combined effects of overgrazing, irrigation/crop conversion of marginal rangelands, and global climate change. Countries of this region are starting to implement large-scale restoration efforts. In order for these efforts to be successful, land managers need a better understanding of which species are likely to survive under various growing conditions. *Artemisia herba-alba* is a dominant perennial plant species that occurs across a wide range of ecosystem types, provides a valuable forage source, and is browse tolerant. Thus, the use of *Artemisia herba-alba* should be considered a priority species on low to moderate salinity sites and across a variety of soil moisture conditions. Special consideration must be taken when designing, implementing and analyzing salinity experiments as salt will accumulate in the soil medium.

Acknowledgement

This research was supported by the International Center for Agricultural Research in the Dry Areas (ICARDA), the CGIAR Research Program (CRP) on Dryland Systems and the CRP on Climate Change, Agriculture and Food Security (CCAFS).

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