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RESEARCH

Evaluation of Alfalfa (*Medicago sativa* L.) Populations' Response to Salinity Stress

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ABSTRACT

Alfalfa (*Medicago sativa* L.) is a moderately salt-tolerant crop with high economic return and is therefore more suitable for production with lower quality water than most high-value crops. This study was conducted to examine the effect of water composition types (Cl^- or SO_4^{2-}) of irrigation water and five salinity levels (electrical conductivity of irrigation water [EC_{iw}] = 0.85, 8, 13, 18.3, and 24.5 dS m^{-1}) on biomass production, salt tolerance, and ion concentration of 15 alfalfa populations. The plants were grown in a greenhouse in 60 sand tanks for 347 d under salt treatment. There was no significant effect of water composition type on shoot and root biomass production. Water composition type × EC and water composition type x population interactions were also not significant. Salinity impact was population dependent (EC × population: $P < 0.05$), except at EC_{iw} 18.3 dS m^{-1}. Across all populations, shoot biomass was significantly reduced with increasing salinity to 77, 50, and 27% of the control at 13, 18.3, and 24.5 dS m^{-1}, respectively. The 'SISA14' and 'SW 8421S' populations were the most productive under saline conditions with the highest degree of salt tolerance. The results showed that alfalfa biomass response to salinity did not depend on the type of salts (Cl^- or SO_4^{2-}). Shoot Cl^- also did not correlate with relative biomass response. Thus, Cl^- ion toxicity does not appear to be a factor in alfalfa salt tolerance for these populations. Although there was a correlation between salt tolerance and shoot Na^+, the shoot ion concentration provides only a partial explanation of the relative salt tolerance of the alfalfa populations.

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Abbreviations: EC, electrical conductivity; SAR, sodium adsorption ratio.

Increasing soil salinization around the world is an important issue limiting land use for crop and animal production. The need to have forage crops that are able to cope with this complex stress has become one of the priorities for plant production around the world (Rengasamy, 2006; Bhardwaj et al., 2010). Alfalfa (*Medicago sativa*, L), which is one of the most valuable forage crops, is widely grown worldwide and currently competes with other crops for land and water. In the United States, alfalfa is the most important forage crop for dairy farming, and it is the fourth crop in area harvested for hay, having a total of 7,194,580 ha (USDA–NASS, 2016b). California, which is ranked seventh in the United States in area harvested, has become the first-ranked national producer of alfalfa hay in terms of tonnage and represents 9.25% of the national production. The average annual yield of California is 2.09 times higher than the national average (15.54 vs. 7.44 Mg ha^{-1}) and almost 100% of its alfalfa crop is irrigated. The forecast for 2016 is that the area of alfalfa to be harvested will increase by 10% in California (USDA–NASS, 2016a). It can thus be expected that the water used to grow alfalfa will also increase by 10% in 2016. Due to the high cost and increasing scarcity of high-quality water, use of low-quality water (such as saline groundwater) for irrigation is being promoted to productively use water that is currently underutilized. More than 70% of the alfalfa production in California is in the Central Valley (Putnam et al., 2008), where

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sulfate (SO_4^{2-}) is the predominant anion in the drainage water. The Imperial and Palo Verde Valleys produce about 17% of the alfalfa in California using irrigation water from the Colorado River. This water has a relatively higher concentration of chloride (Cl^-) in its composition than the drainage water from the Central Valley.

Argentina is another country with an extensive surface area cultivated to alfalfa, with approximately 3.7 million ha (Basigalup, 2015) growing under different conditions (rainfed or irrigated). Argentina has experienced rapid and expansive land use changes (Aizen et al., 2009; Jayawickreme et al., 2011). As a result of these changes, some of the better lands, historically under alfalfa production, have shifted production toward other more profitable crops (i.e., soybean [*Glycine max* (L.) Merr.]). Therefore, some of the alfalfa production is shifting to marginal areas, which include irrigated semiarid and arid areas that are salt affected and where lower quality waters are available for irrigation (Lavado, 2008; Prieto et al., 2015). In the Río Dulce Irrigation System in Santiago del Estero (northwest of Argentina, subtropical semiarid climate), salinization and sodification are among the main threats to sustained agriculture (Prieto et al., 2005). In this area, surface-irrigated alfalfa is a traditional crop that is economically viable. Since the degree of soil salinization is variable depending on water availability and management (Prieto et al., 2008), so is the alfalfa biomass production and persistence (Cornacchione, 2015). Thus, in both California and northwestern Argentina, both Cl^- and SO_4^{2-} dominant waters are used or have the potential to be used for alfalfa production.

Alfalfa is a Perennial Legume, an Autotetraploid, and Highly Heterozygous

The cultivars are synthetic populations obtained after generations of open pollination from a number of parents (Flajoulot et al., 2005). The inherent variability of the species has enabled breeding programs to obtain a number of improved cultivars to ameliorate different stresses (biotic and abiotic). In the United States, there are 182 certified alfalfa cultivars (with different fall dormancy), of which 57 cultivars are currently registered as salt tolerant (NAFA, 2016). From these cultivars, 19.3% have salt tolerance in the germination and forage production stages, while 70.2% have salt tolerance in the germination stage and the rest are listed as tolerant in the forage production stage only.

Alfalfa has been reported as moderately sensitive to NaCl, intermediate in salt tolerance among forages (Maas and Hoffman, 1977; Maas, 1987), and very tolerant within legumes (Munns and Tester, 2008). In general, for a wide range of species, the most salt-tolerant plants have the ability to restrict higher accumulations of Na^+ and Cl^- in the shoots (Munns and Tester, 2008). McKimmie and Dobrenz (1991) studied the physiological basis of variability within alfalfa populations in response to salinity stress. They found that the shoots of the more vigorous group contained lower concentrations of Na^+ and Cl^-, while the roots of the same group contained higher concentrations of Na^+. Kapulnik et al. (1989) found that superior growth under salt stress was associated with exclusion of Na^+ and Cl^- from the alfalfa leaves.

Most of the studies evaluating alfalfa salt tolerance have used increasing levels of NaCl (single salt) in the salinizing solution (Ashraf et al., 1986; Noble and Shannon, 1988; Kapulnik et al., 1989; McKimmie and Dobrenz, 1991; Khorshidi et al., 2009; Bhardwaj et al., 2010; Mezni et al., 2012). Few studies that include alfalfa have been conducted using a mixture of salts in the salinizing solutions. Such solutions are more representative of soil and water salinity under field conditions (Rogers et al., 1998; Anand et al., 2000; Grattan et al., 2004; Suyama et al., 2007; Cornacchione and Suarez, 2015). However, there is limited information on alfalfa about the comparative effect of the mixed salts having Cl^- or SO_4^{2-} as predominant anions. The only study we found that compared Cl^- versus SO_4^{2-} solutions was that of Soltanpour et al. (1999). However, this study was conducted in the absence of Na^+ ions, the primary cation in saline waters. Rogers et al. (1998) reported that alfalfa is more salt tolerant under SO_4^{2-} as compared with Cl^- conditions, though these authors only studied salt response in a SO_4^{2-} system conducted in southern California and compared their results with earlier studies with Cl^-, different populations, and different climatic and experimental conditions in Australia. As with Cl^- versus SO_4^{2-} response, advances in genetic breeding have resulted in the development of a large number of purportedly salt-tolerant cultivars, which have not been compared under the same experimental conditions.

In greenhouse studies, plant response can also be affected by position in the greenhouse. Soltanpour et al. (1999) found that biomass production of alfalfa plants depended on their position in the greenhouse related to the proximity to evaporative cooling pads. They reported high variability at the same electrical conductivity (EC) level. The plants at the EC 11 dS m^{-1}, which by chance were placed closer to evaporative pads than the plants at EC 7 dS m^{-1}, experienced cooler temperatures and higher relative humidity that could have made the plants less subject to water and salt stress. Peel et al. (2004) reported that alfalfa plants placed near the exhaust fan died approximately half as often as those in the other replicates, but they only reported this problem in 1 of the 2 yr of the study. Although not generally corrected, it is important to consider the effect of greenhouse position on plant response.

Additionally, most studies of alfalfa have been conducted for relatively short duration, thus not evaluating the possible changes in response to salinity over the time. This effect is of potential importance because alfalfa is a

perennial crop. Cornacchione and Suarez (2015) observed differences in plant response to salinity, as related to time and climatic changes, over the course of a year. Thus, it seems desirable to conduct alfalfa salt tolerance studies over a number of cuttings and seasonal changes.

The objectives of this research were to evaluate the salt response of different alfalfa populations from both the United States and Argentina to compare their biomass production, salt tolerance, and shoot ion concentration as related to irrigation water salinity. An additional objective was to further evaluate the response of alfalfa to ion composition of water (Cl^- or SO_4^{2-} dominant) in a mixed cation salt system under saline conditions.

MATERIALS AND METHODS

A total of 15 nondormant alfalfa populations were included in this study. Seven of them ('SISA1', 'SISA9', 'SISA10', 'SISA11', 'SISA13', 'SISA14', and 'SISA15') were experimental populations obtained from the Instituto Nacional de Tecnología Agropecuaria (INTA Argentina) from the alfalfa breeding program through a cooperative project between Santiago del Estero and Manfredi Experiment Stations in Argentina. Using the nondormant cultivars 'Salado' and 'Salinera INTA' and germplasm 'AZ-97MEC-ST' as breeding populations, one or two phenotypic recurrent selections were performed to increase the level of salt tolerance. Selection criteria were germination and forage and seed production under natural conditions of saline and saline-sodic soil in Isla Verde, Santiago del Estero, Argentina (selection site: 28°38′41.9″ S, 64°05′03.8″ W; soil taxonomy: Typic Natracualf; Vargas Gil, 1990).

The other populations from the United States included four commercial cultivars (Salado, 'SW 9215', 'SW 9720', and 'SW 8421S') purported to be salt tolerant for forage production (NAFA, 2016), two check cultivars (tolerant 'AZ-90ST' and salt-susceptible 'AZ-88NDC'; Smith, 1991), and two commercial cultivars that did not have an indication of improved salt tolerance ('Cibola' and 'CUF 101'; seeds were sourced from University of California, Davis).

All plants were grown in a greenhouse located in Riverside, CA (33°58′24″ N, 117°19′12″ W), from October 2011 to November 2012. A randomized design with split-plot arrangement was used with two water composition types and five salinity levels as main plot (tank) and 15 populations as subplots. There were six replications (a total of 60 tanks) with three plants of each population per replication (a total of 45 plants per tank). The tanks measure 120 cm long by 60 cm wide by 50 cm deep. The entire experiment had 2700 plants (two water types × five EC levels × 15 populations × three plants per population × six replications).

The plants were irrigated twice daily with a base nutrient solution made up from Riverside tap water (EC of the irrigation water [EC_{iw}] = 0.60 dS m^{-1}): 3.4 Ca^{2+}, 0.8 Mg^{2+}, 1.6 Na^+, 0.1 K^+, 1.3 SO_4^{2-}, 0.83 Cl^-, and 0.48 NO_3^- (in $mmol_c$ L^{-1}). The base nutrient solution was a modified Hoagland's solution consisting of the micronutrients (in μmol L^{-1}) Fe (50) added as Fe-DTPA (Sprint 330®), $(ZnSO_4)7H_2O$ (0.4), $(CuSO_4)5H_2O$ (0.2), H_2MoO_4 (0.1), H_3BO_3 (23), and $MnSO_4$ (5) and of the macronutrients (in mmol L^{-1}) KNO_3 (4.0) KH_2PO_4 (0.34), and $(MgSO_4)7H_2O$ (1.5). In the middle of the experimental period, additional KNO_3 (3 mmol L^{-1}) was added to all reservoirs to maintain adequate K^+ and NO_3^- concentrations. Each tank was irrigated twice daily by pumping approximately 115 L of irrigation water from the reservoirs (890 L) to each of the sand tanks. The leached water drained back into the reservoirs during and after the irrigation for reuse in the next irrigation. At saturation, the sand has an average volumetric water content of 0.36 (cm^3 cm^{-3}), corresponding to 100 L of water stored per tank and 47 L of stored water per tank at field capacity of the sand. The water lost by evapotranspiration was replenished in the reservoirs by adding deionized water to maintain essentially constant water composition and EC in the irrigation water and in the soil water in the sand tanks.

The salt treatments started 30 d after sowing, in the early vegetative stage when the plants had four to five leaves (Mueller and Teuber, 2008). The treatments were imposed by adding specific amounts of salts $MgSO_4$, Na_2SO_4, $CaCl_2$, NaCl, and KCl to tap water in increasing quantities every 3 d until the solutions reached the target EC_{iw} after 2 wk. No additional salts were added during the experiment (except nutrients).

We evaluated two water composition types, one of them dominated by Cl^- (Cl^-/SO_4^{2-} = ~2 when concentration is expressed in $mmol_c$ L^{-1}) and the other dominated by SO_4^{2-} (SO_4^{2-}/Cl^- = ~2). For each water composition type, we tested five EC_{iw} levels with average EC values of 0.85, 8.0, 13.0, 18.3, and 24.5 dS m^{-1} over the course of the experiment. The lowest EC_{iw} was in the control, as it had only tap water and base nutrient solution. The same targets of EC_{iw} for both water types were developed to correspond to drainage water compositions with subsequent increasing concentrations of salts, considering mineral precipitation (calcite and/or gypsum), using the UNSATCHEM model (Suarez and Simunek, 1997), which simulates typical soil water interactions. The two ion compositions simulated typical saline drainage waters of the Central Valley with SO_4^{2-} as dominant anion and of Colorado River water used in the Imperial and Coachella Valley with Cl^- as the dominant anion. These concentrated drainage waters are elevated in SAR [sodium adsorption ratio, defined as $Na/(Ca + Mg)^{0.5}$ where units are expressed in mmol L^{-1}], ranging from 2.3 in the control to up to 40 at EC_{iw} 24.5 dS m^{-1}. Increasing SAR with increasing salinity is typical of saline waters, and these values can be used without hazard to soil structure in the absence of rain (Suarez, 2012). In regions where there is measureable rainfall, producers would likely need to add a calcium amendment to the soil or water if the soils are not sandy. However, these waters are of much lower SAR than the NaCl dominant waters used in most salt tolerance studies. Because the experiment was of long duration, we reported the ion composition during the experiment (Table 1). In our system, the relationship between EC_{iw} and EC_e (saturated paste) was calculated as $EC_e = 0.472\ EC_{iw}$ (Cornacchione and Suarez, 2015).

The environmental conditions were as follows: air temperature was maintained (by heater and evaporative cooler) at 30 ± 5°C (day) and 18 ± 5°C (night), relative humidity ranged from 30 to 70%, and photoperiod ranged from 8 to 13 h with an averaged photosynthetic photon flux of 550 μmol m^{-2} s^{-1}. We periodically applied Enstar® (active ingredient S-kinoprene) and TriStar® (active ingredient acetamidiprid) to control thrips and aphids.

Table 1. Electrical conductivities (EC_{iw}), pH, and ion concentrations of irrigation waters used in this study

	Control	Chloride-dominated				Sulfate-dominated			
EC_{iw}, dS m^{-1}	0.85	7.9	12.8	18.2	24.3	7.9	12.9	18.4	24.7
pH	7.4	7.5	7.7	7.6	7.7	7.5	7.6	7.7	7.8
SAR, mmol L^{-1}†	2.8	16.2	24.1	32.5	37.8	15.2	23.4	31.8	41.1
	Ion concentration								
	$mmol_c$ L^{-1}								
Ca^{2+}	1.9	13.4	16.0	20.6	27.5	15.4	19.5	20.8	21.7
Mg^{2+}	1.2	9.9	15.7	19.8	27.4	13.3	22.2	32.3	49.0
Na^{+}	2.7	55.4	96.0	146.3	198.1	57.7	107.1	163.9	244.7
K^{+}	1.1	1.8	2.7	4.8	5.9	1.6	3.1	4.8	7.2
SO_4^{2-}	2.5	21.8	50.0	66.0	90.9	57.8	107.8	147.8	214.5
Cl^{-}	0.6	54.6	91.6	130.8	174.4	26.5	45.3	71.5	108.7

† Sodium adsorption ratio.

Fresh weight of plant shoots (clipped 6 cm above the crown) was determined 10 times, when most of the control plants reached the 10% flowering stage (harvests were performed from April to October) and at the late vegetative stage (harvests done in the absence of flowering). Shoot dry weight was recorded after drying at 70°C for 48 h. Total shoot biomass per plant (g plant^{-1} dry wt.) was calculated for a total of 10 harvests.

Population salt tolerance was calculated by dividing the total shoot biomass at each salinity level by the mean shoot biomass of the control. This ratio is referred to as relative biomass ratio and is a criterion usually used to measure salt tolerance (Shannon, 1985). For example, for a given salinity level, a ratio close to 1.0 indicates high tolerance and 0.5 indicates low tolerance (biomass reduced to half of the control). After the last harvest, the plants were removed from the sand tanks and the crown portions with residual stems were separated from the roots. We recorded the root dry weight and kept the dry roots for analysis of ion composition.

Plant samples of each population from each replication (n = 6) were taken in each of two different harvests (the third and seventh harvests) to evaluate the possible changes in salt accumulation over time. The samples were washed with deionized water immediately after harvesting and dried in a forced-air oven at 70°C for 72 h. Chloride was determined from nitric-acetic acid extracts by amperometric titration. The concentrations of Na, K, Mg, Ca, and total S were determined from nitric acid digestions of the dried, ground plant material by ICP-OES (inductively coupled plasma optical emission spectrometry). Each harvest was analyzed separately. Root samples from three replications (n = 3) were analyzed in the same way.

Preliminary exploratory analysis supported our observations of the biomass variability. First, for an open-pollinated crop such as alfalfa, each plant in a population is a different genotype (Flajoulot et al., 2005). Thus, at the same condition, the variability among individual plants per population, as we found, is to be expected. The variability by population was higher in the controls and low salinity treatments than in the high salinity treatments, which is a common response to any environmental stress (Shannon, 1985). To stabilize some of the data variability (Fig. 1), the measurements of total shoot biomass per plant were transformed to natural logarithmic (ln), because the standard deviation was proportional to the mean, and the salt tolerance ratios were transformed to square roots (Gomez and Gomez, 1984). The variability in shoot biomass by population was approximately equal to the variability of the entire population of alfalfa plants evaluated. This result suggests that we can utilize the high variability of existing cultivars to improve alfalfa salt tolerance. The variance among and within populations in response to salt was reported earlier for the shoot lengths of alfalfa seedlings from 35 cultivars (Al-Khatib et al., 1994).

Second, some of the variability among our replicates at the same salinity level was related to the position in the greenhouse, as was mentioned by Soltanpour et al. (1999). To test this possibility, we performed the statistical analysis by including the positions of each tank in the greenhouse. The spatial location both parallel and perpendicular to the cooling pads was considered, and only the perpendicular axis was significant. All subsequent statistical analyses were performed using this covariable.

Total biomass and ion concentrations were analyzed using a mixed-model ANOVA with water composition type, salinity level, population, and all double interactions as fixed factors, replicates as a random factor, and spatial location as a covariable. The same test was conducted for salt tolerance ratio within EC level. Any P-values $\leq$0.05 were considered to be significant throughout. Means were compared using the Fisher-protected LSD (P = 0.05) test. Partial correlations between two variables, as mentioned in the text, were also adjusted for the spatial location. All analyses were performed using InfoStat (Di Rienzo et al., 2012).

RESULTS AND DISCUSSION

All ANOVA results for biomass and for shoot and root ion concentrations are presented in Table 2.

Shoot and Root Biomass

The saline treatments having either Cl^{-} or SO_4^{2-} type irrigation water affected shoot and root biomass production equally (Table 2). There was no difference in effect of water composition type on biomass up to EC_{iw} 24 dS m^{-1}. Since it could be argued that the toxic ion effect would only be present at the highest salinity levels, we also performed t-tests at the individual salinity levels, which led to the same conclusion. Our results with different populations are consistent with the results of Soltanpour et al.

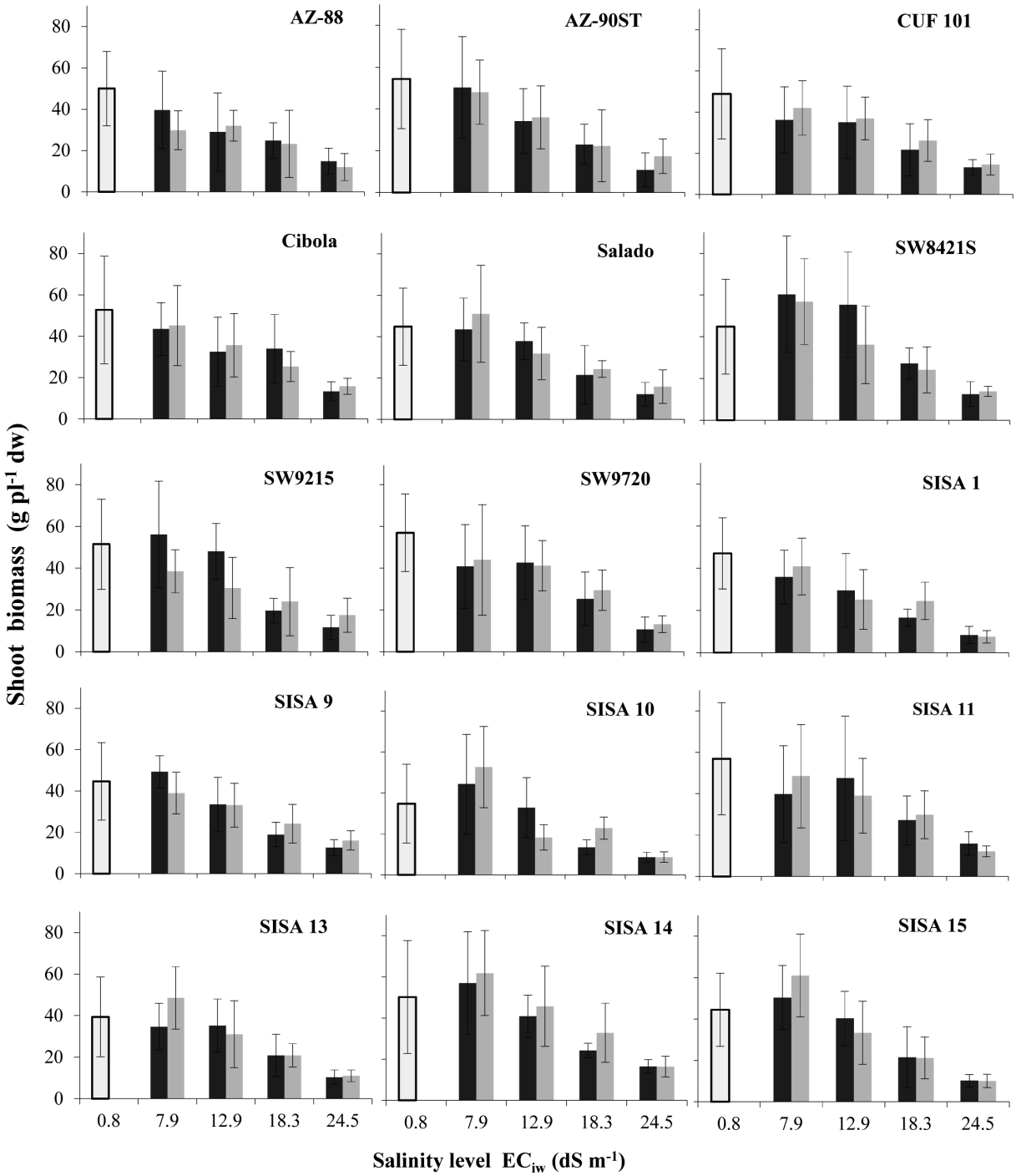

Fig. 1. Total shoot biomass of alfalfa populations at different electrical conductivity (EC_{iw}) levels according to salt type. The white bar is the control, the dark bar is water dominated by Cl, and the light bar is water dominated by SO_4. Data means and standard deviations are untransformed.

(1999), who found no differences in the alfalfa biomass in isoconductive Cl^- or SO_4^{2-} solutions up to EC_{iw} 11 dS m^{-1}. For four rootstocks of roses, the growth reduction varied with the rootstock and dominant salt type (Niu and Rodriguez, 2008).

There was no irrigation water composition type × EC and water composition type × population interaction for the shoot and root biomass. Subsequently, biomass data are presented with the combined water composition types at each EC level. There was an EC × population interaction for the shoot and root biomass (Table 2). Therefore, each EC was analyzed separately.

There were significant differences for shoot and root biomass among populations, except at 18.3 dS m^{-1}. At EC_{iw} 8 dS m^{-1}, SISA 14 and SW 8421 had the highest shoot biomass (Table 3). Both populations had 33 to 70% more biomass per plant than SISA 11, SISA 13, SW 9720, SISA 1, CUF 101, and AZ-88 (rank 10 to 15, Table 3). At

Table 2. Probability values for ANOVA of biomass and ion concentrations for both shoots and roots. Any different values between shoot and root are cited.

Effect	Biomass	Ion concentration						
		Cl^-	Na^+	Ca^{2+}	Mg^{2+}	K^+	Total S	K:Na
Water type (WT)	ns†	0.0001	0.0001‡	0.0001	0.0001‡	0.0006	0.0001	ns§
Salinity (EC)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Population	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0047§
WT × EC	ns	0.0001	0.0001‡	0.0001	0.0001	0.0001	0.0001	0.0498§
WT × population	ns	ns	ns	ns	ns	ns	ns	ns
EC × population	0.0020	0.0010§	ns	0.0005‡	0.0123	ns	0.0011¶	0.0419‡

† ns, nonsignificant.

‡ Nonsignificant for roots at the 0.05 probability level.

§ Significant for roots at the 0.05 probability level.

¶ Nonsignificant for shoots at the 0.05 probability level.

Table 3. Total shoot biomass per plant at each salinity level for 15 alfalfa populations.

Population†	Salinity level (EC_{iw} in dS m^{-1})									
	0.85		8.0		13.0		18.3		24.5	
	g $plant^{-1}$ (rank‡)									
SISA 14	50.2^{abc}§	(6)	59.4^{a}	(1)	43.2^{a}	(1)	28.5 ns¶	(2)	16.4^{a}	(1)
SW8421	44.8^{cd}	(12)	58.6^{a}	(2)	45.7^{a}	(2)	25.7	(5)	13.2^{abcd}	(8)
SISA 11	56.9^{ab}	(2)	44.2^{bcd}	(10)	43.3^{a}	(3)	28.5	(3)	14.1^{abc}	(4)
SISA 15	44.4^{cd}	(13)	55.7^{ab}	(3)	36.8^{a}	(6)	21.3	(14)	10.1^{def}	(13)
SW9720	56.9^{a}	(1)	42.4^{bcd}	(12)	41.9^{a}	(4)	27.4	(4)	11.9^{bcde}	(11)
SW9215	51.5^{abc}	(5)	47.3^{abcd}	(7)	39.2^{abc}	(5)	21.8	(13)	14.6^{abc}	(5)
Cibola	52.7^{abc}	(4)	44.3^{abcd}	(9)	34.1^{a}	(11)	29.6	(1)	14.5^{ab}	(3)
AZ-90ST	54.3^{abc}	(3)	49.2^{abc}	(4)	35.1^{abc}	(9)	22.7	(10)	14.1^{abcd}	(9)
Salado	44.8^{bcd}	(10)	44.2^{abcd}	(6)	34.8^{ab}	(7)	22.9	(8)	14.0^{abcd}	(7)
SISA 9	44.6^{bcd}	(11)	44.2^{abcd}	(8)	33.3^{abc}	(10)	21.6	(9)	14.4^{ab}	(2)
CUF 101	48.8^{abcd}	(9)	39.1^{cd}	(14)	35.9^{abc}	(8)	23.9	(7)	13.8^{f}	(6)
SISA 13	39.3^{de}	(14)	41.6^{bcd}	(11)	33.1^{abc}	(12)	20.8	(11)	10.7^{cde}	(12)
AZ-88	50.0^{abc}	(7)	34.7^{d}	(15)	30.7^{abc}	(13)	24.0	(6)	13.4^{abcd}	(10)
SISA 10	34.7^{e}	(15)	48.5^{abcd}	(5)	25.6^{c}	(15)	18.2	(15)	8.7^{ef}	(14)
SISA 1	47.0^{abc}	(8)	38.3^{cd}	(13)	27.3^{bc}	(14)	20.5	(12)	7.9^{f}	(15)

† Populations arranged from greater to lower average biomass across salinity treatments (from 8.0 to 24.5 dS m^{-1}).

‡ Rank of means at each salinity level according to biomass.

§ Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

¶ ns, nonsignificant at the 0.05 probability level.

EC_{iw} 13 dS m^{-1}, SISA 14 and SW 8421 were again in the top-producing group. However, they only differed from SISA 1 and SISA 10 (rank 14 and 15) by an average of 63 to 74% more biomass per plant. At 24.5 dS m^{-1}, SISA 14 also ranked in the top and it had 38 to 107% more biomass per plant than SW9720, SISA 13, SISA 15, SISA 10, and SISA 1 (rank 10 to 15, Table 3).

The rank of the shoot biomass of the populations did not remain constant across salinity levels, but we observed that some populations were consistently near the top of the rankings and others consistently near the bottom of the rankings. Thus, we averaged the biomass from the salinity treatments of 8 to 24.5 dS m^{-1} to determine if there was a relationship between the biomass produced under saline conditions and that produced under nonsaline conditions. We found no correlation between them (Fig. 2); thus, selection for salt-tolerant plants or populations based only on the shoot biomass performance in the absence of salinity, as was argued by Richards (1983), would not be successful. However, a positive correlation was reported by Veatch et al. (2004) in 20 *Medicago truncatula* Gaertn. accessions and one alfalfa population.

Root biomass, as could be expected, was highly correlated with shoot biomass ($P < 0.0001$); therefore, only shoot biomass data are shown. However, the degree of association between these variables tended to decrease as salinity increased (EC_{iw} 0.85: $r = 0.75$; EC_{iw} 8: $r = 0.73$; EC_{iw} 13: $r = 0.73$; EC_{iw} 18.3: $r = 0.63$; EC_{iw} 24.5: $r = 0.39$; Fig. 3).

Salt Tolerance

Analyses of variance of salt tolerance based on the ratios of the relative biomass at each EC indicated that there were significant differences among populations at low and high salinity levels. At 8 dS m^{-1}, salinity caused

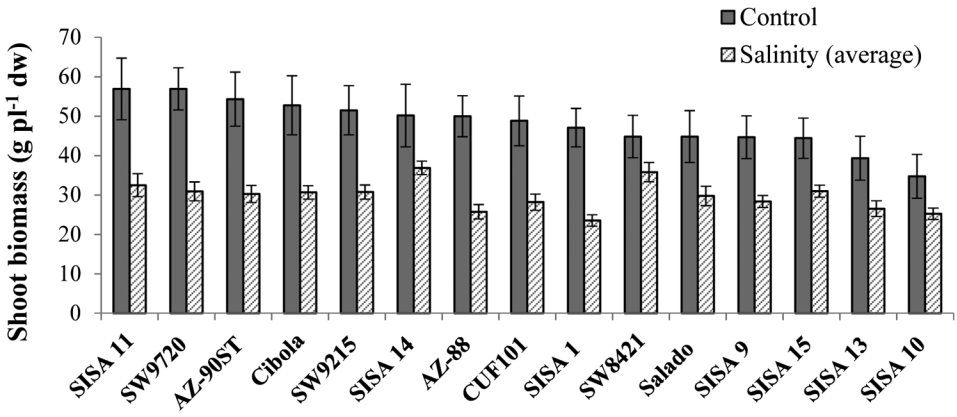

Fig. 2. Average shoot biomass of the 15 alfalfa populations under control and under saline irrigation (average data from electrical conductivity [EC_{iw}] 8.0 to 24.5 dS m^{-1}).

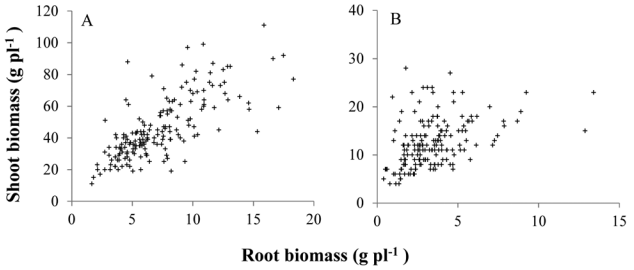

Fig. 3. Correlations between root biomass (g $plant^{-1}$ dry wt.) and cumulative shoot biomass (g $plant^{-1}$ dry wt.) (A) at electrical conductivity (EC_{iw}) 8 dS m^{-1}, r = 0.73 and (B) at EC_{iw} 24.5 dS m^{-1}, r = 0.39.

Table 4. Salt tolerance ratios of the populations

	Salt tolerance ratios‡			
	Salinity level (EC dS m^{-1})			
Population†	8.0	13.0	18.3	24.5
SISA 10	1.40[a]§	0.74 ns¶	0.52 ns	0.25[abc]
SW8421	1.31[ab]	1.02	0.57	0.29[ab]
SISA 15	1.25[ab]	0.83	0.48	0.23[bcd]
SISA 14	1.18[abc]	0.86	0.57	0.33[a]
SISA 13	1.06[abcd]	0.84	0.53	0.27[abc]
Salado	1.06[abcd]	0.78	0.51	0.31[ab]
SISA 9	0.99[bdef]	0.75	0.48	0.32[a]
SW9215	0.92[cdefg]	0.76	0.42	0.28[abc]
AZ-90ST	0.91[cdefg]	0.65	0.42	0.26[abc]
Cibola	0.84[defg]	0.65	0.56	0.28[abc]
CUF 101	0.80[dg]	0.74	0.49	0.28[abc]
SISA 1	0.81[dg]	0.58	0.44	0.17[d]
SISA 11	0.78[efg]	0.76	0.50	0.25[abc]
SW9720	0.75[fg]	0.74	0.48	0.21[cd]
AZ-88	0.69[g]	0.61	0.48	0.27[abc]

† Populations arranged by ratios at the first EC_{iw} level.

‡ Salt tolerance ratios were calculated by dividing the total shoot biomass at each EC by the mean shoot biomass of the control.

§ Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

¶ ns, nonsignificant at the 0.05 probability level.

either no decrease or even a slight increase (salt tolerance ratios above unity) in shoot biomass, as some populations showed high salt tolerance at this level (Salado, SISA 13, SISA 14, SISA 15, and SW 8421S; Table 4). For other populations, salinity caused a decrease in biomass, with the lowest ratio being 0.69 for AZ-88. Despite the fact that no significant differences were detected among ratios at EC_{iw} 13.0 dS m^{-1}, we observed a large variation in the ratios (from 1.2 to 0.58) at that EC, suggesting that some populations were less affected by salinity than others. At 18.3 dS m^{-1}, there were no differences among populations and the average ratio was 0.5. This means that biomass was reduced to half of the control values. At 24.5 dS m^{-1}, there were significant differences among population ratios. However, a small range in variation was observed between the highest salt tolerance ratios of SISA 14, SISA 9, and Salado and the lowest ratios of SW9720 and SISA 1.

The largest separation of the salt tolerance ratios of the populations occurred at 8 dS m^{-1}. We consider that, from a practical viewpoint, the range of EC_{iw} 8 to 13 dS m^{-1} is of most interest to producers, as biomass loss for some populations was less than 20% relative to the control, and at EC_{iw} 18 dS m^{-1}, biomass loss was always greater than 40%. The relationships between biomass and salt tolerance at EC_{iw} 8 and 13 dS m^{-1} placed SW 8421S and SISA 14 as the most salt-tolerant populations with high biomass, and SISA 1 and AZ-88 with low tolerance and low biomass. Other populations, such as SISA13, showed the same salt tolerance as SISA 14 and SW 8421S but less biomass (Fig. 4).

Our results indicate that some populations are indeed more salt tolerant than others, not just more vigorous under all conditions. In agreement with our alfalfa results, Teakle et al. (2010) found no significant correlations between salt tolerance and shoot biomass in controls for 40 *Lotus tenuis* Waldst. & Kit. Ex Willd. lines, and they also found differences in salt tolerance among the lines. As an example, at low salinity, we found SISA 13 to have low shoot biomass for controls and high salt tolerance compared with SW 9720, which was the most vigorous control and showed low salt tolerance (Tables 3 and 4). Few populations had both high biomass under saline conditions and high tolerance, the desirable combination that we detected in SISA 14 and SW 8421S. High salt tolerance suggests that the plants have the ability to persist in

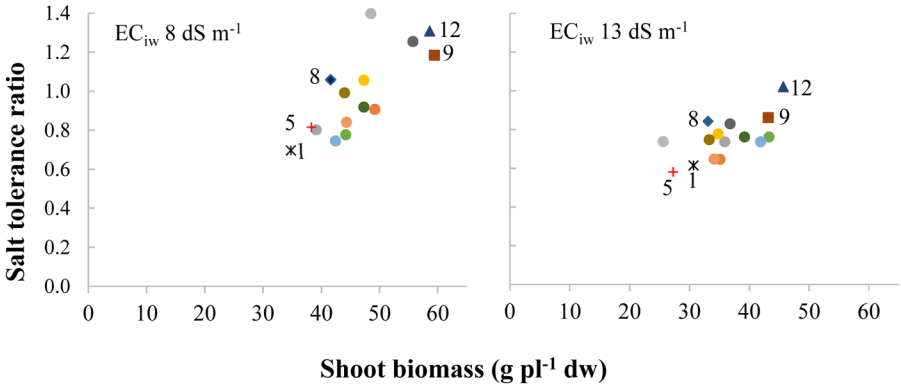

Fig. 4. Scatter plots exploring the relationship between salt tolerance ratios (shoot biomass at electrical conductivity [EC] level to shoot biomass of control) and shoot biomass of 15 alfalfa populations at EC_{iw} 8 and 13 dS m^{-1}. The populations are numbered 9 (SISA 14), 12 (SW 8421S), 1 (AZ-88), 5 (SISA 1), and 8 (SISA 13).

a saline environment; however, vigor will also be critical for a productive pasture (Teakle et al., 2010).

Our salt tolerance results are in contrast to those reported by Maas and Hoffman (1977). They reported a threshold of EC_e 2 dS m^{-1} where yield loss would start to decline, while our results indicated no biomass loss until EC_{iw} >8.0 for 6 of the 15 populations that we examined. In our system, EC_{iw} 8.0 dS m^{-1} corresponds to EC_e 3.8 dS m^{-1} (the relation between EC_{iw} and EC_e is mentioned earlier and given in Cornacchione and Suarez, 2015). Thus, some of our alfalfa populations showed much more tolerance in terms of their threshold salinity than cited by Maas and Hoffman (1977). Soltanpour et al. (1999) reported 50% biomass reduction at EC_{iw} 11 dS m^{-1} from both Cl^- and SO_4^{2-} solutions, while we observed such a reduction at EC_{iw} 18.3 dS m^{-1}. Results from various experiments (Hussain et al., 1995; Isla and Aragüés, 2009; Cornacchione and Suarez, 2015) support the idea that the threshold value of EC_e 2 dS m^{-1} would be considered low, at least for some newer cultivars that have increased salt tolerance.

Based on our results, we conclude that a larger root system is related to greater plant vigor, but this is not the dominant factor in explaining tolerance at the highest salinity level. Salinity decreased both shoot and root dry weight. However, shoot growth has been found to be more adversely affected by salinity than root growth (Munns and Termaat, 1986). We measured a reduction in shoot–root ratio with increasing salinity, as at EC_{iw} 18.3 and 24.5 dS m^{-1}. The root biomass per plant was reduced by 18 and 49%, respectively, while the shoot biomass was reduced by 50 and 73%, respectively. Similar observations were made for other alfalfa plants (Khan et al., 1994; Serraj and Drevon, 1998). In studies with other crops, researchers have also observed decreased shoot–root ratios with increasing salinity (Pearen et al., 1997; Bayuelo-Jiménez et al., 2003; Acosta-Motos et al., 2015). However, there are some reports of increased shoot–root ratios (Bernstein et al., 2004; AbdElgawad et al., 2016). The observation that salinity has less of an adverse effect on root growth compared with shoot growth is consistent with other observations related to stress, such as P deficiency (Fredeen et al., 1989), N deficiency (Sattelmacher et al., 1990), and drought stress (Sharp et al., 1988; Buwalda and Lenz, 1992).

For water stress, the decrease in shoot growth relative to root growth has been related to an increase in abscisic acid (ABA), as the shoots have greater suppression of extension growth than roots with increased ABA (Creelman et al., 1990). Salinity stress has also been reported to increase ABA; thus, a similar response can be expected (Munns and Cramer, 1996).

Ion Concentrations

Ion results from each of the two sampling times led to the same conclusion. Therefore, we will discuss the average data for each ion to simplify the paper. Shoot and root ion concentrations were significantly affected by water composition type, except for Na^+ and Mg^{2+} in the roots (Table 2). Interactions between EC and water composition type were significant for all ions in shoots and roots, except for root Na^+. The water composition type × population interaction was not significant (Table 2). These effects could be expected because of the difference in ion composition between the two irrigation water compositions (Table 1). For instance, shoot and root Cl^- concentrations were significantly higher for plants irrigated with the Cl^- water type, and shoot and root total S concentrations were significantly higher for the plants irrigated with SO_4^{2-} water type (Fig. 5). Also, the concentrations of other ions (Na^+, Ca^{2+}, Mg^{2+}, and K^+) were different between water composition types at the same EC. For instance, the shoot Na^+ concentrations were significantly lower in Cl^- type than SO_4^{2-} type waters at the highest EC (Fig. 5). This was in accordance with the comparatively lower Na^+ concentration in the Cl^- type water (Table 1). Shoot Ca^{2+} concentrations increased significantly at the highest EC in the Cl^- type water, while Mg^{2+} concentrations increased significantly in the SO_4^{2-} type water (Fig. 5).

Although we measured the shoot and root ions at different times, in general, we observed lower concentrations in the roots than in the shoots, except for Na^+. We considered that the greater Na^+ concentrations in roots at all levels of salinity were related to restricted Na^+ translocation from root to shoot. Alfalfa is included in the group

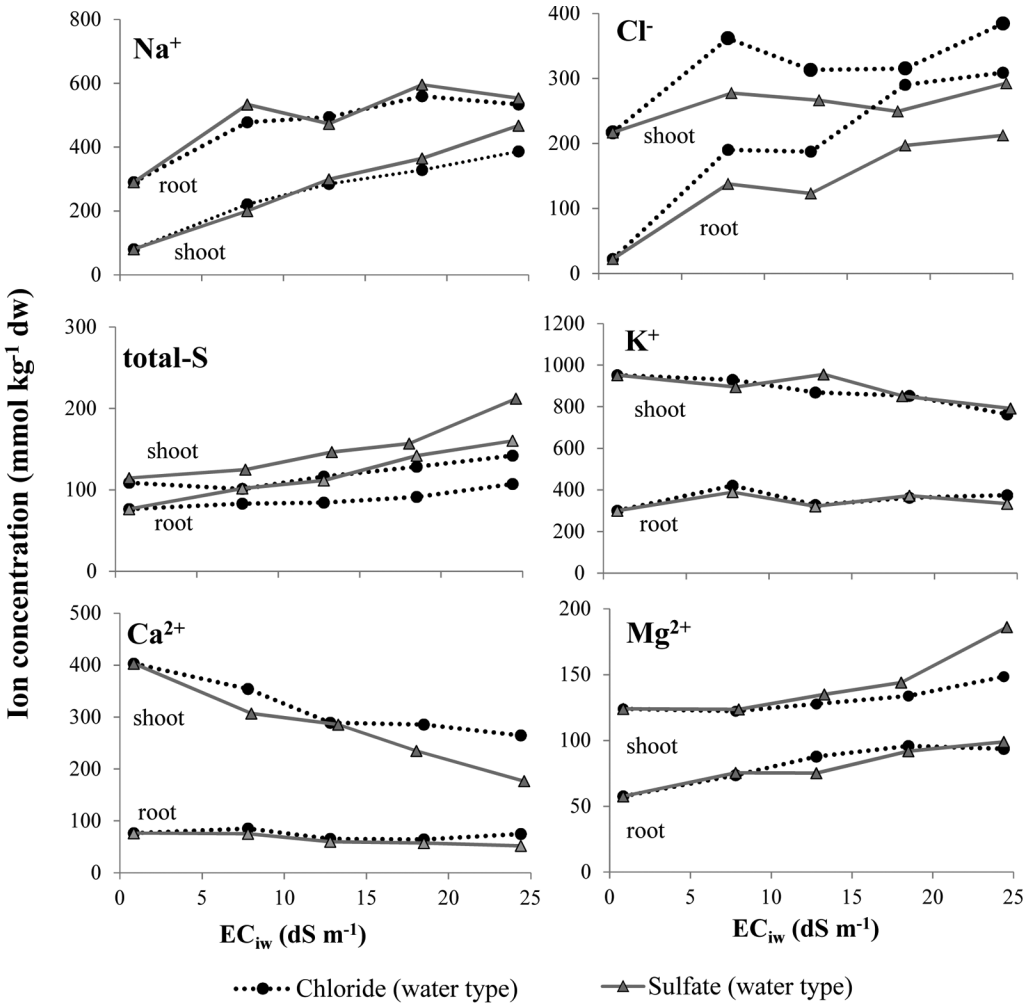

Fig. 5. Shoot and root ion concentrations at different salinity levels (electrical conductivity, EC_{iw}) of Cl^- and SO_4^{2-} water composition type. The plotted data are the means of the shoot ion concentrations across all populations from two harvests (May and July 2012) and the root ion concentrations at the end of the experiment (November 2012).

of the natrophobic species (Smith et al., 1978), which can concentrate Na^+ in their roots, thereby decreasing the translocation to shoots. Exclusion of Na^+ from alfalfa shoots under salt stress was reported by McKimmie and Dobrenz (1991) and exclusion of Na^+ from alfalfa leaves by Kapulnik et al. (1989). Lower concentrations of Na^+ in alfalfa roots than in aerial parts (leaves plus stems) has been reported in the presence of increasing NaCl (Ashraf et al., 1986; Wang and Han, 2007; Mezni et al., 2010). However, in these studies, the mechanism related to salt tolerance was attributed to Cl^- accumulation. Based on the lack of differences in shoot and root biomass due to water composition type and their interactions, we focused on the ion differences among the populations in response to salinity.

Shoot Ion Concentration and Salt Tolerance

As salinity increased, the concentrations of Na^+ and total S increased and K^+ decreased in the shoots of all populations (Fig. 5). For these ions, there were no EC × population interactions (Table 2). Therefore, we explored the correlations between ion concentrations and salt tolerance ratios at all salinity levels. The associations between variables were significant ($P < 0.0001$); the association was higher with shoot Na^+ ($r = -64$; Fig. 6), intermediate with total S ($r = -0.49$), and lower with shoot K^+ ($r = 0.28$).

There was a significant difference in shoot Na^+ concentrations among populations. Salado accumulated less Na^+ in the shoots than the other populations, except SISA 9, SISA 15, and SISA 14 (Table 5). These four populations showed relatively high tolerance at low (EC_{iw} 8 dS m^{-1}) and at high salinity (EC_{iw} 24.5 dS m^{-1}, except SISA 15; Table 4), while within the first group of populations that accumulated more Na^+, SISA 1 and SW9720 had comparatively less salt tolerance (at both low and high salinity). One of the exceptions to this association was SW 8421S, which showed the same salt tolerance as SISA 15, SISA 14, Salado, and SISA 9 at both EC levels but accumulated significantly more Na^+ in the shoot than SISA 1 and SW 9720 (Table 5). Some of the populations with low Na^+ also had low total S and comparatively high salt tolerance; however, a small range of variation was observed between

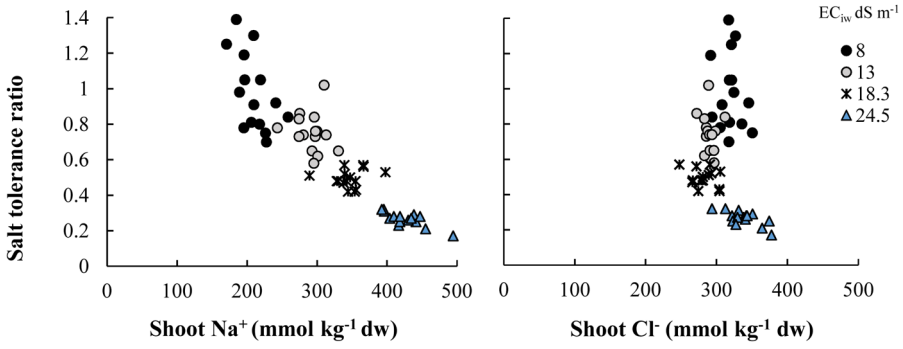

Fig. 6. Scatter plots exploring the relationship between salt tolerance ratios and shoot Na^+ and Cl^- concentrations of 15 alfalfa populations under saline treatments (electrical conductance [EC_{iw}] 8, 13, 18.3 and 24.5 dS m^{-1}).

the highest and the lowest total S concentration (Supplemental Table S1).

As the salinity increased, the concentrations of Mg^{2+} and Cl^- increased and Ca^{2+} decreased in the shoots of all populations (Fig. 5). For these ions, there were EC × population interactions (Table 2). We explored the correlations between concentrations and salt tolerance ratios at each EC level. Differences in these ion concentrations were also observed in the controls.

Shoot Cl^- was highly correlated with salt tolerance ratio at EC_{iw} 18 and 24.5 dS m^{-1} ($r = -0.45$ and -0.40, respectively; $P < 0.0001$; Fig. 6). However, at EC_{iw} 18.3, there were no differences in shoot Cl^- concentrations (Table 5) and salt tolerance among populations (Table 4). At 24.5 dS m^{-1}, there were differences among populations, where SISA 14 and SISA 9 had significantly less shoot Cl^- and higher salt tolerance (Table 4) than SISA 1 and SW 9720.

Shoot Ca^{2+} was weakly correlated with salt tolerance ratio at EC_{iw} 8 ($r = 0.20$) and 13 dS m^{-1} ($r = 0.23$). However, at EC_{iw} 8, there were no differences in shoot Ca^{2+} among populations (Supplemental Table S1), and at 13 dS m^{-1}, there were no differences in salt tolerance among populations (Table 4). Shoot Mg^{2+} was only correlated with salt tolerance at EC_{iw} 8 dS m^{-1} ($P < 0.01$, $r = 0.22$), but there were no differences among populations at that level (Supplemental Table S1).

The K to Na ratio decreased as salinity increased in all populations. The K to Na ratio was correlated with salt tolerance at EC_{iw} 13 ($r = 0.22$), 18.3 ($r = 0.19$), and 24.5 dS m^{-1} ($r = 0.34$). However, only at EC_{iw} 8 dS m^{-1} were there differences in K to Na ratios of the populations (Supplemental Table S1). Among populations that showed the same high tolerance at low EC, only SISA 10 and SISA 15 had higher K to Na ratios than Cibola, AZ-88, and SW9215, which showed comparatively less tolerance (Table 4). Overall, there was very little relative change in shoot K^+, so changes in K to Na ratio related primarily to changes in shoot Na^+.

Root Ion Concentration and Salt Tolerance

As salinity increased, the concentrations of Na^+ increased and Ca^{2+} and K^+ decreased in the roots of all populations (Fig. 5, Supplemental Table S2). The correlation with salt tolerance ratios was only significant for Na^+ ($P < 0.0001$; $r = -0.33$). There was a significant difference in the root Na^+ among populations. For instance, a SISA 1 population that showed low salt tolerance had less Na^+ compared with SISA 14 and SW 8421S (more tolerant). However, SISA 1 did not differ from the other populations that also showed high tolerance at EC_{iw} 8 dS m^{-1}—Salado, SISA 9, and SISA 15.

As salinity increased, the concentrations of Cl^-, Mg^{2+}, and total S increased in the roots of all populations (Supplemental Table S2). Some populations accumulated more Cl^- in their roots than others, but there were no correlations of root Cl^- and total S with salt tolerance ratios. Root Mg^{2+} was correlated with salt tolerance ratio only at EC_{iw} 8, but the association was low ($P < 0.01$, $r = 0.22$).

Na^+ and Cl^- Concentration Related with Salt Tolerance

Toxicity to Cl^- varies widely among plant species and among cultivars. Some studies using only NaCl solutions have focused on the salt tolerance of alfalfa as being controlled by Cl^- uptake (Noble et al., 1984; Noble and Shannon, 1988). Critical concentrations have been estimated between 4 to 7 and 15 to 50 mg g^{-1} for sensitive and tolerant species, respectively. For alfalfa shoots, 6.1 mg g^{-1} was cited as the critical concentration where plant yields decline or where plants show visible symptoms of leaf burn (Xu et al., 1999). However, our results showed that, even at EC_{iw} 0.85 dS m^{-1} (irrigation water having 0.6 $mmol_c$ L^{-1} of Cl^-), the average shoot Cl^- concentrations of all genotypes was 7.6 mg g^{-1} dry wt., higher than the critical value cited by Xu et al. (1999). At EC_{iw} 18.3 dS m^{-1} (average of 100 $mmol_c$ L^{-1} of Cl^-) across all alfalfa populations, the shoots also accumulated 10 mg g^{-1} dry wt. and exhibited an average of 50% reduction in biomass without showing burn symptoms in the leaves. Based on these data, we conclude that shoot Cl^- is not a good indicator of salt damage in alfalfa, at least in mixed salt systems. This result may vary as when only NaCl is present. The critical level of Cl^- could differ according with experimental conditions.

Table 5. Shoot Na^+, K^+, and Cl^- concentrations of alfalfa populations at different salinity levels.

Population	Shoot concentrations at salinity levels (EC_{iw})															
	Na^+					K^+					Cl^-					
	0.85	8.0	13.0	18.3	24.5	mean†	0.85	8.0	13.0	18.3	24.5	mean	0.85	8.0	13.0	18.3	24.5
	mmol kg^{-1} dry wt.																
SISA 10‡	82 ns§	185 ns	275 ns	340 ns	442 ns	265^{cd}¶	1003 ns	951 ns	951 ns	897 ns	802 ns	907^{ab}	214^{dg}	317^{cdef}	286 ns	293 ns	375^{ab}
SW 8421	82	227	313	329	455	281^{abc}	1005	949	944	874	811	902^{abc}	213^{dg}	327^{abcd}	289	291	351^{abcd}
SISA 15	78	196	276	339	393	256de	894	835	870	805	716	810^{h}	208^{efg}	322^{bcde}	283	281	328^{cde}
SISA 14	77	171	274	343	417	256de	937	881	841	829	740	832^{fgh}	230^{abc}	292^{f}	272	248	313de
SISA 13	82	219	296	398	404	280^{abc}	977	992	929	857	764	890^{bc}	223^{bcd}	321^{bcd}	312	305	330^{bcde}
Salado	74	197	244	289	396	240^{e}	928	933	948	875	794	881^{bcd}	207^{fg}	318^{bdef}	286	289	331^{bcde}
SISA 9	75	190	281	330	396	254de	888	851	891	803	744	821^{gh}	213^{dg}	324^{abcd}	290	279	294^{e}
SW9215	84	241	298	344	419	277^{abc}	966	906	926	868	792	877^{cd}	237^{ab}	345^{ab}	299	274	342^{abcd}
AZ-90 ST	82	210	293	354	431	273^{bcd}	928	917	885	840	793	858de	222^{bcde}	308^{def}	291	304	341^{abcd}
Cibola	78	259	331	367	447	296^{a}	915	874	864	853	775	842^{efg}	202^{g}	294^{ef}	296	272	343^{abcd}
CUF101	90	218	298	355	410	274^{bcd}	995	922	923	840	798	881^{bcd}	218^{cf}	336^{abc}	286	280	322^{cde}
SISA 1	83	206	296	356	495	287^{ab}	900	917	925	872	760	861de	205^{fg}	319^{bdef}	297	304	378^{a}
SISA 11	78	195	300	348	418	268^{bcd}	939	912	932	816	786	863de	204^{dg}	305^{def}	288	281	323^{cde}
SW9720	74	210	310	366	439	281^{abc}	1050	970	965	900	808	925^{a}	243^{a}	351^{a}	294	267	364^{abc}
AZ-88	88	228	301	338	435	278^{abc}	919	874	890	847	787	849^{ef}	211^{efg}	318^{cdef}	283	266	339^{abcde}

† Means for shoot Na^+ and K^+ are presented (there were differences among populations and there was no EC × population interaction).

‡ Populations arranged from greater to lower salt tolerance ratios at EC_{iw} 8 dS m^{-1}.

§ ns, nonsignificant at the 0.05 probability level.

¶ Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

At the whole-plant level, the relationship between salt tolerance and low shoot Na^+ involves several processes, including uptake of Na^+ and its distribution within the plant (Tester and Davenport, 2003). Concentrated solutions of Na^+ caused disruptions of root membrane integrity and changes in ion selectivity in roots because K^+ is replaced by Na^+ (Marschner, 1986). Therefore, when salinity increases, a smaller reduction in shoot K^+ and the K to Na ratio can be expected in the most salt-tolerant plants (Maathuis and Amtmann, 1999). Our results are in agreement with that, as the populations exhibiting greater tolerance had lower shoot Na^+, as we found for SISA 15, SISA 10, SISA 9, Salado, and SISA 14, with the exception of SW 8421S. Because there were no significant differences in root Na^+ concentrations among these populations at the end of the experiment (except between SW8421 S and SISA 9) and the concentrations tended to be low, our data are consistent with the idea that Na^+ exclusion from the root is another mechanism that may contribute to salt tolerance. Previous studies with alfalfa plants have reported an association of lower shoot Na^+ and a higher K to Na ratio with salt tolerance (Rogers et al., 1998; Grieve et al., 2004; Cornacchione and Suarez, 2015). The tolerant population SW 8421S accumulated more Na^+ in the shoots than SISA 14 while maintaining the same biomass under stress and relative tolerance, suggesting that other mechanisms may have operated, as mentioned by Munns and Tester (2008).

SISA 1, which exhibited low tolerance and low shoot and root biomass under saline conditions, showed consistently high shoot Na^+ and Cl^-. The cultivar AZ-90 ST showed significantly more biomass (+41%) than the sensitive parental population AZ-88 and a greater K to Na ratio at EC_{iw} 8 dS m^{-1} (Table 3). Both populations did not differ in their shoot Na^+ concentrations across the range of salinity treatments, but AZ-90 ST accumulated significantly more Na^+ in the roots compared with AZ-88. These results suggest that AZ-90ST restricted Na^+ translocation to the shoots compared with its sensitive pair AZ-88. The restriction of Na^+ translocation of to the shoots has been reported as one of the mechanisms related with alfalfa salt tolerance (Ashraf et al., 1986).

Restricted Na^+ translocation to the shoots is thus a necessary but not sufficient condition for a salt-tolerant alfalfa plant. Our future research will therefore consider an alfalfa selection and breeding program of individual plants based on low shoot Na^+ and high biomass under saline conditions.

CONCLUSION

Salinity reduced shoot and root growth of all alfalfa populations, but the magnitude of growth reduction varied with the population. Most of the populations exhibited increased salt tolerance relative to older published values for alfalfa.

Most importantly, this increased salt tolerance expressed at moderate salinity (EC_{iw} 8 to 13 dS m^{-1}) corresponded to EC_e 3.8 to 6.1 dS m^{-1} in our experiment. At EC_{iw} 24.5 dS m^{-1} (EC_e 11.6 dS m^{-1}), little or no growth can be expected.

The lack of a relationship between water composition types (Cl^- or SO_4^{2-} dominant) in a mixed cation system and shoot and root biomass indicates that Cl^- toxicity is not the cause of biomass loss in alfalfa. This finding is also supported by the lack of an overall relationship between shoot Cl^- and relative biomass production.

There was a good correlation between salt tolerance per plant and shoot Na^+ concentrations. However, this appears to provide only a partial explanation of the relative salt tolerance among the different populations. This is not unexpected, because at these salinity levels, plant response to osmotic potential must be considered in addition to ion toxicity. Development of a larger root system was related to overall plant growth but not to improved salt tolerance.

We observed that two commercial cultivars and four Argentinean populations showed good overall salt tolerance and no biomass loss at EC_{iw} 8 dS m^{-1}. However, they showed different responses in their absolute biomass. The population SISA 14 is promising for future study in a breeding program based on high biomass under saline conditions, high relative salt tolerance, and physiological attributes of Na^+ and Cl^- exclusion. Additional populations could be incorporated into the breeding program if the process of selection were to focus directly on ion exclusion. The high variability in response to salinity within the populations makes it worthwhile to select and cross individual plants rather than cross various tolerant populations.

Conflict of Interest

The authors declare there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

Acknowledgments

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