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Growth, Nutrients Concentrations, and Enzymes Involved in Plants Nutrition of Alfalfa Populations under Saline Conditions

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ABSTRACT

In order to assess the effect of salinity constraint on some agro-physiological and biochemical traits in *Medicago sativa* L., four Alfalfa populations (Tafilalet 1, Tafilalet 2, Demnate and Tata), originated from mountains and oasis of Morocco, were tested. The plants were grown under greenhouse conditions in pots filled with sand and peat under three salt treatments (0, 100 and 200 mM NaCl). Thereafter, plants were harvested 45 days after salt treatment and some agro-physiological and biochemical parameters related to salt tolerance, such as plant biomass, water content, membrane permeability, nutrients contents, nitrate reductase and acid phosphatase activities, were measured. Results showed that increase in NaCl concentration gradually reduced plant biomass, which displayed significant differences among the tested populations. Thus, Tata population appeared to be the most tolerant population to salinity, Tafilalet 1 population was the least tolerant one, while Tafilalet 2 and Demnate displayed moderate salinity tolerance. Variations in plant growth were associated with changes in physiological and biochemical parameters. Indeed, salinity caused a decrease in relative water content, perturbation of membrane permeability, and nutrients concentrations. Results also showed that salinity inhibited nitrate reductase activity in leaves of all tested populations, but acid phosphatase activity was increased in both leaves and roots of stressed plants. Salt tolerance of alfalfa populations was associated with high inorganic ion accumulation and the maintenance of membrane integrity and an adequate level in terms of nitrate reductase and acid phosphatase activities.

Keywords: Acid phosphatase, Biomass, *Medicago sativa* L., Membrane permeability, Nitrate reductase, Salt tolerance.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) constitutes the first forage crop in Mediterranean area (Bouizgaren, 2007). In Morocco, this crop occupies over 22% of the total area devoted to forage crops (Bouizgaren *et al.,* 2011) and over 80% of forage area in oasis agro-ecosystems (Janati, 1990). Local populations of this species are widely used in the Moroccan traditional agro-ecosystems, oasis and mountains (Bouizgaren, 2007), and it strongly contributes to socio-economic development of local families.

However, water and soil salinity recorded in many world regions is a major environmental factor limiting plant growth and productivity and constitutes an important constraint to alfalfa (*Medicago*

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sativa L.) production in Morocco (Farissi *et al.,* 2011) and in many parts of the world (Zhang *et al.,* 2007). According to Szabolcs (1994), more than 954 million hectares worldwide, 80 million ha in Africa, were affected by this constraint. In our country, 37% of total cultivated areas surveyed are affected by salinity (Ftouhi, 1981), most of which are under alfalfa. Indeed, this constraint has affected many groundwater resources of the main agricultural areas such as Souss Massa, Moulouya, Gharb, Tafilalet, Loukous, Tadla, Haouz, Doukkala, and it also affected some rivers (Oum Er Rbia in Tadla, El Malh in Ouarzazate, etc.). In general, the constraint will continue to worsen. The salt concentration increases and the affected areas expand. There are very fast damaging effects on soils, crops, and hydrobiology. In many regions, salt concentrations have reached values that make them unsuitable for irrigation (Debbarh and Badraoui, 2002).

Salinity affects alfalfa growth and development by way of osmotic stress and injurious effects of toxic Na^+ and Cl^- ions (Farissi *et al.,* 2011). These effects can be observed at the whole plant level as decreases in productivity and/or the death of plants (Parida and Das, 2005). Most of these changes are associated with activation of physiological and biochemical processes allowing an adaptation to osmotic and ionic stress (Ghoulam *et al.,* 2002; Singh, 2004; Kafi, 2009; Koyro, 2006; Loépez *et al.,* 2008; Chen *et al.,* 2009; Faghire *et al.,* 2011). However, many essential processes for plant growth and development are negatively affected by this constraint. In general, It reduces water availability, nitrate reductase and causes nutritional imbalance in plants (Ghoulam *et al.,* 2002; Bybordi and Ebrahimian, 2011, Faghire *et al.,* 2011).

To exploit saline lands, the selection of tolerant genotypes could be a promising way to ensure adequate forage yield in the soil affected by this constraint. Meanwhile, a reliable selection must be based not only on agronomical parameters but also on physiological and nutritional aspects. Selection of tolerant genotypes based on physiological and nutritional parameters will be helpful to enhance the productivity of the crop in areas adversely affected by this constraint. However, there is not enough information on the effect of salinity stress on enzymes involved in nitrogen and phosphorous metabolism as nitrate reductase (Bybordi and Ebrahimian, 2011). In this context, the present study aimed to evaluate the salinity tolerance in four Moroccan alfalfa populations based on their abilities to adjust some physiological and biochemical parameters such as relative water content, membrane permeability, nutrients uptake, nitrate reductase, and acid phosphatase activities.

MATERIALS AND METHODS

Plant Material and Growth Conditions

This study was carried out during 2010 under greenhouse conditions with an approximate temperature of 30/20°C (day/night) and 16h photoperiod at the National Institute for Agronomic Research (INRA-Marrakech, Morocco). Four Moroccan alfalfa populations, namely, Tafilalet 1 (Taf1), Tafilalet 2 (Taf 2), Demnate (Dem), and Tata were studied. These populations originated from different Moroccan regions: mountains (Dem), south east oasis (Taf1 and Taf 2), and south west oasis (Tata), where they have been cultivated for many centuries and are still widely used by farmers in these traditional agro-ecosystems. Continuous natural and human selection has led, with time, to their adaptation to the local habitats, with the distinction in the agro-morphological characteristics of the landraces which have reached the Hardy–Weinberg equilibrium. Seeds were supplied by INRA-Marrakech and the cultures were established in March. Seeds were germinated in pots 20 cm in diameter and 30 cm high filled with sterile sand (previously rinsed with distilled water) and peat at 2:1 ratio, respectively. After

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emergence of the first true leaves, 15 days after germination, the number of plants was adjusted to six per pot and they were irrigated from the top with 300 mL of distilled water every other day. The pots (six plants) were arranged in a simple randomized design and each one was considered as one replicate with three pots per treatment per population. At 20 days after germination, a half strength Hoagland's nutrient solution was given once a week. Then, one month after sowing, three NaCl concentrations; 0 (control), 100 and 200 mM were applied. To avoid osmotic shock, NaCl concentrations were increased gradually by 50 mM every 2 days until the desired concentration. After 45 days of salt treatment, the plants were harvested, measured, and subjected to different physiological and biochemical analyses.

Biomass Measurements

Shoots and roots were separated and their fresh weights (FW) were directly determined. For dry weight (DW) determination, the shoots and roots were dried at 70°C for 48 hours and weighed. Three replicates of six plants per population per treatment were studied. To standardize the data, the results were expressed as the relative reduction of yield in comparison to the control using the following formula (Ghoulam *et al.,* 2002) :

Relative reduction (%)=[(1−(Salinized/ Control)]×100 (1)

Relative Water Content (RWC)

Relative water content of leaves was estimated by recording the turgid weight of 0.1g fresh leaflet samples by keeping in water for 4 hours (TW), followed by drying in hot air oven till constant weight was achieved (DW). Three replicates per population per treatment were measured and the *RWC* was determined as follows (Ghoulam *et al.,* 2002):

$$RWC\ (\%) = ((FW-DW)/(TW-DW)) \times 100 \quad (2)$$

Membrane Permeability (Electrolyte Leakage)

Electrolyte leakage (EL) was assessed as described by Lutts *et al.* (1996) using young leaves. Samples were washed three times with deionized water to remove surface-adhered electrolytes, then, they were placed in closed vials containing 10 mL of deionized water and incubated at 25°C on a rotary shaker for 24 hours, subsequently, electrical conductivity of the solution (Lt) was determined. Samples were then autoclaved at 120°C for 20 minutes and the last electrical conductivity (L_0) was obtained after equilibration at 25°C. Three replicates per population per treatment were measured and the electrolyte leakage was defined as follows:

$$\text{Electrolyte leakage } (\%) = (L_t/L_0) \times 100 \quad (3)$$

This technique has two limitations (Lutts *et al.,* 1996): Firstly, it cannot allow determination of the direct effect of salt stress on leaves by adding NaCl to the incubating solution, as it would interfere with electrolyte leakage measurement. Secondly, the apoplastic accumulation of ions in salt stressed leaves will contribute to electrical conductivity increase, although they are not involved in cellular efflux.

Considering these limitations, the salt effect on membrane permeability was also quantified by leakage of UV-absorbing substances (UVAS) (Lutts *et al.,* 1996). Leaves prepared as above were incubated with 10 mL of either deionized water or 250 mM NaCl. After 24 hours incubation, a 3 mL aliquot of bathing solution was removed from the vials and the absorbance was determined spectrophotometrically at 280 nm (A_{280}). This aliquot was then added back to its original solution and vials were frozen at -20°C for 12 hours to break the cells. The final absorbance (A'_{280}) was measured after thawing and relative leakage ratio (RLR) of the UVAS was defined as follows:

$$RLR\ (\%) = (A_{280}/A'_{280}) \times 100 \quad (4)$$

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Nitrate Reductase Activity (NRA)

The nitrate reductase (NR, EC 1.6.6.1) is a key enzyme in nitrogen nutrition. It catalyzes the reaction of nitrate reduction and constitutes an indicator of the damaging effects of NaCl (Ghoulam *et al.,* 2002). NRA was determined *in vivo* in leaves (0.1g) according to Heuer and Plaut (1978). The leaf samples were infiltrated under vacuum in 10 mL of 50 mM phosphate buffer, pH 7.5, containing 0.1M KNO_3, and 0.1% Triton X-100. After 5 minutes, the samples were transferred into an identical solution, but without Triton X-100, and incubated for 1 hour at 28°C. For determination of the nitrite formed, 1 mL of the solution was supplemented with 0.25 mL of 1.5M HCl, containing 1% sulfanilamide and 0.25 mL of a 0.02% solution of N-(1-naphtylethylenediamine) dihydrochloride. The absorbance was measured at 540 nm and the NRA was calculated from a standard curve established with $NaNO_2$ concentrations and expressed in µmol NO_2^- g FW^{-1} h^{-1}. Three replicates per population per treatment were analyzed.

Nitrate Content Determination (NO_3^{-1})

The nitrate contents of leaves were estimated as described by Agbaria *et al.* (1996). Leaf samples of 100 mg *FW* were extracted for 60 minutes in deionized water at 45°C. After centrifugation at 6,000g for 15 minutes, 200 µL of the supernatant was incubated at ambient temperature (around 24°C) with 0.8 mL 5% salicylic acid in concentrated sulfuric acid for 20 min. After adding 12 mL of 2 N NaOH for pH adjustment to 12, the samples were cooled to ambient temperature and the coloration was measured spectrophotometrically at 410 nm. The nitrate content was determined using a standard curve established with solutions of KNO_3.

Three replicates per population per treatment were analyzed and results were expressed in mg per g of fresh matter.

Acid Phosphatase Activity (APA)

Leaves or roots (100 mg) were ground in 2 mL of sodium acetate-buffer (0.1M pH 5.8). Homogenates were centrifuged at 13,000g at 4°C during 30 minutes, and aliquots of 50 µl of the supernatants were used for APA assay (ACP, EC 3.1.3.2).

The APA was assayed according to the method described previously by Mandri *et al.* (2012), using p-nitrophenyl phosphate (pNPP) as substrate. A total reaction volume of 1 mL was prepared for each sample and incubated at 30°C for 30 minutes. The reaction was stopped with 3 mL of 0.2 M NaOH, and the ACP activity was measured spectrophotometrically at 405 nm. A standard curve was established with p-nitrophenol solutions. Three replicates per population per treatment were analyzed and the APA was expressed in µg of p-NP per g *FW* per mn.

Analysis of Inorganic Ions

For sodium (Na^+), potassium (K^+), and phosphorus (P) analysis, samples (0.5 g) of dried leaves or roots were ashed in a furnace for 6 hours at 500°C. The ash was dissolved in chloride acid. This solution was diluted with distilled water and filtered on Whatman paper. The Na^+ and K^+ contents were determined by flame emission photometry. The P content was determined colorimetrically using the molybdate blue method (Murphy and Riley 1962). P concentration was measured by reading the absorbance at 820 nm after color development at 100ºC for 10 minutes. A standard curve was established with KH_2PO_4 solutions.

The chloride (Cl^-) contents were determined as described by Boursier *et al.* (1987). Dry samples were digested in a nitric acid and acetic acid mixture (3:1 v/v). Cl^- contents were determined volumetrically using silver nitrate (0.1N) and potassium dichromate (10% p/v) as indicator. Inorganic ion contents were expressed as mg per g of dry weight and three replicates per population per treatment were tested.

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Statistical Analysis

The statistical analysis was performed using SPSS (10.0) software. It concerned analysis of variance (ANOVA II) and Student test (t test).

RESULTS

Effect of NaCl on Growth Parameters

Results (Figure 1) indicated that salinity caused a significant reduction ($P<0.001$) in plant biomass in the studied alfalfa populations compared to their controls (0 mM). The decrease was more pronounced when NaCl concentration was increased, the strongest reduction occurred under the high concentration of NaCl (200 mM). ANOVA test (Table 1) showed a significant difference in growth of the tested populations ($P< 0.001$). Under the high salt concentration, Tata populations showed reductions of 30.97, 22.03, 24.86, and 22.03%, respectively, in shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), and root dry weight (RDW) (Figure 1). The biomass reduction in Taf 2 reached 36.19 and 28.45% in *SFW* and *RFW*, respectively. For *SDW* and *RDW* the reduction attained 35.12 and 28.45%, respectively. Dem population presented the same variations with reductions of 37.10 and 27.98 % for *SFW* and *RFW*, respectively, and of 25.61 and 27.98% for *SDW* and *RDW*, respectively. The growth reduction was more pronounced in Taf1, which was the least tolerant to this constraint according to biomass reduction (49.20, 49.99, 48.71, and 50.03% for SFW, RFW, SDW, and RDW, respectively). ANOVA test also showed that the interaction effect between salt treatment and alfalfa populations was significant ($P< 0.001$).

Relative Water Content (RWC)

Generally, relative water content of the

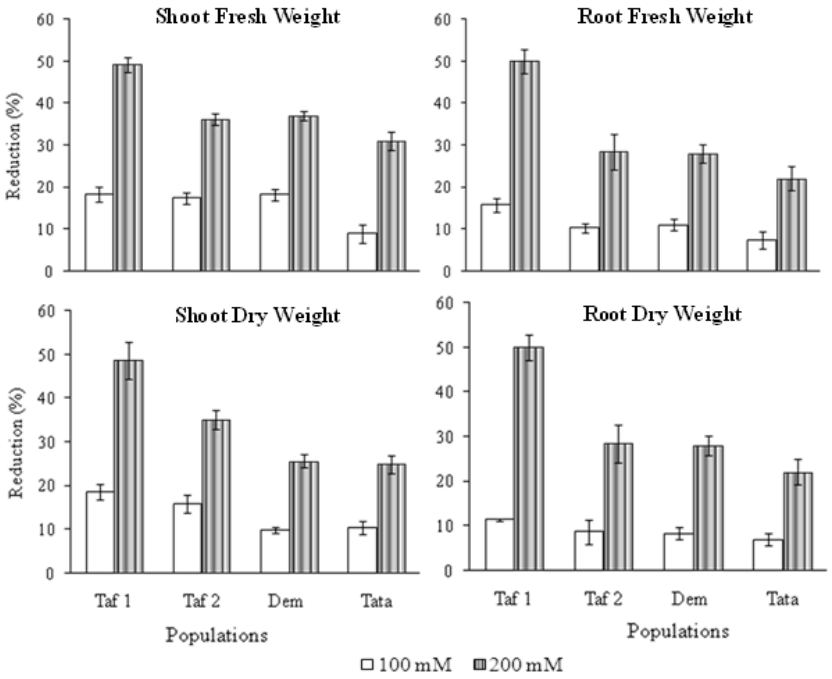

Figure 1. Effect of salt treatment on growth parameters in plants of four Moroccan alfalfa populations. Results are expressed as reduction percentage compared to the controls. Results are mean of three replicates of six plants per population per treatment and bars represent the SE.

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Table 1. Results of two-way analysis of variance (ANOVA II) of salt treatment and population effects and their interaction (salt treatment×population) for the agro-physiological and biochemical parameters studied.

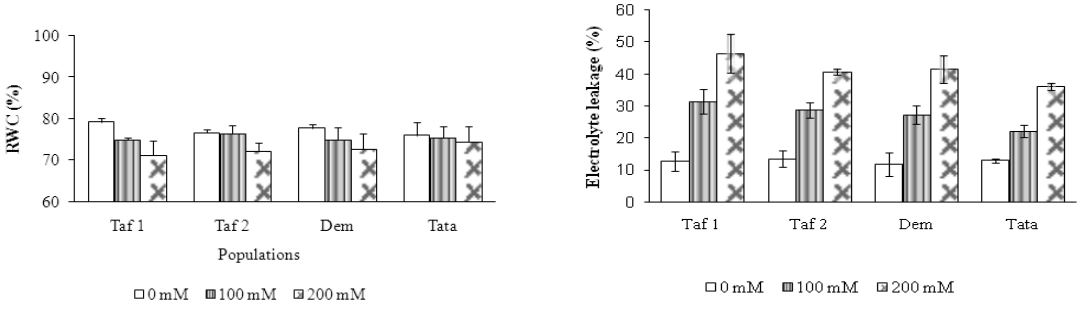
Dependent variable	Independent variable					
	Salt treatment		Population		Interaction	
	F	*Sig.*	*F*	*Sig.*	*F*	*Sig.*
Shoot fresh weight	2 223.11	***	138.61	***	34.78	***
Root fresh weight	875.91	***	121.72	***	39.61	***
Shoot dry weight	948.29	***	139.84	***	29.81	***
Root dry weight	1 030.50	***	95.02	***	51.34	***
Electrolyte leakage	120.20	***	3.08	*	0.87	NS
Relative water content	3.69	*	0.05	NS	0.35	NS
Leaves Na^+	1 322.31	***	13.10	***	3.75	**
Roots Na^+	792.47	***	0.83	NS	0.48	NS
Leaves K^+	306.11	***	14.72	***	4.63	**
Roots K^+	82.45	***	3.32	*	4.79	**
Leaves Cl^-	2 465.66	***	28.56	***	14.97	***
Roots Cl^-	2 881.04	***	4.05	*	2.38	NS
Leaves P	604.76	***	5.86	**	1.72	NS
Roots P	93.18	***	1.79	NS	0.89	NS
Leaves nitrate	12.36	**	0.49	NS	0.15	NS
Root APA	121.38	***	3.37	*	0.33	NS
Leaf APA	184.48	***	5.95	**	1.96	NS
NRA	19.26	***	3.89	**	0.19	NS

*P< 0.05; ** P< 0.01; *** P< 0.001; NS: Not significant, Numbers represent F values with their signification (Sig).

leaves was lower in plants grown under higher salinity treatment (200 mM) compared to the corresponding control (Figure 2-a). However, the significant decrease in this parameter was only noted in Taf1 and Taf 2 at 200 mM NaCl (P< 0.05.). At this NaCl concentration, Taf1 recorded reduction of 10.22% and Tata population appeared to be the least affected one basing on their weak reduction (2.08%). However, Taf 2 and Dem displayed reductions of 5.66 and 6.24%, respectively.

Electrolyte Leakage (EL) and Relative Leakage Ratio (RLR)

Data in (Figure 2-b) show that salt treatments caused a highly significant increase in *EL* in all of the tested populations when compared to the control



(a) (b)

Figure 2. Effect of salt treatment on (a) relative water content (RWC) of leaves (b) electrolyte leakage in plants, of four Moroccan alfalfa populations. Bars are SE of three replicates.

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groups. *EL* was increased as salt concentration increased in the rooting medium and reached the maximum values at 200 mM NaCl. ANOVA test (Table 1) approved that the population effect on this parameter was significant (P< 0.05) and comparison among the studied populations that received 200 mM NaCl indicated that Tata was the least affected population (36.20%) by this constraint and Taf1 was the most affected one (46.13%) (Figure 2-b). For RLR, data of Table 2 showed that salt treatment increased this parameter. At 200 mM NaCl, Taf1 population marked the highest values in hypo and hyper-osmotic shocks, suggesting that its membrane permeability was more affected than that of Tata population.

Effect on Nitrate Reductase Activity and Nitrate Content

NaCl treatment caused a significant inhibition (P< 0.001) of nitrate reductase activity, particularly at 200 mM NaCl (Figure 3). A significant difference among the considered populations was noted (P< 0.01). In 200 mM NaCl treatment, the inhibition of NRA was markedly noted in Taf1 that recorded 0.62 µmol NO_2^- g FW^{-1} h^{-1}. Taf 2 and Dem populations showed almost the same NRA (0.70 and 0.73 µmol NO_2^- g FW^{-1} h^{-1}, respectively). However, the highest activity (0.96 µmol NO_2^- g FW^{-1} h^{-1}) was registered in the most tolerant population in terms of biomass.

Nitrate contents in leaves of these populations decreased under salt treatment (Figure 3). This decrease was more pronounced when the NaCl concentration was increased and the largest decreases occurred under high salt concentration (200 mM NaCl) compared to the controls (P< 0.01). Population and interaction effects were not significant (P> 0.05).

Effect on Acid Phosphatase Activity and Plant P Nutrition

Under salt treatment, the activity of APA significantly (P< 0.001) increased compared to the control (Figure 4). On the whole, APA varied significantly between the populations and also between plant parts. Indeed, APA was preferentially stimulated more in roots than in leaves. Tata population showed relatively high activity, i.e. 3.05 and 2.73 µg p-NP g^{-1} FW mn^{-1} in plant roots and leaves, respectively, at 200 mM NaCl. Taf1, Taf 2, and Dem showed 2.90, 3.02 and 2.98 µg p-NP g^{-1} FW mn^{-1}, respectively, in their roots. However, the activity in their leaves reached 2.48, 2.61 and 2.52 µg p-NP g^{-1} FW mn^{-1}, respectively.

According to Table 3, salt treatment significantly (P< 0.001) decreased the P contents of plants. A significant difference among the studied populations was also noted. For both plant parts, the P contents were less affected in Tata population and decreased more in Taf1 and Taf2, particularly under 200 mM NaCl treatment.

Effect on K^+, Na^+ and Cl^- Concentrations

Table 3 shows that the salinity induced a significant accumulation (P< 0.001; Table 1)

Table 2. Effect of salt treatment on relative leakage ratio in leaves of two contrasting alfalfa populations Taf 1 and Tata. Values are mean of three replicates±S.E

Alfalfa Pop'n	Hypo-osmotic solution			Hyper-osmotic solution		
	0 mM NaCl	100 mM NaCl	200 mM NaCl	0 mM NaCl	100 mM NaCl	200 mM NaCl
Taf 1	14.97 ± 1.74	27.64 ± 2.90	44.08 ± 2.44	46.35 ± 4.09	55.45 ± 2.12	66.61 ± 1.50
Tata	15.29 ± 2.08	23.56 ± 1.80	37.34 ± 2.10	42.88 ± 5.19	50.28 ± 2.92	60.00 ± 3.73

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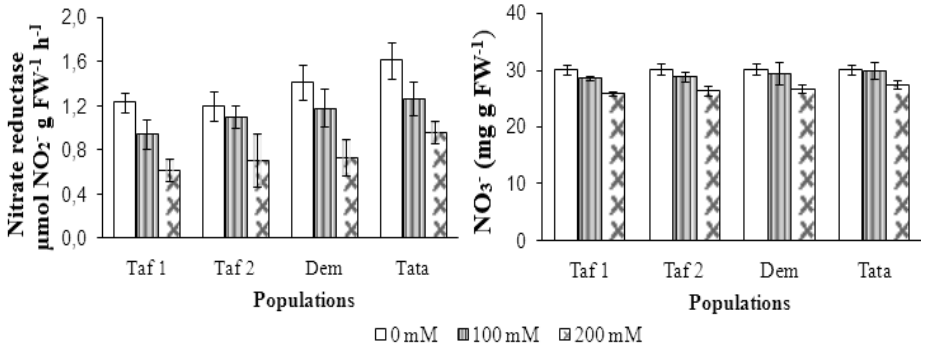

Figure 3. Nitrate reductase activity and nitrate contents in leaves of four Moroccan alfalfa populations under salt treatments of 0, 100 and 200 mM NaCl. Bars are SE of three replicates.

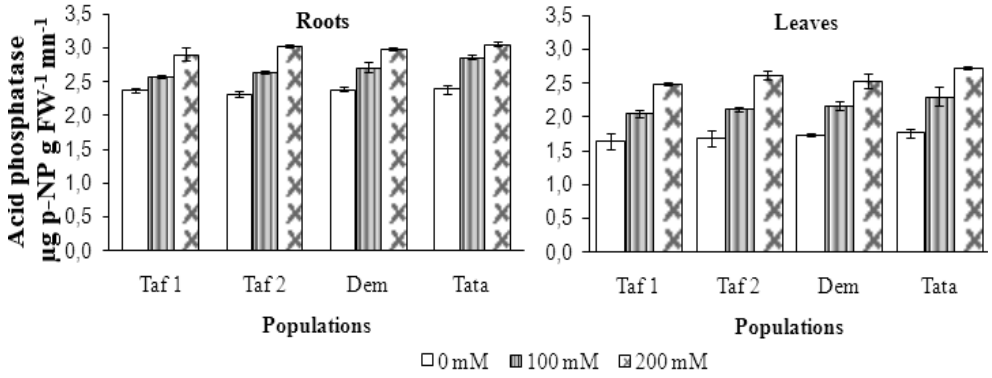

Figure 4. Acid Phosphatase activity in roots and leaves of four Moroccan alfalfa populations under salt treatments of 0, 100 and 200 mM NaCl. Bars are SE of three replicates.

Table 3. Concentration of Na^+, K^+, Cl^- and P in roots and leaves of four Moroccan alfalfa populations under salt treatments. Data are means of three replicates±SE.

Population	NaCl mM	Na+ (mg/ g DW)		Cl- (mg/ g DW)		K+ (mg/ g DW)		P (mg/ g DW)	
		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Taf 1	0 mM	4.18 ± 0.45	5.91±0.86	13.33±1.35	30.66±1.33	31.00±1.08	57.00±1.08	10.08±0.27	6.21±0.28
	100 mM	18.91±1.53	25.51±1.65	30.66±1.33	54.66±1.31	29.46±1.53	48.46±1.53	9.03±0.48	5.50±0.11
	200 mM	24.99±1.99	34.60±1.15	59.11±2.03	74.66±2.65	19.61±1.62	33.94±1.95	7.24±0.09	3.35±0.13
Taf 2	0 mM	4.48±0.61	6.09±0.59	12.22±1.38	30.22±1.26	32.58±1.22	57.25±2.02	10.06±0.22	6.76±0.16
	100 mM	19.00±1.33	27.82±0.76	30.44±1.67	56.00±2.66	30.50±2.01	50.17±0.97	9.21±0.68	5.43±0.17
	200 mM	25.10±0.95	37.90±2.41	57.33±1.33	88.00±2.66	20.50±1.50	39.84±2.51	7.38±0.42	3.43±0.09
Taf 3	0 mM	4.00±0.33	5.96±0.79	13.55±1.01	28.44±2.77	31.20±2.91	58.20±2.91	10.17±0.96	6.59±0.10
	100 mM	19.63±2.41	27.49±0.71	30.04±1.26	56.88±1.53	30.33±0.81	52.33±0.81	9.11±0.27	5.40±0.20
	200 mM	25.49±1.50	37.22±1.08	55.02±1.89	86.22±1.53	25.54±0.54	41.87±2.10	7.83±0.34	3.58±0.11
Taf 4	0 mM	3.92±0.62	5.87±0.94	13.24±0.67	30.52±1.19	30.60±1.28	56.93±1.50	10.05±0.63	6.67±0.18
	100 mM	20.24±1.71	31.03±1.66	28.66±0.66	60.44±1.53	29.27±2.62	53.27±1.02	9.35±0.48	5.64±0.52
	200 mM	26.8±1.12	42.77±3.43	54.40±1.60	94.22±1.53	26.19±0.96	44.19±0.80	8.11±0.65	4.01±0.09

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of Na^+ and Cl^- in both plant organs (roots and leaves). The accumulation increased gradually with the increase of NaCl concentration. However, this constraint caused a significant decrease in K^+ in both organs ($P< 0.001$; Table 1). Indeed, K^+ contents decreased gradually with increase of NaCl concentration, in rooting medium. In all of the tested populations, Na^+, Cl^- and K^+ ions preferentially accumulated in leaves more than in roots. Except for roots Na^+ contents, the differences between populations used were significant for the other ions (K^+ and Cl^-) and in both organs (Table 1). The interaction effect was not significant only for root Na^+ and Cl^- (Table 1). Thus, Tata population presented the highest ions contents under high NaCl concentration, while Taf1 showed the lowest ones, except root Cl^- contents.

DISCUSSION

Salinity, with adverse effects on crops growth and productivity, has emerged into a global threat among the agricultural communities by affecting approximately 20% of the globally irrigated agricultural land (Munns, 2002). Plant growth is one of the most important agricultural indices of salt stress tolerance as indicated in many studies (Parida and Das, 2005). In the present work, salt treatment of 100 mM NaCl induced reductions in biomass not exceeding 20%, whereas, the biomass was highly reduced in all of the tested populations grown under 200 mM NaCl. A significant difference was registered between alfalfa populations in their behaviors under salt-stressed conditions. Indeed, Tata population appeared to be the most tolerant population based on its higher biomass production compared to the remaining ones, especially Taf1 which showed the weakest growth. In fact, similar effect of salt treatment on growth yield is widely documented in many plant species such as *Medicago truncatula* L.(Loépez *et al.,* 2008; Ghasem *et al.,* 2012), *Zea mays* L. (Azevedo Neto *et al.,* 2004), *Beta vulgaris* L. (Ghoulam *et al.,* 2002), *Plantago coronopus* L. (Koyro, 2006), *Cynodon dactylon* L. (Hameed and Ashraf, 2008), *Saccharum officinarum* L.(Suriyan and Chalermpol, 2008), *Delonix regia* (Patel *et al.,* 2009) and *Lablab purpureus* L. (D'Souza and Devaraj, 2010).

The relative water content is a determinant factor for the metabolic activity and survival of leaves, and maintaining it at an adequate level seems to be salt stress tolerance criteria (Hassani *et al.,* 2008). Our results indicated that salt treatment induced a reduction in *RWC*. Under the high salt concentration, this reduction was relatively lower in the most tolerant population (Tata), as judged in terms of plant biomass, than in the least tolerant population (Taf1) that showed appreciable biomass reduction. The decrease in *RWC* indicated a loss of turgor that resulted in limited water availability for cell expansion process (Katerji *et al.,* 1997). Thus, the growth inhibition in Taf1 could be related to the decrease of *RWC* provoked by the presence of salt in rooting medium.

At the 200 mM NaCl treatment, the highest values of solute leakage and UVAS leakage were recorded in Taf1 population and the least values in Tata population. This clearly indicated that the membrane system of Tata plants was less affected under salt stress. Thus, Tata plants could keep their turgor pressure at a level high enough to ensure adequate growth even under salt treatment. Consequently, these observations could be partially the reason of the Tata population tolerance to this environmental constraint. Similar result was reported in *Oryza sativa* L. (Lutts *et al.,* 1996), *Cucumis sativus* L. and *Capsicum annuum* L. (Kaya *et al.,* 2001), *Beta vulgaris* L. (Ghoulam *et al.,* 2002), *Linum usitatissimum* L. (Nacir Khan *et al.,* 2007) and *Rosmarinus officinalis* L. (Hejazi-Mehrizi *et al*., 2012).

Nitrate reductase activity was found markedly inhibited under salt treatment, especially in Taf1 population. Gouia *et al.* (1994) noted that the negative effect of NaCl on NRA was more pronounced for salt sensitive plants of bean than for those of salt tolerant cotton. The presence of salts in the rooting medium inhibits nitrate uptake and,

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consequently, at lower nitrate concentration in the leaves, the NRA decreases (Abd Elbaki *et al.,* 2000). The increase in the levels of NaCl decreased NRA in both plant parts of *Medicago sativa* (Khan *et al.,*1995), in leaves of *Bruguiera parviflora* (Parida and Das, 2004), in wheat seedling (Carillo *et al*., 2005) and in *Brassica napus* L. (Bybordi and Ebrahimian, 2011). The decrease of NRA could be due to the low content of nitrate reductase protein and /or to a limiting nitrate transport to shoots. Indeed, in our experiments, we noted that the inhibition was associated with a decrease of nitrate content in the leaves. Thus, the effect of salt stress on NRA inhibition could be explained by the limited nitrate availability and /or the toxicity exerted by Na^+ and Cl^-. Indeed, the nitrate could be stored in the vacuoles as compatible solute, thus, reducing its availability in the metabolic pool in the cytosol.

However, phosphatase acid activity increased gradually with the increase of NaCl concentrations. Similar observation was documented in *Brassica napus* L. ((Bybordi and Ebrahimian, 2011). This increase was accompanied with a change in phosphorus level. Hence, our data are in agreement with the hypothesis suggesting that activity of this enzyme in plants and plant parts typically increases when the plants become phosphorus deficient (Lefebvre *et al.,* 1990, Parida and Das, 2004). Our results showed a reduction of phosphorus contents under salt treatment in both plant parts (shoot and root). This reduction could be behind the increase of acid phosphatase activity. In *Medicago sativa* L., the same observation was noted by Arab and Ehsanpour (2006). The significant difference in Na^+ and Cl^- accumulations between the contrasting populations suggests that they could play an important role in osmotic adjustment, in stressed alfalfa plants. The significant decrease of K^+ and nitrate contents in plants tissues with increasing salinity could be explained by an antagonistic effect that Na^+ and Cl^- exerted on K^+ and nitrate uptake, respectively. Alian *et al.* (2000) reported that, under salt stress, one of the mechanisms of salt tolerance is accomplished by uptake and accumulation of inorganic ions and the increase of Na^+ in salt tolerant species is generally associated with a decrease in K^+ (Amini and Ehsanpour, 2005; Patel *et al.,* 2009; Dadkhah, 2011; Saleh and Maftoun, 2008).

The decrease of nitrate uptake is accompanied by a high Cl^- uptake and translocation to the shoots (Parida *et al*., 2004). Similar observation was reported for *Beta vulgaris* L. (Ghoulam *et al.,* 2002), *Lycopersicon esculentum* Mill. (Juan *et al.,* 2005), and *Schinopsis quebracho* (Meloni *et al.,* 2008). Mahajan and Sonar, (1980) noted that phosphorus uptake in wheat crop was retarded under saline conditions. Adams (1991) also noted that leaves phosphorus concentration decreased in tomato plants with increase in NaCl concentration in nutrient solution.

CONCLUSIONS

We concluded that the salt treatment of 100 mM NaCl did not cause a great inhibition of plant growth. However, the strongest growth inhibition occurred at the higher salt concentration, i.e. 200 mM NaCl. Significant differences in the behaviors of the studied alfalfa populations were observed: Tata population was the most salt tolerant and Taf1 was the least tolerant, while Taf2 and Dem displayed moderate salinity tolerance. Salt tolerance of alfalfa populations was associated with inorganic ion accumulation and the maintenance of membrane integrity, and an adequate level of nitrate reductase and acid phosphatase activities.

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رشد، غلظت عناصر غذایی، و آنزیم های دخیل درتغذیه گیاه چند جمعیت یونجه در شرایط شور

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چکیده

به منظور ارزیابی اثر شوری روی چند صفت فیزیولوژیکی و بیو شیمیایی یونجه ***Medicago sativa* L.** ، چند جمعیت مختلف (شامل Tafilalet 1, Tafilalet 2, Demnate و Tata) که مبداء آنها مناطق کوهستانی و واحه مراکش بود مورد آزمایش قرار گرفتند. گیاهان آزمایش در شرایط گلخانه در گلدان هایی کاشته شدند که حاوی شن وپیت بودند و سه تیمار شوری (0, 100 , 200 mM NaCl) برآنها اعمال شد.سپس، گیاهان ۴۵ روز بعد از تیمارهای شوری برداشت شدند و چند صفت فیزیولوژیکی و بیو شیمیایی که در پیوند با تحمل شوری بودند(از قبیل زیست توده، محتوی آب گیاه، نفوذ پذیری ممبران، غلظت عناصر غذایی در گیاه، نیترات رداکتاز وفعالیت اسید فسفاتاز) اندازه گیری شد.بر پایه نتایج به دست آمده، زیست توده گیاه تفاوت معنی داری در بین جمعیت های یونجه داشت و در اثر افزایش غلظت NaCl به تدریج کم شد. به این قرار، جمعیت Tata بیشترین تحمل به

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شوری را نشان داد در حالی که Tafilalet 1 از همه تحمل کمتری داشت و جمعیت های Tafilalet 2وDemnate تحمل متوسطی نشان دادند. تغییرات رشد گیاهان با تغییرات در پارامترهای فیزیولوژیک و بیوشیمیایی همراه بود. در واقع، شوری باعث کاهش محتوی نسبی آب در گیاه، ایجاد دگرگونی در ممبران و غلظت عناصر غذایی شد.همچنین، نتایج نشان داد که شوری از فعالیت نیترات ردوکتاز در برگ همه گیاهان مورد آزمون جلوگیری کرد ولی فعالیت اسید فسفاتاز در برگ و ریشه گیاهان تحت تنش افزایش یافت. تحمل تنش شوری در جمعیت های یونجه همراه بود با انباشت زیاد یونهای معدنی و حفظ یکپارچگی و استحکام ممبران و فعالیت نیترات ردوکتاز و اسید فسفاتاز درسطح کافی .

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