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REGULAR ARTICLE

MINERALS UPTAKE, ORGANIC OSMOTICA CONTENTS AND WATER BALANCE IN ALFALFA UNDER SALT STRESS

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SUMMARY

Organic osmotica contents (proline, soluble sugars), minerals (sodium and potassium), osmotic and water potentials were investigated in leaves, stems and roots of three alfalfa varieties, at the late bloom-early pod stage. Varieties (*Gabès*, *Hunterfield*, and *Hyb.555*) were irrigated with water having four NaCl concentrations: (0g.l^{-1}; 2.5g.l^{-1}; 5g.l^{-1} and 10g.l^{-1}). Results showed that all varieties accumulated high Na^+ and low K^+ contents in leaves and stems. However, the local variety *Gabès* differed from the other ones by significantly lower Na^+ and higher K^+ contents in leaves at the highest salt concentration. Furthermore, its proline content at 5 and 10g.l^{-1} NaCl was significantly higher in leaves, stems and roots than in *Hunterfield* and *Hyb.555* varieties. Nevertheless, proline content in the different plant parts increased with increasing salt concentration in the medium, reaching a significantly higher level for *Gabès* at the greatest salt concentration (10g.l^{-1}). Leaf tissue soluble sugar contents in *Gabès* were higher than those recorded for *Hunterfield* and *Hyb.555* varieties at 5 and 10g.l^{-1} treatments. However, stems had similar sugar contents in the three varieties. Soluble carbohydrate contents in root tissues of *Hunterfield* and *Hyb.555* were higher than in those of *Gabès*. This could probably be related to the difficulty in generating new leaves in both introduced varieties. Water potential (Ψw) decreased in three varieties with increasing of NaCl. The Ψw for *Gabès* variety was lower, compared to the introduced alfalfa. In the same way, osmotic potential (Ψs) decreased with the increase of the salt at the three varieties. The Ψs of *Gabès* reached a lower level in the most stressful treatment, compared to the introduced varieties.

Key words: Alfalfa, Osmotic potential, Potassium, Proline, Salinity, Sodium, Soluble sugar, Water potential

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1. Introduction

According to Flowers and Flowers (2005), naturally occurring salt-affected soils cover about a billion hectares. In this area, only halophytes have evolved to grow. However, most of our cultivated plants are sensitive to the salt. Attempts to improve the salt tolerance of crops through conventional breeding programs have met with very limited success, due to the complexity of the trait: salt tolerance is complex genetically and physiologically (Flowers, 2004). Salinity imposes two stresses on plant tissues: one, a water deficit resulting from the relatively high solute concentrations of the soil (Tester et Davenport, 2003; Munns et al., 2006); and two, ion-specific stresses resulting from altered K^+/Na^+ ratios and Na^+, Cl^- ion concentrations that are inimical to plants (Blumwald, 2000; Rejili et al., 2007; Haro et al., 2010). Osmotic constraint is the first difficulty which plants are confronted in saline medium. Soluble salts in the soil reduce the osmotic potential which according to Strogonov (1964) becomes in a

state of "physiological drought", particularly for plants that cannot adjust their osmotic potential.

Salt stress increase Na^+ (Huang et al., 2009) and decrease K^+ uptake in shoots. Activation of K^+ flux and Na^+ efflux from the cell, compartmentation into the vacuole and utilization of Na^+ for osmotic adjustment are strategies used by plants to maintain desirable K^+/Na^+ ratios in the cytosol. Osmotic and ionic homeostasis are established by Na^+ compartmentation or by biosynthesis and accumulation of compatible solutes (Chinnusamy et al., 2005; Sanchez et al., 2005).

Salinity reduces the ability of plants to take up water (Munns, 2002 and 2005). However, the absorption of mineral ions and the synthesis of soluble organic compounds mainly proline, glycine betaine (Meloni et al., 2004; Slama et al., 2008) and soluble sugars (Klages et al., 1999; Sakamoto and Murata 2002; Abebe et al., 2003; Ashraf and Bashir 2003; Murakeözy et al., 2003), allow the plant to overcome this failure and re-establish a water potential gradient which in turn gives the possibility to absorb water and restore plant turgor (Xiong and Zhu, 2002).

Proline has a higher solubility in water, and at higher concentrations it has little or no perturbing effect on macromolecule-solvent interactions (Rathinasabapathi, 2000). Proline accumulation is a common metabolic response of higher plants to salinity stress or water stress (Ben Khaled et al., 2003; Khedr et al., 2003; Kavi Kishor et al., 2005). Proline is localized in the cytoplasm of cells leaves of many higher plant species grown in saline environments (Ashraf and Bashir, 2003; Rabie and Almadini, 2005). In salinity or water stress, osmolyte accumulations in cells contribute substantially to cytoplasmic osmotic adjustment (Hare et al., 1998; Chinnusamy et al., 2005).

Huang et al., (2009), were found that an exogenous application of proline by foliar spray alleviated salt stress in *Cucumis sativus*. Organic solutions, while participating to the osmotic adjustment, they protect plant physiological reactions (Abraham et al., 2003) and constitute a non toxic source of carbon, nitrogen and energy reserves (Joyce et al., 1992). Moreover, these organic osmotica reduce excessive accumulation of Na^+ in aerial plant parts. Among "includer", the most salt tolerant species are those that limit the Na^+ transport to leaves in many glycophyte (Shi et al., 2003). To compensate for this mineral deficiency, however, plants synthesize soluble organic substances of low molecular weight in order to re-establish osmotic balance. In saline condition, Kerepesi and Galiba (2000) and Azevedo Neto et al., (2004) found that tolerant varieties of wheat and different maize genotypes respectively accumulated more soluble carbohydrate than did sensitive ones.

The aim of the present study is to evaluate the sensibility or the tolerance in three alfalfa varieties grown under salt stress, throw some physiological parameters for screening several lines of alfalfa tolerant for salinity.

2. Materials and Methods

Plant material

Alfalfa varieties were chosen from a preliminary field trial conducted for two consecutive years using nine introduced varieties, compared to a native material of the Tunisian oasis: *Gabès*. Throughout the study, all varieties were irrigated by submersion using the Medjerda river water which has a salt concentration varying from $1g.l^{-1}$ in winter to $3g.l^{-1}$ in summer. The choice of these varieties was based on (i) winter dormancy, (ii) cumulative dry matter production and (iii) population density at the end of two growing seasons.

Experimental conditions

Plants were cultivated in greenhouse (natural light; maximal temperatures of 30 to 42°C and minimal of 15 to 28°C; maximal relative humidity of 80 to 95% and minimal of 25 to 45%). The seedling was carried out in plastic pots (volume = 10litres) containing an equilibrated soil with a basic pH (8.2), rich in active chalk (13%). It has a humidity fluctuating between: 12.1% at wilting point and 22.6% at field capacity (table 1). Pots were daily irrigated and continuously drained and drainage water was collected in small bottles. The quantities of water

necessary for each irrigation treatment were determined using mini-lysimeters. The soil was maintained at the field capacity throughout the experiment.

Table 1. Physical and chemical composition of soil

Measured parameters	Corresponding value
Clay	35.5%
Silt	14%
Sand	47.5%
Organic matter	3%
pH	8.2
Active chalk	13%
Wilting point	12.1%
Field capacity	22.6%

The chemical composition and the sodium adsorption ratio (S.A.R.) of the soil at the beginning and at the end of the experiment are summarized in table 2.

Table 2. Chemical composition and Sodium Adsorption Ratio (S.A.R.) of soil

	At the beginning of experiment						At the end of experiment					
NaCl ($g.l^{-1}$)	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	SAR	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	SAR
0	22.2	32.0	0.6	23.1	6.9	5.8	23.2a	32.2a	0.8a	25.8a	7.7a	5.7a
2.5							112b	167b	0.8a	82.6b	22.4b	15.4b
5							183c	319c	1.9b	117c	33.7c	21.0c
10							234d	393cd	1.9b	118c	28.7c	27.3d

Averages of every column followed by the same letter are not significantly different at $\alpha = 5$ %

Each experimental pot contained ten plants of the same variety. Before achieving this density, an initial sowing of twenty seeds per pot was carried out, followed by a thinning at the four leaves trifoliate stage to leave only the ten best plants, while respecting a uniform spatial distribution between individuals. Seedlings were first irrigated with tap water during establishment (20 days). Two water samples were taken monthly to monitor their chemical composition. The average concentration was: 0.2meq.l^{-1} for K^+, 12 to 17meq.l^{-1} for Ca^{2+}, 2 to 5meq.l^{-1} for Na^+, and 2 to 6meq.l^{-1} for Cl^-. At the four leaves trifoliate stage, seedlings were irrigated with NaCl added water at 4 concentrations (0g.l^{-1} - 2.5g.l^{-1} - 5g.l^{-1} and 10g.l^{-1}). The experimental design included 12 treatments (3 varieties x 4 levels of salinity), with randomized 4 replications, resulting in a total of 48pots and 480 plants. The choice of salt levels was based on the observed variability in salt concentration in the main Tunisian water, commonly used for irrigation. That concentration varied from 1 to 3g.l^{-1} in the Medjerda water (UNESCO, 1968) and from 4 to 6g.l^{-1} in the Souassi plain areas (Franclet and Le Houerou, 1971).

To avoid osmotic shocks, the salt was supplied with irrigation water using increasing gradual fractions until the desired level of each treatment is reached (Hamza, 1977). It took 12 days to fully establish the saline treatments. Thereafter, weekly analyze were done either on soil or on excess drainage water to insure that NaCl concentration in medium was constant. This procedure allowed us to monitor NaCl quantities taken up by alfalfa plants or adsorbed by the exchangeable complex in the soil.

Chemical analysis was carried on plants harvested at the late bloom-early pod stage, corresponding to a salt stress period of three months. The measurement of the soluble

sugars and the proline was done on lyophilized samples of leaves, stems and roots from the three alfalfa varieties. Sugars were extracted with boiling alcohol at 70° using a spectrophotometer set at 520 nm wavelengths. The extraction and the dosage of the proline were based on the method developed by Troll and Lindsley (1955), and simplified by Dreier and Goring (1974). Readings were determined by spectrophotometer at a wavelength of 528 nm.

Extraction of ions was achieved using the nitric extraction technique (nitric acid at 0.5%) of dry matter sample. The sodium and potassium were measured by a flame photometer (Eppendorf photometer). To avoid interference, dilutions were made so that Na^+ concentrations were lower than 30mg.l^{-1}.

The water potential was determined using a pressure chamber type Wescor's, on eight mature leaves, with their petiole. The manipulations were performed in the morning from ten o'clock.

The osmotic potential was measured by an automatic cryoscopic osmometer Roebling type. The machine is calibrated with a solution of 300mOsm.kg^{-1}.

Statistical analysis

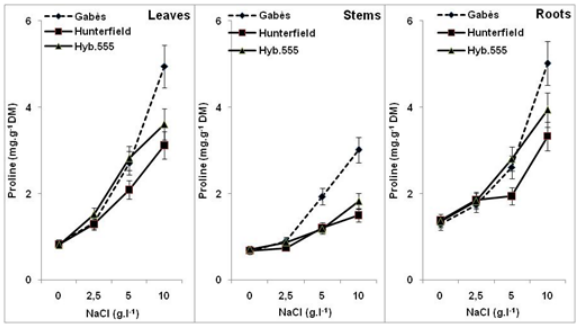
Confidence intervals were calculated to the threshold of 95% probability. General Linear Models of SAS was used to explain the degree of significance of each factor and of the interactions between different factors. The Duncan test was used to compare treatment means for all studied parameters.

3. Results

Proline contents

The proline content increased in the different organs of the three alfalfa varieties with increasing NaCl concentrations in the medium (figure 1). It was quantitatively more abundant in leaves and roots. Stems had the lowest concentration of this amino acid. Compared to the two introduced alfalfa, *Gabès* variety had a significantly higher level of proline content in its leaves, stems and roots under the 10g.l^{-1} treatment. This accumulation of proline in *Gabès* was highly correlated with increasing salt concentrations in the medium (r = 0.99). *Hunterfield* and *Hyb.555* had similar proline contents, particularly in stems.

Figure 1. Proline contents (mg.g^{-1} DM) in leaves, stems and roots, of three alfalfa varieties, in presence of increasing NaCl (0 - 2.5 - 5 and 10g.l^{-1}), after three months of salt treatments. Confidence intervals were calculated at α = 95% probability level



The analysis of variance showed a highly significant effect for variety, salinity and their interaction on proline contents in the different organs of the three varieties (table 3). The Duncan test ranked the varieties in the following order for all three organs: *Gabès* ≥*Hyb.555*>*Hunterfield*.

Table 3. Variance analysis of 2 factors (varieties, salinity) of proline contents in roots, stems and leaves of the 3 alfalfa varieties, after three months exposure to salt stress

Effect	Probability level of differences in proline contents in :		
	Roots	Stems	Leaves
Varieties (1)	0.0004	0.0001	0.0001
Salinity (2)	0.0001	0.0001	0.0001
(1) x(2)	0.0001	0.0001	0.0001

Soluble sugars

Reduced sugars

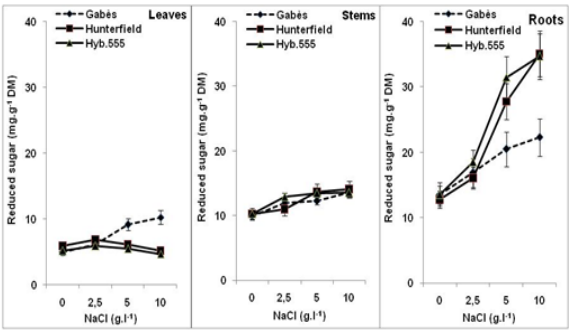
The content of reduced sugars in leaves, stems and roots of the three alfalfa varieties depended on the plant part and the salt concentration in pots (figure 2). Indeed, the increase in reduced sugar contents in leaves of *Gabès* was highly correlated with the increasing of NaCl in the medium (r = 0.95). This content reached a significantly higher level in *Gabès* than in *Hunterfield* and *Hyb.555* at 5 and 10g.l^{-1} of NaCl treatments. The introduced varieties had less reduced sugar contents which were negatively correlated (r =-0.63) and less affected by the increase in NaCl in the medium. In stems, however, the three alfalfa varieties had practically similar reduced sugar contents. In roots, we observed a significant difference between *Hunterfield* and *Hyb.555* on one hand, and *Gabès* on the other hand at the most stressful treatment. Indeed, the reduced sugar contents in the two introduced varieties increased quickly and with similar intensity according to the enrichment of the medium in NaCl. On the other hand, reduced sugar contents of *Gabès* varied slowly and reached a significantly lower level at the most stressful treatment. On a quantitative basis, roots were the plant parts which had the highest level of reduced sugar.

The variance analysis for the reduced sugar contents in the different organs of the three alfalfa varieties showed a significant "variety" and "salinity" interaction in leaves and roots and only a significant "salinity" effect in stems (table 4). The Duncan test relative to variety effect gave the following ranking for the reduced sugar contents in roots: *Hyb.555* = *Hunterfield*> *Gabès*. The order is different in leaves: *Gabès*> *Hyb.555* = *Hunterfield*. The same test relative to the effect of the "salinity" on the reduced sugar contents in roots ranked the three varieties in the following order : T0 <T1 <T2 <T3.

Table 4 . Variance analysis of reduced sugar contents in roots, stems and leaves of three alfalfa varieties, after three months of salt stress

Effect	Probability level of reduced sugar contents in :		
	Roots	Stems	Leaves
Varieties (1)	0.0001	0.0576	0.0001
Salinity (2)	0.001	0.0001	0.0355
(1) x (2)	0.0001	0.0007	0.0001

Figure 2. Reduced sugar contents (mg.g^{-1} DM) in leaves, stems and roots, of three alfalfa varieties, in presence of increasing NaCl (0 - 2.5 - 5 and 10g.l^{-1}), after three months of salt treatments. Confidence intervals were calculated at α = 95% probability level

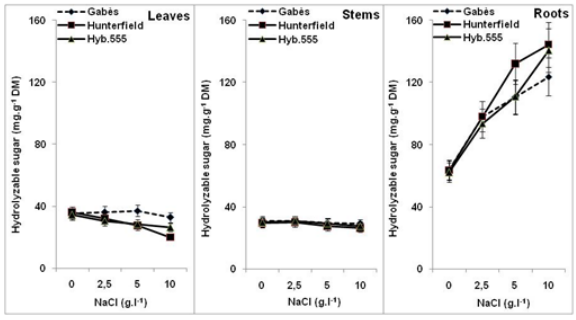


Hydrolyzable sugar contents (H.S.)

As for reduced sugars, the accumulation of hydrolyzable sugars varied with the intensity of saline stress and depended on the plant parts of the variety (figure 3). Indeed, at the most stressful treatment, the H.S. in leaf tissue of *Gabès* was significantly higher than those in the two introduced varieties. Besides, the evolution of these sugars in *Gabès* was practically not affected by the enrichment of the medium in NaCl (R^2 = 0.37). In contrast, the increasing salt in culture pots resulted in a linear decrease in H.S. contents in leaves of *Hyb.555* and especially *Hunterfield*. Determination coefficients (R^2) for *Hunterfield* and *Hyb.555* attained 0.998 and 0.915, respectively.

The increase in salt concentration of the medium resulted in a low change of H.S. contents in stems, whereas it caused a strong increase in root tissues of all three varieties. However, the H.S. content in *Gabès* remained significantly lower than in the two other varieties at 10g.l^{-1} of NaCl. Roots of the three alfalfa varieties had the highest H.S. contents.

Figure 3. Hydrolyzable sugar contents (mg.g^{-1} DM) in leaves, stems and roots, of three alfalfa varieties, in presence of increasing NaCl (0 - 2.5 - 5 and 10g.l^{-1}), after three months of salt treatments. Confidence intervals of were calculated at α = 95% probability level



The analysis of variance of H.S. contents in the different organs showed a highly significant effect for "variety", "salinity" and their interaction in roots, leaves, but not in stems (table 5). In leaves, the Duncan test ranked the varieties in the following order: *Gabès*> *Hyb.555* ≥ *Hunterfield*. The order is, however, reversed for roots: *Hunterfield* ≥ *Hyb.555*>*Gabès*.

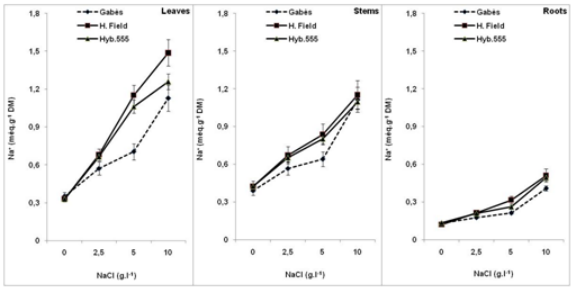
Table 5. Variance analysis of hydrolyzable sugar contents in roots, stems and leaves, of three alfalfa varieties, after three months of salt treatments

Effect	Probability level of hydrolysable sugar content in :		
	Roots	Stems	Leaves
Varieties (1)	0.0001	0.0281	0.0001
Salinity (2)	0.0001	0.0019	0.0001
(1) x (2)	0.0001	0.9611	0.0001

Sodium content

The sodium content in leaves, stems and roots of the three varieties increased with increasing salt in the medium, (figure 4). Results showed an important difference in the accumulation of Na^+ with the organs on one hand and between the three varieties on the other hand. Indeed, compared to the introduced varieties, *Gabès* accumulated less Na^+ in all parts and especially leaves. *Hyb.555* and particularly *Hunterfield* accumulated more Na^+ in leaves. Compared to other organs, roots had the lowest content of Na^+ in the three varieties. For *Hunterfield*, the invasion of leaf tissue by Na^+ was proportional to the NaCl concentration in the medium.

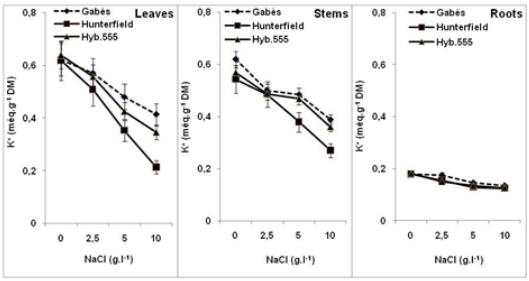
Figure 4. Na^+ contents (meq.g^{-1} DM) in leaves, stems and roots, of three alfalfa varieties, in presence of increasing NaCl (0 - 2.5 - 5 and 10g.l^{-1}), after three months of salt treatments. Confidence intervals were calculated at α = 95% probability level



Potassium content (K^+)

The potassium content of the leaf, stem and root organs of the three varieties is presented in figure 5. In the three varieties, salinity had a significantly drastic effect on K^+ content in leaves and stems. *Hunterfield* K^+ content in leaves and stems was dramatically reduced by increasing NaCl concentrations in the medium. In roots, K^+ content of the three alfalfa varieties was very low, compared to the other organs.

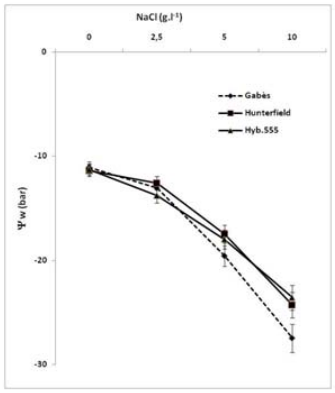
Figure 5. K^+ contents (meq.g^{-1} DM) in leaves, stems and roots, of three alfalfa varieties, in presence of increasing NaCl (0 - 2.5 - 5 and 10g.l^{-1}), after three months of salt treatments. Confidence intervals were calculated at α = 95% probability level



Water potential (Ψw)

The results showed that the water potential (Ψw) in leaf tissue, in three varieties, decreased significantly with increasing of NaCl in the culture medium (Fig. 6). For the control and treatment T1, the decrease of Ψw is almost identical among the three varieties. Beyond T1, the Ψw of *Gabès* variety is significantly lower than the two introduced varieties. At 10g.l^{-1} NaCl, the water potential, compared to the control, is about -16.4, -14 and -12 bars in *Gabès*, *Hunterfield* and *Hyb.555* varieties respectively. In all cases, the decrease in water potential is highly correlated with increasing salinity in the medium. Similar results were found by Hameed and Ashraf, (2008) on two populations of *Cynodon dactylon* who differ by their tolerance and their sensibility in the salt.

Figure 6. Water potential (Ψw) in leaves of three alfalfa varieties, in presence of increasing NaCl (0 - 2.5 - 5 and 10g.l^{-1}), after three months of salt treatments. Confidence intervals were calculated at α = 95% probability level



Osmotic potential (Ψs)

The figure 7A represented the osmotic potential measured. The result showed that the three alfalfa varieties presented the same osmotic potential, in the absence and presence of 2.5g.l^{-1} NaCl. In the treatment of 10g.l^{-1}, osmotic potential of *Gabès* variety is significantly lower, compared to the other two varieties.

Variance analysis of the measured osmotic potential revealed a highly significant effect of varieties and salinity. For the varietal factor, DUNCAN classification gives the following order: *Gabès* < *Hyb.555* = *Hunterfield*. The DUNCAN test for salinity effect gives the following classification: T0 < T1 < T2 < T3 for the three varieties.

The figure 7B illustrated the osmotic potential estimated by the formula (1); **C** indicated the cellular osmolarity contents of the mobile K^+ and Na^+ ions and estimated by the sum of the average tissular concentrations of these ions, obtained by dividing their ionic contents by the water contents.

$$\Psi s = 22.4*C \quad (1)$$

With $C=2*(K^+ + Na^+)$

Results showed that the osmotic potential estimated of the three varieties, is significantly higher than that measured (fig. 7B and table 6). This confirmed that the organic osmotica (proline and soluble sugars) participated in the osmotic adjustment. At 10g.l^{-1} of NaCl, a significant difference between *Gabès* variety and *Hunterfield* and *Hyb.555* were observed. The osmotic potential estimated of *Gabès* was significantly higher, compared to the introduced varieties. Osmotic potential estimated of *Hunterfield* and *Hyb.555* was appreciably equal up to the treatment 5g.l^{-1} of NaCl. Variance analysis of the osmotic potential estimated showed a different classification order: *Hunterfield* = *Hyb.555* < *Gabès*. The "salinity" effect was significant for the three varieties and kept the same classification order.

Figure 7. Osmotic potential (Ψs) measured (A) estimated (B) in leaves of three alfalfa varieties, in presence of increasing NaCl (0 - 2.5 - 5 and 10g.l-1), after three months of salt treatments. Confidence intervals were calculated at α = 95% probability level

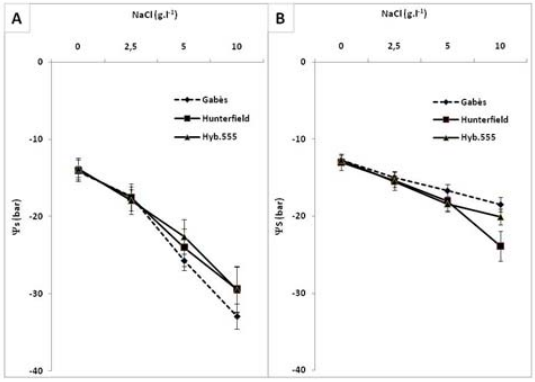


Table 6. Potential osmotic measured and estimated of three alfalfa varieties in absence and in presence of increasing NaCl concentrations (0 – 2.5 - 5 and 10g.l-1), after three months of salt stress. ΔΨs = Ψs measured - Ψs estimated

NaCl	*Gabès*			*Hyb.555*			*Hunterfield*		
	Ψs (bars)			Ψs (bars)			Ψs (bars)		
(g.l-1)	measured	estimated	ΔΨs	measured	estimated	ΔΨs	measured	estimated	ΔΨs
0	-14.2	-12.7	-1.5	-13.8	-12.7	-1.1	-14.0	-13.0	-1.0
2.5	-17.4	-15.0	-2.4	-17.9	-15.5	-2.4	-17.5	-15.4	-2.2
5	-25.7	-16.7	-9.0	-22.6	-18.4	-4.2	-24.0	-18.0	-6.0
10	-32.9	-18.5	-14.4	-29.5	-20.1	-9.4	-29.4	-23.9	-5.5

4. Discussion

Salt stress affected plant growth by perturbing physiological and biological process (photosynthesis, ion homeostasis and osmolytes accumulation). Results showed that the three alfalfa varieties exported high quantities of Na^+ into leaves and stems, which confirms the "includer" character of these varieties (Slama, 1982). These quantities were, however, more important than those accumulated in roots. In *Hunterfield*, the excessive accumulation of Na^+ ions reached toxic levels in leaf tissue, thus causing an important reduction in growth followed by a drying of aerial plant parts and the subsequent death of plants (data not shown).

In contrast, the Na^+ accumulation in *Gabès* leaf tissue was lower compared to the introduced varieties (Mezni et al., 2002; Munns, 2002; Munns et al., 2003; Sibole et al., 2003; Davenport et al., 2005; Byrt et al., 2007; Hameed and Ashraf, 2008). In addition, the Na^+ content in *Gabès* stems exceeded that in leaves. This suggested that part of the sodium in the leaves of this variety is reexported (reflux) towards stem through the phloem sap (Mühling and Läuchli, 2002; Berthomieu et al., 2003; James et al., 2006; Rodriguez-Navarro and Rubio, 2006; Apse and Blumwald, 2007). This type of "trapping" of Na^+ in *Gabès* stems constitutes an efficient way to preserve the physiological activities such as photosynthesis in leaves. Our results confirmed those found by Lohaus et al., (2000), by Mühling and Läuchli (2002), in maize, by Davenport et al., (2005) in wheat, where the salt tolerant plants accumulated less Na^+ in the shoot and also accumulated the major proportion of shoot Na^+ in leaf sheaths, by Keutgen et Pawelzik, (2008) on strawberry plant and by Misra et Saxena (2009) on lentil.

Regarding the organic osmotica, the proline content increased in all organs of the three alfalfa varieties with increasing NaCl in the rooting media, but with a higher intensity for *Gabès*. The increase of the proline in *Gabès* tissue, in response to the medium enrichment with sodium chloride

allows it (i) to attenuate the invasion of its organs by the sodium and (ii) to adjust its osmotic potential.

As with proline, the soluble sugars (reduced and hydrolysable sugars) participate to the osmotic adjustment in plants exposed to the salt stress. For the *Gabès* variety and at the most stressful treatments, the reduced and hydrolysable sugar contents in leaves were higher than those of *Hunterfield*. In the roots, the situation is very different than what has been observed. Root tissues of the two introduced varieties had higher soluble sugar contents. For *Hunterfield* and *Hyb.555*, the richness in soluble sugars in roots resulted from the acceleration of leaf senescence and the difficulty to generate new leaves (data not shown). According to Smith and Marten, (1970) and Hall, et al., (1988), the soluble sugars of the collar and roots were mobilized to support the growth and the establishment of new photosynthetic active leaves.

The water potential of the three varieties decreased with the increasing of NaCl, but with an intensity more important for *Gabès* variety, especially at 5 and 10g.l^{-1} of the NaCl. This state allowed to the local variety, to keep a gradient of the water potential between tissues organs and medium, assuring a better water supply for plants.

The comparison of the osmotic potential measured and estimated of the three alfalfa varieties, showed a difference equal 14.4 bars for *Gabès* variety, at 10g.l^{-1} of NaCl (table 6). Certainly, this difference between Ψs measured and estimated was partially due, in the fact that we did not take into account in the calculation, of the participation of the proline and the soluble sugars in the osmotic adjustment. These substances, while protecting the plant physiological functions and limited the excessive accumulation of Na^+ toxic ions in tissues. These explained the absence of necroses in *Gabès* leaves, accompanied with a higher accumulation of dry matter (data not shown).

This study also showed the prominence of *Gabès* variety in the selection program of alfalfa varieties for salt tolerance.

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