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Growth and Water Status in Narbonne Vetch (Vicia Narbonensis L.) Under Salt Stress

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Abstract: *Plants are known for their ability to resist or tolerate various types of stress (water and salt stresses, heat, frost, etc.). Particularly, water or soil salinity is a major environmental stress which limits plant growth and crop productivity. Salt alters wide metabolic and physiologic processes in plants during their growth and induces changes. In this context, an experiment was conducted in greenhouse to study the effect of increasing NaCl concentrations (0 - 2.5 - 5 and 7.5g.l^{-1}) on growth parameters (dry matter, leaf area, plant height) and water status (WC, RWC, Ψw) in Narbonne vetch (Vicia narbonensis* ***L.****). Results showed that salt stress reduced dry matter of shoot and root, plant height and leaf area which was accelerated by premature leaf senescence on one hand, and the difficulty in producing new leaves, on the other hand. NaCl decreased water content, relative water content and water potential. Pearson's correlation coefficient showed a positive and a significant relationship between the LA, plant height and the root growth. For, water status, a positive and significant relationship was observed between LA, on one hand, and RWC and WC on the other hand. This study showed that in spite of the large geographical distribution of the Narbonne vetch in Tunisia (arid to the subhumid bioclimatic level). This specie was very sensitive to the salt, and does not tolerate NaCl concentrations greater than 2.5g.l^{-1}.*
Keywords - *Dry matter (DM), leaf area (LA), Narbonne vetch, plant height, relative water content (RWC), salt stress, water content (WC), water potential (Ψw).*

I. Introduction

Vetch species (*Vicia narbonensis*, *sativa* and *villosa*) are widely used for fodder production in Tunisia (Haffani et al., 2013). Narbonne vetch has a wide ecological distribution ranging from subhumid to arid zones (Zoghlami and Hassen, 2004). The salinity in soil or water constitutes a major constraint to the development and growth of cultivated plants in the world (Ashraf and Harris, 2004; Shahid et al., 2011). Salt incidence is more severe in arid and semiarid regions where higher evapotranspiration compared to precipitation aggravates the situation. According to Athar and Ashraf, (2009) more than 100 million hectares of land throughout the world are salt affected.

In Tunisia, soils affected by salts cover approximately 1.5 million hectares, about 10% of the total area of the country (Hachicha, 2007). These areas are characterized by halomorphic soils which contain high concentrations of soluble salts, especially Na^+ and Cl^-, but also others salts such as: Na_2SO_4, $CaSO_4$, $MgSO_4$, KCl, (Zhang et al., 2013). According to Van Schilfgaarde, (1993), Shannon, (1998) and Ben Hassine, (2005) inadequate management of irrigation with a drainage system badly conceived leads to land salinity. In fact, in Tunisia more than half of irrigated lands are affected with salinity which disturb plant growth and destroy soil structure (Mezni et al., 2010). Irrigation using waters with considerable quantities of NaCl provokes an excessive accumulation of Na^+ and Cl^- and decreases soil permeability to water and air (Hachicha et al., 1997). The soil physicochemical aspects have harmful effects and a negative influence on the plant development and growth: decrease in plant height and fresh weight in wheat (Afzal et al., 2006) and in soybean (Amirjani, 2010), decrease in flower yield of saffron (Torbaghan et al., 2011), reduction in leaf area and photosynthesis in whole plant (Chaves et al., 2009) and wheat (Jamal et al., 2011). Salinity affects wheat genotypes according to its intensity and duration (Munns and James, 2003).

Salt affects plants in two ways (i) through the osmotic phase resulting from the high concentrations of salts in the soil, which make it difficult for roots to extract water and gave to plant a condition of "*physiological drought*" due to the low water potential in root medium (Dajic, 2006; Leksungnoen, 2012), and (ii) through the toxic phase due to the high accumulation of salts in the plant organs (Afzal et al., 2006; Munns and tester, 2008). The salinity causes many adverse effects on plant growth, because of the low osmotic potential of soil solution (osmotic stress), specific ion effects (salt stress), nutritional imbalances, or a combination of these factors (Ashraf, 2004; Athar and Ashraf, 2009).

Salinity inhibits shoot growth by disturbing the photosynthesis (Jamil et al., 2007; Dadkhan, 2011) in radish and sugar beet respectively, or by declining the turgor of expanding tissues due to an insufficient osmoregulation. Salt stress affects water status in plants. Indeed, leaf water potential of *Pueraria lobata* (Al-Hamdani, 2004) and Brassica species (Singh et al., 2010) decreased with the increase in NaCl within the growth medium due to the increase of solute accumulation in the cell. The objective of this study was to determinate the extent of the Narbonne vetch tolerance to salt, as it constitutes an interest plant for livestock feeding and soil fertility due to its capacity to fix atmospheric nitrogen through symbiosis.

II. Materials And Methods

Plant material and growth conditions

Vetch plants were cultivated in greenhouse under natural light, temperatures and relative hygrometry. The seedling was carried out in plastic pots (volume = 2litres) containing an equilibrated soil, composed with a culture substrate (2/3 soil and 1/3 sand) to facilitate plant extraction and to keep maximum roots. Chemical and physical characteristics of the soil are given in table 1. Each pot was sown with two germinated seeds. Seedlings were first irrigated with tap water during establishment. At the two leaves stage, salinity stress was induced by adding NaCl into irrigation water at 4 concentrations (0 - 2.5 - 5 and 7.5g.l^{-1}). The experimental design included 4 treatments with 4 complete randomized replications. To avoid osmotic shocks, the salt was supplied with irrigation water using gradually increasing fractions until the desired level is reached for each treatment. Every two days, the pots are irrigated to compensate water losses by evapotranspiration.

Plant Measurements

Growth parameters, including plant height, leaf area (LA) and biomass were measured using whole plants harvested after a salt stress period of twenty-four days. The LA was calculated by the method of weighting, on leaves completely developed. Leaves are quickly photocopied and their imprints carefully cut and weighed. The LA was then deducted, with the weight of a known surface area. The LA per plant was obtained by multiplying the average LA by the corresponding leaves number (Mezni et al., 2012).

The fresh weight of each organ (roots, stems and leaves) was determined on plant carefully pulled up to keep the maximum of the root system. The plant was separated in shoot and root at the level of the crown. The shoots were separated in leaves and stems. Dry matter of leaves, stems and roots was obtained by desiccation at 105°C for 48 hours. Plant height was measured with a graduated ruler in mm.

Table 1. Physical and chemical characterization of culture substrate

Parameters	Values
Clay (%)	30.0
Silts (%)	22.0
Sands (%)	46.7
pH (1/2.5)	8.3
Saturation (ml/100g)	40.0
Conductivity (mmho/cm)	5.2
Total chalk (%)	12.3
Organic matter (%)	0.8
Carbone	0.5

Water parameters were determinate after a salt stress period of sixteen days. *Relative water content* (*RWC*) was measured in eight fully expanded leaves without petiole. Leaves were immediately weighed after the harvest to determine their fresh weight (*FW*); they were then immersed on distilled water in Petri dishes for 4 hours (required time to regain turgidity after many essays). Then, leaves were carefully wiped with filter paper to remove droplets water on their surface and weighed to determine turgid weight (*TW*). The samples were dried at 80°C for 24 h to determine the dry weight (*DW*). RWC was defined as follows:

$$RWC (\%) = [(FW - DW)/(TW - DW)]x100$$

Water content (*WC*) was given by the following formula:

$$WC (g\ H_2O.g^{-1}\ DM) = (FW - DW)/DW$$

The ***water potential*** (Ψw) was measured using a Scholander pressure chamber (type PMS Instrument CO, Corvallis, Oregon, USA).

Statistical analysis

Variance analysis of all parameters was conducted using the SAS software (version 8.0) via ANOVA procedure. The comparison between treatments was made using the Duncan test. The association between the various traits was assessed using Pearson correlation coefficients estimated via proc. Corr. of SAS. Confidence intervals were calculated to the threshold of 95% probability.

III. Results

Shoot dry matter (DM)

Figure 1 shows the variation of shoot dry matter. Result revealed that the aerial dry matter was significantly affected by increasing NaCl concentrations in pot experiment. Indeed, at 7.5g^{-1} NaCl, the decrease reached 29.5 % in comparison with the control treatment.

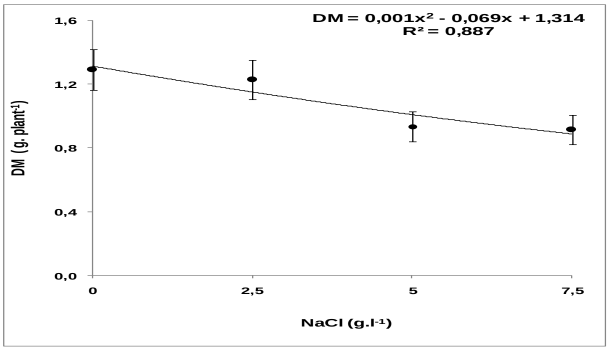

Figure 1. Shoots dry matter of Narbonne vetch (*Vicia narbonensis* L.), harvested after a salt stress period of 24 days in presence of increasing NaCl (0 - 2.5 - 5 and 7.5g.l^{-1}). Each point is the mean of 4 individual values. Confidence intervals were calculated at α = 95%.

Root dry matter

Figure 2 gives the variation of the root dry matter under increasing salt concentrations. The root mass decreased significantly from one treatment to another. The decrease in root dry matter reached 52.8% at the most stressful treatment (7.5g.l^{-1} NaCl). Compared to shoot dry matter, the root biomass was more sensitive to salt stress.

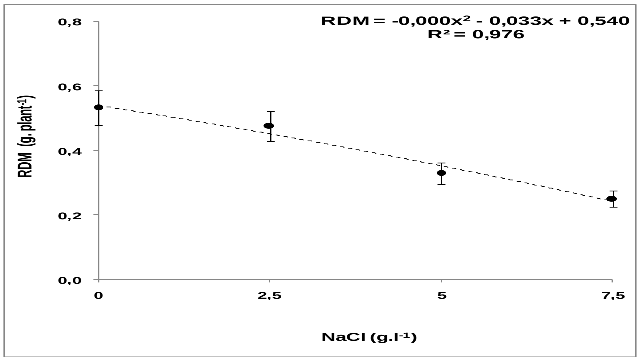

Figure 2. Roots dry matter (RDM) of *Vicia narbonensis* L., harvested after a salt stress period of twenty-four days in presence of increasing NaCl (0 - 2.5 - 5 and 7.5g.l^{-1}). Each point is the mean of 4 individual values. Confidence intervals were calculated at α = 95%.

Shoot to Root (S/R) ratio

Figure 3 shows the variation in the shoot to root ratio. The S/R ratio increased with the rise of NaCl concentration in the soil medium. The distribution of photoassimilats between shoot and root organs reflects the sensibility of roots towards Na^+ and Cl^- aggression in salt environments.

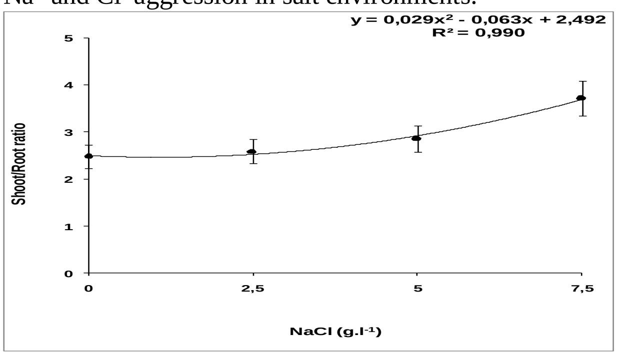

Figure 3. Shoot/Root ratio of Narbonne vetch, harvested after a salt stress period of twenty-four days in presence of increasing NaCl (0 - 2.5 - 5 and 7.5g.l^{-1}). Each point is the mean of 4 individual values. Confidence intervals were calculated at α = 95%.

Leaf area (LA)

Figure 4 represents the variation in leaf area, in absence and in presence of increasing NaCl concentrations. Results showed that, after 24 days of salt stress, leaf area was significantly reduced with the increase in salt concentrations. Indeed, the dramatically decrease in leaf area per plant, in response to the increase of salt in the soil medium is the result of the acceleration of premature senescence of old leaves and of the difficulty to regenerating new photosynthetically active leaves.

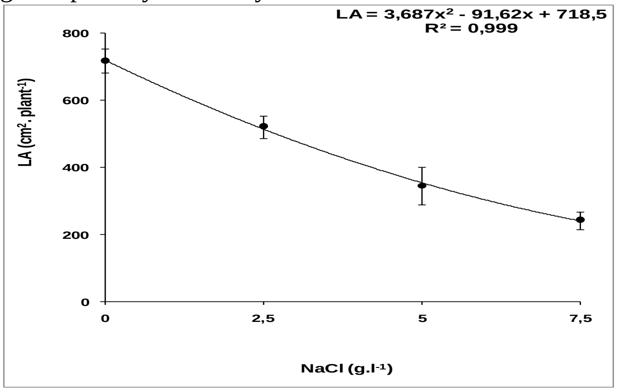

Figure 4. Leaf area (LA) of Narbonne vetch (*Vicia narbonensis* L.), harvested after a salt stress period of 24 days in presence of increasing NaCl (0 - 2.5 - 5 and 7.5g.l^{-1}). Each point is the mean of 4 individual values. Confidence intervals were calculated at α = 95%.

Plant Height

Narbonne vetch height is an important parameter of biomass production (figure 5). Results showed a significant decrease in plant height with the increase in salt concentrations. The decrease reached 38.9% at the most stressful treatment (7.5g.l^{-1}). It is admitted that height growth, in non limiting conditions (sufficient water and mineral supply) is the result of the increase in cell number and of cellular expansion (increase in cell volume) as reported by Neves-Piestun and Bernstein, (2001) in maize.

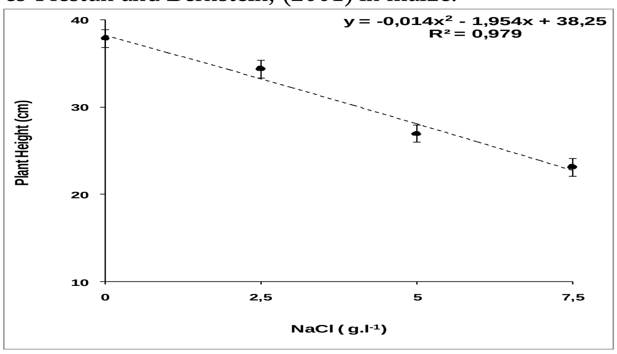

Figure 5. Plant height of Narbonne vetch, harvested after a salt stress period of twenty-four days in presence of four different levels of salinity (0 - 2.5 - 5 and 7.5g.l^{-1}). Each point represents the mean of 4 individual values. Confidence intervals were calculated at α = 95%.

Water Content (WC)

Water content of leaves is a good indicator of the water status in the plant. It decreased with the increase in NaCl concentration. The decrease in WC can be attributed to the difficulty of this specie to control its osmotic adjustment (Fig. 6). Narbonne vetch was classified in the category of glycophytes sensitive, especially for higher salt concentrations (7.5 g l^{-1}). However, plants remained enough hydrated with a water content of 4.7 gH_2O per gram of DM under the most stressful treatment.

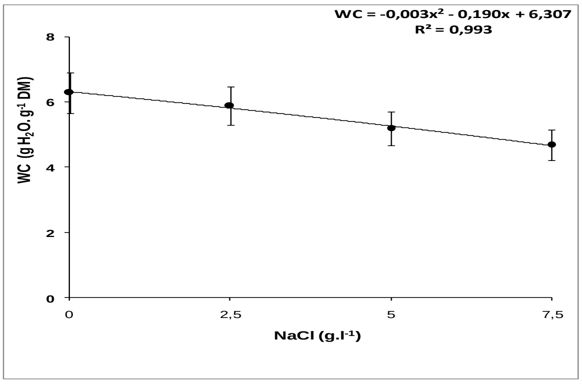

Figure 6. Water content (WC) in *Vicia narbonensis* L., measured after a salt stress period of 16 days, in presence of four different levels of salinity (0 - 2.5 - 5 and 7.5g.l^{-1}). Each point is the mean of 4 individual values. Confidence intervals were calculated at α = 95%.

Relative water content (RWC)

Figure 7 gives the variation of RWC in response to the increase in the NaCl concentrations in the culture medium. RWC represents the difference between leaf water status in ambient conditions and that under water saturation conditions. RWC decreased with the increase in NaCl. However, vetch plants remained enough hydrated as the RWC in the most stressful treatment (7.5g l^{-1}) was 81%.

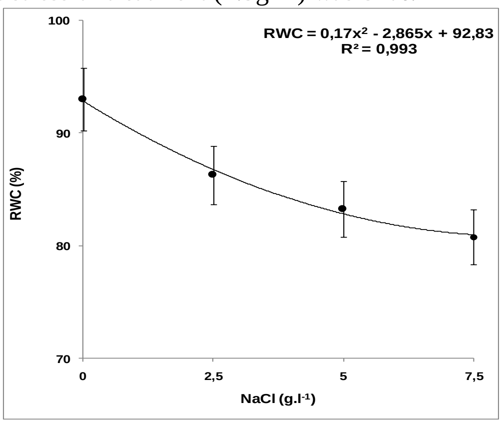

Figure 7. Relative water content (RWC) of Narbonne vetch, measured after a salt stress period of 16 days, in presence of four different levels of salinity (0 - 2.5 - 5 and 7.5g.l^{-1}). Each point is the mean of 4 individual values. Confidence intervals were calculated at α = 95%.

Water potential (Ψw)

Figure 8 shows that leaves water potential decreased from one treatment to the other with the rise of NaCl in soil. The decrease in Ψw allowed the Narbonne vetch to keep a gradient of the water potential between tissue organs and the medium, they ensuring a better water supply for plants.

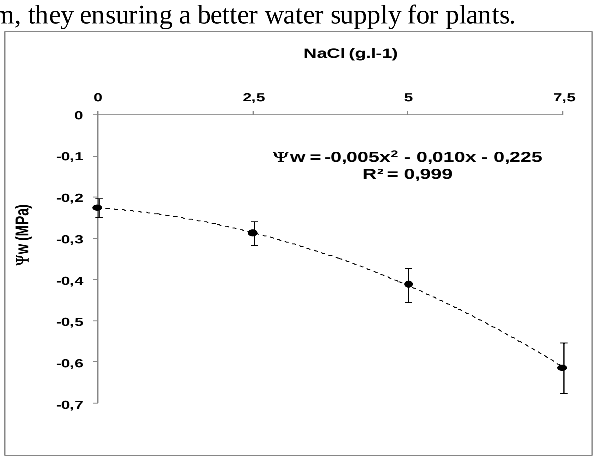

Figure 8. Water potential (Ψw) of Narbonne vetch, measured after a salt stress period of 16 days, in presence of four different levels of salinity (0 - 2.5 - 5 and 7.5g.l^{-1}). Each point is the mean of 4 individual values. Confidence intervals were calculated at α = 95%.

IV. Discussion

Salinity is a major environmental factor that limits plant growth and crop productivity, and different crops may have varying in salt-tolerant mechanisms (Xiao-shan and Jian-guo, 2009). This work was conducted in order to evaluate the effect of salinity stress on growth parameters and water changes in Narbonne vetch grown under controlled conditions with and without increasing NaCl concentrations. During the experimentation, we studied the effects of salinity duration and intensity on growth and water parameters measured respectively at 24 and 16 days, to determine the degree of sensibility or tolerance of the Narbonne vetch.

Results showed that growth parameters (dry matter, plant height, leaf area, leaf/stem ratio), measured during a short salt exposure, decreased with the increase in salt concentrations. In Narbonne vetch, LA was the most affected by salt stress. Hence, plant reduced the leaf area in response to salt stress using two mechanisms: first by regenerating young small leaves; secondly, by accelerating the senescence of old leaves. Our results agree with those found by Kaddour et al., (2010) on *Arabidopsis thaliana*; Dolatabadian et al., (2011) on soybean; Tavakkoli et al., (2011) on barley; Mezni et al., (2012) on alfalfa. Under salt stress, Narbonne vetch is characterized by the drying of old leaves, which eventually fall because of premature senescence. Moreover, newly formed leaves undergo a strong reduction in their size. Sun et al., (2013) reported in *Arabidopsis* that *GASA14* could depress reactive oxygen species (ROS) accumulation. These authors demonstrated that *GASA14* regulates leaf expansion and salt stress resistance by modulating ROS accumulation, which is responsible for leaf elongation in maize (Rodríguez et al., 2002).

Starting at a concentration of 5g and especially at $7.5g.l^{-1}$ of NaCl, we observed a drying of the leaves which become severe under the most stressful treatment (photo 1). The salt stress has two effects: (1) an osmotic effect, resulting from a bad absorption of water supply, and/or (2) a toxic effect due to the excessive accumulation of Na^+ and Cl^-. To determine which of both effects is involved, withered plants were transferred into a medium without salt, if plants resume their growth, this is an osmotic effect. If plants remain withered, that means that organs were invaded by Na^+ and Cl^- and reached the toxicity threshold. Result showed, that after transferring Narbonne vetch plants in a salt free environment, plants exposed to $5g.l^{-1}$ of NaCl resumed their growth, while those cultivated under the stressful treatment ($7.5g.l^{-1}$) remained withered, suggesting that plants accumulated an excessive Na^+ and Cl^- in their leaves, which exceeds the threshold of toxicity. Hu et al., (2011) found that NaCl treatments caused toxicity to perennial ryegrass. Our results agree with those found by Wang et al., (2012) and by Kravchik and Bernstein, (2013) on rice and maize respectively where old leaves accumulated more Na^+ and Cl^- than the younger one.

Photo 1. Plants of Narbonne vetch (*Vicia narbonensis* L.), after 24 days of salt stress, at presence of four different salinity levels (N1=0 – N2=2.5 – N3=5 and N4=$7.5g.l^{-1}$).

Our study showed the importance of LA of Narbonne vetch in the root growth and the plant height (table 2). Indeed, Pearson's correlation coefficient showed a positive and significant relationship between the LA and the root growth ($r = 0.972$, $P<0.05$) and the LA and plant height ($r = 0.983$, $P<0.05$).

The decrease in growth (plant height and DM of shoots and roots) was the result of a progressive aggression of Na^+ and Cl^- which invaded the leaves, causing burns and necrosis highly visible with the stressful treatment ($7.5g.l^{-1}$). Similar results were found by Oztekin and Tuzel, (2011) and by Zhang et al., (2012) on tomato genotypes and cotton respectively. Narbonne vetch exhibited various degrees of damage and its growth was limited in presence of sodium chloride. Shoot dry matter was significantly and positively correlated with root DM ($r = 0.977$, $P<0.05$). Plant height, was highly and positively correlated with root and shoot DM ($r = 0.998$, $P<0.01$ and 0.976, $P<0.05$ respectively). The decrease in growth was reported by Borowski, (2008) on four perennial ryegrasses and by Boughalleb et al., (2012) on *Nitraria retusa*.

In saline condition, the osmotic constraint is the first difficulty which plant is confronted. The salt stress disturbs plant water status and mineral nutrition. To resist for excessive NaCl in root medium, plant should maintain their turgidity and be able to support a positive growth. In Narbonne vetch, RWC and WC decreased in response to the increase of NaCl in the culture medium. Our results agree with those found by Qin et al., (2010) on *Shepherdia argentea* seedlings, by Heidari et al., (2011) on twelve sunflower lines and by Saleh, (2013) on cotton. In spite of the decrease in WC, however, the Narbonne vetch remains enough hydrated with 4.7g of water by g of DM (fig. 6).

RWC and WC were positively and significantly correlated with LA (r = 0.989, P<0.05 and r = 0.984, P<0.05, respectively). Indeed, the decrease of the two water parameters reduced plant leaf area by decreasing young leaves growth and by accelerating premature senescence of old leaves. Mutually, the decrease in LA, limits the organ dehydration of Narbonne vetch via leaf transpiration. Suriya-arunroj et al., (2004) used the leaf relative water content as a criterion for screening in rice. For Boyer et al., (2008), Barley and wheat relative water contents were used extensively to determine the water status of plants relative to their fully turgid in saline condition.

WC, was highly and positively correlated with root DM and plant height (r = 0.997, P<0.01 and 0.998, P<0.01 respectively). Indeed, the root growth entails the exploration of an additional soil volume and thus gives to the plant a good supply of water. For plant height growth, this parameter is related to cellular division and expansion (increase in volume) which is strongly bounded with the water content in plant.

Leaf water potential decreased with the rise of NaCl in soil medium. The decline in Water potential allows the Narbonne vetch to have a water gradient between the leaf tissues and the culture medium, allowing it to have a water supply. Water potential is positively correlated with root DM, plant height and WC (r = 0.970, P<0.05, r = 0.966, P<0.05 and r = 0.977, P<0.05, respectively) and negatively correlated with shoot to root ratio (r = -0.983, P<0.05).

In conclusion, in spite of its vast geographical distribution in Tunisia, the Narbonne vetch is better adapted to the drought than to salt stress (Haffani et al., 2013). Indeed, the plant does not support NaCl concentration greater than to 2.5g.l^{-1} and therefore, this specie is not adapted to the salt soil.

Table 2. Pearson's correlation coefficients (r) between the measured parameters and their level of significance.

	Shoot DM	Root DM	Sh/R ratio	Plant height	LA	RWC	WC	Ψ_W
Shoot DM	1							
Root DM	0.977*	1						
Sh/R ratio	-0.805NS	-0.911NS	1					
Plant Height	0.976*	0.998**	-0.901NS	1				
LA	0.949NS	0.972*	-0.857NS	0.983*	1			
RWC	0.898NS	0.925NS	-0.809NS	0.945NS	0.989*	1		
WC	0.963*	0.997**	-0.920NS	0.998**	0.984*	0.949NS	1	
Ψ_W	0.897NS	0.970*	-0.983*	0.966*	0.935NS	0.891NS	0.977*	1

Sh/R ratio=Shoot/Root ratio; LA=leaf area; DM=dry matter; RWC=relative water content; Ψw=water potential. Means followed by NS are not significant (P > 0.05); *: (P < 0.05) and **: (P < 0.01).

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