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MOHAMED FARISSI

ABDELAZIZ BOUIZGAREN

MUSTAPHA FAGHIRE

ADNANE BARGAZ

CHERKI GHOULAM

TÜBİTAK

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Agrophysiological and biochemical properties associated with adaptation of *Medicago sativa* populations to water deficit

Mohamed FARISSI[1,2,*], Abdelaziz BOUIZGAREN[2], Mustapha FAGHIRE[3], Adnane BARGAZ[1], Cherki GHOULAM[1]

[1]Unit of Plant Biotechnology and Agrophysiology of Symbiosis, Faculty of Sciences and Techniques, Gueliz, Marrakesh, Morocco
[2]Unit of Plant Breeding, National Institute for Agronomic Research, Gueliz, Marrakesh, Morocco
[3]Department of Biology, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco

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Abstract: The effect of water deficit on growth and some physiological and biochemical parameters related to water deficit tolerance was studied in 4 Moroccan alfalfa *Medicago sativa* L. populations that originated from the mountains and oases of Morocco. The experiment was conducted in greenhouse conditions. Seeds were allowed to germinate in pots filled with sand and peat at a 2:1 ratio, respectively. After 1 month of sowing, the plants were subjected to 3 water regimes: optimal irrigation (75% of field capacity, FC), moderate water deficit (50% FC), and severe water deficit (25% FC). At 45 days of stress, the plants were harvested and subjected to some agrophysiological and biochemical analyses related to tolerance. Results showed that the water deficit negatively affected dry biomass, membrane permeability, leaves' relative water contents, and nitrate and phosphorus contents. The behaviors of tested populations were significantly different for most of the considered parameters. The tolerance was positively correlated to the ability of plants to overcome oxidative stress by the induction of antioxidant enzyme activity, accumulation of inorganic ions (Na^+ and K^+), and maintenance of an adequate level of nitrate reductase and acid phosphatase activities.

Key words: Alfalfa, water deficit, membrane permeability, oxidative stress, nitrate reductase, phosphatase

1. Introduction

In the arid and semiarid areas, the availability and the management of irrigation water have become priorities of great importance. In Morocco, the successive years of drought, induced by climate change and population growth, increasingly reduced the amount of water reserved for agriculture. Consequently, a rational and efficient use of irrigation water will be helpful to solve this problem.

Medicago sativa L. (alfalfa) is one of the forage species endowed with many socioeconomic and environmental advantages. It is the main forage legume in crop–livestock systems of Morocco, and in many European countries and North America, owing to its contribution to sustainable agriculture and its production of feed proteins per unit area (Farissi et al., 2011; Bouizgaren et al., 2013). However, this plant requires water. Hence, the determination of minimum irrigation amount and the tolerance mechanisms of plants under water deficit can be helpful to solve the problem of water deficit, which constitutes the majority of the environmental constraints and affects great areas of Morocco, inducing consequently an important decline of yields.

Under water deficit, plants develop many agrophysiological and biochemical changes (Pérez-Pérez et al., 2009; Carmen Antolín et al., 2010). This constraint engendered reduction of plant growth by affecting various processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism, and growth promoters (Jaleel et al., 2008; Bouizgaren et al., 2013). Understanding plant responses to drought is of great importance and also a fundamental part of making crops stress-tolerant (Reddy et al., 2004). In this context, the objective of our study is to evaluate the effect of water deficit occurring during the vegetative phase on growth and yield forage in some Moroccan populations of *Medicago sativa* L. and the impact of this environmental constraint on some physiological and biochemical process.

2. Materials and methods

2.1. Plant material and growth conditions

Four Moroccan alfalfa populations, Tafilalet 1 (Taf 1), Tafilalet 2 (Taf 2), Demnate (Dem), and Tata, were used in this study. Seeds were supplied by National Institute for Agronomic Research (INRA, Marrakech, Morocco). These

* Correspondence: farissimohamed@gmail.com.

populations originated from different Moroccan regions of mountains and southeastern and southwestern oases, where they have been cultivated for many centuries and are still widely used by farmers in traditional agroecosystems. Continuous natural and human selection has led, by this time, to their adaptation to the local habitats with distinction in the agromorphological characteristics of the landraces, which have reached Hardy–Weinberg equilibrium. The experiment was conducted in a greenhouse at 32/22 °C day/night, 50%–80% relative humidity, and a photoperiod of 16 h (18,000 lx). Seeds were allowed to germinate in pots 20 cm in diameter and 30 cm high filled with sterile sand (previously rinsed with distilled water) and sterile peat at a 2:1 ratio, respectively. After emergence of the first true leaves at 15 days after germination, the number of plants was adjusted to 6 per pot and they were irrigated every 2 days. The pots (6 plants) were arranged in a simple randomized design and each pot was considered as 1 replicate with 3 pots per treatment per population. At 20 days after germination, a half-strength Hoagland's nutrient solution was given once a week. After 1 month, plants were subjected to 3 water treatments: optimal irrigation (75% of field capacity, FC), moderate water deficit (50% FC), and severe water deficit (25% FC). After 45 days of stress, the plants were harvested, measured, and subjected to different physiological and biochemical analyses.

2.2. Biomass measurements

The plants were removed from the pots at 45 days of stress. Shoots and roots were separated. For dry weight (DW) determination, the shoots and roots were dried at 70 °C for 48 h and weighed. For standardizing data, the results were expressed as the relative reduction of yield in comparison to the optimal irrigation (75% of FC), using the following formula:

Relative reduction (%) = [(1 – (stressed / optimal irrigation)] × 100 (Ghoulam et al., 2002).

2.3. Relative water content

Relative water content (RWC) was estimated by recording the turgid weight of 0.1 g of fresh leaflet samples by keeping the samples in water for 4 h (TW), followed by drying in hot air oven until a constant weight was achieved (DW).

RWC = ((FW – DW) – (TW – DW)) × 100 (Ghoulam et al., 2002).

2.4. Electrolyte leakage

Electrolyte leakage was determined according to the method described by Ghoulam et al. (2002) with minor modification. First, 0.3 g of fresh leaves tissue was rinsed with distilled water and immersed in 20 mL of distilled water overnight at 25 °C on a rotatory shaker. Conductivity of the solution (C_1) was measured using a conductivity meter. Samples were then heated in boiling water for 15 min and cooled to room temperature. Conductivity of killed tissues (C_2) was measured again. Electrolyte leakage was calculated as the ratio of C_1 to C_2.

2.5. Nitrate reductase activity

The nitrate reductase activity (NRA; E.C. 1.6.6.1) was used as an indicator of the damaging effects of stress. NRA was measured in leaves (0.1 g) according to the method of Heuer and Plaut (1978). The leaf samples were infiltrated under vacuum in 10 mL of 50 mM phosphate buffer, pH 7.5, containing 0.1 M KNO_3, and 0.1% Triton X-100. After 5 min, the samples were transferred into an identical solution but without Triton X-100 and were incubated for 1 h at 28 °C. For determination of the nitrite formed, 1 mL of the solution was supplemented with 0.25 mL of 1.5 M HCl, containing 1% sulfanilamide and 0.25 mL of a 0.02% solution of N-(1-naphthylethylenediamine) 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 produced μmol NO_2^- h^{-1} g^{-1} FM.

2.6. Nitrate content determination

The leaves' nitrate contents (NO_3^-) were estimated as described by Agbaria et al. (1996). Leaf samples of 100 mg FW were extracted for 60 min in deionized water at 45 °C. After centrifugation at 6000 × *g* for 15 min, 200 μL of the supernatant was incubated at ambient temperature (around 24 °C) with 0.8 mL of 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.

2.7. Acid phosphatase activity

Leaves or roots (100 mg) were ground in 2 mL of sodium acetate buffer (0.1 M, pH 5.8). Homogenates were centrifuged at 13,000 × *g* at 4 °C for 30 min, and aliquots of 50 μL of the supernatants were used for acid phosphatase activity (APA) assay.

The APA (ACP, E.C. 3.1.3.2) was assayed according to the method described previously by Mandri et al. (2012), using p-nitrophenyl phosphate as the substrate. A total reaction volume of 1 mL was prepared for each sample and incubated at 30 °C for 30 min. The reaction was stopped with 3 mL of 0.2 M NaOH, and the APA was measured spectrophotometrically at 405 nm. A standard curve was established with p-nitrophenol solutions.

2.8. Inorganic ions analysis

For sodium (Na^+), potassium (K^+), and phosphorus (P) analyses, samples (0.5 g) of dried leaves or roots were ashed in a furnace for 6 h 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 phosphorus content was determined calorimetrically using the molybdate blue method (Murphy and Riley, 1962). P concentration was measured by reading the absorbance at 820 nm after color development at 100 °C for 10 min. A standard curve was established with KH_2PO_4 solutions.

2.9. Lipid peroxidation

The lipid peroxidation was evaluated by the contents of malonyldialdehyde (MDA) that were determined according to the method previously described by Dhindsa et al., (1981). The leaves or roots (100 mg) were crushed in 1.5 mL of thiobarbituric acid at 0.1%. The homogenate was then centrifuged at 10,000 × *g* for 10 min. The supernatant obtained (1 mL) was added with 1 mL of trichloroacetic acid at 20% containing 0.5% thiobarbituric acid. The mixture was heated at 95 °C for 30 min. The reaction was stopped by a bath of ice followed by centrifugation at 10,000 × *g* for 10 min. The absorbance of the supernatant obtained was determined at 532 nm. Nonspecific absorbance at 600 nm was measured and subtracted from the 532 nm readings. The MDA contents were calculated using the molar extinction coefficient of the MDA (1.55×10^5). The results were expressed as µmol MDA/g fresh weight (FW).

2.10. Peroxidase and Catalase (CAT) activities

The leaves or roots (100 mg) were crushed in 1 mL of phosphate buffer (20 mM, pH 7). The homogenate was centrifuged at 15,000 × *g* for 20 min at 4 °C. The supernatant obtained was used for the determination of the guaiacol peroxidase (POD; EC 1.11.1.7) enzymatic activity according to the technique previously described by Hori et al. (1997). The reaction mixture consisted of 200 µL of H_2O_2 at 0.3%, 300 µL of guaiacol at 20 mM, 2 mL of phosphate buffer (0.1 M, pH 6), 1 mL of distilled water, and 10 µL of enzymatic extract. After 2 min, the POD activity was determined at 470 nm against a control, where the enzymatic extract was replaced by distilled water. The activity of POD was presented as µmol H_2O_2 min^{-1} mg $protein^{-1}$.

The catalase (CAT; EC 1.11.1.6) activity was determined according to the method described by Gong et al. (2001). First, 100 mg of leaves or roots was homogenized in 1.5 mL of Tris-HCl buffer (pH 8.5), including 2 mM EDTA and 10% (w/v) PVPP. The homogenate was centrifuged at 16,000 rpm for 14 min at 4 °C. Supernatant was used for the activity measurement. Enzyme extract (250 µL) was added to 2 mL of assay mixture (50 mM Tris-HCl buffer, pH 6.8, containing 5 mM H_2O_2). The reaction was stopped by adding 250 µL of 20% titanic tetrachloride (in concentrated HCl, v/v) after 10 min at 20 °C. A blank was prepared by addition of 250 µL of 20% titanium tetrachloride at zero time to stop the enzyme activity. The absorbance of the reaction solutions was read at 415 nm against water. CAT activity was determined by comparing absorbance against a standard curve of H_2O_2 from 0.25 to 2.5 mm. The activity of CAT was presented as µmol H_2O_2 min^{-1} mg $protein^{-1}$.

3. Results

3.1. Effect of water deficit on dry biomass

Figure 1 indicates that water deficit caused a significant reduction ($P < 0.001$) in dry biomass of the studied alfalfa populations compared to the optimal irrigation (75% FC). The decrease was more pronounced when the severity of water deficit was greater. The behavior of the tested populations was significantly different for the considered agronomical parameter (Table 1). Indeed, at severe water deficit (25% FC), the Tata population appeared to be the more tolerant population, showing reductions of 22.48% and 29.23% for shoot dry weight (SDW) and root dry weight (RDW), respectively. However, the Taf 1 population showed reductions of 34.45% and 40.22% for

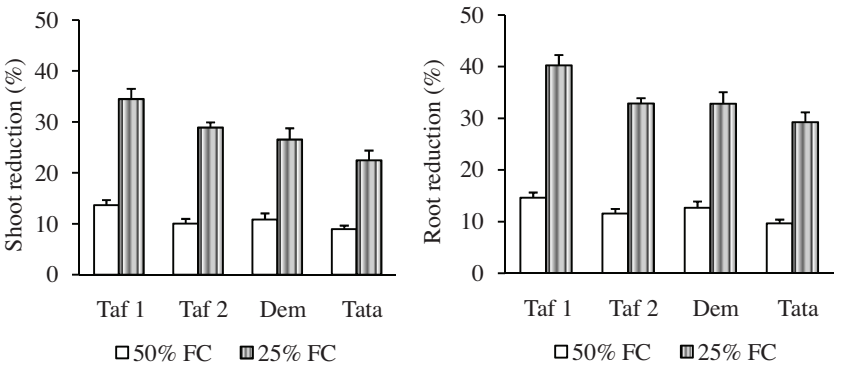

Figure 1. Effect of water deficit on dry biomass in plants of 4 Moroccan alfalfa populations. Results are expressed as reduction percentage of optimal irrigation (75% FC). Values are means of 3 replicates, bars are SE.

Table 1. Results of 2-way analysis of variance (ANOVA II) of water deficit and population effects and their interactions for the considered parameters.

		Independent variables	
Dependent variables	Water deficit	Population	Water deficit × population
Shoots' dry weight	206.00***	5.19*	0.71NS
Roots' dry weight	400.09***	9.33**	1.58NS
Relative water content	9.45**	0.59NS	0.06NS
Electrolyte leakage	128.94 ***	0.38 NS	0.68 NS
Shoots' nitrate	30.35***	3.12*	1.07NS
Shoots' Na^+	362.60***	14.81***	3.933**
Roots' Na^+	420.21***	21.19***	30.21***
Shoots' K^+	161.48***	11.30***	5.06**
Roots' K^+	215.81***	8.08***	3.13*
Shoots' P	525.27***	8.63***	5.25**
Roots' P	289.68***	7.20**	5.47**
Nitrate reductase activity	65.58***	9.66**	1.36 NS
Roots' acid phosphatase activity	81.22***	4.21*	1.12NS
Leaves' acid phosphatase activity	48.73***	1.59NS	0.24NS
MDA - roots	1071.05***	3.91*	4.23**
MDA - leaves	353.50***	4.72*	6.16**
POD - roots	276.70***	13.53***	3.67*
POD - leaves	291.25***	13.26***	3.49*
CAT - roots	212.85***	23.03***	11.60***
CAT - leaves	598.28***	33.06***	10.86***

*: Significance at 0.05 probability level; **: significance at 0.01 probability level; ***: significance at 0.001 probability level; NS: not significant at 0.05.

SDW and RDW, respectively, and it is considered the less tolerant population in terms of DW compared to the rest populations that showed intermediate reductions, with preference for the Dem population.

3.2. Effect on RWC

The results obtained (Figure 2) for the effect of water deficit on RWC showed that this constraint, especially at 25% FC, caused a significant reduction on this parameter ($P < 0.01$) compared to optimal irrigation. Significant reduction was noted in Taf 1 compared to the other considered populations. However, ANOVA testing showed that there was no significant difference among the tested populations ($P > 0.05$). The interaction effect was also not significant ($P > 0.05$; Table 1).

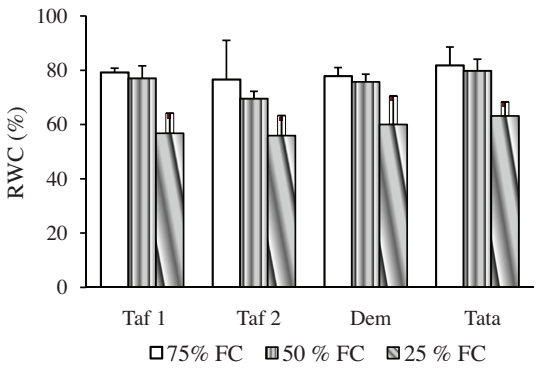

Figure 2. Effect of water deficit on leaves' relative water content in plants of 4 Moroccan alfalfa populations. Values are means of 3 replicates, bars are SE.

3.3. Effect on membrane permeability

The effect of water deficit on membrane permeability was estimated according to the electrolyte leakage. Data in Figure 3 show that the water deficit caused a highly significant increase in electrolyte leakage in all tested populations ($P < 0.001$). The lower values for electrolyte leakage were recorded in the optimal irrigation. At severe stress, comparison between the used populations showed that Tata was less affected (40.64%) and Taf 1 was more affected by this constraint (46.41%). However, Dem and Taf 2 showed 41.09% and 41.10%, respectively.

3.4. Effect on NRA and nitrate content

Figure 4 shows that the water stress caused a great reduction in NRA and nitrate content ($P < 0.001$) with a significant difference among the used populations. Indeed,

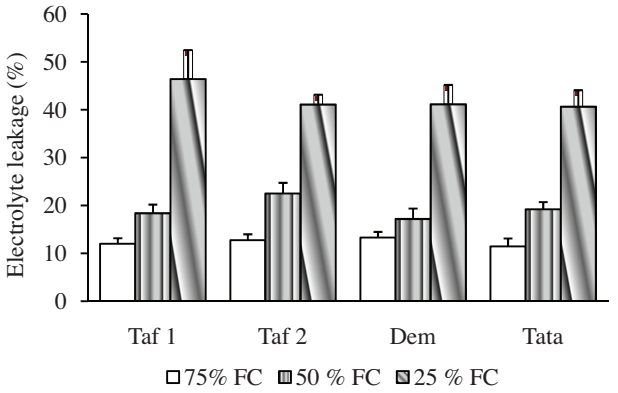

Figure 3. Effect of water deficit on leaves' electrolyte leakage in plants of 4 Moroccan alfalfa populations. Values are means of 3 replicates, bars are SE.

the lower values were noted in the Taf 1 population (0.95 µmol NO^-_2 min^{-1} g^{-1} FM and 22.98 mg g^{-1} FM for NRA and nitrate, respectively). Meanwhile, Tata showed higher NRA (1.25 µmol NO^-_2 min^{-1} g^{-1} FM) and nitrate content (27.59 mg g^{-1} FM). The values recorded for Dem and Taf 2 were intermediary.

3.5. Effect on APA and phosphorus content

Data reported in Table 2 signal a significant decrease in roots' and leaves' P contents in the considered alfalfa populations under water deficit conditions ($P < 0.001$). P content was significantly varied among the tested populations and between both plant organs. The roots appeared to have more P content than shoots. Table 2 also shows that this constraint caused a significant increase in the APA ($P < 0.001$) in all considered populations and in both organs, with significant differences between populations. The Tata population appears to have the highest activity of the populations, reaching, at severe stress, 3.64 and 2.89 µg p-NP min^{-1} g^{-1} FM in the roots and leaves of plants, respectively. For Dem and Taf 2, the APA reached 3.49 and 3.39 µg p-NP min^{-1} g^{-1} FM in roots, respectively, and 2.78 and 2.80 µg p-NP min^{-1} g^{-1} FM in leaves, respectively.

3.6. Effect on Na^+ and K^+ uptake

Figure 5 indicates the effect of water deficit on Na^+ and K^+ uptake. Under this environmental constraint, the data indicate an increase in the uptake of these inorganic ions in both plant organs ($P < 0.001$). The increase was proportional to the severity of the stress. Generally, high values were recorded in roots but not shoots. In both organs, the behaviors of the tested populations were significantly different ($P < 0.001$). At the used severe stress (25% FC), the Tata population accumulated more Na^+ and K^+ in both organs compared to other populations that showed almost the same values, with a slight diminution in the Taf 1 population. The interaction effect was also significant (Table 1).

3.7. Effect on lipid peroxidation

The effect of water deficit on lipid peroxidation was estimated by MDA accumulation in organs of the studied populations. Results noted in Table 3 signal that the application of water stress caused a significant increase of MDA in roots and shoots of plants ($P < 0.001$). This accumulation was more important at the severe stress. A significant difference was noted ($P < 0.05$) among the used populations and in both organs. Higher values were recorded in roots than in shoots. During the used severe stress, the Taf 1 population accumulated a higher content of MDA compared to the other populations (29.37 and 23.20 µmol MDA g^{-1} FM in roots and leaves, respectively). However, lower values were shown in Tata populations,

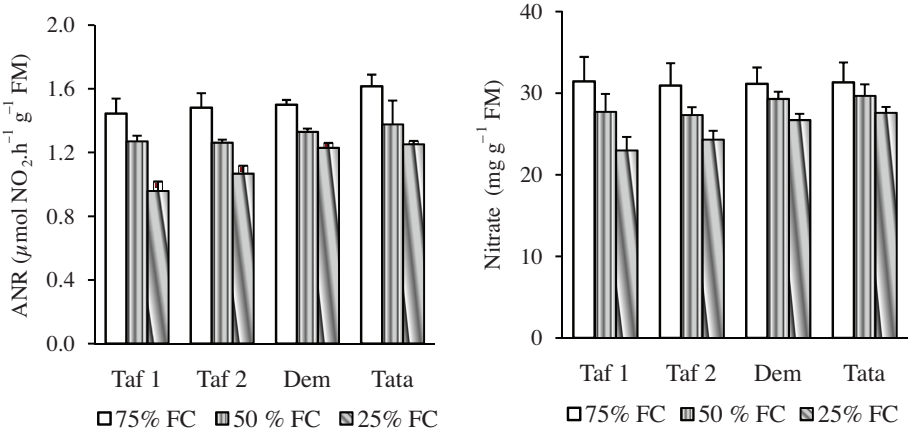

Figure 4. Effect of water deficit on leaves' nitrate reductase activity, expressed in µmol of NO^-_2 h^{-1} g^{-1} of fresh matter, and on leaves' nitrate contents in 4 Moroccan alfalfa populations. Values are means of 3 replicates, bars are SE.

Table 2. Effect of water deficit on phosphatase acid activity (APA) and on phosphorus contents (P) in roots and leaves of 4 Moroccan alfalfa populations. Values are means of 3 replicates ± SE.

		P (mg/g DM)		APA (µg p-NP min^{-1} g^{-1} FM)	
Water deficit	Population	Roots	Leaves	Roots	Leaves
75% FC	Taf 1	8.95 ± 0.27	6.54 ± 0.27	2.65 ± 0.03	1.91 ± 0.31
	Taf 2	8.76 ± 0.27	6.92 ± 0.21	2.61 ± 0.11	1.97 ± 0.27
	Dem	8.94 ± 0.81	6.82 ± 0.25	2.75 ± 0.14	2.00 ± 0.04
	Tata	8.81 ± 0.48	6.89 ± 0.33	2.64 ± 0.05	2.02 ± 0.15
50% FC	Taf 1	7.77 ± 0.58	5.70 ± 0.02	2.99 ± 0.07	2.27 ± 0.09
	Taf 2	8.04 ± 0.68	5.72 ± 0.18	2.94 ± 0.07	2.33 ± 0.04
	Dem	8.42 ± 0.36	6.18 ± 0.84	3.14 ± 0.14	2.43 ± 0.15
	Tata	8.18 ± 0.26	5.93 ± 0.75	3.23 ± 0.10	2.61 ± 0.30
25% FC	Taf 1	4.85 ± 0.10	2.42 ± 0.19	3.25 ± 0.29	2.73 ± 0.05
	Taf 2	5.02 ± 0.58	2.52 ± 0.07	3.40 ± 0.08	2.80 ± 0.04
	Dem	6.08 ± 0.34	3.29 ± 0.56	3.50 ± 0.09	2.79 ± 0.12
	Tata	6.63 ± 0.57	4.02 ± 0.34	3.64 ± 0.08	2.90 ± 0.11

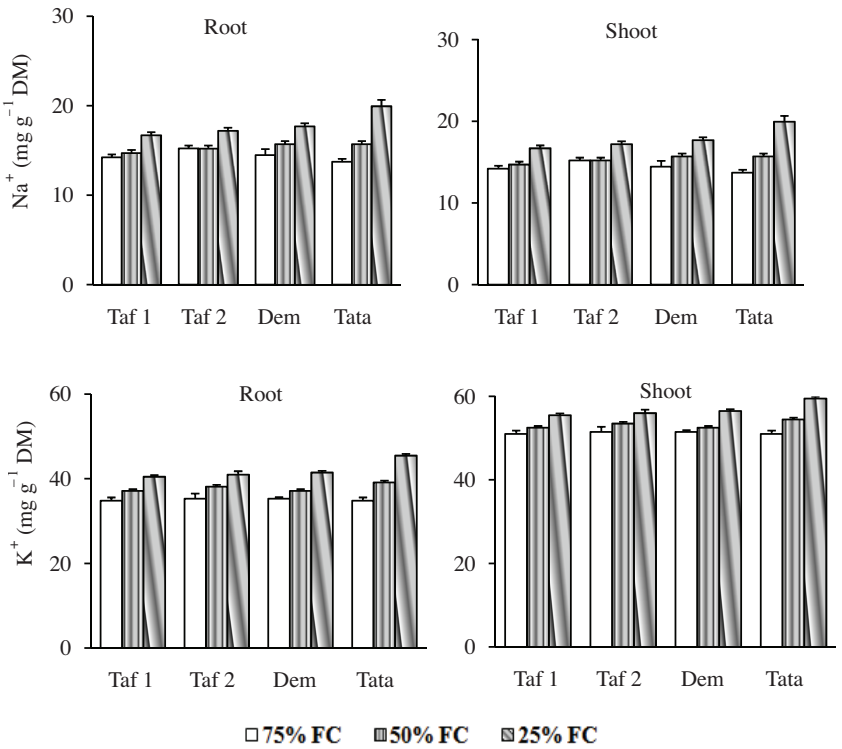

Figure 5. Effect of water deficit on Na^+ and K^+ concentrations in roots and shoots of 4 Moroccan alfalfa populations. Values are means of 3 replicates, bars are SE.

with 25.54 µmol MDA g^{-1} FM in roots and 18.25 µmol MDA g^{-1} FM leaves. The intermediate values were recorded in the 2 resting populations (Dem and Taf 2). In both organs, the interaction effect was also significant ($P < 0.01$).

3.8. Effect on antioxidant enzyme activities

With the 2 studied antioxidant enzymes, data (Table 3) showed that the water deficit caused an increase in their enzymatic activities ($P < 0.001$) such that they appeared more important under the used severe stress. For POD,

Table 3. Effect of water deficit on MDA content (nmol/g FM), POD activity (μmol H_2O_2 min^{-1} mg $protein^{-1}$), and CAT activity (μmol H_2O_2 min^{-1} mg $protein^{-1}$) in roots and leaves of 4 Moroccan alfalfa populations. Values are means of 3 replicates ± SE.

Roots		75% FC		50% FC		25% FC	
		Leaves	Roots	Leaves	Roots	Leaves	
Taf 1	MDA	9.29 ± 0.85	8.55 ± 0.58	19.86 ± 1.48	15.85 ± 0.58	29.37 ± 0.80	23.20 ± 1.00
	POD	12.01 ± 2.08	7.14 ± 1.89	20.56 ±1.42	13.40 ± 1.58	28.52 ± 2.04	21.89 ± 2.07
	CAT	1.57 ± 0.40	3.00 ± 0.25	2.95 ± 0.32	5.56 ± 0.72	3.62 ± 0.17	7.78 ± 1.29
Taf 2	MDA	8.54 ± 0.51	9.24 ± 0.72	20.51 ± 1.44	15.51 ± 1.33	28.50 ± 0.72	22.14 ± 1.02
	POD	12.08 ± 2.84	8.11 ± 2.82	21.27 ± 1.58	13.27 ± 0.92	29.58 ± 1.48	23.82 ± 1.60
	CAT	1.86 ± 0.30	2.97 ± 0.17	3.20 ± 0.49	5.65 ± 0.31	4.39 ± 0.50	8.19 ± 1.19
Dem	MDA	9.96 ± 0.87	9.44 ±1.65	19.89 ± 0.46	14.66 ± 1.50	26.20 ± 1.04	19.36 ± 0.96
	POD	12.30 ± 0.93	7.74 ± 2.27	26.26 ± 1.69	19.19 ± 2.58	35.62 ± 3.27	27.25 ± 0.90
	CAT	1.83 ± 0.33	2.87 ± 0.43	3.63 ± 0.38	6.74 ± 0.36	5.93 ± 0.65	10.4 ± 1.85
Tata	MDA	8.35 ± 0.99	10.27 ± 0.98	20.18 ± 0.85	14.71 ± 0.51	25.54 ± 1.12	18.25 ± 0.93
	POD	11.96 ± 3.48	7.55 ± 1.42	26.97 ± 2.44	19.40 ± 0.89	38.27 ± 1.05	28.87 ± 1.39
	CAT	1.53 ± 0.44	3.14 ± 0.25	4.02 ± 0.28	7.23 ± 0.25	7.19 ± 0.57	11.48 ± 1.11

the activity was more important in plant roots compared to the leaves, which showed the higher activity of CAT. ANOVA testing indicated that the population behavior was significantly different ($P < 0.001$) and the interaction effect was also significant. At 25% FC, Tata populations showed the highest enzymatic activity in both organs, followed by Dem and Taf 2, whereas Taf 1 had less antioxidant activity in both organs.

4. Discussion

Drought causes changes in a number of physiological and biochemical processes governing plant growth and productivity (Alexieva et al., 2001). It affects both elongation and expansion growth (Shao et al., 2008). In the present study, we have noted that water deficit caused a significant reduction in shoots' and roots' biomass. The comparison between the used populations showed that the Tata population is affected the least. However, Taf 1 is the most affected population. The reduction in growth and yield by water deficit has been documented in *Medicago sativa* L. (Bouizgaren et al., 2013) and in many other species, such as *Medicago truncatula* (Chebouti et al., 2001), *Phaseolus vulgaris* L. (Emam et al., 2010), and *Helianthus annuus* L. (Baloğlu et al., 2012)

Application of water stress induced a reduction in leaves' RWC (Fig. 2). This reduction was more important at 25% FC. The reduction in leaves' RWC was stimulated by the water deficiency in the soil, because during photosynthesis water loss occurs through the stomatal mechanism and the water assimilation rate is negatively affected during water stress (Verslues et al., 2006). The decrease indicated a loss of turgor that resulted in limited water availability for the cell extension process (Katerji et al., 1997). Thus, the great growth inhibition at 25% FC could be related partially to the decrease of RWC stimulated by the water restriction in rooting medium.

Drought stress caused a marked decrease in NRA and the leaves' nitrate content. Similar results under water deficit were reported earlier in *Helianthus annuus*, *Lupinus albus* L. (Correia et al., 2005), and *Phaseolus vulgaris* and *Sesbania aculeata* (Ashraf and Iram, 2005). Lobato et al. (2008) noted that NRA had an accumulated reduction of 70% after 6 days of water deficit and that rehydration promoted an increase of 96% in this parameter. The seedlings of *Hordeum vulgare* and *Triticum durum* that were subjected to water stress lost 30%–85% of their NRA (Smirnoff et al., 1985). The decrease noted in NRA was positively correlated to the decrease of leaves' nitrate contents and that could be the cause of the NRA reduction. Indeed, Gouia et al. (1994) noted that NRA was not sensitive to osmotic effects.

The decrease in phosphorus contents was accompanied with a change in acid phosphatase activity in both plant organs. This observation confirms the hypothesis suggested that activities of this enzyme in plants and plant parts typically increase when the plants become phosphorus-deficient (Parida and Das, 2004; Li et al., 2009). Many studies reported the increase of APA under drought conditions, such as those of Barrett-Lennard et al. (1982) and McLachlan (1984) in wheat leaves.

The significant accumulation of sodium and potassium in both plant organs under water deficit conditions and the difference between the contrasting populations (Taf 1 and Tata) permits us to suggest that they could play an important role in osmotic adjustment. Indeed, Morgan (1984) marked that the higher accumulation of ions was

associated with tolerance to water stress. In the same sense, Noaman et al. (2004) pointed out that the PEG-selected line of sunflower accumulated more K^+ and Na^+ than the nonselected line. Additionally, Wang et al. (2004) reported that K^+ is not only an essential macronutrient for plant growth and development, but is also a primary osmoticum in maintaining low water potential of plant tissues.

MDA and electrolyte leakage can indirectly reflect the degree of cell membrane integrity in plant organs. Our results indicated that relatively lower MDA and electrolyte leakage were observed in leaves under optimal irrigation, suggesting a higher degree of membrane integrity still maintained when compared with the water deficit. The increase of MDA content indicates that the bulk oxidative lipid metabolism in plants was enhanced by drought, suggesting a relationship between drought and oxidative stress (Munne-Bosch et al., 2001). Abedi and Pakniyat (2010) noted that water deficit is inevitably associated with increased oxidative stress due to enhanced accumulation of ROS, particularly O_2^- and H_2O_2 in chloroplasts, mitochondria, and peroxisomes. As a result, the induction of antioxidant enzyme activities is a general adaptation strategy that plants use to overcome oxidative stresses (Foyer and Noctor 2003; Sekmen Esen et al., 2012). In our experiment, the results showed significantly enhanced POD and CAT activities in both plant organs exposed to water deficit. Lee et al. (2009) mentioned that the activity of POD increased mostly under progressive water stress and was correlated with increase in lipid peroxidation and growth restriction in leaves of *Trifolium repens* L. The increase in POD activity under water deficit was also documented in *Capsicum annuum* L. (Awad Hamad et al., 2004) and in *Oryza sativa* L. (Lin and Kao, 2002). The increase in CAT activity under drought conditions was noted in a tolerant genotype of pea by Gillham and Dodge (1987). Similar observations have also been reported in sorghum (Jagtap and Bhargava, 1995), bean (Zlatev et al., 2006), common bean (Terzi et al., (2010), sunflower (Pourtaghi et al., 2011), and wheat (Sairam et al., 1998).

We concluded that the water deficit caused a significant reduction in dry plant biomass. The strongest reduction occurred during the applied severe stress (25% FC). The behavior of the tested populations was significantly different; the Tata population was more tolerant and Taf 1 was less tolerant. Taf 2 and Dem were intermediate. Tolerance of Moroccan alfalfa populations to water deficit was associated with the accumulation of inorganic ions (Na^+ and K^+), the ability of plants to overcome oxidative stress, the induction of antioxidant enzymes and maintenance of membrane integrity, and some physiological and biochemical reactions such as leaves' relative water content and the acid phosphatase and nitrate reductase activities.

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