Alfalfa nutritive quality as influenced by drought in South-Eastern Oasis of Tunisia

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ABSTRACT

Medicago sativa is the main perennial legume in many places in the world such as South-East Tunisian Oases. The widespread use of this species is due to its high adaptability, forage yield potential and to its good quality. Furthermore, the succession of drought years in South-East Tunisian Oases especially in Gabes influenced fodder distribution and digestibility. To a better understanding of the variation of the nutritive value of (Medicago sativa L.) plants, the effect of four drought levels (25%; 50%; 75% and 100% of field capacity) on nutritive quality was investigated for three populations [P1 (Gannouch); P2 (Chenini) and P3 (Tebelbou)] cultivated in these oases. Results showed that drought reduced leaves, shoots and roots dry matter, while this effect was more pronounced for above organs (values reached 1.2 mg) than below organs (values reached 1.8 mg). However, drought decreased phosphor, calcium, magnesium and nitrogen contents, neutral detergent fibre, acid detergent fibre and in acid detergent lignin content for P1 and P2, this stress enhanced potassium uptake, leaves shoots and roots shoots ratios especially for P3, values passed from 1.56 for control treatment to 2.06 for 25% of field capacity. This population seems to be the most resistant to drought by maintaining reduced leaf area and elevated ratios. This result indicates that Medicago sativa succeed to maintain a good nutritive value under stress conditions. To the best of our knowledge, it is important to study the effect of this stress on others varieties to more understand its effect on nutritive value.

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Introduction

Alfalfa (Medicago sativa L.) is considered native of north-western countries of Asia and it was spread through the world gradually (Essghaier 1980). It is the main perennial pasture legume in many places in the world such as Australia (Humphries & Auricht 2001), Canada (Lin et al. 2007); Italy (Iannucci et al. 2002) and the North of America (Huyghe 2003). In Tunisia, the introduction of alfalfa dates to very antique time (Le Houerou 1969). This genotype is well represented in all countries and it constitutes a genetic inheritance extremely rich and diversified (Seklani et al. 1990). The main production centres are the East coastal oases, especially, the oases of Gabes. This plant is also cultivated in the western continental oases in the region of Jerid (Kebili and Tozeur). Approximately 75% of oasis surfaces reserved for alfalfa are in the Southern Tunisia (Kassah 1996) and its surface average reaches 12,410 ha (CRDA 2007).

The widespread use of this species is due to its high adaptability, to its forage yield potential and to its good quality. In addition, alfalfa’s deep-root system can help in preserving soil and water loss in dry lands (Maurie`s 2004). Alfalfa is also known for its symbiotic relationship with bacteria, taking part in collecting its nutritive needs.

However, most climates scenarios predict a worldwide increase in arid areas (IPCC 2007) and subsequent water stress for the crops. Drought becomes the major environmental determinant of plant growth and productivity. The succession of drought years in these oases influenced fodder distribution (Sghaier 2010) and the lack of water decrease its digestibility of fodder (Marsh et al. 1994).

Generally, plants have developed a wide diversity of morphological and physiological drought tolerance mechanisms. Water stress can affect the growth of plant organs differently (Spollen et al. 1993) which...
may result in the alteration of the morphological features of the plants (Cox & Conran 1996). Indeed, in order to diminish consumption and increase absorption of water, plants in dry conditions often decrease their growth rate and biomass production. This is contributing more biomass to roots, so that they could maintain a higher root/shoot ratio (R/S) (Yin et al. 2005; Erice et al. 2007). This has been considered as one of the mechanisms involved in the adaptation of plants to drought stress (Turner 1997). In addition, some studies showed that the competition for water and the light is accompanied for alfalfa with a reduction of the production and an increase in the leaves/shoot ratio. This ratio is used to determine the energetic value or digestibility of Lucerne (Mauries 1994).

As the nutritive value is a very important criteria when buying or selling alfalfa, there are many predictors of the quality of forages, such as the proteins content (Lin et al. 2007), the concentrations of cell-wall components, like the neutral detergent fibre (NDF) fraction (primarily cellulose and hemi-cellulose), the acid detergent fibre (ADF) fraction [including the less digestible and indigestible (e.g. lignin)] (Van Soest 1994) and the nutritional elements contents like N (nitrogen); P (phosphorus); K (potassium); Ca (calcium) Mg (magnesium) and Na (sodium). Several studies have shown that environmental factors such as drought modify forage quality (Thorvaldsson & Andersson 1986; Buxton & Fales 1994).

Although the effects of drought on growth and production, photosynthesis parameters and osmotic adjustment are relatively well studied, little is known about effects of water stress on nutritive quality of crops. In order to more understand the variation of nutritive value and the nutrient disturbance in alfalfa plants, this study aim to investigate the effect of four drought levels on dry matter production (DM), mineral composition, crude proteins (CP) and fibre contents of alfalfa. In addition, the present study is a first step to evaluate and compare the tolerance of three Medicago sativa populations, that frequently exist in the southern oasis of Tunisia, to drought and to identify adaptive mechanisms that enable alfalfa plants to affront this stress.

Materials and methods

Growth conditions and water stress application

Experiment was conducted in the Institute of Arid Lands in the South of Tunisia in controlled conditions of a glasshouse with a thermo-period of 26 °C (day) and 18 °C (night). Daily maximum relative humidity (RH) ranged from 65% to 70%. Average daily photosynthetically active radiation inside the greenhouse was 500 μmol m⁻² s⁻¹. The species used is Medicago sativa and the seeds of three populations (Chenini (P1), Gannouch (P2) and Tebelbou (P3)) were collected from Gabes Coastal Oases in the South-East of Tunis (Latitude 33°35′ N, Longitude 10°48′3″ E, Altitude 105 m).

Plants were grown in 101 plastic pots filled with a mixture of 2/3 sand and 1/3 of oasis soil. All the samples were well watered until complete leaf sprouting occurred and fully expanded leaves were available. After 30 days of sowing, treatments were initiated. Four stress levels were applied: 25%, 50%, 75% and 100% (control) of field capacity with four replicates for each treatment. The study was undertaken for in spring period (corresponding to early vegetative stage in alfalfa plants).

Dry matter determination

The dry matter of the collected material (leaves, shoots and roots) was measured after drying at 85 °C for 48 h. The completely dry samples were weighted and the dry weight is taken after stabilisation.

Measurement of leaf area

To measure leaf surface [cm²], five fresh leaves were collected from five plants in the same pot and placed on a scanner machine. The images obtained were treated with Mesurim (7.0, Germany) to determine the total leaf area (LA).

Mineral composition

For the determination of potassium (K) and sodium (Na) content, 10 g of each sample was heated at 105 ± 5 °C, than 1 g of each dry sample was incinerated during 4 h at 550 °C. Ashes were mixed with 4 ml of distilled water and 1 ml of concentrated hydrochloric acid (HCl). The sodium and potassium contents were determined with a Sherwood 410 flame photometer (Germany) regulated on the filter of sodium or potassium. The contents of sodium (% Na) or of potassium (% K) in the dry matter plant were calculated as (% Na or % K = C × DF)/(100 × m), where C is the concentration of sodium or potassium (mg/l) and DF is the factor of dilution and m is the mass of the extract (g).

Phosphorus content (P) is carried out by a spectrophotometer (Germany). For 1 ml of each extract (dry matter mixed with sulphuric acid), 5 ml of vanadomolybdic reagent were added. The mixture was kept...
in darkness, at ambient temperature during 10 min. The phosphorus content is expressed by the following relation \[\% P = \left(\frac{C \times DF}{100 \times m}\right)\] with: \(C\): P content in mg l\(^{-1}\) of the diluted aliquot part of extract and DF: factor of dilution. Calcium (Ca) and magnesium (Mg) content were determined using proportioning colorimetric method.

**Nitrogen determination**

The quantity of nitrogen (N) was determined by the method of micro-Kjeldahl (Kjeldahl 1883). It consists of an attack of 0.5 g of crushed matter (in a tube of digestion or mineralisation) by 20 ml of hot sulphuric acid concentrated with a catalyser. The percentage of the total nitrogen expressed in mg of dry matter was calculated by the following formula: \(N\% = \frac{V_{HCl} \times 14 \times 0.05}{m \times 100}\) (With: \(V_{HCl}\): volume of HCl obtained when the colour was moved from the green to purple, 0.05 is the normality of HCl and 14 is the molar mass of nitrogen and \(m\) is the weight of samples).

**Fibres content determination**

Forage concentrations on NDF, acid detergent lignin (ADL) and ADF were determined sequentially in the Ankom\(^{220}\) Fibre Analyzer (Netherlands) using Ankom filter bags F57 (USA) according to procedures of Van Soest et al. (1991).

**Statistical analysis**

Different parameters were subjected to an analysis of variance using (ANOVA) tests. Significant differences between means were evaluated by LSD method. Data were expressed as mean ± SE. Relationships using Regression lines between different parameters were established. Statistical analyses were performed using SPSS 16.0 for (Chicago, IL) Windows.

**Results**

**Drought impact on dry matter production**

Results showed that drought influences significantly the dry matter of above ground (leaves and shoots) and below ground organs (roots). There was a significant reduction \((p < 0.001)\) in response to progressive stress levels. For above ground organs, values obtained on the severe level of water deficit (25% of field capacity) were reduced to the half of those obtained on the control treatment (Figure 1(a) and (b)).

However, between organs, shoots and leaves were more affected by stress than roots. Values for roots were between 0.8 and 1.8 mg for plants subjected to 25% of field capacity while values did not exceed 1.2 mg for leaves and 0.9 mg for shoots (Figure 1(c)).

Significant differences in response to drought were also observed between the three studied populations; P1 exhibited the highest dry matter production under progressive stress levels and P3 exhibit lowest values for the three studied organs.

These differences allowed a significant difference in R/S ratio in response to increasing stress levels (Figure 2). Nevertheless, the effect was less marked especially for P1 which maintain a higher ratio even under severe stress level (2.5 for 25% of field capacity) as compared to control conditions (3.2 for 100% of field capacity). For P3, the situation was different because water deficit enhanced significantly the R/S ratio. In fact, values passed from 1.56 for control treatment to 2.06 for 25% of field capacity. There is no difference between the other treatments.

Drought induced a high significant reduction on leaf area \((p < 0.001)\). Differences between populations were also highly significant. The highest values were registered for P1 while the lowest values were registered for P3. The most severe stress level (25% of field capacity) affected strongly the leaf area for the three studied populations. Values varied from 4.92 to 1.6 cm\(^2\) for P1; from 4.88 to 1.1 cm\(^2\) for P2 and from 4.28 to 0.85 cm\(^2\) for P3 under the same treatment (Figure 3).

As a response to drought conditions, leaves shoots ratio (L/S) increased significantly \((p < 0.001)\) mainly under severe water stress levels (25% and 50% of field capacity) (Figure 4).This effect was observed for the three studied populations. However, P3 (Tebelbou) has the highest ratio under several treatments. Values increased for this population from 1.3 under 100% of field capacity, to 1.55 under 75%, to 1.84 under 50%, to finally reach 2.04 under 25% of field capacity.

**Drought impact on mineral composition**

The increasing drought conditions enhanced the potassium uptake by leaves \((p < 0.001)\). Thereby, its average content increased from 3% to 5% in stressed plants. A significant difference was observed between populations in response to drought, P2 showed the highest rates.

Drought stress has no significant effect on Na content. There was no significant difference between treatments. Sodium rates were similar in all treatments under drought or control conditions. Between studied populations, there was also no significant difference in sodium accumulation also (Table 1).
P content in Lucerne leaves decreased under drought conditions significantly \((p < 0.001)\). Under control conditions, leaves had 3-fold the concentration of P than those under severe stress level. Significant difference was also observed between populations. P2 showed the highest values compared to P1 and P3 which showed the lowest values (0.07% for 25% of field capacity).

The Ca concentration was very affected by water stress \((p < 0.001)\). A progressive reduction was observed for the three studied populations. Furthermore, P3 seemed to be the most affected by drought, the means passed from 2.1% for 100% of field capacity to 1.67% for 75% of field capacity, to 1.21% for 50% of field capacity and to 0.78% for 25%...
of field capacity. The last is the lowest value compared to the values registered for P1 (1.42%) and P2 (1.09%) under the same treatment.

Increasing stress levels led to a significant reduction in Mg content in *Medicago sativa* leaves (**p < 0.001**) for the three populations. Values registered for the severe stress level are reduced more than the 1/4th of those registered under control conditions.

### Drought impact on nitrogen (N) content

N content declined significantly (**p < 0.001**) in Lucerne leaves especially, the high stress level. N concentration was lower in P3 leaves than P2 and P1 which has the highest values of nitrogen under stress and control conditions (Table 2).

### Drought impact on fibres content

Drought has a significant effect on fibres contents. Results showed that this content was reduced especially for P1 and P2. The effect of drought in fibres content was not observed for P3.

The NDF content decreased from 41.33% for P1 under control conditions to 35.73% under 25% of field capacity, for P2, values passed from 37.27% to 32.97% and for P3, values are 39.13% and 38.20% for the same treatments, respectively. For ADF content, values are reduced under severe stress for P1 from 31.00% to 20.00%, for P2, from 28.00% to 19.00% while for P3 values the reduction is slight (24.00% and 23.00%). The result for ADL content is not significant for P3, values are, respectively, 9.00% for 100% of field capacity; 8.49% for 75%; 9.27 for 50% and 8.95% for 25% of field capacity (Table 2).

### Relationships and correlations

Relationships between root dry mass and leaf area for the three studied populations are represented in Figure 5. This figure showed that leaf area was correlated positively with root dry mass produced under the four treatments. Whereas, this correlation was significantly higher with root dry mass produced for P2 and P3 (**R² = 0.94** and **0.95**, respectively).

Regressions coefficients between leaf area and mineral composition were shown in Table 3. These coefficients were variables between nutrients. Highest values were recorded for nitrogen (N) in P1 and P3 leaves, calcium (Ca) in P3 and magnesium (Mg) in P1, **R² = 0.8**, **0.85**, **0.91**, respectively. The slopes of the linear functions indicated that leaf area was negatively correlated to potassium (K) content while the correlation with the others minerals was positive.

![Figure 4. Leaves/shoots ratio (L/S) variations of three *Medicago sativa* populations (P1: Gannouch, P2: Chenini and P3: Tebelbou) grown under four stress levels (25%, 50%, 75% and 100% of field capacity).](image-url)

**Table 1.** Effect of different stress levels (25%, 50%, 75% and 100% of field capacity) on nutrient contents (%) (K: potassium; Na: sodium; P: phosphorus; Ca: calcium; Mg: magnesium) in alfalfa leaves for the three populations (P1: Gannouch, P2: Chenini and P3: Tebelbou).

| Nutrient | Stress levels (SL) | Significance |
|----------|-------------------|-------------|
| K content | 100%  | 75%  | 50%  | 25%  | SL P SL X P |
| P1 | 0.61 ± 0.03 | 0.59 ± 0.02 | 0.67 ± 0.03 | 0.78 ± 0.06 | ** ** ns |
| P2 | 0.36 ± 0.04 | 0.41 ± 0.01 | 0.51 ± 0.04 | 0.59 ± 0.01 | ** ** ns |
| P3 | 0.31 ± 0.02 | 0.35 ± 0.04 | 0.45 ± 0.05 | 0.59 ± 0.06 | ** ** ns |
| Mean | 0.43 ± 0.15 | 0.45 ± 0.12 | 0.54 ± 0.11 | 0.52 ± 0.15 | ** ** ns |
| Na content | P1 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.01 ± 0.00 | ** ** ns |
| P2 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | ** ** ns |
| P3 | 0.01 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | ** ** ns |
| Mean | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | ** ** ns |
| P content | P1 | 0.39 ± 0.01 | 0.28 ± 0.02 | 0.19 ± 0.01 | 0.10 ± 0.01 | ** ** ** |
| P2 | 0.29 ± 0.01 | 0.21 ± 0.01 | 0.14 ± 0.01 | 0.08 ± 0.01 | ** ** ** |
| P3 | 0.16 ± 0.01 | 0.11 ± 0.01 | 0.08 ± 0.00 | 0.05 ± 0.01 | ** ** ** |
| Mean | 0.28 ± 0.10 | 0.20 ± 0.08 | 0.14 ± 0.05 | 0.08 ± 0.02 | ** ** ** |
| Ca content | P1 | 4.56 ± 0.04 | 2.66 ± 0.04 | 1.62 ± 0.21 | 1.42 ± 0.06 | ** ** ** |
| P2 | 3.71 ± 0.02 | 2.15 ± 0.04 | 1.46 ± 0.04 | 1.09 ± 0.01 | ** ** ** |
| P3 | 2.10 ± 0.01 | 1.67 ± 0.03 | 1.21 ± 0.01 | 0.78 ± 0.05 | ** ** ** |
| Mean | 3.46 ± 1.08 | 2.16 ± 0.43 | 1.43 ± 0.26 | 1.10 ± 0.28 | ** ** ** |
| Mg content | P1 | 2.02 ± 0.01 | 1.62 ± 0.10 | 0.63 ± 0.06 | 0.38 ± 0.05 | ** ** ** |
| P2 | 1.45 ± 0.23 | 0.88 ± 0.06 | 0.55 ± 0.02 | 0.27 ± 0.05 | ** ** ** |
| P3 | 1.37 ± 0.07 | 0.69 ± 0.01 | 0.44 ± 0.02 | 0.10 ± 0.01 | ** ** ** |
| Mean | 1.61 ± 0.37 | 1.06 ± 0.44 | 0.54 ± 0.10 | 0.25 ± 0.14 | ** ** ** |

Each value represents mean ± SE of *n = 3*. The meaning of the symbols used in ANOVA test are: **Significant at 1%**, *significant at 5%*, ns: not significant; P1, P2, P3: the three populations; Na: sodium; K: potassium; Ca: calcium; Mg: magnesium; P: phosphorus; SL: stress levels; P: populations.
Table 2. Effect of four levels of drought (25%, 50%, 75% and 100% of field capacity) on N (nitrogen, %), crude protein (CP, %) and fibre contents (NDF, ADF and ADL, %) in *Medicago sativa* leaves for the three populations (P1: Gannouch, P2: Chenini and P3: Tebelbou).

| Nutrient | 100%       | 75%       | 50%       | 25%       | SL  | SL  | SL x P |
|----------|------------|-----------|-----------|-----------|-----|-----|--------|
|          |            | 25%       | 50%       | 75%       |     |     |        |
| N        |            |           |           |           |     |     |        |
| P1       | 10.90 ± 0.12 | 9.45 ± 0.35 | 6.11 ± 0.05 | 5.65 ± 0.03 | ** | ** | **     |
| P2       | 7.47 ± 0.27  | 5.68 ± 0.07  | 4.87 ± 0.32  | 4.13 ± 0.08  | ** | ** | **     |
| P3       | 4.40 ± 0.07  | 4.16 ± 0.03  | 3.60 ± 0.21  | 3.21 ± 0.06  | ** | ** | **     |
| Mean     | 7.59 ± 0.94  | 6.43 ± 0.79  | 4.86 ± 0.38  | 4.33 ± 0.36  | ** | ** | **     |
| NDF      |            |           |           |           |     |     |        |
| P1       | 41.33 ± 1.20 | 36.66 ± 1.45 | 35.88 ± 0.88 | 35.73 ± 0.88 | ** | ** | ns     |
| P2       | 37.27 ± 0.88 | 35.60 ± 1.16 | 34.83 ± 0.88 | 32.97 ± 1.20 | ** | ** | ns     |
| P3       | 39.13 ± 0.88 | 38.60 ± 1.53 | 38.80 ± 0.58 | 38.20 ± 0.58 | ** | ** | ns     |
| Mean     | 39.24 ± 2.96 | 36.95 ± 3.25 | 36.5 ± 2.91  | 35.63 ± 2.41 | ** | ** | ns     |
| ADF      |            |           |           |           |     |     |        |
| P1       | 31.00 ± 1.73 | 26.00 ± 0.58 | 24.33 ± 0.88 | 20.00 ± 0.58 | ** | ** | ns     |
| P2       | 28.00 ± 0.58 | 25.00 ± 0.58 | 22.00 ± 0.58 | 19.00 ± 1.16 | ** | ** | ns     |
| P3       | 24.00 ± 0.33 | 22.00 ± 0.58 | 23.00 ± 0.58 | 23.00 ± 0.58 | ** | ** | ns     |
| Mean     | 27.66 ± 1.45 | 24.33 ± 1.05 | 23.11 ± 1.14 | 20.66 ± 1.36 | ** | ** | ns     |
| ADL      |            |           |           |           |     |     |        |
| P1       | 10.07 ± 0.07 | 9.83 ± 0.09  | 9.27 ± 0.07  | 8.29 ± 0.06  | ** | ** | **     |
| P2       | 9.47 ± 0.03  | 8.53 ± 0.03  | 7.20 ± 0.06  | 6.83 ± 0.03  | ** | ** | **     |
| P3       | 9.00 ± 0.02  | 8.49 ± 0.02  | 9.27 ± 0.03  | 8.95 ± 0.08  | ** | ** | **     |
| Mean     | 9.51 ± 0.16  | 8.95 ± 0.24  | 8.58 ± 0.33  | 7.99 ± 0.32  | ** | ** | **     |

Each value represents mean ± SE of *n* = 3. The meaning of the symbols used in ANOVA test are: **significant at 1%**. ns: not significant; SL: stress levels; P: populations.

Figure 5. Relationships between leaf area and roots dry matter of three populations (P1: Gannouch, P2: Chenini and P3: Tebelbou) under control conditions (100% of field capacity) and stress treatments (25%, 50% and 75% of field capacity) with the equation associated to each treatment from linear regressions.
Discussion

As reported in many studies, water deficit has been recognised as one of the most important environmental factors limiting plant development and metabolism. Results showed that leaves, shoots and roots dry matters were reduced significantly mainly under severe stress levels (25% and 50% of field capacity). These results are similar to those obtained by Maurie`s (1994) and Erice et al. (2010) indicating that water stress caused a significant reduction of the quantity of dry matter in the aboveground and below ground organs in alfalfa plants. These finding are the same also for many species such as pearl millet (Pennise tumgaucum L.) (Kusaka et al. 2005), Phaseolus vulgaris (Ashraf & Iram 2005) and maize (Chimenti et al. 2006). This reduction observed especially in leaf DM may allow these cultivars to maintain relative water content (RWC) under drought conditions by decreasing the size of transpiring organs (Kramer & Boyer 1995).

Additionally, results showed that leaf area is reduced by stress. Tebelbou population had the lowest leaf area than the two others populations. This can be considered as a strategy to allow the accumulation of nutrients in roots and may allow these plants to maintain their water status by a low rate of water uptake. These results are in agreement with those of (Erice et al. 2006) in nodulated alfalfa and in four alfalfa genotypes. Comparative results had been obtained for a number of plants (Navas & Garnier 2002; Lei et al. 2006; Markesteijn & Poorter 2009). The relationships between root dry mass and leaf area for P2 and P3 reinforce this idea (R²= 0.94 and 0.95, respectively).

The below organs accumulate a larger proportion of dry matter than the aerial parts with more reduced volume. This accumulation is due to their structures able to store non-structural sugars in order to ensure pro-growth after defoliation (Avice et al. 2003). Thereby, this reduction interpreted as a tolerance mechanism in alfalfa leaves, leaded consequently to a higher R/S ratio observed especially for P3. Similarly, many studies showed that plants in drought conditions often decrease the biomass production and contribute more biomass to roots, maintaining a higher R/S ratio (Yin et al. 2005; Erice et al. 2007) as an adaptation trait to drought resistance.

In addition, leaves shoots ratio (L/S) increased significantly mainly under severe water stress levels for the three studied populations. This may reflect the resistant character of alfalfa plants in response to water deficit. These results corroborate with others showing that alfalfa irrigated to 65% of field capacity (moderate stress) increased leaf: stem ratio and digestibility (Halim et al. 1989).

It is commonly known that drought can affect nutrient uptake and im prohibition of some nutrients and some studies indicate that under drought conditions, wilting in plants suggests possible K deficiency (Beringer & Trolldenier 1978). However, these experiments showed that the potassium content increased under progressive drought treatments in leaves. This increase is probably due to the important role of K in plants. Further, potassium plays a crucial role in osmotic adjustment for alfalfa plants such as Medicago truncatula and Medicago laciniata (Yousfi et al. 2010). This increase in potassium content in the three populations was also attributed to the good selectivity of Gabes variety for K (Mezni et al. 2002) as compared to Na. This conclusion corroborate with our results which indicate that Na content was not significantly altered in the leaves or in the roots by any water-deficit treatment. However, some studies show that in contrary, Na concentration significantly increased under water deficit stress in Medicago populations from the arid climate (Yousfi et al. 2010).

This stress has a negative effect on P, Ca and Mg content. These results are in agreement with others indicating that drought leads to a reduction on P, Mg and Ca uptake content in alfalfa plants (Goicoechea et al. 1997).

Nitrogen is the mineral element that plants require in the largest amounts and it is a constituent of many plant cell components, including amino and nucleic

### Table 3. Relationships between leaf area (LA) and mineral composition of the three populations (P1: Gannouch, P2: Chenini and P3: Tebelbou) under four stress levels (25%, 50%, 75% and 100% of field capacity).

| Parameters | $R^2$ | Regressions equations | Significance |
|------------|------|----------------------|-------------|
| **P1**     |      |                      |             |
| LA–K       | 0.58 | $y = -0.040^* + 0.84$ | **          |
| LA–P       | 0.892| $y = 0.0755^* + 0.026$| **          |
| LA–Ca      | 0.66 | $y = 0.751^* - 0.10$  | **          |
| LA–Mg      | 0.916| $y = 0.480^* - 0.583$  | **          |
| LA–N       | 0.88 | $y = -1.533^* + 2.59$  | **          |
| **P2**     |      |                      |             |
| LA–K       | 0.765| $y = -0.054^* + 0.648$ | **          |
| LA–P       | 0.872| $y = 0.043^* + 0.02$   | **          |
| LA–Ca      | 0.699| $y = 0.534^* + 0.325$  | **          |
| LA–Mg      | 0.671| $y = 0.245^* - 0.029$  | **          |
| LA–N       | 0.719| $y = 0.69^* + 3.24$    | **          |
| **P3**     |      |                      |             |
| LA–K       | 0.719| $y = -0.060^* + 0.619$ | **          |
| LA–P       | 0.777| $y = 0.0248^* + 0.028$| **          |
| LA–Ca      | 0.851| $y = 0.030^* + 0.586$  | **          |
| LA–Mg      | 0.71 | $y = 0.26^* - 0.093$   | **          |
| LA–N       | 0.801| $y = 0.29^* + 3.017$   | **          |

With the equation associated to each element linear regressions. The meaning of the symbols used in ANOVA test are: **significant at 1%. (the relationship between minerals and leaf area is significant at 1%).

Minerals: K: potassium; N: nitrogen; Ca: calcium; Mg: magnesium; P: phosphorus; significance: P.
acids. Results of this experiment showed that drought conditions reduce N content of the leaves mainly under the most severe stress level (25% of field capacity). This is in agreement with several studies about alfalfa plants (Goicoechea et al. 1997) and others crops such as maize plants (Yuncal & Schmidhalter 2005) and pea plants (Mahieu et al. 2009). In fact, drought conditions may reduce soil-N mineralisation, thus lowering the N availability. Besides, a reduced N uptake in the crop may also be attributed to a decreased transpiration rate, to transport N from roots to shoots (Tanguilig et al. 1987).

Thus, it is well established that drought reduces both nutrient uptake by the roots and transport them from the roots to the shoots, because of restricted transpiration rates and impaired active transport and membrane permeability (Alam 1999) probably a result to the leaf area restriction. Positive and elevated relationships between leaf area and Mg, N, Ca, P and Na contents were observed, indicating and reinforcing this idea. Indeed, as a deep rooted plant, Lucerne is able to reduce water table recharge (Latta et al. 2002), when soil was dried, which eventually led to a large decrease in leaf area (a strong sink for nutrients). However, for K, the correlation was negative indicating the role of potassium accumulation in drought stress tolerance.

In this study, fibre contents declined under severe stress conditions for P1 and P2. These results are in accordance with other studies showing that soil moisture deficits increased alfalfa (Medicago sativa L.), red clover (Trifolium pratense L.), birdsfoot trefoil (Lotus corniculatus L.) and cicer milkvetch (Astragalus cicer L.) forage quality while NDF, ADF and ADL values were decreased (Peterson et al. 1992). This reduction is attributed to the amount of carbon (C) incorporated into the cell wall which decreases during water deficits (Buxton & Fales 1994), to a delayed maturity and a higher leaf:stem ratio (Peterson et al. 1992; Van Soest 1994).

Conclusions

The data obtained from the present study suggests that alfalfa plants can maintain to a certain level a good nutritive value in response to drought. Further, although leaf area, leaves and shoots dry matter; nutrients content and fibres content were reduced; L/S ratio (the important criteria to evaluate nutritive quality) was increased under progressive stress conditions. This result indicates that alfalfa is an adapted plant to stress and it can endure different stress levels especially Tebelbou population.

The differences between cultivars in chemical composition forage yield and fibre characteristics provide the possibility to select genotypes able to survive under stress growing conditions. This study is a part of many aiming to preserve deteriorated regions by promoting the sowing of resistant cultivars to expected severe stress conditions in spite of sensible ones.

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Disclosure statement

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