



Contribution to the study of the effect of water stress on the accumulation of a metabolite species of annual clover spontaneous in Algeria

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Abstract

Our study is part of the work of characterization and development of local plant genetic resources, particularly those on medics, which may play a role in improving forage production in our country, where the water deficit and erratic rainfall are key factors limiting agricultural production. It is in this sense that we have studied the effect of a post floral water stress on phenological and the accumulation of proline in populations of four species of local annual medics. Our results indicate that *M. laciniata* variety is the most resistant to the applied stress.

Key words: water deficit, *M. laciniata*, *M. Lttoralis Rohde*, *M. minima Grufb*, *M. Secundiflora Dur*, *alfalfa annual*.

Introduction:

Algeria, the countries of North Africa, covers nearly 2.4 million km², from east to west along the Mediterranean Sea on 1200 km and sinks, from north to south, about 2000 km at the heart of the Sahara desert (DGF,1998).

Through this vast territory are divided 3139 plant species (Quezel and Santa, 1962). Of all these species in Algeria, the legumes are represented by fifty kinds mainly belonging to the subfamily of the Fabaceae (Quezel and Santa, 1962) and a significant number are of interest pastoral and food. Despite all the rich flora, the problem of feeding livestock remains unresolved in our country.

Among these plant genetic resources, the Medics (alfalfa or annual), legumes annual cycle comprising about 55 species (Damerval, 1983) and 39 in Algeria (Quezel and Santa, 1962). They produce a high protein feed (Huguet and Prospreri, 1995), enrich the soil with nitrogen through symbiotic fixation of atmospheric nitrogen (60kg/ ha/year) and could help solve the problem of fodder shortage.

This is indeed a very important reservoir for genetic variability of alfalfa populations to adapt to possible changes in the environment (Ayala, 1978). By quality adaptation and productivity, Medics can enter a biennial rotation "Medics - Cereals" replacing fallow both in the highlands at the level of arid regions.

Their introduction in vineyards and orchards limit soil erosion reduces weed growth and reduces the use of herbicides. Annuals improve the physical and biological properties of soil and involved in the degradation of certain parasites, in the end, because of their short growing season, they do not compete with the tree layer for water and nitrogen (Abdelkefi and Merrakchi 2000).

Water resources are limited and the search for plants more adapted to drought is a key factor for agricultural production in the coming decades.

The ability to quantitatively evaluate the performance of crop plants undergoing water stress is very important in research programs aimed at the rehabilitation and improvement of forage production. Many researchers are working on varieties adapted to these regions can produce the maximum biomass or seeds under conditions of water deficit. This selection focuses on coping mechanisms that could be used for genetic improvement.

The installation of a forage crop Algeria suffers not only from low increase (due to lack of material and financial resources) but also weather conditions (drought). The decline in rainfall accumulated from a given period of the year is growing year. Nowadays, it is considered the major abiotic stress affecting crops.

Our experimental work is to characterize the effect of water stress corresponding to a rate of 95 % of the usable soil water reserve on the development during the flowering phase to total desiccation of plants of four species of annual alfalfa (*M. minima*, *M. secundiflora*, *M laciniata* and *M. littoralis*), and the identification of two main physiological criteria associated with tolerance to water deficit. The aim of our study was to estimate the influence of water deficit on two physiological characteristics that are relative water content and proline accumulation in populations of *M.laciniata*, *M.secundiflora*, and *Mr. M.littoralis minima*.

Materials and Methods

The site of the experiment

The trial was conducted at the experimental station Mehdi Boualem Baraki the National Institute of Agronomic Research (INRAA) . This region is characterized by an average temperature between 26 ° C (min) and 41 ° C (Max) and a sub-humid bioclimatic floor.

The plant material Our experimental work has focused on four species of the genus *Medicago* L., *Medicago laciniata* (L.), *Medicago littoralis* Rohde., *Medicago minima* Grufb., *Medicago secundiflora* Dur.

Each species is represented by a number of local people gathered at the Algerian steppe.

Table 2.1: Species and populations studied with Abbreviations

Species	Abbreviations
<i>M. laciniata</i> (L.) All.	Mla 1
	Mla 2
	ML/a 3
	Mla 4
<i>M. littoralis</i> Rohde	Mli 1
	Mli 2
	Mli 3
	Mli 4
<i>M. minima</i> Grufb.	Mm 1
	Mm 2
	Mm 3
<i>M. secundiflora</i> Dur.	Mse 1
	Mse 2
	Mse 3
	Mse 4

The conduct of the trial

Sowing was done manually November 11, 2013. The test was conducted under glasshouse



Figure 1.2: The glasshouse (Original photo 1)

In plastic pots with a volume of 4.7 to 5 kg of an upper diameter of 22.9 cm, a bottom diameter of 12.8 cm and 21.5 cm and a depth of weight 200 g. These pots were filled with 4.7 kg of a mixture of 1/2, 1/4 and 1/4 respectively loam, peat and sand and gravel 2cm. Other manipulations for determining the retention capacity were carried out at the Laboratory of Soil Science in the Department of Agronomy of the University of Blida.

Our experiment is to study the effect of water stress on floral post behavior and the identification of some physiological traits under drought conditions in populations of *M. laciniata* and *M. secundiflora* *M. littoralis* and finally *Mr. minima*. For this two methods of water treatments were applied:

The non- stressed or control treatment (NS)

This is the unstressed treatment, where plants are carried out in maximum evapotranspiration regime (ETM) (they do not lack water at any point in their growth cycle). The determination of the water requirements of plants is made from the average of the maximum evapotranspiration lysimeter pots that are grown in the same conditions as the rest of the test pots. It consists in a water balance between the quantity of water supplied and the drained at each pot lysimeter:

$$ETM = A - D$$

Inputs of irrigation water are all 2-3 days depending on the cycle.

The stress treatment (RS)

This treatment is severely stressed from the beginning of flowering until the end of the growing cycle. Water stress corresponds to a rate of depletion of 95% of the useful reserve. The pots are irrigated as soon as the threshold of 95 % of the rate of depletion is reached. To determine the rate of depletion , the principle is to weigh regularly (every two days) pots severe stress from an initial weight (P_i) corresponding to the retention capacity until the final weight (P_f) corresponding to the rate of depletion of 60 % of the usable soil water reserve. The final weight is détermined by the following formula:

$$P_f = P_i - 0,95 RU$$

Where: $P_i = P_s + C_r$

Ps: dry weight of the soil of the pot (g)

Cr : weight of water to the pot holding capacity (g / g of dry soil) = 37.4%

Let Cr = 0,315g / g = 0,315Ps

Pi = Ps + 0,315Ps = 1,315Ps

Ps = Pi / 1.315

The water reserve (UK) is 50% of the withholding water holding capacity ; from where :

RU = 0.5x (0,315Ps) = 0.157 Ps

RU = 0,157xPi / 1.315 = 0.119 Pi

Pf = Pi - 0, 95 (0,119Pi)

Pf = Pi (1 - (0, 95x0 , 119))

Mp = 0.887 Pi

For the value of the holding capacity, we have determined as follows:

Were weighed three empty capsules and their average was calculated: mP = 37,75g noted.

Second step was weighed three filled capsules, after centrifugation of the soil samples. We calculated the average weight recorded M'P = 90,83g

The final step was weighed weight of the three capsules filled after a night in an oven at 105 ° C and calculated the average weight noted m''Pe = 76,37g

Using the following formula:

$$H_c = (m'p - m''Pe) / (m''Pe - mp)$$

Calculating the retention capacity

The parameters sought

Rate proline

Dosage of proline

To study the evolution of the levels of proline populations in ETM and under the stress regime, three assays were performed according to the following schedule:

- First of sampling was conducted March 23, 2014.
- Second sampling was conducted April 2, 2014.
- Third sampling was conducted April 13, 2014.
- Add 5 ml of toluene in each tube
- After vortexing two phases appear
- Collect the upper phase to which is added 5 mg of sodium sulfate
- Allow to stand for 48 hours

Is performed by reading the optical density of the samples with the spectrophotometer at a wavelength of 528 nm.

Measurement of relative water content (TRE)

It expresses the amount of water present in% of the amount measured saturation and allows a physiological assessment of water status of the plant as well as water potential.

It was determined on a sample of three leaves well developed by the method of (Hanson et al., 1982), which involves cutting the sheet at the base of the blade and weighed immediately. (Fresh weight; PF), the severed end result is placed in distilled water at 4 ° C in the dark for a minimum of 18-20 hours to obtain a maximum rate of hydration water (weight saturation Psat). The sheet is weighed again after drying in an oven (80 ° C / 24 hours) (dry weight; PS). The dates when samples coincide with those of the previously cited proline.

His estimate is the following formula:

$$TRE = (PF - PS) \cdot 100 / (P_{Sat} - PS)$$

PF = fresh weight
PSAT = weight saturation
PS = dry weight

The equipment used and sowing

The experimental design is a randomized complete block design with four replications for all populations of the species studied.



Figure 2.2: the device used for the experiment (Original photo 2)

Statistical Treatment:

The results have been the following statistical treatments:

An Analysis of variance two criteria classifications (populations- water regimes) for the 3 samples with a comparison of means by the Newman -Keuls test , which was performed by the Statistica software, version 8 (2007).

Results and discussion

The proline content:

The first sample was taken (Pro1)

The content of the first sample taken proline is shown in Figure 3.1 and Table 3.1 ANOVA.

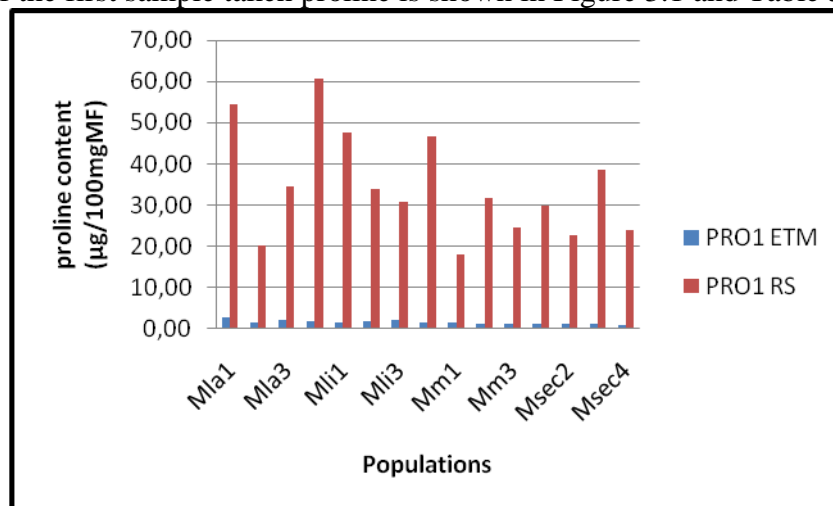


Figure 3.1: The proline content of the first sample taken in populations: *M.laciniata*, *M.secundiflora* , and *M.littoralis M.minima* .

Table 3.1: Analysis of variance for the regime and ETM NS regime two characters and four species studied.

	average	standard deviation	CV %	F	P
Pro1 NS	1,62	0,44	26,94	5,2533	0,0171
Pro2 NS	1,99	0,57	28,78	3.28	0.062
Pro3 NS	3,06	1,33	43,66	0.83	0.50
TRE1 NS	59,66	9,34	15,66	1.78	0.21
TRE2 NS	51,75	5,80	11,22	1.16	0.37
TRE3 NS	48,92	4,84	9,90	1.39	0.2
Pro1 RS	34,57	12,82	37,11	1,8990	0.188
Pro2 RS	44,67	12,42	27,80	1.187	0.36
Pro3 RS	70,76	22,05	31,16	2.82	0.06
TRE1 RS	41,05	5,38	13,11	1.013	0.42
TRE2 RS	32,01	5,17	16,15	0.73	0.55
TRE3 RS	27,31	4,37	16,01	0.20	0.89

For the first sample taken (Pro1), analysis of variance indicates an insignificant effect between species and populations and even the interaction between people and the water regime but a very highly significant effect between the two water regimes .

For the unstressed system, the highest population in proline content was MLA1 population with a grade of 2,7µg / 100mg; by against the Mse4 population had the lowest content not exceeding 1 .mu.g / 100mg.

Upon observation of the results of stress regime, we find that the population Mla4 had the highest content (60,81µg/100mg) in contrast to the Mm1 population with low 17,94µg / 100mg . (Table 3.1).

The Newman -Keuls test gives three groups of overlapping averages (Figure 3.2).

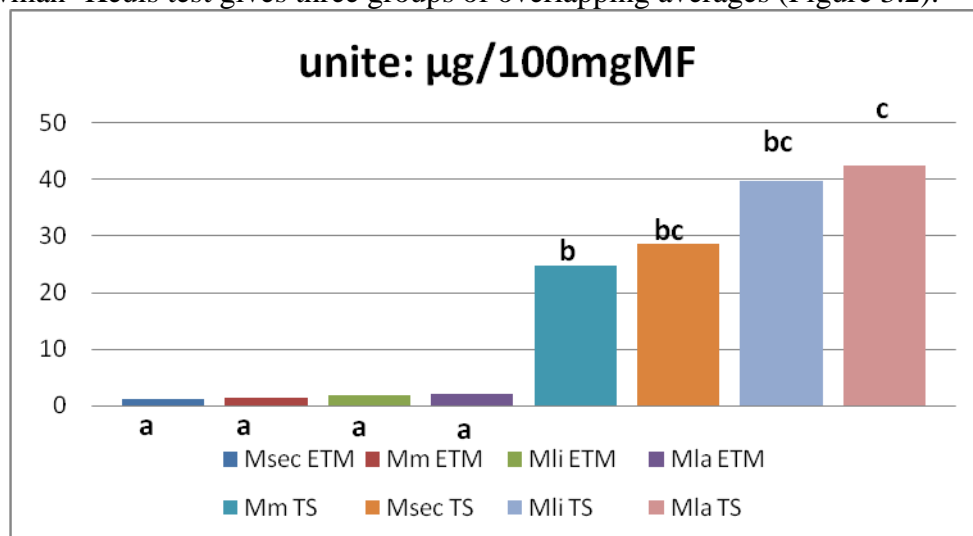


Figure 3.2 : Le test de Newman et Keuls pour Pro1

The second sampling (Pro2) :

The content of the second sample taken proline is shown in Figure 3.3 and Table 3.1 ANOVA

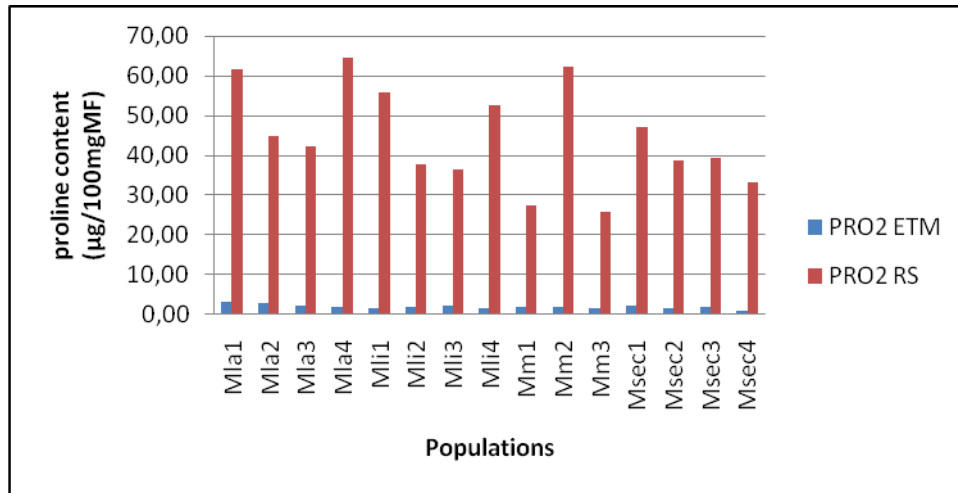


Figure 3.3: The proline content of the second sampling in populations *M.laciniata*, *M.secundiflora*, and *M.littoralis M.minima*.

The test of comparison of means shows a very highly significant effect between the two water regimes with F obs equal to 178.04, Thus, the effect was not significant between the people and even the interaction between the two variables (populations / water regime).

For the unstressed regime MLA1 the population remains strongest in proline content with 3,26µg / 100mgMF and Mse4 population is lowest with 1,07µg / 100mgMF.

At the stress regime, and 64,63µg / 100mgMF the Mla4 population is still the largest value, Mm3 has the lowest population in content (Table 3.1).

The Newman -Keuls test highlights two groups of medium that does not overlap (Figure 3.4).

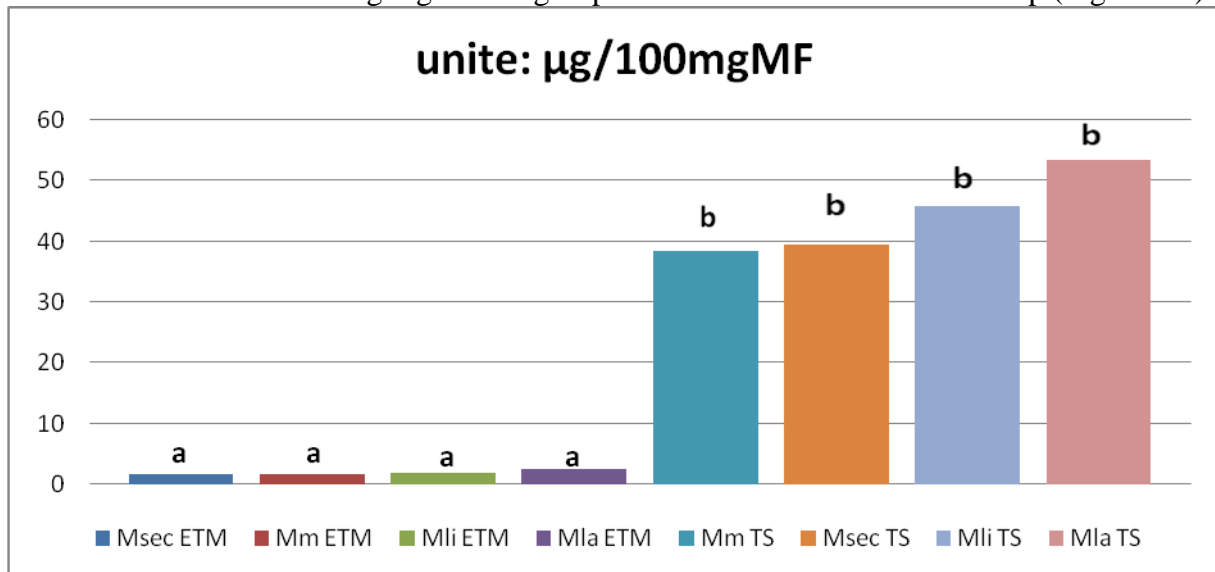


Figure 3.4: The Newman -Keuls test for Pro2

The third sampling (Pro3)

Very highly significant differences between the two water regimes. Analysis of variance revealed significant effects between populations (Figure 3.6) where *M. laciniata* (TS) has a distinct group with an average of 93.08 species mcg / 100mgMF

For the unstressed regime, the population had a high content of proline was Mm2 population with a grade of 6,85µg / 100mgMF, and Mm3 population was characterized by a low- proline with 1,72µg / 100mgMF (Table 3.1 and Figure 3.5).

For the stressed system, people who had a low population was Mse3 with 41,06 μg / 100mgMF by Mla4 against the population is always characterized by the highest proline content (Table 3.1 and Figure 3.5).

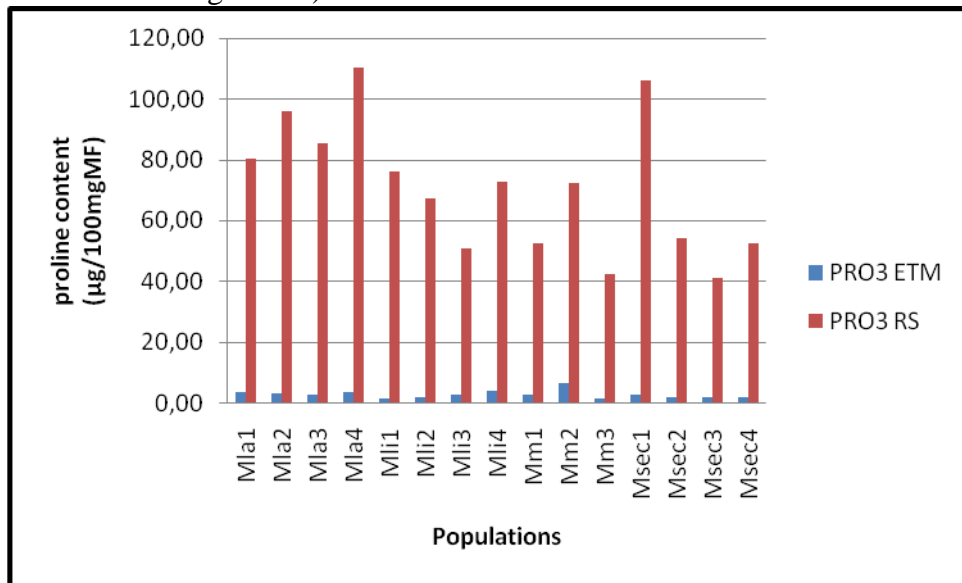


Figure 3.5: proline content of the third sampling in populations: *M.laciniata*, *M.secundiflora*, and *M.littoralis* *M.minima*.

The Newman -Keuls test highlights three groups averages that do not overlap (Figure 3.6).

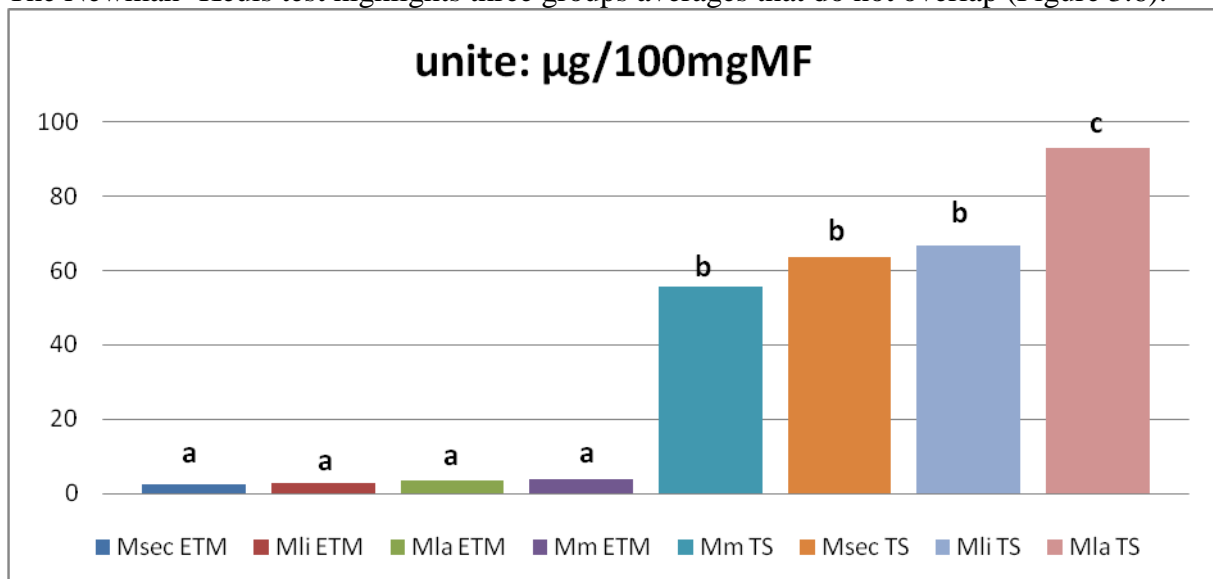


Figure 3.6 : Le test de Newman et Keuls pour Pro3

The relative water content

First measurement taken:

The results obtained during this test tap revealed a non-significant effect between populations (Table 3.1) ; For the stressed system , the population characterized by a high water content is MSE2 population with content that reaches 50.79 % , on the other hand, Mli4 population is characterized by low water content with a percentage of 32.83 %.

For the unstressed regime the lowest level characterizes Mli1 population (45.27 %). Furthermore, the population is characterized by a high relative content is Mm1 the population (Figure 3.7).

For interaction between the two water regimes and populations analysis was not significant, for the cons are very highly significant differences between the two different water regimes. The Newman -Keuls test shows three groups of averages that do not overlap (Figure 3.8).

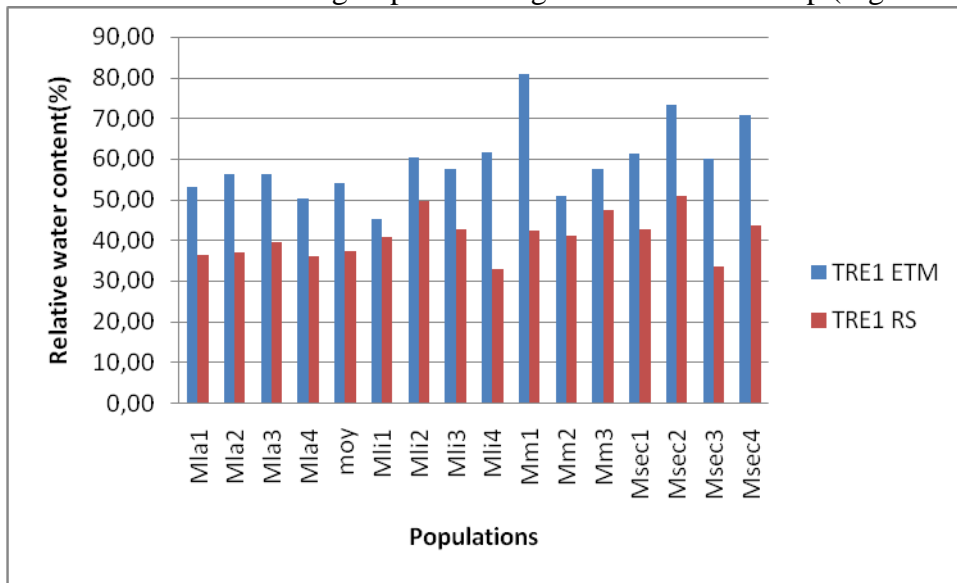


Figure 3.7: the relative water content of the first sample taken in populations: *M.laciniata*, *M.secundiflora*, and *M.littoralis M.minima*.

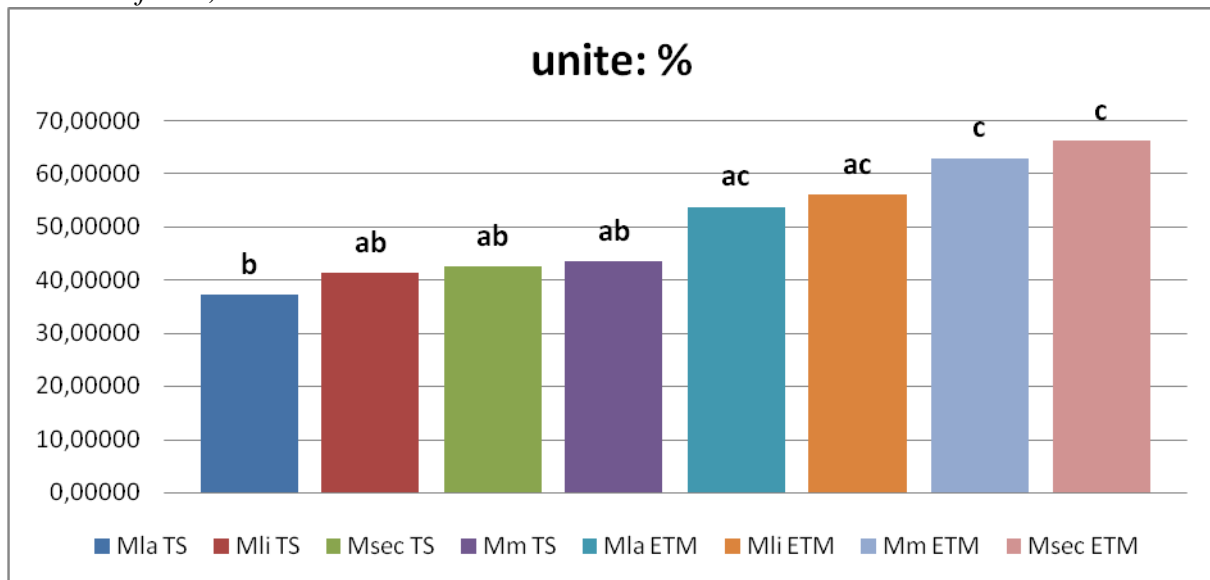


Figure 3.8: The Newman -Keuls test for TRE1

Second power measurement:

The analysis of variance reveals no significant differences between the populations. We note that unstressed regime Mm1 the population has the greatest water content reached 64.05 % in contrast to the Mli1 population is characterized by low (41.62 %) (Table 3.1).

Regarding the stress regime, the MSE2 population is still characterized by greater relative water content (42.88 %), and Mm1 a low population (22.04 %) (Figure 3.9).

Analysis of the results reveals more insignificant differences between water regimes and very highly significant for the interaction of two variables.

The Newman -Keuls test revealed two groups of averages that never overlap (Figure 3.10).

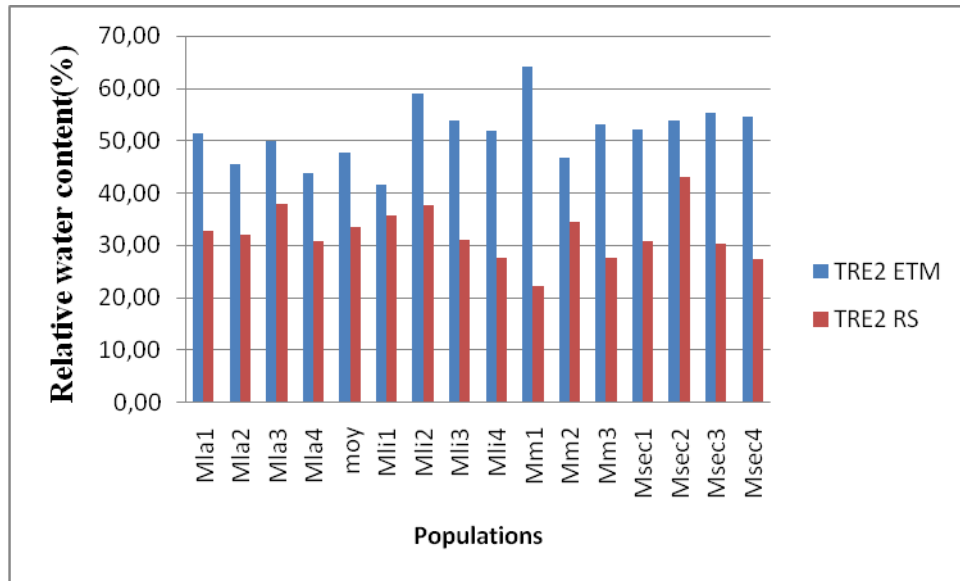


Figure 3.9: the relative water content of the second sampling in populations : *M.laciniata* , *M.secundiflora* , and *M.littoralis M.minima*.

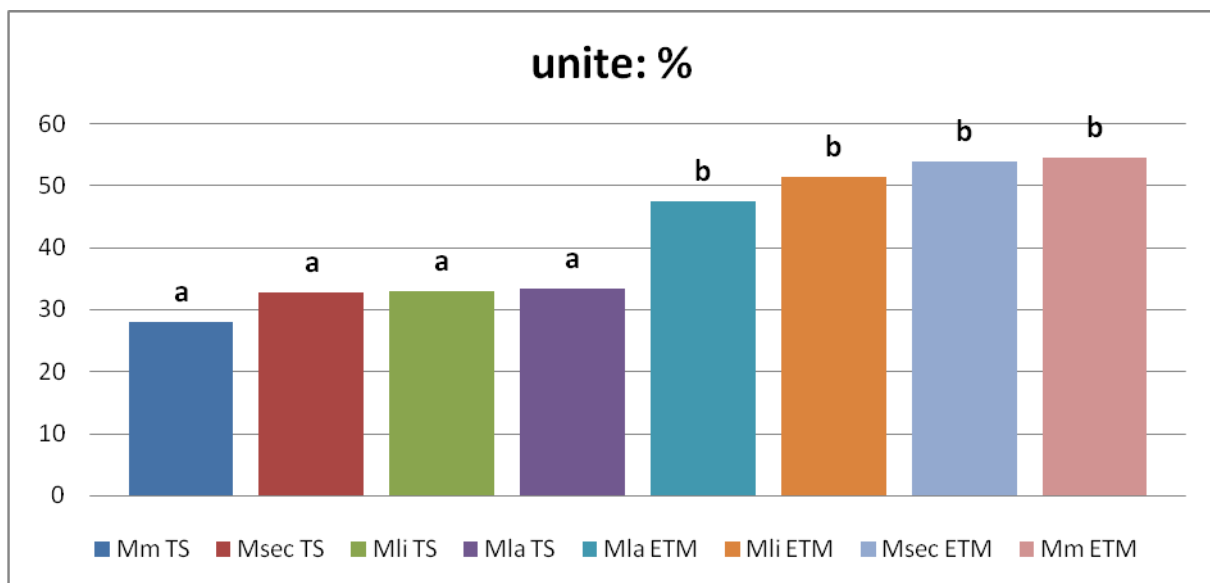


Figure 3.10: The Newman -Keuls test for TRE2

Third taking measurements:

Analysis of variance highlights very highly significant differences between the two regimes applied differences. Between the people and for the people * water interaction regimes, against the effect was very highly significant (Table 3.1).

Populations with the highest levels for water are Mm1 population of ETM with 55.98 % and MSE2 stressed the diet with 36.79 % and Mli1 populations ETM (40.63 %), Mm1 the stress regime (20.66%) are the lowest (Figure 3.11).

There are two groups of averages that never overlap (Figure 3.12).

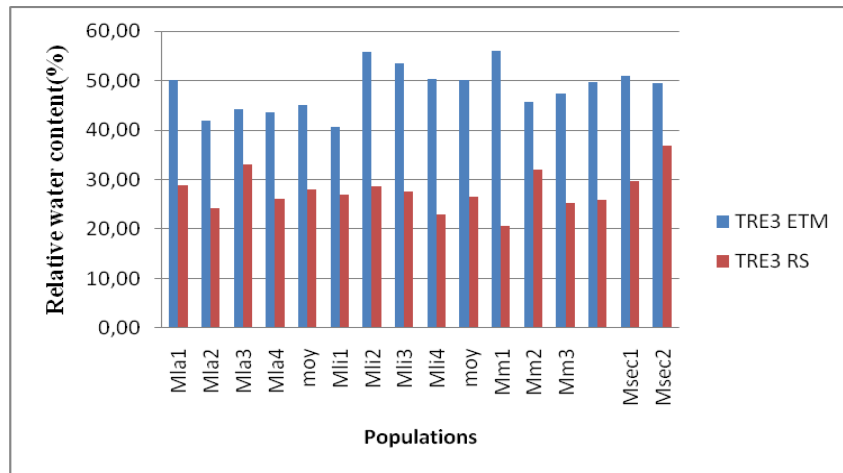


Figure 3.11: The relative water content of the third sampling in populations : *M.laciniata* , *M.secundiflora* , and *M.littoralis M.minima*.

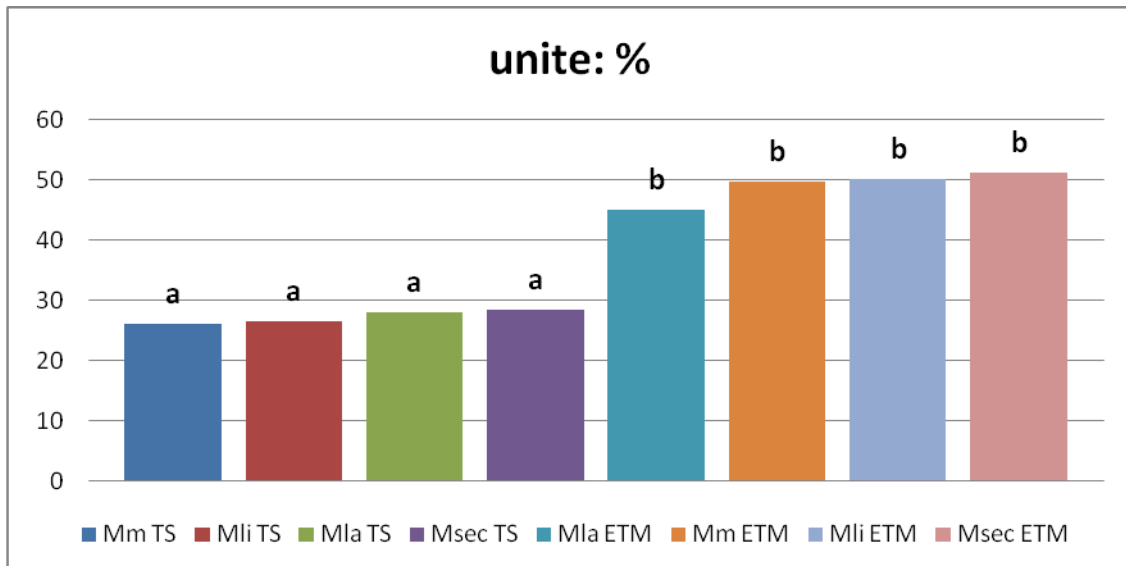


Figure 3.12: The Newman -Keuls test for TRE3

Discussion

Regimes in severe water stress, the four species react by an average increase more or less important in the synthesis of proline. This reaction is amplified with the duration of the water stress. The process of concentration of proline in leaf tissues of stressed plants is recognized as an adjustment feature (Deraissac, 1992; and Kameli Losel, 1995). This is an equally important component of the osmotic adjustment observed in many species cultivated as wheat (Munns and Weir, 1981; Gaudillière and Barcelo, 1990; Johnson et al Flanagan ., 1992; Adjab , 2002; and Adjab Khezane , 1998) and in alfalfa (Mefti et al., 2000).

This strong synthesis of proline is a sign of lack of tolerance to water in the sense that there was a significantly higher rate of proline in the four species compared to their non-stressed controls.

The accumulation of proline under deficit conditions has been reported by several researchers, on different types of plants; in hard and soft wheat (El Jaafari, 1993; Nouri, 2002), tomato (Claussen, 2005), Tea (Chakraborty et al., 2002), Bean (Bousaba, 2001).

Simultaneous or non accumulation of solute depending on the variety and the degree of stress, allows plants to withstand lack of water, maintaining their leaf relative turgidity least interference as possible and preserved cellular integrity (Philadelphia, 1993).

However, Claussen (2005), working on tomato, under conditions of salt and water stress, suggesting that the accumulation of proline would be due to induction or activation of the enzyme involved in the biosynthesis of proline, or due to a lowering of its oxidation to glutamate, and improved protein turnover.

Passive cell dehydration plants tested, induced a loss of turgor at the cellular level, to overcome this, the plant tries to limit water losses that resulted, in large part to decreased sweating after stomatal closure (Ykhlef, 2001; Nouri, 2002).

Water stress applied in our experiment caused a sharp decrease in relative water content, which is closely associated with the ability of osmotic adjustment, which is about 32.7% for populations of *M. laciniata*, 50.5% for *M. secundiflora*, 56.2% and 67.5% *M. littoralis*. *M. minima*.

The analysis of the relative water content (TRE) is used to describe in a comprehensive manner the water status in response to water stress and to assess the ability to achieve good osmo-regulation, and maintain cell turgor (El Jaafari, 2000).

In our work, the levels of water stress applied led to a decline in the relative water content of the populations tested. A similar behavior was observed in a number of plants: barley and fescue (Bennaceur, 1994), wheat (El Jaafari, 1993; Ykhlef, 2001; Nouri, 2002), and in many wood such as *Casuarina glauca* (Albouchi et al., 2003).

The greater the intensity of water stress is growing more ERR falls, while keeping relatively high values compared to the ETM, reaching minimum values for the level of the most severe water stress (95%). This lowering is due to the phenomenon of dehydration, so a loss of water affecting plant cells.

Maintaining the TRE higher or lower compared to control for populations under water stress, is probably due to active regulation osmo, following the establishment of a mechanism for tolerance to water stress namely osmotic adjustment. Indeed, the same type of work carried out by Turner (1979); Bennaceur (1994) and Nouri (2002) on different types of plants subjected to water stress of different intensity, confirmed that indeed in the latter, the implementation of some mechanisms of drought tolerance as the osmotic adjustment has been increased.

Général conclusion

The installation of a forage crop in Algeria suffers not only from low increase (due to lack of material and financial resources) but also weather conditions (drought). The decline in rainfall accumulated from a given period of the year is growing year. Nowadays, it is considered the major abiotic stress affecting crops.

The aim of our study was to estimate the influence of water deficit on two physiological characteristics that are relative water content and proline accumulation in populations of *M. laciniata*, *M. secundiflora*, and *M. littoralis minima*.

The results we have achieved show that these two parameters are affected by water stress; however, the responses of people facing the stress is different.

Furthermore, unstressed (ETM conduit) and leads to stress regimes 95% of the rate of drying, the four species reacted on average by a more or less significant increase in the synthesis of proline, the longer the stress s 'being more proline content increases.

Furthermore, cash teneures reduced their relative water , every time the length of the water stress lengthens the relative water content decreases.

Finally, and according to our results, we can conclude that the two parameters studied there is a significantly negative correlation therefore, when the proline content increases it is always followed by a decrease in the relative water content.

At the end of this study, we suggest that research is ongoing to verify our results.

Behavior of plants on land should be studied in order to validate the results obtained under controlled conditions. This will see the difference between an undisturbed when the management of ground water, as well as the growth and resistance of plants to drought stress, The amount of water made during this study was calculated based on the capacity of retention, we suggest that the study be conducted according to the actual needs of Medicago water and apply this study vegetative stage up at the end of the life cycle.

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