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Influence of the environment on the content of primary metabolites in the aerial part of *Tamarix* sp. in the arid zones of the Khenchela region (Eastern Algeria)

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Abstract

In this work, we tried to determine the influence of the environment on the content of the main primary metabolites in *Tamarix sp.* in different areas of the arid region of Khenchela located in the Algerian East where different species of this genus of the Tamaricaceae family represent a remarkable adaptation, where we recorded rather variable values according to the environment. The dosage of chlorophylls (a+b) gave values ranging from 10.05 (µg/g MF) for the site of Guerat El Taref, 10.78(µg/g MF), for the site of Ouazerne 19.02 (µg/g MF), for the site of Oued El Arab. The determination of total sugars gave 0.178 (µg/g MF) for the site of Guerat El Taref, 0.182 µg/g MF) for the site of Ouazerne and 0.184 µg/g MF) for the site of Oued El Arab. The results of the proline and total protein assay followed the same fluctuation with the lowest values in the Guerat El Taref site, the average values in the Ouazerne site and the highest values in the Oued El Arab site. On the other hand, the DNA and RNA quantification gave a high value of 10.85 (µmol/g MS) for Guerat El Taref, 5.62(µmol/g MS) for the site of Oued El Arab and the lowest value that is 1.79(µmol/g MS) for the site of Ouazerne. Statistical analysis by one-way analysis of variance revealed a significant link between environment and the synthesis of these secondary metabolites as an adaptation strategy adopted by *Tamarix*.

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Introduction

Arid and semi-arid lands represent one-third of the world's surface, especially in the Mediterranean basin. The current data are summarized in 16 million hectares of saline soils. In this region, soil salinization is one of the major abiotic factors that limit plant productivity and agricultural yield (Zid and Grignon, 1991; Baatour *et al.,* 2004; UNCCD, 2009; UNEMG, 2011).

Algeria, where a large part of the agricultural regions is characterized by an arid and semi-arid climate, is affected by the process of salinization, soil erosion and drought. Currently, nearly 3.2 million hectares are affected (Hamdy, 1999; Meradi *et al.*, 2016; Daoudi *et al.*, 2021).

These ecosystems are characterized by a scarcity of rainfall with a high irregularity (Mnif and Chaieb, 2004; Rezgui *et al.*, 2004) associated with significant evaporation promoting the accumulation of salts in the soil (Hayek and Abdelly, 2004).

This phenomenon affects nearly 7% of the global surface area in the world (Munns, 2002; Rechachi *et al.*, 2020).

It is possible to limit the extent of salinization, erosion and drought of the land by exploring saline ecosystems and identifying halophytic species with economic and/or ecological potential to use these species naturally tolerant to these abiotic constraints for the rehabilitation and enhancement of degraded ecosystems (Belkhodja and Bidai, 2004; Ghernaout *et al.*, 2020). Varietal selection requires knowledge of the mechanisms responsible for plant tolerance to salinity. Thus, several halophytes express high potentialities of growth, salt uptake and storage in their aerial parts are interesting for soil fixation and desalination in arid and semi-arid areas, as well as in the fight against erosion in these regions (Messedi and Abdelly, 2004).

The Tamaricaceae constitute a very important family of halophytes, especially the genus *Tamarix*. Unlike glycophytes that cannot tolerate the presence of salts, halophytes grow best in saline soil (Calu, 2006; Soundararajan *et al.*, 2019). They trigger tolerance mechanisms that help adapt to osmotic and ionic stress caused by high salinity (Lee *et al.*, 2008). These mechanisms adjust internal osmotic pressure through electrolytes and organic solutes (Driouich *et al.*, 2001), mainly soluble sugars and amino acids, such as proline (Taji *et al.*, 2004; Denden *et al.*, 2005).

Tamarix sp., a plant well adapted to aridity and salinity, is considered among the plant species that best valorize the water of saline soils, thanks to its high vacuolar osmotic pressure due to high salt concentrations. It also has a highly developed root system fixing the upper layers of the soil and can be used as a means of combating desertification (Khabtane *et al.*, 2017).

The objective of this work is the evaluation of the effect of the environment on the accumulation of the main primary metabolites: chlorophyll (a+b), total sugars, proline, total proteins, NDA and RNA in *Tamarix sp*., the quantification of these parameters in the aerial part is done by optical spectrophotometry.

The samples are taken from three sites extremely different from the climatic and edaphic point of view of the arid zone of Khenchela, which are chosen according to a North-South transect :
Garat El Taref in the extreme North, is a sebkha salty and flooded with water more than half the year;
Oued El Arab, in the center of the study area, is a transition zone between the desert and the North ;
Ouazerne located in the extreme South (Sahara), is a total desert area.

Material and methods

Presentation of the study area

Location of the study area: The region of Khenchela is located in the North of Algeria, in the South-East of Constantinois; and in the foothills of the Aurès mountain between 34° 06' 36" and 35° 41' 21" of North latitudes; and between 06° 34' 12" and 07° 35' 56" of East longitudes.

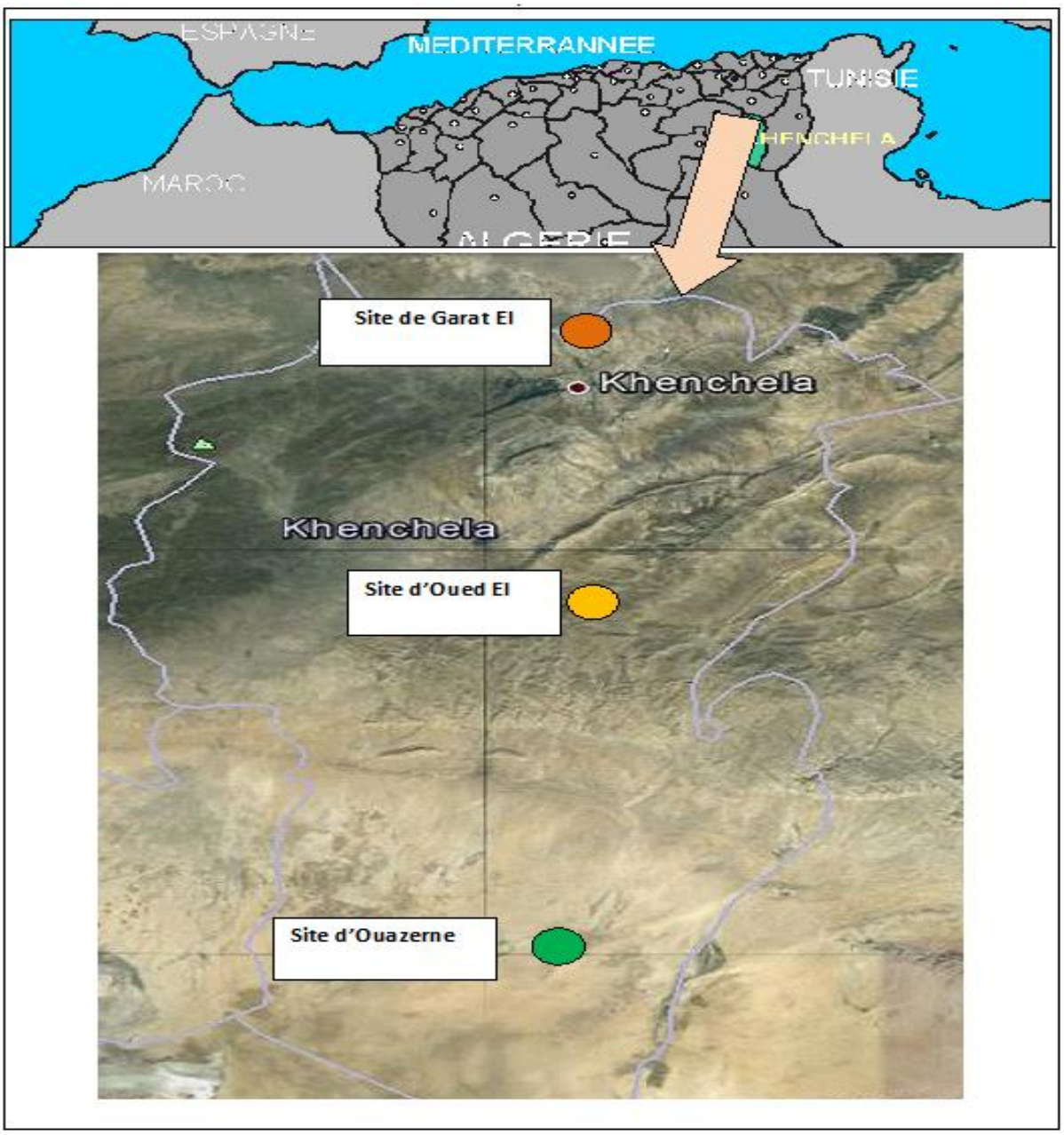

Fig. 1. The geographical position of the Khenchela region and the distribution of the study sites (source: In Khabtane *et al.*, 2014).

It is distinguished by its very diversified and multifaceted physical and natural environments, according to (Djebaili, 1984), ranging from:

Tellian landscapes (high mountain areas, well watered and well wooded with green landscapes): Mountains of the Aurès occupying the western part of the wilaya;

High plains landscapes (high semi-arid cereal plains) for the northern part of the wilaya;

Steppe and Saharan landscapes composed of: completely bare and eroded mountains (Némenchas mountains in the east), oases (Siar, Khirane and El Ouldja) and low plains (El Meita and Ouazerne)**.**

Choice of sites: For the realization of our study, we have chosen three (03) sites across the region according to a North / South transect whose order is as follows:

Wet salt site, in the North in the area of Garat El Taref

in the limit between the wilaya of Oum El Bouaghi and the wilaya of Khenchela;

Site in a transitional zone between the desert and the such, in the center in the town of Babar on Oued El Arab near the dam of Babar;

Desert site, in the South located in the area of Ouazerne in the commune of Babar (Fig.1).

Climatic characterization

The bioclimatic study conducted by Khabtane in 2010 confirms that the climate of the study area shows a very significant irregularity between its different zones where it goes from a semi-arid climate in the North to a purely desert climate in the South. The first area, representing respectively the site of Garat El Taref and the site of Oued El Arab, is characterized by two clear-cut seasons: a relatively wet season of about 8 months and a summer season, dry and hot about 4 months with an average annual rainfall varies between 462.8 mm and 393.8 mm. The second desert zone (Ouazerne site) is characterized by a dry period that extends over the twelve months of the year with an average annual rainfall of 124mm.

Following the study of different climatic indices, we can conclude that the study area represents two different bioclimatic stages; one semi-arid with cool winter (the site of Garat El Taref and the site of Oued El Arab), the second desert with mild winter (site of Ouazerne) (Khabtane, 2010).

Edaphic characterization

The study of the physical parameters of the soil of the three sites reveal that the texture is variable; sandy-silty to clayey with the dominance of sandy elements with the highest rate then clays in second place, so the study of the chemical parameters of the soil in the three sites shows a very large variability from one site to another (Khabtane, 2010).

Plant material

The plant material that was the subject of this study concerns the aerial part (leaves and branches) collected from the three study sites according to the vegetation sampling techniques (Archaux *et al.*, 2005); the samples were taken during the first flowering season, i.e., in March. The plants were collected very carefully so as not to damage the organic and mineral elements present.

The experimental protocol

Determination of total chlorophyll

The extraction of chlorophyll is carried out according to the method of HOLDEN (1975), which consists of maceration of the plant in acetone; the plant is cut into small pieces and crushed with a mortar in acetone (80%) while adding a few milligrams of calcium carbonate. After the total grinding, the solution obtained is filtered and put in black boxes (to avoid the oxidation of chlorophyll by the light), then one proceeds to the reading of the optical densities of the solutions with a spectrophotometer, at two wavelengths: (645 and 663 nm), after calibration of the apparatus with the control solution of acetone to 80%.

According to Arnon (1949) in (Porra, 2002) the formula for the solvent allows us to calculate the value of chlorophyll as follows:

Total chlorophyll (a+b) (µg/g FM) =8.02 x OD_{663} + 20.2 × OD_{645}

Wher :
FM : Fresh Matter
OD : wavelengths

Determination of soluble sugars

We proceeded to the determination of soluble sugars in plant leaves according to the method of Dubois (1956). For the extraction of soluble sugars: Put 100 mg of fresh plant material in test tubes, then add 2 ml of ethanol at 80%. Leave the tubes closed to rest for 48h.

Evaporate the alcohol by putting the test tubes in a water bath at 70°C. After cooling, add 20 ml of distilled water to each test tube. Take 1 ml of the

solution and add 1 ml of 5% phenol and shake well. Add 5 ml of concentrated sulphuric acid in each test tube, then pass them to the vortex, let rest for 10mn, then put the tubes in the water bath for 15 mn at 30°C. Proceed to the reading with a spectrophotometer at the wavelength of 490 nm. The determination of the content of soluble sugars is done according to the formula:

Soluble sugars (µg/g DM) = DO_{490} x 1.657

Wher:
DM : Dry Matter
OD : wavelengths

Determination of proline

Proline is determined according to the technique used by Troll and Lindesly (1955), simplified and developed by Dreier and Goring (1974) and modified by Monneveux and Nemmar (1986).

The principle is the quantification of the proline-ninhydrin reaction by spectrophotometric measurement.

Proline couples with ninhydrin forming a colored complex. The intensity of the coloration is proportional to the amount of proline in the sample. 100 mg of fresh plant material is placed in test tubes and 2 ml of 40% methanol is added. The covered tubes (to avoid volatilization of alcohol) are boiled in a water bath at 85 °C for 60 min.

After cooling, 1 ml of the solution was taken from each tube and put into new tubes, to which we added 1 ml of acetic acid and 25 mg of ninhydrin. Then, 1 ml of a mixture containing: 120 ml of distilled water, 300 ml of acetic acid, 80 ml of ortho-phosphoric acid was added to each tube.

The test tubes are boiled in a water bath for 30 min. After cooling the solutions, 5 ml of toluene is added to each tube. After vortexing, two phases appear. The upper phase is taken and 5 mg of sodium sulphate is added to it, then left to stand for 48 hours. The optical density of the samples is read with the spectrophotometer at a wavelength of 528 nm. The determination of the content of proline is carried out according to the formula:

Proline (µg/g DM) = DO_{528} x 0.62

Wher:
DM : Dry Matter
OD : wavelengths

Determination of total proteins

Protein determination was performed according to the colorimetric method of Bradford (1976) as follows: each sample consisting of 0.5g of fresh leaf material is cut and macerated in 10ml of trichloroacetic acid acetic (20%).

After grinding and filtration in test tubes, 1ml of the extract is taken and centrifuged at 5000rpm for 10min. The supernatant is poured out and the pellet is kept in the same tube, to which 1ml of ether/chloroform mixture (1/1) is added, then second centrifugation is carried out at 5000rpm which gives two phases, the supernatant is recovered to which 1ml of NaOH (0.1N) is added and energetically shaken for the dissolution of proteins.

Then we take, by means of a micropipette, a volume of 100µl to which we add 4ml of BBC reagent (brilliant blue of Coumassie (50mg BBC + 50ml of orthophosphoric acid 85% and we complete 500ml with distilled water), a blue color appears and we pass directly to the reading with the spectrophotometer to a wavelength of 595nm.

The calculation of the real concentrations is done by the equation deduced from the calibration range and by conversions taking into account all the dilutions carried out (Fig.2).

Nucleic acid quantitation

DNA and RNA were assayed according to the technique used by Burton (1956). Put 0.2g of dry plant material in test tubes to which 2 ml of perchloric acid (0.5N) was added. Incubate in a water

bath for 20 min at 90°C. Then put the test tubes in the centrifuge for 10 min at 2000 rpm. At the end of the centrifugation, we obtain a solution containing both DNA and RNA.

Determination of DNA

0.5 ml of the recovered solution is taken, to which 0.5 ml of perchloric acid and 2 ml of diphenylamine are added in test tubes.

The tubes are covered and left to stand in the dark for 18 hours, after which the optical density of the samples is read with the spectrophotometer at a wavelength of 600 nm.

Preparation of diphenylamine reagent:

Dissolve 500mg of diphenylamine in 49 ml of acetic acid and 1 ml of concentrated chloride acid HCl. The determination of the DNA content is performed according to the formula:

ADN (µmol/g DM) = (DO_{600} – 0.015) / 0.005986

Wher:

DM : Dry Matter

OD : wavelengths

RNA quantitation

1 ml of distilled water is added to 0.5 ml of recovered solution and 1.5 ml of orcinol in test tubes, which are boiled in the water bath for 45 min. After cooling with tap water, the optical density of the samples is read with the spectrophotometer at a wavelength of 675 nm.

Preparation of the orcinol reagent: In an Erlenmeyer flask, 3 mg of copper chloride is added to 100 mg of orcinol, onto which 50 ml of HCl is poured. The determination of the RNA content is carried out according to the formula:

ARN (µmol/g DM) = (DO_{675} – 0.0071) / 0.0061

Wher:

DM : Dry Matter

OD : wavelengths

Processing and statistical analysis

All the trials were repeated at least three times, the results presented in the form of histograms, most often join mean values and their standard deviations, the latter two were carried out by the Excel 2007 software.

The data obtained are subjected to an analysis of variance with two fixed factors; the averages are compared according to the method of Newman and Keuls (Dagnelie, 1999), based on the smallest significant value, carried out by the software MINITAB version 13.31 for Windows.

Results are considered significant when $p \leq 0.05$. The graphs presented and the tables of the groupings of the means are made by Excel 2007.

The obtained means were compared with each other by analysis of variance (ANOVA) performing Fisher's multiple tests for a significance level of 5%.

The homogeneous groups were created with the "XLSTAT 2009" software using the Newman-Keuls test.

Results and discussion

Variation in chlorophyll content (a+b)

The analysis of variance at a classification criterion shows that there is a very highly significant difference between the different measured means of total chlorophyll content (chl a+b).The Newman and Keuls test at the threshold α = 5% shows three homogeneous groups A, B and C. The first group (A) dominant is represented by the study site Oued El Arab with a maximum average of total chlorophyll content of 19.02 µg/g MF; the second group B represents the study site Ouazerne with an average of 10.78 µg/g MF and the last group C represents the study site Garaet El Taref with an average of 10.05 µg/g MF (Table 1) The results we obtained show the effect of the biotope on the concentrations of total chlorophyll (Fig. 3). The increase in total chlorophyll

content is the consequence of the reduction in leaf cell size under water stress, which leads to a higher concentration (Siakhene, 1984). On the other hand, the fall in chlorophyll content is the consequence of the reduction of the opening of stomata to limit water losses by evapotranspiration and by increasing the resistance to the entry of atmospheric CO_2 necessary for photosynthesis (Bousba *et al.*, 2009).

Table 1. Determination results of major primary metabolites in *Tamarix* sp. at the three study sites.

	paramètrs					
Sites	Chlorophyll (a+b) in (µg/g FM)	Total sugars in (µg/g DM)	Proline in (µg/g DM)	Total Protein in (µg/g DM)	RNA in (µmol/g DM)	DNA in (µmol/g DM)
Oued El Arab	19.026	0.184	1.178	1.178	1.047	5.623
Garat El Taref	10.050	0.178	0.596	0.596	1.513	10.858
Ouazerne	10.783	0.182	0.515	0.515	3.999	1.765

Variation in total soluble sugar content

The analysis of variance at a classification criterion shows that there is no significant difference (Table 2) between the different measured means of total sugar content, which indicates that there is no difference between the three varieties in the accumulation of soluble sugars in their leaves. Exceptionally, the leaf content of total sugars characterized the site of Oued El Arab, which recorded the highest level, 0.184µg/g MF (Fig.4).

Table 2. Results of the one-way analysis of variance at the 5% significance level (Legend: ns: Non-significant effect, *: Significant effect, **: Highly significant effect and***: Very highly significant effect at the 5% threshold, ddl: degree of liberty).

Source	ddl	Chlorophyll (a+b)	Total sugars	Proline	Total Protein	DNA	RNA
Site effect	2	74,469 ***	0,000029 ns	0,27716 ***	0,39251 ***	62,490 ***	7,551 **
Error	6	0,859	0,000453	0,00781	0,00241	0,610	0,303

The test of Newman and keuls at the threshold α= 5% brings out a single homogeneous grouping A. Which groups the three varieties in the three study sites.

The accumulation of soluble sugars is a means adopted by plants under stress to resist environmental constraints (Loretti *et al.*, 2001).

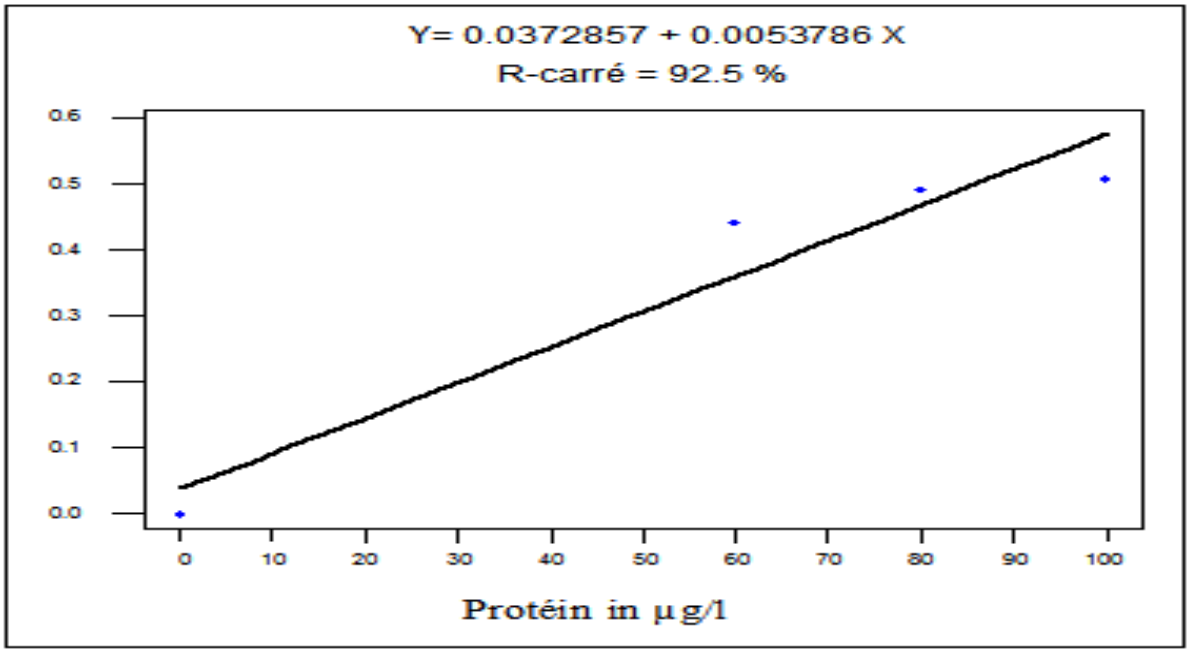

Fig. 2. The protein calibration curve.

Soluble sugars are indicators of the degrees of stress; because of their significant increase in severity, metabolic sugars (glucose, galactose, sucrose, and fructose) allow the resistance to different stresses Zerrad *et al.*, 2006). In contrast, the lowering of sugar concentrations is explained by their storage in a complex form in reserve substances. Corte and Sinclair (1987) and others attributed the increase in soluble sugars to degradation of starch reserves following their rapid conversion to sucrose, which could also be attributed to inhibition of starch synthesis.

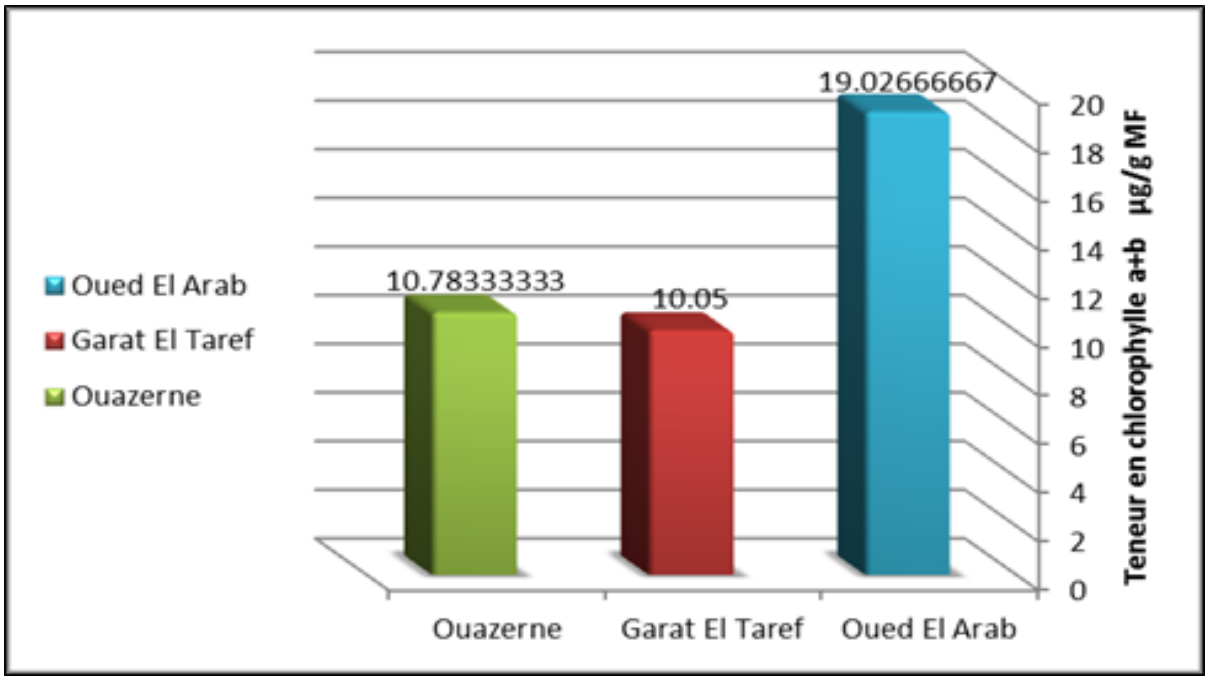

Fig. 3. Results of chlorophyll content (a+b) in (µg/g FM) in the aerial part of *Tamarix* sp. in the three study sites.

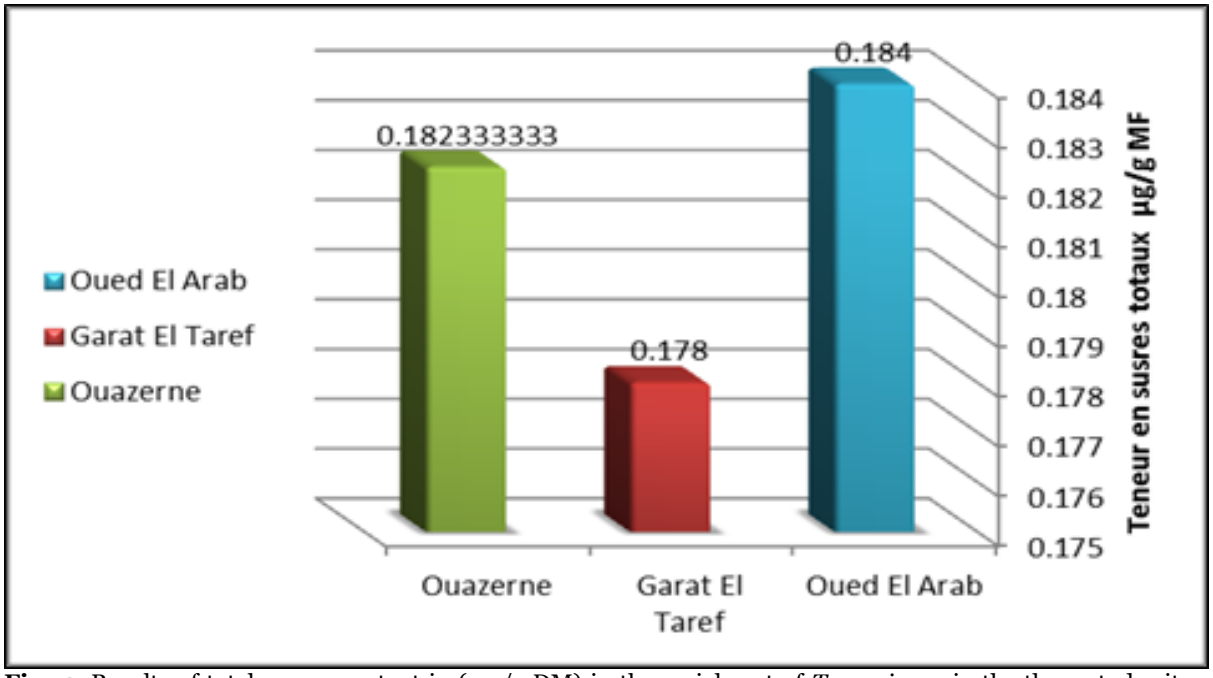

Fig. 4. Results of total sugars content in (µg/g DM) in the aerial part of *Tamarix* sp. in the three study sites.

Soluble sugars protect the membranes against dehydration; under water deficit conditions, they participate to a large extent in the lowering of the osmotic potential.

Variation in proline content

The statistical analysis of the variance of the results obtained reveals the existence of very highly significant differences between the three varieties of the plant in the three different study sites (Table 2).The Newman-Keuls test at the 5% threshold classifies the effect of biotope into three groups A, B and C; Groups A characterizes the highest accumulation of proline with an average of 1.06 µg/g MF. Group B is the site of Garaet El Taref with an average of 0.55µg/g MF. While the last group C, contains the site of Ouazerne with a low average proline content of 0.50µg/g MF (Fig.5).

This variation in proline accumulation observed in Tamarix sp. plants Experimented would be due to compartmentalization of the amino acid, hence the expression of sites of plant resistance to salt stress.

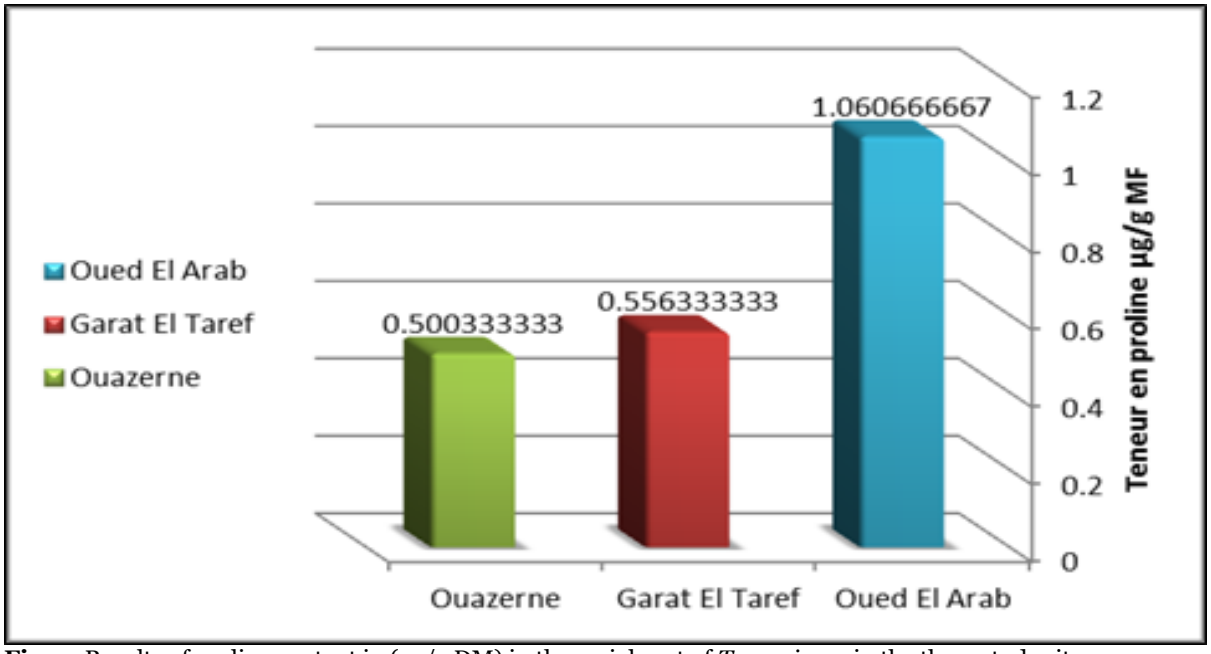

Fig. 5. Results of proline content in (µg/g DM) in the aerial part of *Tamarix* sp. in the three study sites.

The results show the halophilic character of the plant, which responded to abiotic conditions by increasing the proline content. One of the main physiological traits of tolerance to environmental stress is an osmotic adjustment. This is achieved through an accumulation of osmoregulatory compounds leading to a reduction of the osmotic potential, thus allowing the maintenance of the turgidity potential (El Midaoui *et al.*, 2007).

Variation in total protein content

The statistical analysis of the variance of the results obtained reveals the existence of very highly significant differences between the three varieties of the plant in the three different study sites.

The Newman-Keuls test at the 5% threshold classifies the biotope effect into three groups A, B and C; Groups A characterizes the site of Oued El Arab by the highest accumulation of total protein with an average of 1.17µg/g MF. Group B represents the site of Garat El Taref with an average of 0.59µg/g MF. While the last group C contains the site of Ouazerne with a low average protein content of 0.51µg/g MF (Fig.6).

Variation in RNA content

Analysis of variance at a classification criterion shows that there is a highly significant difference between the different measured means of plant RNA content in the presence of biotope constraints.

The Newman and keuls test at the α = 5% threshold highlight two homogeneous groupings A and B. The first group (A) dominant is represented by the sites of Ouazerne with a value of 3.99 µmol/g MS, the second group (B) is represented by Garaet El Taref and Oued

El Arab by successive values of 1.513 µmol/g MS and 1.047µmol/g MS (Fig.7).

The Newman and Keuls test at the α = 5% threshold shows three homogeneous groupings.

Variation in DNA content

The analysis of variance at a classification criterion shows that there is a very highly significant difference (Table 1) between the different measured means of plant DNA content in the presence of biotope constraints.

The first dominant group (A) is represented by the sites of Garaet El Taref with a value of 10.85 µmol/g DM, the second group (B) is represented by Oued El Arab with a value of 5.62µmol/g DM and the third group (C) is represented by the site of Ouazerne with a value of 1.765µmol/g DM (Fig.8).

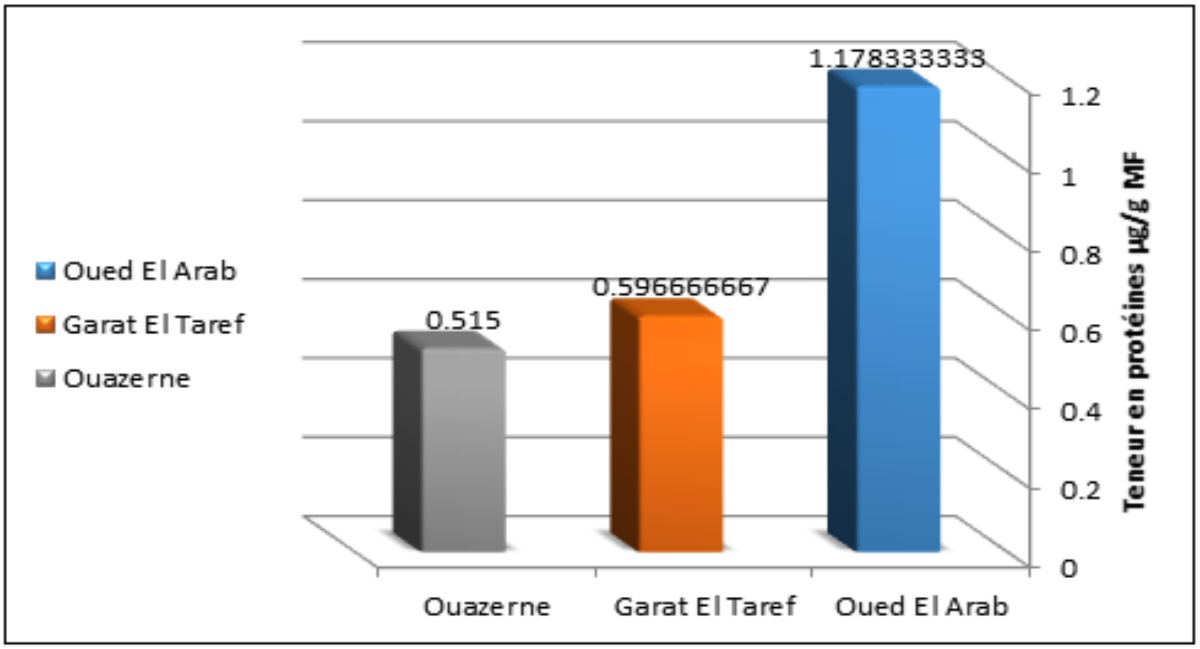

Fig. 6. Results of total protein content in (µg/g DM) in the aerial part in *Tamarix* sp. at the three study sites**.**

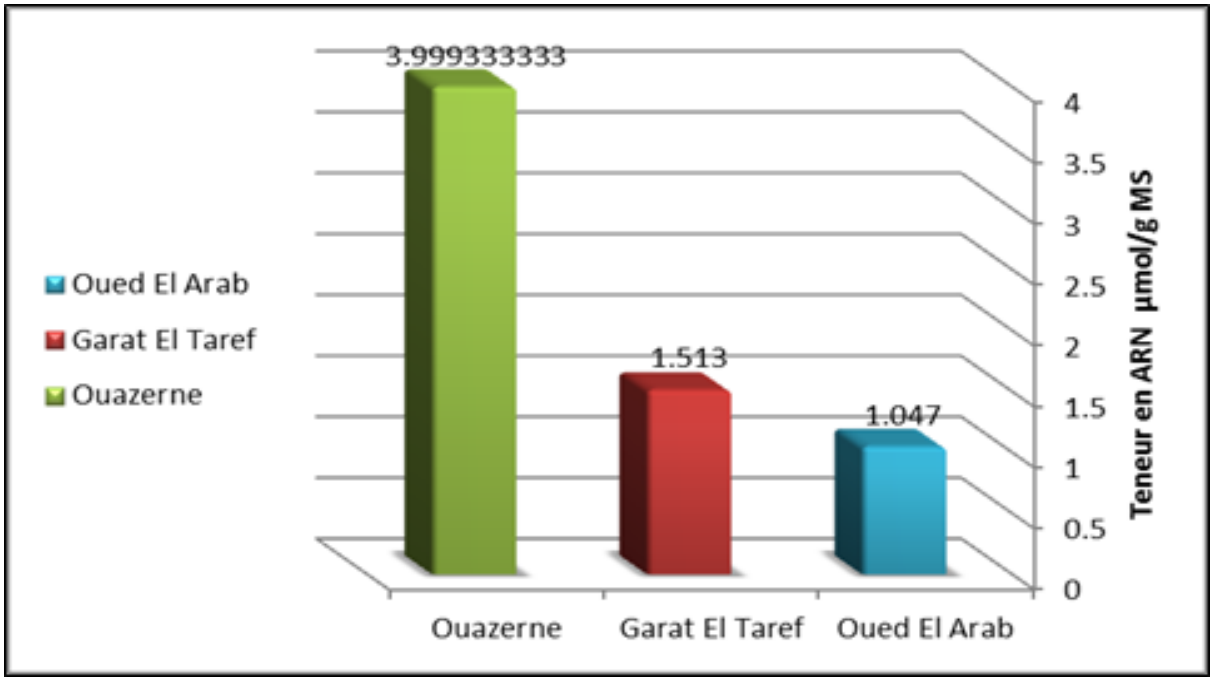

Fig. 7. Results of RNA content in (µmol/g DM) in the aerial part in *Tamarix* sp. at the three study sites.

The levels obtained for the RNA assay are inversely proportional to those obtained for the nitrogenous protein assay, which is probably due to the effects of abiotic constraints on the mechanisms of transcription and protein synthesis. Similar results were obtained in studies by (Nieman, 1965 in Katterman, 1990) on the effects of salinization on beans.

The results obtained show a reduction in the rate of cell development and the synthesis of proteins and RNA in the leaves of bean (*Phaseolus vulgaris* L.).

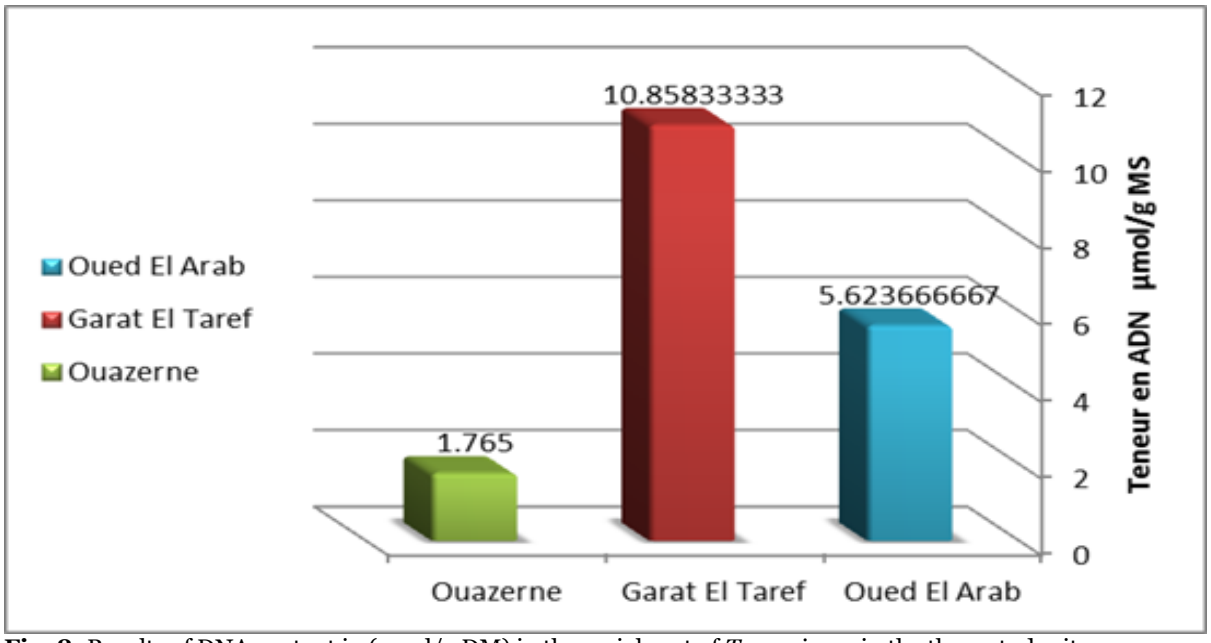

Fig. 8. Results of DNA content in (µmol/g DM) in the aerial part of *Tamarix* sp. in the three study sites.

Conclusion

The results of our study on the effect of the environment on the contents of primary metabolites in *Tamarix sp*. in the arid region of Khenchela located in the east of Algeria, reveal an important physiological adaptation in the stressful environments, whether it is: hydric stress, thermal stress or stress of water excess. This tolerance is reflected in the highest levels of DNA and RNA content that we measured in the stress environments, which leads us to believe that stress resistance mechanisms are triggered.

The effect of the environment is confirmed by the statistical study using analysis by one-way analysis of variance, which demonstrated a significant relationship between the variability of *Tamarix* content in primary metabolites and the environment. These adaptive abilities make *Tamarix* species among those that can be used on a large scale in programs to combat desertification, as well as the exploitation of their genetic patrimony in the improvement of plants of economic interest by deepening research in this field.

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