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Eco-physiological and biochemical characterization of *Rhus tripartita* (Ucria) Grande growing in Algerian Sahara under arid climate

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Abstract: *Rhus tripartita* (Ucria) Grande, is an Anacardiaceae autochthonous shrub of the Algerian Sahara. Its ecological, pastoral and therapeutics interests prompted us to carry out an eco-physiological and biochemical behavior in relation to aridity. Therefore, relative water content of shrub leaves were found on average 81.55% and the maximum of electrolyte leakage recorded was 14.29. The biochemical determination of proteins and sugars shows that leaves are a true source of protein (33.76 mg/g FM) and sugars (938.93 µg/g FM) while the proline assay gave a value of 824.40 µg/g. The quantitative study of flavonoids in the leaves gave a result of 36.53 mg/g. The analysis of photosynthetic pigments content showed respectively results of 28.1 µg/g, 31.24 µg/g, 56.47 µg/g and 11.23 µg/g for chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. The total antioxidant capacity was evaluated and gave result of 95.5 mg GAE/g. Therefore, *Rhus tripartita* was found to accumulate high proportions of primary and secondary metabolites which showed a good adaptation to its arid environment. In conclusion, the plant can be considered as a xeromorphic plant, that is, a desert-adapted plant that is not limited by the water availability.

1. Introduction

Rhus tripartita (Ucria) Grande is synonymous with *Searsia tripartita* (Ucria) Moffett, an Anacariaceae forage plant; it is traditionally used by the Tuaregs (local inhabitants) of Ahaggar (Algeria). This Saharo-Mediterranean shrub is widespread from North Africa to Egypt (Sahki and Sahki, 2004). Ferchichi (1999) has described the shrub as a very drought-resistant species characterized by abundant foliage throughout the year despite the soil's moisture status, and it can be planted successfully on poor and marginal lands. On the geomorphological level of the Algerian

Saharan environment, this plant belongs to the grouping of the Ahaggar mountainous massifs where it grows on shallow soils.

Plant growth is strongly influenced by many biotic and abiotic factors. Ecologists and physiologists have long been interested in the effects of environmental factors that lead to plant adaptation and distribution. Therefore, phyto-ecophysiological studies are important to understand the species response in extreme environments and especially their valuation in these areas. By staying in extreme environments, the Sahara is one of the driest and hottest deserts in the world (Dutil, 1971). The desert fact is explained by extreme values of climatic parameters which constitute the essential factors of vegetation's distribution and the grounds evolution (Daoud and Halitim, 1994). Moreover, aridity is a natural selection force that influences plant adaptive strategies to water stress (Sayed, 1998). Hence different adaptive strategies of plants to aridity have been developed through considerable survival mechanisms. These strategies are divided into three general categories: drought escape, drought avoidance and dehydration tolerance (Levitt, 1972; Turner, 1979). Drought escape is employed by plants under conditions where water limitation late in the growing season is likely, and it ensures that the plants can complete their life-cycle quickly during the brief period of favorable conditions. Drought avoidance is a strategy for avoiding lower water status during drought by maintaining relatively high tissue water content due to reduced evaporatory water loss and an efficient water uptake (Levitt, 1972). The third category, drought tolerance, is the ability of plants to withstand water deficit and maintain metabolism at low tissue water content (Valliyodan and Nguyen, 2008). In this strategy, osmotic adjustment, osmoprotection, antioxidation and scavenging defense system have been the most important physiochemical and biochemical bases responsible for drought tolerance. Cell tissue and water conservation, antioxidant defense, cell membrane stability, compatible solutes and plant growth regulators mainly contributes in above said physiochemical and biochemical mechanisms. The three strategies are not mutually exclusive, and the same plant may use more than one strategy in order to adapt to periods of drought stress (Nilsen and Orcutt, 1996).

Therefore, to adapt with these natural constraints, plants have had to produce primary metabolites such as proteins and soluble sugars. This includes photosynthetic mechanisms, osmoregulation and antioxidant enzymes (Liu *et al.*, 2011; Guo and Gan, 2012). Furthermore, water stress has a positive effect on the production and accumulation of secondary plant metabolites (Ncib *et al.*, 2018). Their biosynthesis is often stimulated in response to biotic or abiotic stress (Naczk and Shahidi, 2004).

The aim of this work is to study the adaptive physiological response of *Rhus tripartita* to its naturally arid ecosystem (Ahaggar, Algeria). For this purpose, we will content to discuss our results in relation to water deficit and oxidative stress.

2. Materials and Methods

Presentation of study area

Localisation. The native plants of *Rhus tripartita* (Fig. 1) are located in the Ilamane region (100 km away from Tamanrasset city) which is situated in the Ahaggar National and Cultural Park (22°49'59" north, 5°19'59" east). This park with an area of 4.500.000 ha, was created by the decree n. 87-231 of 03 November 1987. It is located in the mountainous center part of the volcanic Ahaggar in the extreme Algerian south (Fig. 1).

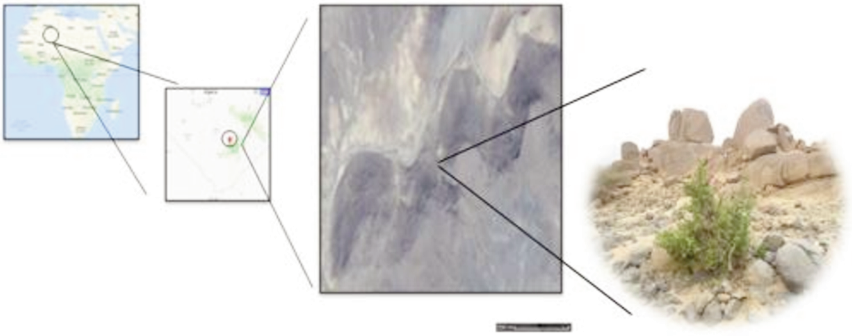

Fig. 1 - Location of *Rhus tripartita* growing in the rocky mountains of Ilamane in the Ahaggar National and Cultural Park.

Features edapho-climatic. Before looking more specifically at plant physiology that interests us, it is necessary to present the main characteristics of the Ahaggar. The bioclimate is arid, with very varied winter and summer temperatures and very low rainfall. The rains occur from May to September and usually increase in August. The annual rainfall varies between 180 mm and 250 mm. The thermal regime varies between the two very short winter and longer summer seasons. Dubief (1959) noted the maximum average on the ground, in July, at 52°C towards 2700 m of altitude (Assekrem) and estimates at 60°C this value at 1376 m (Tamanrasset). These high temperatures condition the biology of plants in this environment.

On the edaphic plane, soil encountered in this area is very little evolved because of a low rainfall. However, this park is marked by a geomorphological distinction (mountainous mass, wadis, hammadas, gueltates, plains and rocky ravines); this has resulted in a high heterogeneity in the soil composition (sandy, sandy-loamy, sandy-clayey, shallow and rocky). Therefore, in the Ilamane region, edaphic variability is a result of shallow skeletal soils that characterize this area.

Determination of physiological parameters

In order to study the *Rhus tripartita* physiology and biochemistry characteristics in relation to its arid environment, different measurements of primary and secondary metabolites are performed. All analyzes were performed on three replicates of six shrubs naturally growing in this area. The sampling was conducted during December 2017 (winter season). Therefore, we consider that 6 individuals are largely sufficient given the physiognomic homogeneity of the population.

Relative water content (RWC)

The RWC measurement is an old method (Slatyer, 1967) which is currently widely used to estimate the plant water status and in particular water deficit. A 12 mm leaf disc taken from the second leaf by means of a punch is weighed directly (weight of the fresh vegetable material, WFVM). It is then placed in the refrigerator in a test tube containing distilled water for 24 hours and weighed to obtain the turgor weight (TW). The fragments are finally placed in an oven at 80°C for 48 hours and then weighed to obtain the weight of dry vegetable matter (WDVM). The RWC is calculated according to the following equation:

$$RWC (\%) = (WFVM - WDVM) \times 100/(TW - WDVM)$$

Metabolites compositions

For the analysis of sugars, proteins and proline, 100 mg of fresh material from each repeat was used.

Total Hydro-soluble protein. Soluble proteins are assayed according to Bradford method (Bradford, 1976). This method is a colorimetric assay based on the color change of Coomassie blue after binding to aromatic amino acids (tryptophan, tyrosine and phenylalanine) and the hydrophobic residues of amino acids present in proteins.

Proline free foliar. The technique used for proline determination is that of Troll and Lindsley (1955) modified by Magné and Larher (1992). It is based on the ability of proline to react in acidic and hot conditions with ninhydrin (revealing aromatic amino acids including proline) to give a pink compound soluble in organic solvents such as toluene.

Ethanol soluble sugars. The soluble sugars assay method is based on the technique of McCready *et al.* (1950). In the presence of 91% sulfuric acid (H_2SO_4) and hot conditions, the oses produce furfural derivatives that react with the anthrone to give a blue-green compound.

Photosynthetic pigments. The assay was performed by colorimetric method; the pigments being naturally colored. To do this, read the OD of the samples using the spectrophotometer previously calibrated with 80% acetone (corresponding to OD= 0). The OD readings were carried out at wavelengths 647 and 663 nm for chlorophylls and 470 nm for carotenoids. The concentrations were determined according to Lichtenthaler (1987).

Antioxidant system

Total Anti-oxidant Capacity (CAT). The total antioxidant capacity of Rhus *tripartita* leaf was estimated using the method described by Prieto *et al.* (1999). The amount of 0.1 ml of methanolic extract (2 g of dry matter in 10 ml of methanol) was mixed with 1 ml of molybdate reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance was measured at 695 nm after incubation in boiling water bath about 90 min. The total antioxidant capacity was expressed as the milligramme gallic acid equivalent per gram of dry matter (mg GAE/g DM).

Lipid peroxidation. Lipid peroxidation is estimated according to the method of Cakmak and Horst (1991) which consists in grinding 100 mg of fresh plant material in 1 ml of 1% Trichloroacetic acid (TCA) followed by centrifugation at 12000 rpm/20 min. Then, 1 ml of Thiobarbituric acid (TBA) at 0.5% (prepared in 20% TCA) is added to 500 µl of extract and incubated with Marie-bath at 95°C/30 min. After cooling, the optical densities (OD) reading is carried out at 532 and 600 nm. The MDA measurement content is calculated using its molar extinction coefficient (ع= 155 mmol/l cm).

Electrolyte Leakage. Electrolyte Leakage (EL) was estimated by measuring the electrolytic conductivity according to modified method of Pike *et al.* (1998). This technique consists in placing ten foliar disks 12 mm in diameter in 20 ml of distilled water. A first measurement of the conductivity (EC) is carried out after stirring for 3 hours. Then, a second measurement of the conductivity (ET) is conducted, after Marie-bath heating (95°C) for one hour. Electrolyte leakage (%) is determined by the ratio (EC/ET).

Total flavonoid content. Total flavonoid content was estimated using the method reported by Dewanto *et al.* (2002). It consists to mix 250 µl of vegetable methanolic extract with 25 µl of 5% $NaNO_2$, added with 150 µl of $AlCl_3$ (2%). After 5 min, 0.5 ml of 1M NaOH is added to the solution. After 10 min of incubation, the absorbance was measured at 510 nm.

Statistical analysis

The data were subjected to statistical analysis using the Microsoft Excel 2010 program. All values of biochemical compounds and secondary metabolites are the mean ± SE (standard error) of three replicates of a single sample. Unidirectional analysis of variance (ANOVA) was used and differences between individual means were considered significant at P<0.05.

3. Results and Discussion

Aridity depends on several climatic factors (temperature, wind, radiations) and above all on evaporation, an essential factor to calculate the water deficit. Therefore, a rocky environment such as that of our study site, expresses accentuated drought effects because the soil scarcity. Moreover, relatively rapid soil drought after rains causes water stress even during the rainy season (Pimienta-Barrios *et al.*, 2001). To this end, we will discuss our results in relation to the influence of hydric and oxidative stress as the main parameters of aridity.

Therefore, plant eco-physiological study depends on several criteria, depending on environmental changes and their adaptation to them. Indeed, plant survival in arid environments depends on different coping mechanisms. In this work, we have analyzed the variation of physiological parameters of *Rhus tripartita* in relation to the arid climate of Ahaggar. These parameters are often measured to study the different plants responses to abiotic stress.

Relative water content

The low water availability can cause tissue dehydration, for this purpose, plant can control its hydric potential to cope with these high temperatures of arid zones. Therefore, the relative water content of the plant gave a high value ranging from 54.79% to 81.55% (Fig. 2). The highest content is recorded in shrub n. 5 with a significant difference compared to the same species shrubs. Poole and Miller (1975) reported that species of the same genus: *Rhus ovata, Rhus laurina* and *Rhus interguifolia;* showed high water potential in water-deficit conditions.

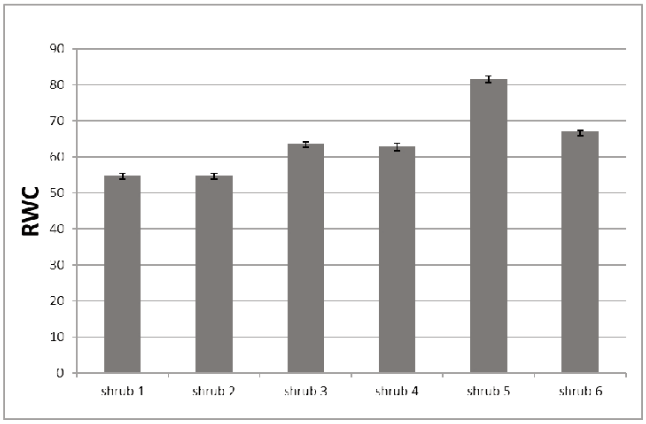

Fig. 2 - Relative water content (RWC%) of *Rhus tripartita* leaves according to climatic aridity conditions.

Metabolite content

In extreme temperatures, the plant can produce metabolites to protect itself from harmful and damaging effects. The total protein assay of *R. tripartita* showed levels that ranged from 20.03 mg/g in shrub n.3 to 33.76 mg/g in Shrub n. 1 (Fig. 3), which represent fairly high levels compared to *Periploca Angustifolia* Labill, a shrub growing in Tunisian arid areas with a rate of 5.204 mg/g DM (Dghim *et al.*, 2015). However, this amount is twice as high as that of the same species collected in Libya (10.1 mg/g DM) (Le Houérou, 1991) and four times higher than that found in other species of the same genus such as *Rhus lancea* (7.79 mg/g MS) (Aganga and Mosase,

2001) but it is one and a half times higher in other forage species: *Medicago sativa* (19.4 mg/g MS) (Le Houérou, 1991).

On the other hand, proline is an amino acid whose rate increases proportionally more rapidly than other amino acids in plants under water stress. It has been suggested as a study parameter for the selection of drought-resistant plants (Bates *et al.*, 1973). The proline level recorded in *R.tripartita* showed a significant difference ($P<0.05$) between the six shrubs of the same species with an interval of 212.45 µg/g - 851.28 µg/g (Fig. 3). Plants tolerant to stress, have relative stability or low accumulation of proline compared to sensitive plants (Lemzeri, 2006). According to Dix and Pearce (1981), the proline accumulation is not an adaptation reaction to stress, but rather a sign of metabolic disturbance. Alternatively, proline may confer a protective effect on the plant by induction of stress-protecting proteins (Vinocur and Altman, 2005).

However, osmotic stress can produce harmful effects in the plant's cell compartments. A wide range of metabolites can intervene to avoid these effects including a variety of sugars and alcoholic sugars such as mannitol and trehalose (Vinocur and Altman, 2005). The study of sugars compound in *Rhus tripartita* leaves was relatively high (938.93 µg/g) (Fig. 3). Several studies have investigated the soluble sugars accumulation in several stressful species (Garg *et al.*, 2002; Penna, 2003; Silva and Arrabac, 2004).

Ahaggar is a region known for its continuous sunshine all year round. In the presence of strong radiation, several plants use protective mechanisms to reduce the absorption of solar radiation (Harrison *et al.*, 2010). The study of *Rhus tripartita* photosynthetic pigments leaves showed results of 28.1 µg/g, 31.24 µg/g and 53.56 µg/g for chlorophyll *a*, *b* and total chlorophyll respectively (Table 1). Similar to those recorded in *Rhus typhina* under water stress conditions (Liping, 2007), these results indicate that dry conditions do not have significant effects on the degradation of photosynthetic pigments, which explains the dark green colour of the leaves. Nevertheless, photosynthesis of plants in arid zones that believe under permanent conditions of water deficiency; are subject to photoinhibition risk (Voronin *et al.*, 2003). Furthermore, the amount of photosynthetic pigments in the leaves of these plants has been shown to be relatively low (Valladares and Sanchez-Gomez, 2006) which contradicts the findings of this study. The water stress is considered one of the environmental factors limiting photosynthesis, therefore plant growth (Ozturk *et al.*, 2010).

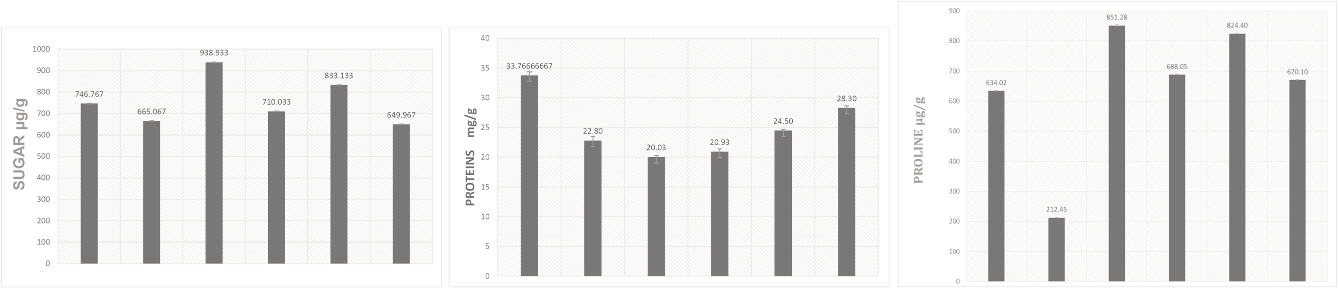

Fig. 3 - Soluble sugar content (µg/g), total proteins content (mg/g) and proline content (µg/g) in *Rhus tripartita* leaves according to climatic aridity conditions.

Table 1 - Content of photosynthetic pigments (µg/g) in the fresh leaves of *Rhus tripartitus* (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids)

Shrub	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll	Carotenoids
Shrub n° 1	17.367 ± 0.284	24.800 ± 0.527	41.300 ± 0.584	8.480 ± 0,006
Shrub n° 2	23.600 ± 0.156	29.967 ± 0.295	53.560 ± 0.383	8.022 ± 0.025
Shrub n° 3	24.133 ± 0.157	18.880 ± 0.271	43.067 ± 0.415	5.797 ± 0.017
Shrub n° 4	24.967 ± 0.151	31.243 ± 0.564	56.473 ± 0.378	11.237 ± 0.079
Shrub n° 5	24.033 ± 0.390	20.783 ± 0.533	44.577 ± 0.443	6.619 ± 0.089
Shrub n° 6	28.100 ± 0.340	21.203 ± 0.363	49.503 ± 0.257	6.841 ± 0.039

Antioxidant system

In this study, the oxidative stress effect is evaluated through the quantification of MDA and the electrolyte leakage since secondary aldehyde products from lipid peroxidation are generally considered to result from oxidative stress (Del Rio *et al.*, 2005). The results presented in figure n. 10 showed that the MDA content in the shrub is significantly higher (7.007 nmol/g) than other shrubs [5.165-5.673 nmol/g]. The electrolyte leakage was studied using conductivity measurement. It varies between 12.39 and 14.293 in the six shrubs of *Rhus tripartita* as shown in figure n. 4. Similary, in *Rhus typhina*, high levels of MDA have been recorded in water stress conditions (Liping, 2007). Therefore, MDA is a degradation product of lipid peroxidation reactions that are formed during the attack of polyunsaturated lipids by reactive oxygen species (ROS). However, this is the most widely used assay to characterize oxidative damage in plants (Shulaev and Oliver, 2006). Moreover, high temperatures can produce metabolic disturbances based on reactive oxygen species and antioxidant systems. However, the present work showed a variability in Total Anti-oxidant Capacity from one shrub to another and is within a range of 19 mg/g - 100 mg/g (Fig. 4). Therefore, it is known that TAC is mainly due to phenolic compound (Tlili *et al.*, 2014). On the other hand, the flavonoids constitute the main group of polyphenols, ubiquitous in plants, they are formed from aromatic amino acids (Hernandez *et al.*, 2009). They act as antioxidant molecules that ensure the binding of ROS produced during stress and thus neutralize their effects before the manifestation of oxidative damage at the cellular level (Lovdal *et al.*, 2010). The determination of total flavonoids contents in *Rhus tripartita* leaves gave result of 35.89 mg/g (Fig. 4). This level is relatively similar to the same species under water stress conditions (39.2 µg/mg) (Ncib *et al.*, 2018) but higher than in other species of the same family such as *Rhus punjabensis* (30.50 µg/mg) (Tabassum *et al.*, 2017). Several studies have demonstrated the richness of *Rhus tripartita* in flavonoids (Mahjoub *et al.*, 2007, 2010; Tlili *et al.*, 2014). Therefore, flavonoid is considered as a phytochemical adaptation to the biotic and abiotic environment (Dixon and Paiva, 1995). In the same perspective, the carotenoids are soluble antioxidant lipids that play a very important role in abiotic stress tolerance (Sieferman-Harms, 1987; Gill and Tuteja, 2010). The carotenoids content found in our shrub leaves is relatively low (11.63 µg/g) (Table 1) compared to another shrub of Tunisian arid zones: *Periploca angustifolia* Labill (Dghim *et al.*, 2015). It is known that carotenoids have a protective role against photooxidation (Ladygin *et al.*, 2008) which may explain the adaptive power of this shrub.

The relationship between aridity and the results found is certainly evident. It can be explained by the

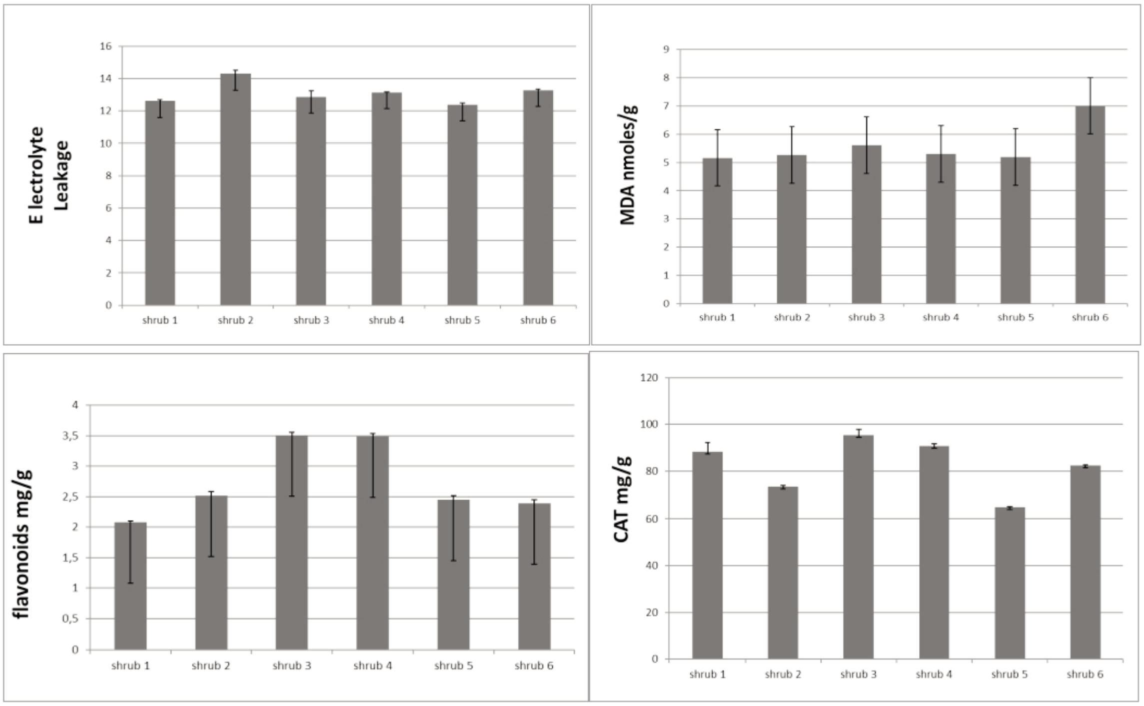

Fig. 4 - Malondialdehyde content (nmol/g), total antioxidant capacity (mg/g), electrolyte leakage and flavonoid content (mg/g), of *Rhus tripartita* leaves in relation to climatic aridity conditions.

fact that the plant increases in both primary and secondary metabolite production under drought conditions. It should be noted that a difference in the metabolites composition has been recorded among different individuals of the same species. That said, not all shrubs had the same orientation towards the sunshine and some were grouped with the species *Myrtis nivellei* and *Periploca laevigata*. These two parameters can explain the difference between the results of the six individuals.

4. Conclusions

In summary, we presented a simple preliminary work on the physiological information of *Rhus tripartita* in arid environments. Therefore, our results have shown that this shrub presents an interesting adaptation to its climatic and edaphic environment. The approaches developed in this study certainly contribute to the understanding of shrub ecophysiology and can be improved plant productivity in arid regions.

In conclusion, and according to classification of Jenks and Hasegawa (2005) of plants from desert and semi-arid zones, *Rhus tripartita* can be considered as a xeromorphic plant, a species adapted to the desert that is not limited by water availability.

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