IMPACT: International Journal of Research in Applied Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(E): 2321-8851; ISSN(P): 2347-4580
Vol. 3, Issue 9, Sep 2015, 127-138

DROUGHT STRESS EFFECT ON SOME BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS; ACCUMULATION OF TOTAL POLYPHENOLS AND FLAVONOIDS IN LEAVES OF TWO PROVENANCE SEEDLING *PISTACIA LENTISCUS*

FATMA CHEHRIT-HACID[1], AREZKI DERRIDJ[2], FARIDA MOULTI-MATI[3] & ABDERRAHMANE MATI[4]

[1]M. Maamri University, Department of Biology, Laboratory of Biochemistry and Biotechnologies, Tizi-Ouzou, Algeria

[2] M. Maamri University, Department of Agronomy, Laboratory of Natural Resources, Tizi-Ouzou, Algeria

[3,4]M. Maamri University, Department of Biochemistry, Laboratory of Biochemistry and Biotechnologies, Tizi-Ouzou, Algeria

ABSTRACT

In Mediterranean climate which characterized by warm, dry summers, cool and wet winters, drought stress affects growth and survival of species. Study of the impact of drought on some biochemical features would contribute to understand strategies of *Pistacia lentiscus* to cope with Mediterranean field conditions. Seedlings of *P. lentiscus* were subjected to summer drought in greenhouse by without watering. Seeds of plants growing in the Azazga region (North Slope of Kabylie) and Bouira one (southern slopes of Kabylie) were used to obtain seedling. Relative water contents, proline (osmotic solute), pigments, lipid peroxidation, total polyphenols, total flavonoids are investigated.Drought induced a slight but significant decrease in relative water content and an increase in the proline level indicating the maintenance of osmotic balance. Total polyphenols and total flavonoids were not influenced by the drought.

KEYWORDS: Drought Stress, Mediterranean, *Pistacia Lentiscus*, Lipid Peroxidation, Total Polyphenols, Total Flavonoids

INTRODUCTION

The Mediterranean climate which characterized by warm, dry summers and cool, wet winters (Daget, 1997; Spano et al 2003) was considered the most vulnerable regions to climate change with a pronounced warming and a sharp decrease in precipitation in the spring and summer seasons (Gao and Giorgi, 2008).In its ecosystems, the summer water deficit is considered the main environmental constraint for plant growth and survival (Galmès et al 2007)that can seriously reduce crop productivity and causes changes in biomass accumulation, grown rate and many other physiological or structural traits (Chatanya et al 2003;Sepanlo et al 2014).

In response to drought stress, plants adopt various mechanisms. Plants witch accomplishestheir life cycle when water is plentiful; those tolerate the drought by accumulating high concentrations of substances, allowing to withstand dehydration such as secondary metabolites (Kleinwächter and Selmar, 2015), compatible solutes(Bartels and Phillips, 2010); and those avoid water deficit by a suite of traits that modulate water utilization such as Root system traits and C3/C4 or CAM photosynthesis (Mullet, 2009).

Pistacia lentiscus L. is a dioecious, evergreen small tree or shrub of the Anarcadiaceae family up to 8 m tall, widely distributed in a variety of habitats in Mediterranean ecosystems (Correiaand Diaz Barradas, 2000); it behaves as a

thermophilous species, growing in warm areas at low altitude and medium altitudes (Flexas et al 2001). Thus, it may be used for reforestation programs under Mediterranean climatic conditions (Garcia-Fayos and, Verdu 1998). It's one of the most important Mediterranean "keystone" species (Naveh, 1995) and is an interesting model species for evaluation of drought effects (AïtSaïd et al 2013). The present study was aimed todetermine drought tolerance among tow populationsof *Pistacialentiscus* with reference to lipid membrane peroxidation, chlorophyll, proline and polyphnenol contents.

MATERIAL AND METHODS

Plant Material and Culture Conditions

The fruits of *Pistacia lentiscus* plants growing in the regions Azazga(North Slope of Kabylie) and Bouira (southern slopes of Kabylie) were used in this study. The two batches of the fruit containing the seeds were soaked in water for 24 hours to remove the pulp. The floated seeds (non-viable) were removed and the remainder was scarified with concentrated sulfuric acid for 15 minutes and immediately rinsed and kept under tap water for 24 hours to remove all traces of sulfuric acid (Ates, 2011). 100 seeds per population were germinated on two layers of filter paper moistened with 10 ml of distilled water in Petri dishes incubated at room temperature. After 15 days of incubation, the seeds of the two provenances germinated with 86.36 ±9.24 81 and 81,82±10.79 % respectively. At the two leaves stage (twenty four seedlings for each provenance were planted in bags containing about 2 kg of soil collected in the area of growth of the species; the bags were placed in a greenhouse at the Institute of Agricultural means specialized technology, under natural conditions with temperatures reaching 51, 6± 3,84 °C in average, and watered as needed, about twice a week.At the beginning of stress (mid-August), half of the plants were subjected to water stress by suspending watering for twelve days. Because two plants were dead, others were watered twice and then stressed for twelve days.

Relative Water Content (RWC)

Five plants from each treatment were randomly selected. The relative water content was evaluated according to the method described by Seelig et al (2009), briefly Fresh weight of the sample leaves (FW) was obtained by weighing them immediately after sampling using a precision balance; Weight at full turgor pressure (TW) was obtained after saturating samples in water for 24 hours, and dry weight (DW) was obtained after oven drying samples at 80C for 24h.

$$RWC\ (\%) = [(FW-DW)/(TW-DW)]\cdot 100$$

Proline Contents

For each sample, 100mg of dry leaves were mixed with 2mL of 80% of methanol (v/v) and heated in a Water bath at 85°C for 1hour. After cooling, 1mL of leaf extract was mixed with 1mL of acidic ninhydrin reagent and 1mL glacial acetic acid. The mixture was well shaked for few seconds and incubated in a water bath at 100°C for 30mintes. After an ice cooling period of 3 minutes, 5mL of toluene were added to the mixture and vortexed again. The upper phase of the mixture was collected and dehydrated with a pinch of anhydrous sodium sulfate. Then, absorbances of leaf samples were measured and calculated. Proline contents were measured by colorimetric method as described by Thiam et al (2013). The amount of proline, on a fresh-matter basis of plant leaves, was determined according to a calibration straight graph constructed from a series of standard proline solutions. The absorbance of toluene phase was estimated at 528 nm using UVmini-1240 UV-Vis Spectrophotometer Shimadzu. Appropriate proline standards were included for the calculation of proline in the samples. Each measure was repeated three times to ensure reproducibility of results.

Leaf Pigment Content

Frozen leaves samples (0.2 g) were analyzed for chlorophyll contents. The leaves were ground in 3 ml cold 80% acetone using a pestle and mortar. Acetone extracts were centrifuged at 3000 g for 10 min and the resulting pellets were extracted in cold 80% acetone. This operation was repeated three times. The successive supernatants were pooled and centrifuged at 4000 g for 5 mintes for clarification. The absorbance of the acetone extracts was recorded at 626, 647, 663 and 470 nm using UV/VIS spectrophotometer. The amounts of Chlorophyll were calculated according to Porra (2002).

Lipid Peroxidation

100 mg of leaves samples were homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 × *g* for 5 minutes at 4 °C. Aliquot of 1 ml supernatant was mixed with 4ml of 0.5% thiobarbituric acid (TBA) prepared in TCA 20%, and incubated at 95 °C for 30 minutes. After stopping the reaction in an ice bath for 5 minutes, samples were centrifuged at 10 000 × *g* for 10 minutes at 25°C. The supernatant absorbance at 532 nm was measured usingUVmini-1240 UV-Vis Spectrophotometer Shimadzu. Lipid peroxidation rate equivalents (nmolmalondialdehyde ml^{-1}) were calculated by using the formulae given by Hodges et al. (1999).

Polyphenol Extraction

The samples of *Pistacia*leaves were dried in the open air and in the dark. Samples were grounded in the blender before the extraction. The mean particle size(d=0.3092±0.16 mm).Plant samples (0.5 g) were extracted by 80% ethanol (5ml) at 50°C for 30minutes. The solutions were centrifuged at 2400 g for 20 minutes, the supernatant was recovered and adjusted with 80% ethanol and stored at - 20 ° c.

Total Flavonoid Determination

The method used to determine total flavonoids is derived from the protocol described by Li et al. (2010). Briefly, 0.5 ml of the sample solution was introduced into a test tube. The volume was made up to 3 ml with distilled water and then 0.3 ml of $NaNO_2$ (1: 20) were added. After 6 minutes, 0.3 ml of $AlCl_3$were added(1: 10). 4.0 ml of 1M NaOH were added later 6min. Then, the whole was adjusted to 10 ml with distilled water. The solution was shaken well and incubated for 15 minutes at room temperature. The solutions were scanned by UVmini-1240 UV-Vis Spectrophotometer Shimadzu, using quartz cuves (1.0 cm) at 506 nm against a blank. The rutin was used as the standard for a calibration curve. The flavonoids content was expressed on mg/g DW.

Total Polyphenolic Compound Determination

300 µl of ethanolic extract were added to test tubes containing 2100 µl of distilled water and 150 µL of FC reagent. The mixtures were vortexed and allowed to equilibrate (20 °C, 10 min) after which, color was stabilized by the addition of sodium carbonate (450 µL of a 0.7 mM solution); then, the mixture was vortexed and incubated at 40 °C for 20 min. Tubes were cooled rapidly in ice. The developed color was read at 765 nm using polyphenol content was expressed as mg/g DW (mean±SD) (Ramirez-Sanchez et al 2010).

Data Analysis

Statistical analyses were performed using Statisica 7.1. All the experiments were conducted with a minimum of three replicates and results were expressed as mean ± standard deviation (SD). Data were subjected to one way ANOVA followed by Newman-Kurls when differences were significant. Statistical significance was set at $P<0.05$. Data were tested for normality by shapirowilk test and for homogeneity by Levene's test.A principal component analysis was used to examine potential correlation between different parameters.

RESULTS

Relative Water Content (RWC)

The relative water content (RWC) is a key indice for drought stress study (Talbi et al 2015). At the beginning of stress, both populations displayed similar values of leaf RWC (Fig. 1). It was 96±3 % and 95±1 % for Azazga and Bouira respectively. However, the difference between the control and the stressed plants was significant for both populations (p=0,002) although leaf RWC decreased weakly, with 4% and 3% respectively during drought stress.

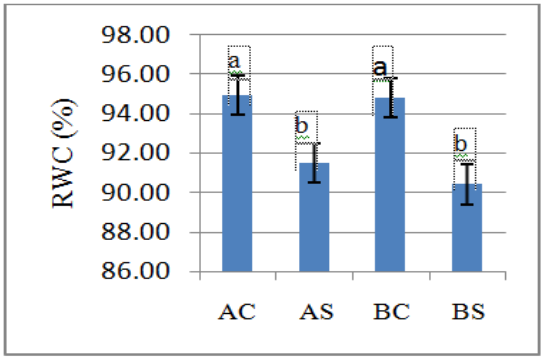

Figure 1: Changes in RWC (%) of Two *P. Lentiscus* Populations (AC: Azazga Control, AS: Azazga Stressed, BC: Bouira Control, BS: Bouira Stressed). Values Are Mean±SE Based on Four Replicates

Proline Content

Under control condition, the results obtained showed that proline contents were similar in both *P. lentiscus* populations with 13,26±2,15 and 11,83±1,57 µmole/g FW in Azazga and Bouira respectively. However, in stressed plants, the increase was about 2 fold in average ($p<0,000001$) (fig.) likely due to an increased need of proline for processes such as increased protein biosynthesis or due to a blockage of proline-consuming processes (Ramadan et al 2014).

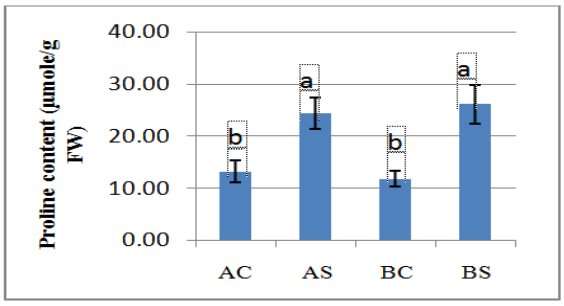

Figure 2: Changes Inproline Contents (µg/Ml FW) of Two *P. Lentiscus* Populations (AC: Azazga Control, AS: Azazga Stressed, BC: Bouira Control, BS: Bouira Stressed). Values Are Means±SE Based on Four Replicates

Pigment Content

Under control conditions, both populations exhibit significant differences in the levels of all leaves pigments (p value<0,05), seedling from Brouira showed higher value than those from Azazga whereas the Chl a/b ratio in Azazga seedling was higher than in Bouira seedling . Water stress induced a conspicuous decrease as well in all the pigments than in chl a/chl b ratio (p value <0,05). All pigments decreased in Bouira population more than in Azazga one; chl a, chlb,and car (c+x) decreased respectively by 42,29%,32,32% and 21,43% in Azazga population while they were 47,05%, 46,00% ,24,49% and in Bouira one. Moreover, the differences of $car_{(c+x)}$/total chl and chla/chlb ratios were insignificant.

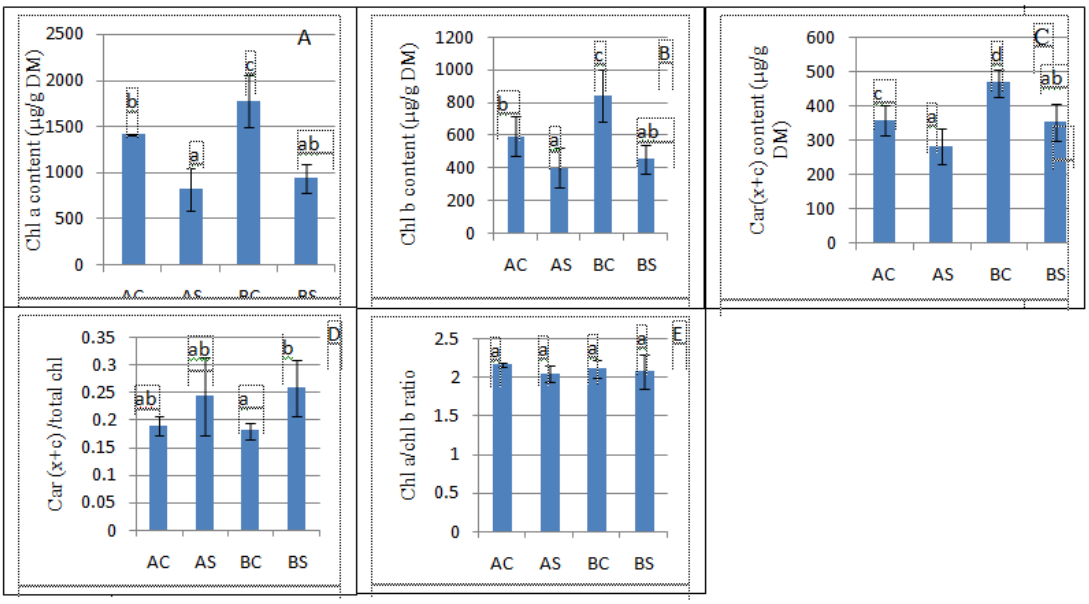

Figure 3: Changes in Pigment Content (µg/G DM) In Leaves of Two *P. Lentiscus* Population Seedlings under Drought Stress. A: Chlorophyll A; B: Chlorophyll B; C: Total Carotenoids; D: Total Carotenoids/Total Chlorophyll Ratio E: Chla/Chb Ratio Values Are Means±SE Based on Four Replicates. Values Are Means±SE Based On Four Replicates

Lipid Peroxidation

Under control conditions, the difference in concentrations of MDA in leaves of the two populations was insignificant p value= 0,28; it was 4,09±0,41 nmol/ml and 2,44±1,02 nmol/ml in Azazga and Bouira populations respectively. Compared to control, stressed seedling showed higly MDA concentrations in both populations (p value =0,0016) where they reached the same level. The increment in Bouira population was 121,31%, whereas it was only 30,80% Azazga population.

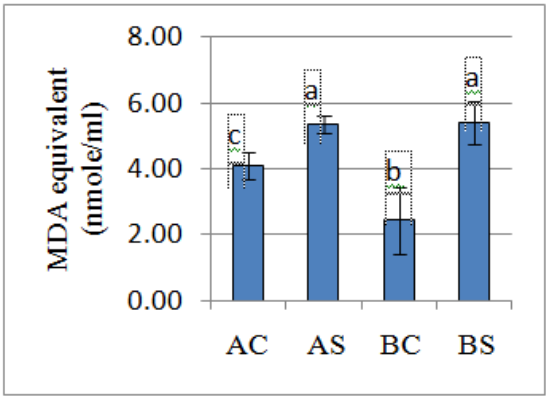

Figure 4: Changes in MDA Content (Nmol/Ml) in Leaves of Two *P. Lentiscus* Population Seedlings Under Drought Stress Values are Means±SE Based on Three Replicates

Polyphenol Content

The differences in flavonoid contents were insignificant between the populations of *P. lentiscus* under control conditions and drought treatment (p=0,196**)** FT levels in Azazga population was higher than in Bouira one (17,%) whereas perspicuous increase was observed in the last one (15,12%).TPP contents were insignificantly different both under condition control and stress treatment (p=0,59). TPP varied from 87,41±5,37 mg/g DW to 105,11±1,2. The same tends was observed in situ (data not shown).

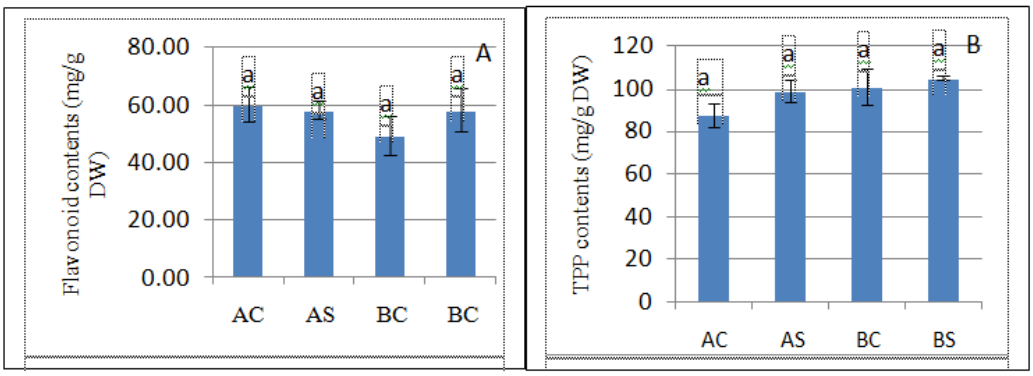

Figure 5: Changes in Polyphenol Contents (Mg/G DW) A: Flavonoid Content; B: TPP Contentin Leaves of Two *P. Lentiscus* Population Seedlings under Drought Stress. Values are Means±SE Based on Four Replicates

DISCUSSIONS

Furthermore the drought stress imposed to *P. lentiscus* seedling, they were exposed to summer environmental conditions including high temperatures and high light irradiance. The results of the various measures showed signs of stress in both treated seedlings.Relative water contents,related to water uptake by the roots and to its loss by transpiration, are considered a way to evaluate plant water status (Anjum et al, 2011). The weak decrease in RWC in both populations was similar with the trend that observed by Catoni et al (2013); *P. lentiscus* was one of the sclerophyllous species maintained highest RWC. Furthermore, the reduction of RWC in *Moringaoleifera*, drought tolerant species, was insignificant (Rivas et al 2013). For these auteurs, the maintained of RWC at high level helps to maintain cellular physiological processes and growth. Prolineplays an important role in stress tolerance. Its accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells (Sankar et al 2007; Anjum et al., 2011).It was

negatively correlated with RWC. Similar results obtained in several drought-stressed plants such as *Cocosnucifera*

(Gomes et al, 2010) and sugarcane (Medeiros et al 2013). Proline is an effective organic substance, functioning as well as an osmolyte conferring osmotic adjust, than in the detoxification of ROS and the cellular stabilization (Gao et al 2008; Kusvuran et al 2013; Ben Rejeb et al 2014). For Gao et al (2008), a direct consequence of a higher proline concentration was the relatively higher water retaining capacity.Drought stress generally causes decrease in photosynthetic pigments (Liu Y. et al 2011; wang et al 2014;Rajasekar and Manivannan, 2015). Nikolaeva et al (2010) reported that chla and b deceased under severe drought stress while chl a/chl ratio remained unchanged. On other hand, Chl a/ b ratio significantly increased in *Broussonetiapapyrifera* and*Platycaryalongipes*, but decreased in *Cinnamomumbodinieri* (Liu C. et al 2011).The decrease in chlorophyll content under drought stress might be due to the increased activity of chlorophyllase leading to chlorophyll degradation (Ajithkumar and Panneerselvam, 2013; Ajithkumar and Panneerselvam, 2014) and thus to degradationof chlorophyll-proteincomplex of the thylakoids under severedrought condition (Wang, 2014). Chlorophyll loss would contribute to the survival of severely stressed plants by reducing the amount of photons absorbed by leaves (Munné-Bosch and Alegre, 2000) reduce the proportion of PSII (Ait-saïd et al 2013).For Jain et al (2013), decrease in chlorophyll was due to decrease in chlorophyll biosynthesis rather than its degradation. Ait-saïd et al (2013), working in situ on adult individuals of *P. lentiscus,*found that carotenoids increase in summer. This difference would be due to the development stage of the plant, intensity, duration and rate of progression of the stress (Chaves et al 2009).The MDA contents were significantly higher in stressed seedling than in the controls(p=0,0016). The increase in Azazga seedling was only 1,31 folds while it was 2,28 folds in Bouira seedling. This increase reached same level in both populations (5,35 and 5,40 nmol/ml). It may be caused by the combined effect of the heat and drought where Bouira population could be more sensitive than Azazga one. The increase has been generally observed in plant stressed. This suggested that under combined environmental stresses such as drought and high temperatures, Azazga population were more adapted than Bouira one. Under drought treatment, both flavonoids and total phenolic compounds differ in various studies; in some ones there was an increase (Trabelsi et al 2013) and in others there was a decrease in the rate of TFC (McKiernan et al, 2014) or no significant difference with favorable conditions (Daniels et al 2015). Flavonoid contents of *Quercus ilex* decreased generally in summer under drought, compared to winter but their behavior varies at individual level of these substances. So, epicatechin, and epicatechingallate showed no seasonal changes whereas seasonal variations in flavonol-hexosides decrease from winter to summer (Brossa et al 2009). Liu et al (2011) studying two desert shrubs**,** found that flavonoids in *Caryopterismongolica* increased while they decreased in *Reaumuriasoongorica*..Moreover,anthocyanin content increased in both shrubs, indicating different regulation responses in the flavonoid pathway. Alexieva et al (2001) found that in pea and wheat plant anthocyanin content was unaffected by drought while soluble phenol content was significantly increased. For Rajabbeigi et al (2014), drought and UV-B treatments affect phenolic profile while total phenolic content remained unchanged.

CONCLUSIONS

In conclusion, *P.lentiscus*tolerate drought stress by maintaining RWC at high level and accumulating osmoregulator substances such as proline conferring osmotic adjust to the plant. However, itexhibitedoxidative stress manifested by the increase of the MDA content and decrease of photosynthetic pigments.Identification and quantification of polyphenols and particularly flavonoids could help to understand the mechanismof tolerance of this species.

REFERENCES

1. Ait said S., Torre F., Derridj A., Gauquelin T., Mevy J.P. (2013). Gender, Mediterranean drought, and seasonality: photosystem II photochemistry in *Pistacia lentiscus* L. PHOTOSYNTHETICA 51 (4): 552–564.
2. Ajithkumar I.P., Panneerselvam R. (2013). Osmolyte accumulation, photosynthetic pigment and growth of Setariaitalica(L.) P. Beauv. under drought stress. Asian Pacific Journal of Reproduction2(3): 220-224.
3. Ajithkumar I. P., Panneerselvam R. (2014**).** ROS Scavenging System, Osmotic Maintenance, Pigment and Growth Status of Panicumsumatrense Roth. Under Drought Stress.Cell BiochemBiophys 68:587–595.
4. Alexieva V.,Sergiev I., Mapelli S.,KaranovE. (2001).The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell and Environment* 24: 1337–1344
5. Anjum S.A., Xie X. Y. , Wang L.C., Saleem M. F.,ManC.and Lei W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research 6 (9): 2026–2032.
6. Ates E. (2011). Influence of some hard seededness-breaking treatments on germination in persian clover (*Trifoliumresupinatum*ssp. *typicum*fiori et paol.) seeds. Romanian Agricultural Research 28: 229–236.
7. Bartels D., Phillips J. (2010). Drought stress tolerance. Genetic Modification of PlantsBiotechnology in Agriculture and Forestry Volume 64: 2010, 139-157
8. Ben Rejeb K., Abdelly C., Savouré A. (2014). How reactive oxygen species and proline face stress together. Plant Physiology and Biochemistry 80 : 278-284
9. Brossa R., Casals I., Pinto´-MarijuanM., Fleck I. (2009). Leaf flavonoid content in Quercus ilex L. resprouts and its seasonal variation. Trees 23:401–408.
10. Catoni R., Varone L., Gratani L. (2013).Variations in leaf respiration across different seasons for Mediterranean evergreen species. PHOTOSYNTHETICA 51 (2): 295–304.
11. Chaitanya K.V., Jutur P.P., Sundar D., Reddy A. R. (2003). Water stress effects on photosynthesis in different mulberry cultivars. Plant Growth Regulation 40: 75–80.
12. Chaves M. M., Flexas J., Pinheiro C. (2009). Photosynthesis under drought and salt stress: regulation mechanismsfrom whole plant to cell. Annals of Botany 103: 551–560.
13. CorreiaO., Diaz Barradas M. C. (2000). Ecophysiological differences between male and female plants of *Pistacia lentiscus* L. *Plant Ecology* 149:131–142.
14. Daget P. (1977). Le bioclimat mediterraneen: caractères généraux, modes de caractérisation Vegetatio, vol. 34, 1 :1-20.
15. Daniels C.W., Rautenbach F., Marnewick J.L., Valentine A.J., Babajide O.J., Mabusela W.T. (2015).Environmental stress effect on the phytochemistry and antioxidant activity of a South African bulbous geophyte, Gethyllismultifolia L. Bolus. South African Journal of Botany 96: 29–36.
16. FlexasJ., Gulíasa J., Jonasson S., Medranoa H., Mus M. (2001). Seasonal patterns and control of gas exchange in

local populations of the Mediterranean evergreen shrub *Pistacia lentiscus* L. ActaOecologica 22: 33−43

17. Galmès J., Flexas J., Savé R.,Medrano H. (2007). Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. Plant Soil 290:139–155.
18. Gao X., Giorgi F. (2008). Increased aridity in the Mediterranean region under greenhouse gas forcing estimated from high resolution simulations with a regional climate model. Global and Planetary Change 62: 195–209.
19. Gao J., Xiao Q., Ding L., Chen M., Yin L., Li J., Zhou S. He G.(2008). Differential responses of lipid peroxidation and antioxidants in Alternantheraphiloxeroides and Oryza sativa subjected to drought stress. Plant Growth Regul 56:89–95.
20. Garcia-FayosP.,Verdu M.(1998). Soil seed bank, factors controlling germination and establishment of a Mediterranean shrub: *Pistucia lentiscus L.* ActaOecologica 19 (4) 351-366.
21. Gomes F. P., Oliva M. A., Mielke M. S., Almeida A.F., Aquino L. A.(2010). Osmotic adjustment, proline accumulation and cell membrane stability in leaves of Cocosnucifera submitted to drought stress. ScientiaHorticulturae126: 379–384.
22. Hodges D. M., DeLongJ.M., Forney C. F., Prange R. K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207: 604–611.
23. Jain M., Mittal M., Gadre R. (*2013).* Effect of PEG-6000 Imposed Water Deficit on Chlorophyll Metabolism in Maize Leaves. Journal of Stress Physiology & Biochemistry, Vol. 9 No. 3
24. Kleinwächter M., Selmar D. (2015). New insights explain that drought stress enhances the quality of spice and medicinal plants: potential applications. Agron. Sustain. Dev. 35:121–131
25. KusvuranS.,EllialtiogluS., Polat Z. (2013). Antioxidative Enzyme Activity, Lipid Peroxidation, and Proline Accumulation in the Callus Tissues of Salt and Drought Tolerant and Sensitive PumpkinGenotypes under Chilling Stress.Hort. Environ. Biotechnol. 54(4):319-325.
26. Li S., Zhu1 R., Zhong M., Zhang Y., Huang K., Zhi X., Fu S. (2010). Effects of ultrasonic-assistant extraction parameters on total flavones yield of *Selaginelladoederleinii*and its antioxidant activity. Journal of Medicinal Plants Research 4(17): 1743–1750.
27. Liu C., Liu Y., Guo K., FanD., Li G., Zheng Y., Yu L., Yang R. (2011). Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of outhwestern China. Environmental and Experimental Botany 71:174–183.
28. Liu Y.B., Liu M.L., Cao B. (2011). Effects of drought stress on antioxidant enzyme, photosynthetic pigment and flavonoid pathway in two desert shrubs. Sciences in Cold and Arid Regions 3(4): 0332–0338.
29. Medeiros D. B., Silva E. C., Nogueira M. C. R. J., Teixeira M. M., Buckeridge M. S. (2013) Physiological limitations in two sugarcane varieties under water suppression and after recovering. Theoretical and Experimental

Plant Physiology, 25(3): 213-222.

30. Mullet J. (2009).Traits and Genes for Plant Drought Tolerance*in Molecular Genetic Approaches to Maize Improvement.* Biotechnology in Agriculture and Forestry, A .L. Kriz, B.A. Larkins (eds.), Vol. 63: 55-64.
31. Munné-Bosch S., Alegre L. (2000).Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinusoffcinalis* plants. Planta 210: 925–931.
32. Naveh Z. (1995). Conservation, Restoration, Research PrioritiesFor Mediterranean Uplands Threatenedby Global Climate Change. *in*: MORENO J, OECHEL WE (Eds.) Global change and Mediterranean–type ecosystems ecological studies VOL. 117. SPRINGER, NEW YORK, PP. 482–508.
33. Nikolaeva M. K., Maevskaya S. N., Shugaev A. G., Bukhov N. G. (2010). Effect of Drought on Chlorophyll Content and Antioxidant Enzyme Activities in Leaves of Three Wheat Cultivars Varying in Productivity. Russian Journal of Plant Physiology Vol. 57 (1): 87–95.
34. Porra R. (2002). The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. Photosynthesis Research 73: 149–156.
35. Rajasekar M.,Manivannan P. (2015). Drought and drought with triazoles induced changes on pigment composition and biochemical constituents of Zea mays L. (Maize). Appl. Transl. Genomic. http://dx.doi.org/10.1016/j.atg.2015.04.001
36. Rajabbeigi E., Eichholz I., Beesk N., Ulrichs C.,KrohL. W, RohnS., Huyskens-KeilS. (2014). Interaction of drought stress and UV-B radiation – impact on biomass production and flavonoid metabolism in lettuce (*Lactuca sativa* L.). Journal of Applied Botany and Food Quality 86, 190 – 197.
37. Ramadan A., Sabir J. S. M., Alakilli S. Y. M., Shokry A.M., Gadalla N. O., Edris S., Al-Kordy M. A., Al-Zahrani H. S., El-Domyati F. M., Bahieldin A., Baker N. R., Willmitzer L. Irgang , S. (2014). Metabolomic Response of CalotropisproceraGrowing in the Desert to Changes in Water Availability. PLOS ONE 9 (2):e87895
38. Ramirez-Sanchez I., Lisandro M., Ceballos G., Villarreal F. (2010). Fluorescent detection of (–)-epicatechin in microsamples from cacao seeds and cocoa products: Comparison with Folin–Ciocalteu method. Journal of Food Composition and Analysis 23: 790–793.
39. Rivas R., Oliveira M. T., Santos M. G. (2013). Three cycles of water deficit from seed to young plants of *Moringaoleifera* woody species improves stress tolerance. Plant Physiology and Biochemistry 63: 200–208.
40. Sankar B., Jaleel C. A., Manivannan P., Kishorekumar A., Somasundaram R., R. panneerselvamR. (2007). Drought-induced biochemical modifications and proline metabolism in Abelmoschusesculentus (L.) Moench. Acta Bot. Croat. 66 (1), 43–56.
41. Seelig H.D.,Hoehn A., Stodieck L. S.,Klaus D. M., Adams III W. W.,Emery W. J.(2009). Plant water parameters and the remote sensing R_{1300}/R_{1450} leaf water index: controlled condition dynamics during the development of water deficit stress. IrrigSci 27:357–365.DOI 10.1007/s00271-009-0152-5
42. Sepanlo N., Talebi R., Rokhzadi A., Mohammadi H. (2014). Morphological and physiological behavior in

soybean (Glycine max) genotypes to drought stress implemented at pre- and post-anthesis stages.ActaBiologicaSzegediensisVol. 58(2):109-113

43. Spano D. , Snyder R. L., and Cesaraccio C. (2003). Mediterranean climates in Phenology: an Integrative Environmental Science, 39:139-156.
44. Talbi S., Romero-Puertas M. C., Hernández A., Terrón L., Ferchichi A., Sandalio L. M. (2015). Drought tolerance in a Saharian plant Oudneyaafricana: Role of antioxidant defences. Environmental and Experimental Botany 111: 114–126.
45. Trabelsi N., Waffo-Te´guo P., Snoussi M., Ksouri R., Me´rillon J. M., Smaoui A., Abdelly C. (2013).Variability of phenolic composition and biological activities of two Tunisian halophyte species from contrasted regions. ActaPhysiol Plant 35:749–761.
46. Thiam M., Champion A., Diouf D., SY M. O. (2013).NaCl Effects on *In Vitro* Germination and Growth of Some Senegalese Cowpea (*Vignaunguiculata*(L.) Walp.) Cultivars. ISRN Biotechnology Volume doi :10.5402/2013/382417
47. Wang L. (2014). Physiological and molecular responses to drought stress in rubber tree (HeveabrasiliensisMuell. Arg.). Plant Physiology and Biochemistry 83: 243-249.