

Acta Scientifica Naturalis

Former Annual of Konstantin Preslavsky University of Shumen: Chemistry, Physics, Biology, Geography

Journal homepage: asn.shu.bg

Effect of seasonal variations on the content of some osmolytes of Aleppo pine and holm oak

M. Rached-Kanouni¹, R. Bousba², H. Khammar¹, L. Redjaimia¹, Z. Kadi¹, L. Ababsa¹

¹ *Laboratory of Functional Ecology and Environment, Department of Life and Nature Sciences, Faculty of Exact Sciences and Life and Nature Sciences, University of 'Larbi Ben M'hidi', Oum El Bouaghi. Algeria*

² *Laboratory of Genetic, Biochemistry and Plant Biotechnology, Department of biology and ecology, Faculty of Natural and Life Sciences, University Mentouri Brothers Constantine 1, Algeria*

Abstract: *The forest of Chettaba is of great ecological importance for the Constantine region. It is characterized by a very heterogeneous forest cover, the dominance of forest species (*Pinus halepensis* and *Quercus ilex*) and an advanced stage of degradation. Vegetation in this region reflects the climate. The summer is dry and hot while the winter is rainy and cold. Indeed, the combination of rain and temperature directly influences the physiology of the vegetation in this forest. The main objective of this work is to characterize the different responses of Aleppo pine and holm oak seedlings and to specify the biochemical variations under the seasonal effect. Biochemical assays of proline, soluble sugars, total proteins and chlorophyll were quantified in the different organs of two species for each of the years 2018–2019. The results obtained show very significant accumulation of total soluble sugars and proline in holm oak and Aleppo pine leaves compared to stems and roots during the winter season (520 ± 20 and $370 \pm 17.98 \mu\text{mol/mg DM}$ respectively). Contrary to the previous results, the highest total protein levels were recorded during the summer with levels of 0.666 ± 0.116 and $1,626 \pm 0.107 \text{ mg/g FM}$. Parallel to the accumulation of these three solutes, a gradual decrease in the chlorophyll pigment content was recorded in winter and summer.*

Keywords: *P. halepensis*, *Q. ilex*, soluble sugar, proline, total protein, chlorophyll pigment

Introduction

The Algerian forest, which is essentially Mediterranean in type, is made up of a certain number of species that are closely linked to the climate [1]. As one move away from the sea, the forest facies changes. The main species in these forests is Aleppo pine (*Pinus halepensis*), which covers an area of 850,000 ha [2]. Like any forest in the Mediterranean basin, the Aleppo pine forest has for centuries been subject to intense human pressure (clearings, illegal cutting, fires, grazing) [3, 4] causing deforestation and regression of the vegetation cover [5, 6]. Also, in recent years, successive and prolonged droughts have caused trees to dry out and die back.

Far behind the Cork Oak (*Quercus suber*) come the Holm Oak (*Quercus ilex*) forests, which cover only 354,000 ha [7], consisting mainly of coppice as a whole degraded by abusive exploitation, disorganized by repeated fires and abandoned to indiscriminate grazing [8]. Holm oak is normally adapted to continental and altitudinal conditions between the semi-arid and sub-humid; it can climb to the altitude limit of 1600 m where it is in direct competition with cedar. However, it overflows on semi-arid stations in the most degraded stations. The northern limit of this distribution area seems to result from the competition with better-adapted species, rather than from maladjustment to climatic conditions since the ecological amplitude of the holm oak remains significant in terms of climate and edaphic conditions.

Thus, forest ecosystems are at the heart of the climate change debate and issue because of their dual role as both carbon sinks and sources [9]. In Algeria, this dual importance is added to the crucial importance of forests for the survival of rural and mountain populations, but above all because of their role in the hydrological balance and the intersectoral implications for socio-economic and human development.

Global climate models converge to estimate probable warming of the Maghreb region of around 2 to 4°C during the 21st century. For the most part, climate projections are characterized by an increase in average temperature from 2.5 to 5.5°C more marked during the summer; an increase in maximum temperatures and the frequency of hot waves, a decrease in the number of cool days and frost days, and a decrease in rainfall of about 20%. In general, droughts will become longer and more severe [10].

Recourse to artificial regeneration is of great necessity to rehabilitate degraded ecosystems and thus develop Algerian forests (oak groves and pine forests). However, several reforestation failures have been observed in recent years; these failures are most often linked to poor knowledge of the ecology of the species and/or poor choice of provenances [11].

Natural regeneration will be preferred to artificial regeneration, as it will allow stands to evolve progressively in a dynamic continuity of maturation, offering a high capacity of resistance to parasitize attacks and environmental aggression [12]. Where the species present only imperfectly meet ecological, landscape

and economic objectives, natural regeneration may be assisted to enrich the composition of the stands with appropriate supplements.

To better understand the effect of climate on the sowing behaviour of these two species and to quantify their degree of adaptation to the environment, biochemical analyses were carried out on a few metabolites such as soluble sugars, total proteins and proline on their various organs.

Material and Methods

Plant material

The plant material used in our experiment is harvested from the Chettaba forest. The forest of Chettaba, with an estimated area of 2398 ha, is located between the coordinates (X1: 36° 18', X2: 36° 21') North latitude and (Y1: 6° 26', Y2: 6° 30') East longitude. Its climate varies from semi-arid to sub-humid. The average annual rainfall is estimated at between 670 and 800 mm/year and the average annual temperature in the study area is 18°C. The brown calcareous modal soils are generally poor in organic matter. *Pinus halepensis* and *Quercus ilex* are the two dominant species of this forest and are commonly used in forest reforestation in Algeria.

Measurements

Biochemical assays were seasonally carried out on one-year-old natural seedlings at the level of the various organs of Aleppo pine (needles, stems and roots) and holm oak (leaves, stems and roots) seedlings. The major plants' osmolytes (chlorophyll pigment, total soluble sugars, proline and total soluble protein) were determined to study their roles in plant response to changes in environmental conditions (seasonal climate fluctuations). Proline concentrations in different organs (leaves, stems and roots) were analysed using the ninhydrin method described by Bates *et al.* [53]. The developed colour with ninhydrin reagent was measured using a spectrophotometer (Mapada UV 1200) at 520 nm. Whereas, the concentrations. Total soluble sugar content in the extracts normally was analysed by a phenol sulphuric acid method [54]. Total soluble protein was determined in the leaves extracts using the colorimetric method described by Bradford [55], based on binding of proteins to Coomassie Brilliant Blue G-250 reagent then reading at 595 nm using bovine serum albumin as the standard.

Statistical analysis

All statistical tests were performed using Minitab software version 18.1. Statistical analysis was performed using the one-factor ANOVA ($P < 0.05$). Based on the results of the analysis of variance, the multiple comparisons of means were performed using Duncan's method, for a 95% confidence interval, to determine significant variations between treatments.

Results and Discussion

Table 1 shows the variations in soluble sugar content in the different organs of Aleppo pine and holm oak seedlings according to the seasons. Compared to Aleppo pine seedlings, holm oak seedlings raised under natural conditions produce more soluble sugars in their organs. The winter further amplifies the accumulation of sugars in the different organs of the seedlings, reaching practically their maximum in holm oak leaves and Aleppo pine needles with values that are 520 ± 20 and $370 \pm 27,984 \mu\text{mol/mg MS}$, respectively; showing again for holm oak seedlings of 40% compared to Aleppo pine seedlings. The soluble sugar contents obtained in the stems and roots of both species are lower than those of the leaves. These results are confirmed by the analysis of variance at a classification criterion that shows very significant differences between the different organs of two species according to the seasons (Table 1).

Table 1. Inter-seasonal variation in soluble sugar contents in the different seedling organs of holm oak and Aleppo pine

		Winter	Spring	Summer	Autumn	F	p
<i>Quercus ilex</i>	Leaves	520a	423.33b	333.3c	316.7c	61.43	0.000
	Stems	315a	231.78b	195.00bc	168.3c	11.32	0.003
	Roots	218a	174.67b	139c	132.3c	28.27	0.000
	Needles	370a	330a	216.78b	206.7b	16.15	0.001
<i>Pinus halepensis</i>	Stems	251.7a	195b	191.7b	165b	10.92	0.003
	Roots	189a	185.7a	158ab	118b	9.87	0.005

Examination of the seasonal results shows that soluble sugar levels in Aleppo pine and holm oak seedlings during the winter are significantly higher than in other seasons (Table 1). This cold period is characterized by low temperatures ranging from 1.25 to 3.05°C between the extremes of altitude in the Chettaba Forest [8]. It reflects variations in soluble sugar levels depending on the organ under consideration, probably reflecting different tolerance mechanisms.

The increase in soluble sugar content in seedling organs during the winter may be the result of increased starch degradation during this season. Laala et al. [13] also show that the increase in summer temperatures favours the accumulation of starch and delays its hydrolysis while low night temperatures during the ripening period accelerate the degradation; this is in agreement with our results.

Concerning the maximum soluble sugar content recorded during the winter period, it can be seen that starch is converted into simple soluble carbohydrates during this season, which contributes to lowering the crystallization temperature by increasing the concentration of solutes (supercooling). The reduction in soluble

sugar levels during the summer reflects its strong translocation to growing organs for metabolism and other needs, which explains the rapid rate of plant growth during the summer [14, 15]. Much research shows that trees' acclimatization to cold, which begins in the fall, is concomitant with an increase in the concentration of sugars in the cells [16, 17]. For example, pot-grown and previously acclimatized poplars can withstand temperatures as low as -196°C , probably due in part to the accumulation of sugars [18, 19, 20].

Several functions have been proposed for sugars in the role of protecting cells against frost damage: they could act as cryoprotectants for certain enzymes [21], participate in the stabilization of cell membranes during water movements related to successive dehydration/expansion phases [22] or act as osmolytes to prevent excessive dehydration of cells during freezing. The idea of an important role of sugars in cold acclimatization has also been reinforced by the study of mutants or transgenic *Arabidopsis* plants.

Table 2 shows the variations in the proline content of the different organs of holm oak and Aleppo pine seedlings as a function of the seasons according to the analysis of variance for one classification criterion. The highest values are obtained in winter with averages of $4,523 \pm 0.341 \mu\text{mol/g DM}$ in holm oak leaves and $4,256 \pm 0.167 \mu\text{mol/g DM}$ in Aleppo pine needles. Proline levels are also important during the summer for both species.

Contrary to the previous result, the lowest values are obtained in autumn in holm oak stems and roots and are 0.646 ± 0.090 and $0.630 \pm 0.153 \mu\text{mol/g DM}$ respectively. Finally, Aleppo pine roots show the highest proline contents compared to holm oak roots throughout the seasons.

Table 2. Inter-seasonal variation in proline levels in the different seedling organs of holm oak and Aleppo pine

		Winter	Spring	Summer	Autumn	F	p
<i>Quercus ilex</i>	Leaves	4.523a	3.090b	2.523b	1.123c	99.35	0.000
	Stems	1.013a	0.946ab	0.816b	0.646c	11.46	0.003
	Roots	0.760a	0.760a	0.760a	0.630a	2.58	0.126
	Needles	4.256a	3.523b	2.256c	1.923c	41.87	0.000
<i>Pinus halepensis</i>	Stems	1.896a	1.866a	1.316b	1.030c	63.91	0.000
	Roots	2.766a	2.466b	1.466c	1.136d	91.40	0.000

The results obtained show that the low temperatures of the winter and the high temperatures of the summer induced a very high accumulation of proline in the different organs (Table 2). The mechanism of proline accumulation suggests the presence of sites of plant resistance to stress. Indeed, the transport of proline from the source to the site of resistance has long been accepted as an important parameter in the

acquisition of this resistance [23]. Costa E Silva et al. [24] reported that proline is synthesized in leaves and transported to these sites; others report that the amino acid migrates in various glycophytic plants to the leaves. The highest values are recorded during the summer in Aleppo pine needles and holm oak leaves.

Indeed, late frosts recorded during the spring also cause an increase in the content of this amino acid in the various organs of seedlings. For this season, the minimum value is recorded at root level $1,681 \pm 0.207$ $\mu\text{mol/mg}$ M.S. Proline accumulation is one of the adaptive strategies triggered by the plant in response to environmental constraints [25]. It accumulates during hydric stress [26], saline stress [27], as well as under the effect of low and high temperatures [28, 29].

Proline is an amino acid with multiple functions. It is one of the osmoprotectants playing a primary role in the adjustment of osmotic pressure [30]. Its most important function is the maintenance of turgidity and the trapping of excess ROS [31]. Proline acts as an antioxidant and regulates the oxidation-reduction status of cells under conditions of stress [32].

Although this work tends to demonstrate a causal link between proline accumulation and cold tolerance, other results suggest that increased proline concentrations are more a consequence of stress than a coping mechanism. Proline, amino acid and a compatible solute have been widely reported to accumulate in response to various abiotic stresses, such as salt [33], drought [34], low and high temperatures [28–35]. Studies have suggested various roles for proline as a mediator of stress tolerance including osmotic adjustment and molecular chaperone [36, 37]. Osmoregulators such as proline and glycine betaine would be involved in high-temperature tolerance mechanisms. For example, exogenous application of proline and glycine betaine improved the response of plants to heat stress at 45 °C compared to control. The role of glycine betaine and proline was observed particularly in the protection of OEC (oxygen-evolving complex) [38]. In addition to its protective effect on the oxygen-evolving complex, these two osmolytes may also be involved in the protection of PSII reaction centres (RC) [39].

The results in Table 3 show that summer is the season with the highest accumulation of total protein in the various organs, while the lowest levels are obtained in the autumn. Leaves have the highest levels of protein in the organs compared to the other organs (stems and roots). These variations are confirmed by the analysis of variance at a classification criterion that shows significant differences between the organs according to the seasons. These proteins are used to participate in the reorganization of cell structures damaged by high temperatures (natural conditions).

Comparison between the four seasons shows that the highest protein content is recorded in the foliage where the number of chloroplasts is high; these values are of the order of 0.660 ± 0.116 mg/g FM in holm oak leaves and $1,626 \pm 0.107$ mg/g FM in Aleppo pine needles.

Table 3. Inter-seasonal variation in total protein levels in the different seedling organs of holm oak and Aleppo pine

		Winter	Spring	Summer	Autumn	F	p
<i>Quercus ilex</i>	Leaves	0.490ab	0.523ab	0.660a	0.423b	3.11	0.044
	Stems	0.313b	0.346b	0.413a	0.246c	12.75	0.002
	Roots	0.253a	0.276a	0.306a	0.250a	2.11	0.177
	Needles	0.760b	1.426a	1.626a	0.626b	42.42	0.000
<i>Pinus halepensis</i>	Stems	0.680c	1.047b	1.380a	0.480c	18.60	0.001
	Roots	0.556bc	0.757b	1.490a	0.390d	50.50	0.001

The analysis of variance of total protein content is highly significant between organs and seasons (Table 3). Thus, the protein accumulates as a result of heat stress. This increase is due to the activation of a set of genes allowing the synthesis of specific proteins associated with stresses such as the “Late Embryogenesis Abundant: LEA” proteins which ensure the protection of the vital set of cellular proteins [40] and heat shock proteins which allow maintenance of the protein and membrane structures of the plant cell [41]. The involvement of HSPs in the phenomenon known as “thermotolerance” has been demonstrated. The HSPs thus synthesized allow the “renaturation” of proteins and the recovery of enzymatic activity [42].

Heat Shock Proteins “HSPs” are chaperone proteins that assist in the folding, assembly and transport of proteins. Often induced by heat, some are also induced by cold and thus protect proteins from denaturation and aggregation [43]. In addition to free amino acids, cold hardening induces important quantitative and qualitative changes in protein composition. An increase in total protein concentration during cold hardening has been demonstrated in several plants [44]. In addition to representing a nitrogen reserve for spring recovery, proteins could play an important role in the frost tolerance of perennial plants.

The cryoprotective role of HSPs would come from protection against cell dehydration, maintenance of transport of cellular metabolites and protection of the integrity of certain proteins [45]. Although the genetics of HSPs and the regulation of their gene expression are gradually being deciphered, relatively little is known about their role in the cell; HSPs are synthesized very rapidly after a sharp increase in temperature and newly transcribed mRNAs are detectable within 3–5 minutes. HSPs account for the bulk of newly synthesized proteins. There is, for example, a Rubisco-binding protein in the chloroplast that allows the assembly of large and small subunits to form the functional protein [46].

Statistical analysis indicates that the leaves of two species have the highest mean total protein (Table 3). The growth of the aerial part, especially the leaves, is generally more sensitive than that of the roots. The latter are not important storage tissues; their continuous growth and low protein concentration

levels may indicate that roots are permanent absorbers with constant requirements during the period of growth of the aerial parts in waves [47] and explains the low protein content at their levels. However, it appears that the synthesis of specific proteins is necessary for hardening.

Indeed, some recent studies suggest that soluble sugars in combination with proteins could protect membrane integrity in plants exposed to freezing temperatures. The accumulation of sugars in cells may increase tolerance to dehydration in plants via their “osmotic effect” which limits water loss from the cell. For example, in oak and dogwood, monosaccharides, particularly glucose and fructose, are believed to be mainly responsible for osmotic adjustment following water stress [48]. Poplars subjected to water stress show an osmotic adjustment of between 0.23 and 0.48 MPa, 48% of which can be attributed to organic solutes, including sucrose, glucose and fructose [49]. It can be noted that the levels of chlorophyll pigments in the needles of Aleppo pine seedlings and holm oak leaves depend on the severity of the season. During the winter, a decrease in chlorophyll (a) and (b) is noted for both species (Table 4).

Table 4. Inter-seasonal variation in chlorophyll pigments levels in the different seedling organs of holm oak and Aleppo pine

		Winter	Spring	Summer	Autumn	F	p
<i>Quercus ilex</i>	Chlorophyll a	4.523a	3.090b	2.523b	1.123c	99.35	0.000
	Chlorophyll b	1.013a	0.946ab	0.816b	0.646c	11.46	0.003
<i>Pinus halepensis</i>	Chlorophyll a	4.256a	3.523b	2.256c	1.923c	41.87	0.000
	Chlorophyll b	1.896a	1.866a	1.316b	1.030c	63.91	0.000

Concerning chlorophyll, a significant decrease in the levels of photosynthetic pigments was recorded during the winter and summer. Among the possible causes of this reduction is the destruction of thylakoid cell membranes: a loss of cell partitioning may indeed inhibit the development of major metabolic functions [50]. It can be seen that the photosynthetic system is damaged by stressful temperatures and the reduction in chlorophyll pigment levels can be explained by decreased chlorophyll content due to destruction of pigment-protein complex which subsequently led to a reduction of dry matter [51, 52].

Conclusions

Holm oak and Aleppo pine are targeted species of choice for their ecological interest. These two thermophilic and xerophilic species show great adaptability to the extreme conditions of their environment. This last point is of particular interest for the study of tolerance mechanisms to seasonal thermal variations, as holm oak and Aleppo pine could implement particular processes to achieve resistance during winter and

summer. The osmoregulation system is one of the defensive responses of plants to abiotic stresses such as the hydric deficit, low and high temperatures, etc.

In conclusion, the study reveals the thermophilic character of Aleppo pine and holm oak seedlings which responded to thermal variations, linked to the winter by increasing their proline and soluble sugar contents and to the summer by a high level of total protein. Indeed, the rates of accumulation of osmolytes in the different organs tested indicate the thermal limit of two species their capacity to adapt to seasonal thermal changes.

References

- [1]. Bentouati, A.; Bariteau M., Une sylviculture pour le pin d'Alep des Aurès (Algérie). *Forêt méditerranéenne*, **2005**, XXVI (4), 315-321.
- [2]. Mezali, M., Rapport sur le secteur forestier en Algérie. 3e session du forum des Nations Unis sur les forêts, **2003**.
- [3]. Vennetier, M., Changement climatique et dépérissements forestiers: causes et conséquences. Changement Climatique et Modification Forestière, CNRS, Paris, **2012**, 50-60.
- [4]. Meddour-Sahar, O. ; Derridj, A., 2012. Bilan des feux de forêts en Algérie: analyse spatio-temporelle et cartographie du risque (période 1985-2010). *Sécheresse*, **2012**, 23 (2), 133-141.
- [5]. Madoui, A. ; Gehu, J.M. ; Alatou, D., L'effet du feu sur la composition des pinèdes de *Pinus halepensis* Mill. dans le nord de la forêt de Bou-Taleb, Algérie. *Ecologia Mediterranea*, **2006**, 32, 5-13.
- [6]. Lemouissi, S. ; Rached-Kanouni, M.; Hadeif, A.; Amine Khoja, A. E. M.; Alatou, D., Adaptation of Holm oak (*Quercus ilex* L.) to seasonal climate variations. *International Journal of Management Sciences and Business research*, **2014**, 3(5), 30–35.
- [7]. Rached-Kanouni, M.; Alatou, D.; Sakr, S., Responses of Cork Oak Seedlings to Short-Term Exposures to Low Temperatures. *American Journal of Scientific Research*, **2012**, 59, 28–41.
- [8]. Megrrouche, R.; Rached-Kanouni, M.; Amine Khodja, A. E. M.; Alatou D., Susceptibility to Fire (Case the Forest of Chettabah, Algeria). *International Journal of Management Sciences and Business Research*, **2015**, 4(4), 8–13.
- [9]. Quézel, P.; Médail, F., Conséquences écologiques possibles des changements climatiques sur la flore et la végétation du bassin méditerranéen. *Bocconea*, **2003**, 16 (1), 397-422.
- [10]. Petit, J. P.; Hampe, A.; Cheddadi, R., Climate changes and tree phylogeography in the Mediterranean. *Taxon*, **2005**, 54 (4), 877-885.

- [11]. Nicault, A. ; Rathgeberg, C.; Tessier, L.; Thomasd, A., Observation sur la mise en place du cerne chez le pin d'Alep (*Pinus halepensis* Mill.): Confrontation entre les mesures de croissance radiale, de densité et les facteurs climatiques. *Ann. For. Sci.*, **2002**, 58, 759-784.
- [12]. Sarir, R. ; Benmahiou B., Etude comparative de la croissance végétative et du développement de jeunes semis de trois espèces de chênes (chêne vert, chêne liège et chêne zéen) cultivés en pépinière. *Agric. For. J.*, **2017**, 1 (1), 42-48.
- [13]. Laala, A. ; Rached-Kanouni, M. ; Alatou, D., Les variations thermiques saisonniers et leurs impacts sur le comportement écophysiological des semis de pin d'Alep. *European Scientific Journal*, **2013**, 9(24), 143–153.
- [14]. Rached-Kanouni, M.; Alatou, D.; Sakr S., Effects of high temperature on concentrations of soluble sugars and quercitol of Cork oak (*Quercus suber*) seedlings. *International Journal of Management Sciences and Business research*, **2012**, 1(6), 1–10.
- [15]. Chantuma, P.; Lacote, R.; Sonnarth, S.; Gohet E., Effects of Different Tapping Rest Periods during Wintering and Summer Months on Dry Rubber Yield of *Hevea Brasiliensis* in Thailand. *J Rubber Res*, **2017**, 20, 261–272.
- [16]. Durbak, A.; Yao, H.; McSteen, P., Hormone signaling in plant development. *Current Opinion in Plant Biology*, **2012**, 15, 92–96.
- [17]. Gechev, TS.; Rille, J., Molecular basis of plant stress. *Cellular and Molecular Life Sciences*, **2012**, 69, 3161–3163.
- [18]. Sakai, A.; Larcher; W., Frost survival of plants-Responses and Adaptation to Freezing Stress. *Ecol. Studies*, **1997**, 62, 112–133.
- [19]. Sakuma, Y.; Liu, Q.; Dubouzet, J. G.; Abe, H.; Shinozaki, K., DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. *Biochemical and Biophysical Research Communications*, **2002**, 290, 998-1009.
- [20]. Sakr, S.; Alves, G.; Morillon, R. ; Maurel, K.; Decourteix, M.; Guilliot, A.; Fleurat-Lessard, P.; Julien, J.L.; Chrispeels M., Plasma membrane aquaporins are involved in winter embolism recovery in walnut tree. *Plant Physiology*, **2003**, 133, 630-641.
- [21]. Welling, A.; Palva, E.T., Molecular control of cold acclimation in trees. *Physiologia Plantarum*, **2006**, 127, 167-181.
- [22]. Silpi, U.; Lacoite, A.; Kasempsap, P.; Thanysawanyangkura, S.; Chantuma, P.; Gohet, E.; Musigamart, N.; Clement, A.; Ameglio, T.; Thaler, P., Carbohydrate reserves as a competing sink: evidence from tapping rubber trees. *Tree Physiology*, **2007**, 27, 881–889.

- [23]. Chinnusamy, V.; Zhu, J.; Zhu, J. K., Gene regulation during cold acclimation in plants. *Physiologia Plantarum*, **2006**, 126(1), 52–61.
- [24]. Costa E Silva, F.; Shvaleva, A.; Broetto, F.; Ortuno, M.F.; Rodrigues, M.L.; Almeida, M. H.; Chaves, M. M.; Pereira J.S., Acclimation to short-term low temperatures in two *Eucalyptus globulus* clones with contrasting drought resistance. *Tree Physiol.*, **2009**, 29, 77–86.
- [25]. Delauney, A. J.; Verma, D. P. S., Proline biosynthesis and osmoregulation in plants. *The Plant Journal*, **1993**, 4, 215–223.
- [26]. Szabados, Z.; Saviouré, A., Proline : a multifunctional amino acid. *Trends Plant Sci.*, 2010, 15, 89–97.
- [27]. Widodo, Patterson, J. H.; Newbigin, E.; Tester, M.; Bacic, A.; Roessner, U., Metabolic responses to salt stress of barley (*Hordeum vulgare* L.) cultivars, Sahara and Clipper, which differ in salinity tolerance. *J. Exp. Bot.*, **2009**, 60(14), 4089–4103.
- [28]. Rached-Kanouni, M.; Alatou, D; Sakr, S., Responses of Cork Oak Seedlings to Short-Term Exposures to Low Temperatures. *American Journal of Scientific Research*, **2012**, 59, 28–41.
- [29]. Rached-Kanouni, M.; Alatou, D.; Sakr S., Effects of high temperature on concentrations of soluble sugars and quercitol of Cork oak (*Quercus suber*) seedlings, *International Journal of Management Sciences and Business Research*, **2012**, 1(6), 1–13.
- [30]. Chinnusamy, V.; Schumaker, K.; Zhu, J.K., Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. *J. Exp. Bot.*, **2004**, 55 (395), 225–236.
- [31]. Zaki, S.S.; Rady, M.M., *Moringa oleifera* leaf extract improves growth, physiochemical attributes, antioxidant defense system and yields of salt-stressed *Phaseolus vulgaris* L. plants. *International Journal of ChemTech Research*, **2015**, 8 (11), 120–134.
- [32]. Parida, A. K.; Dagaonkar, V. S.; Phalak, M.S.; Aurangabadkar, L.P., Differential responses of the enzymes involved in proline biosynthesis and degradation in drought tolerant and sensitive cotton genotypes during drought stress and recovery. *Acta Physiol. Plant.* **2008**, 30(5), 619–627.
- [33]. Chinnusamy, V.; Zhu, J.; Zhu, J. K., Cold stress regulation of gene expression in plants. *Trends Plant Sci.*, **2007**, 12, 444–451.
- [34]. Oraki, H.; Khajani, F. P.; Aghaalikhana, M., Effect of water deficit stress on proline contents, soluble sugars, chlorophyll and grain yield of sunflower (*Helianthus annuus* L.) hybrids. *African J. Biotechnol.*, **2012**, 11, 164–168.
- [35]. Assaha, D.V.M.; Liu, L.; Ueda, A.; Nagaoka, T.; Saneoka, H., Effects of drought stress on growth, solute accumulation and membrane stability of leafy vegetable, huckleberry (*Solanum scabrum* Mill.). *Journal of Environmental Biology*, **2016**, 37, 107–114.

- [36]. Shin, H.; Oh, S.; Kim, K.; Kim, D., Proline accumulates in response to higher temperatures during dehardening in peach shoot tissues. *Hort. J.*, **2016**, *85*(1), 37–45.
- [37]. Lehmann, S.; Funck, D.; Szabados, L.; Rentsch, D., Proline metabolism and transport in plant development. *Amino Acids*, **2010**, *39*(4), 949–962.
- [38]. Kavi Kishor, P. B.; Sreenivasulu, N., Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? *Plant Cell Environ.* **2014**, *37* (2), 300–311.
- [39]. Verbruggen, N.; Hermans, C., Proline accumulation in plants: A review. *Amino Acids*, **2008**, *35*, 753–759.
- [40]. Angelcheva, L.; Mishra, Y.; Antti, H.; Kjellsen, T.D.; Funk, C.; Strimbeck, R.G.; Schröder, W. P., Metabolic analysis of extreme freezing tolerance in Siberian spruce (*Picea obovate*). *New Phytol.* **2014**, *204* (3), 545–555.
- [41]. Baker, S. S.; Wilhelm K.S.; Thomashow M. F., The 5'-Region of *Arabidopsis-Thaliana* Cor15a Has Cis-Acting Elements That Confer Cold-Regulated, Drought-Regulated and Aba-Regulated Gene-Expression. *Plant Mol. Biol.*, **1994**, *24*, 701–713.
- [42]. De Ronde, J.A.; Cress, W.A.; Krüger, G. H. J.; Strasser, R. J.; Van, Staden, J., Photosynthetic response of transgenic soybean plants, containing an *Arabidopsis P5CR* gene, during heat and drought stress. *J. Plant. Physiol.*, **2004**, *161*, 1211–1224.
- [43]. Souren, J. E.; Wiegant, F.A.; Van, wijk, R., The role of hsp70 in protection and repair of luciferase activity in vivo; experimental data and mathematical modelling. *Cell Mol. Life Sci.*, **1999**, *55*, 799–811.
- [44]. Sung, D.Y.; Kaplan, F.; Lee, K. J.; Guy, C. L., Acquired tolerance to temperature extremes. *Trends in Plant Science*, **2003**, *8*, 179–187.
- [45] Dionne, J., Protection hivernale et tolérance au froid du Pâture Annuel *Pea ann. Plant J.*, **2001**, *4*, 215–223.
- [46]. Houde, M. ; Daniel, C.; Lachapelle, M; Allard, F.; Laliberté, S.; Sarhan, F., Immunolocalization of freezing-tolerance-associated proteins in the cytoplasm and nucleoplasm of wheat crown tissues. *Plant. J.*, **1995**, *8*, 583–593.
- [47]. Vierling E., Chloroplast-localized Clp proteins. In: Guidebook to molecular chaperones and Protein-Folding Catalysts. M. J. Gething (ed.), Sambrook and Tooze Publications at Oxford University Press, **1997**, 255–258.
- [48]. Alaoui-Sossé, B.; Parmentier, C.; Dizengremel, P; Barnola, P., Rhythmic growth and carbon allocation in *Quercus robur*. Starch and sucrose. *Plant Physiology and Biochemistry*, **1994**, *32*, 331–339.
- [49]. Gebre, G. M.; Tschaplinski, T.J., Solute accumulation of chestnut oak and dogwood leaves in response to throughfall manipulation of an upland oak forest. *Tree Physiol.*, **2002**, *22*, 251–260.

- [50]. Gebre, G. M.; Kuhns, M. R.; Brandle, J. R., Organic solute accumulation and dehydration tolerance in 3 water-stressed *Populus deltoides* clones. *Tree Physiol.*, **1994**, *14*, 575–587.
- [51]. Cornic, G.; Ghashghaie J., Effect of temperature on net CO₂ assimilation and photosystem II quantum yield of electron transfer of French bean (*Phaseolus vulgaris* L.) leaves during drought stress. *Planta*, **1991**, *185*, 255-260.
- [52]. Santos, C. V., Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Scientia Horticulturae-Amsterdam*, **2004**, *103*, 93–99.
- [53]. Bates L.; Waldren R. P.; Teare I. D., Rapid determination of free proline for water-stress studies. *Plant and Soil*, **1973**, *39*, 205–207.
- [54]. Dubois, M.; Gilles, K.A.; Hamilton J.K; Rebers P.A.; Smith F., Colorimetric method for the determination of sugars and related substances. *Anal. Chem.*, **1956**, *28*, 350–356.
- [55]. Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **1976**, *72*, 248-254.