Available online at www.easletters.com

ISSN No: 2349-2864

Entomology and Applied Science Letters, 2016, 3, 5:8-13

Seasonal changes in the physiological characteristics of Aleppo pine

Malika RACHED-KANOUNI and Karima KARA

National High School of Biotechnology ENSB, University Town AliMendjeli, Constantine/ Algeria

Corresponding Email: kmalikbio@yahoo.fr

ABSTRACT

Aleppo pine (Pinus halepensis Mill.) plays an important role in the ecology and landscape of different countries around the Mediterranean basin. It is an essential component in reforestation strategy for limy soils in the arid or semi-arid climates. In the present study, in order to analyze seasonal physiological and biochemical changes in Aleppo pine trees in forest of Chettaba (region in north-eastern of Algeria), leaf, stems and root samples of the plant were collected in 3 replicates during different season of 2015/2016. The findings suggested that the features under study changed with the season. In summer leaves, there was a significant difference in chlorophyll a contents, soluble sugars, proline and oxidase enzymes activities (catalase, peroxidase) as compared with other seasons samples. In stems and roots, proline rate and soluble sugars had respectively increased in summer and autumn. However, no significant difference was observed in catalase and peroxidase in stems. In summer, catalase and peroxidase content roots was significantly increased compared to the other seasons.

Key words: Aleppo pine, Seasonal changes, Chlorophylls, Soluble sugars, Proline, Catalase, Peroxidase

INTRODUCTION

The Aleppo pine (*Pinus halepensis* Mill.) is a conifer native to the Mediterranean region. In Algeria, Aleppo pine, considered the most important and dominant local forest species covers a surface estimated at more than 800.000 ha. It is a typically Mediterranean species, easily adaptable to various eco-climatic conditions, which grants it the privilege of being the most often used tree species in the country's reforestation programs [1].

In the Mediterranean climate, the rainy season is during autumn and winter. Summers are hot with no rains; winter temperatures are mild (with a wet season starting in October and ending in April or May, followed by 5 months of dry season).Temperature and precipitation have been revealed as important drivers for local adaptation for conifers [2]. Temperature can profoundly influence seed germination, seedling growth, productivity and distribution of conifers, whereas precipitation has a determinative impact on variables such as soil moisture and the length of a wet or dry season [3]. Temporal and spatial variation in temperature and precipitation can also strongly influence the survival of conifers [4].

In extreme situations, high or low temperature stresses and precipitation events will ultimately cause mortality. Plants resort many adaptive strategies in response to abiotic environmental stresses such as dehydration and excessive osmotic pressure. These adaptive mechanisms include changes in physiological and biochemical processes. Among them, the accumulation of compatible solutes according to the metabolic responses has drawn much attention. Adaptation to all these stresses is associated with metabolic adjustments that lead to the accumulation of several organic solutes like sugars, polyols, betaines and proline [5]. Accumulation of sugars in different parts of plants is enhanced in response to the variety of environmental stresses. Various authors point to the role of soluble sugars in the protection against stresses. Metabolization of storage reserves in the endosperm of cereal seeds is tightly regulated and has a primary pivotal role in the interactions among sugars, ABA and gibberellin pathways responsible for the response to drought [6]. A central role of sugars depend not only on direct involvement in the synthesis of other compounds, production of energy but also on stabilization of membranes, action as regulators of gene expression [7] and signal molecules [8].

Exposed to unfavorable conditions, plants will experience oxidative stress which due to generation of reactive oxygen species (ROS) will affect their growth [9]. Through the activity of enzymatic antioxidant systems including superoxide dismutase, peroxidase and properties of peroxidase, catalase, polyphenol oxidase and glutathione reductase, plants fight against stress [10]. Activity of peroxidase and polyphenol oxidase enzymes in pistachio trees increases in winter when trees are dormant due to the cold weather and its resulting oxidative stress. This increase helps the trees to tolerate the cold and resist it. The activity of these enzymes decreased to the minimum during the late winter and early spring [11]. In the present study, in order to delve into the physiological tolerance of pine trees under temperature and climatic changes due to the seasonal variations, physiological and biochemical changes of *Pinus halepensis* plants in forest of Chettabain north east Algeria was studied.

MATERIALS AND METHODS

The Study Area

Forest of Chettaba is located southwest of Constantine (Algeria). The estimate terrain elevation above sea level is 865 meters. The study area is located on the map topographic Constantine Scale 1/200 000 sheet N° 17 and located between the coordinates 36°19'4" north latitude and 6°28'36" East longitude. The forest spreads over an area of 2398ha and 94a. Extreme altitudes of the forest is about 1104 m (maximum altitude) and 652 m (minimum altitude), corresponding to each of them respectively following map coordinates: (x = 839, y = 344), (x '= 839.9, y' = 340.3). Its bioclimatic is semi-arid to sub-humid. The average annual rainfall is estimated between 670 and 800 mm and the mean annual temperature of the region is 18°C, with an average of the warmest month above 35°C and the coldest month varies between 1.25 and 3.05°C. A large plant grouping as the forest of Chettaba can be studied in its entirety, especially when it concerns hundreds of hectares to be treated in the detail. Our choice fell on the Aleppo pine which is one of the dominant species in this forest.

Analytical methods

Experiment was conducted during the year of 2015/2016. The different tissues (leaves, stems and roots) were collected, with 3 replication per treatment during autumn, winter, spring and summer.

Chlorophyll

Chlorophyll a (Chla) and chlorophyll b (Chlb) were determined spectrophotometrically using 80% acetone as a solvent [12].

Extraction and estimation of total soluble sugars

The sugar content in the extracts normally was analyzed by a phenol-sulfuric acid method [13]. This colorimetric method determines only the amount of total sugars. This is the most widely used colorimetric method to date for determination of carbohydrate concentration in aqueous solutions. The basic principle of this method is that carbohydrates, when dehydrated by reaction with concentrated sulfuric acid, produce furfural derivatives. Further reaction between furfural derivatives and phenol develops detectible color. The standard procedure of this method is as follows. A 2 ml aliquot of a carbohydrate solution is mixed with 1ml of 5% aqueous solution of phenol in a test tube.

Subsequently, 5 ml of concentrated sulfuric acid is added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they are vortexes for 30s and placed for 20 min in a water bath at room temperature for color development. Then, light absorption at 490 nm is recorded on a spectrophotometer. Reference solutions are prepared in identical manner as above, except that the 2 ml aliquot of carbohydrate is replaced by DDI water. The phenol used in this procedure was redistilled and 5% phenol in water (w/w) was prepared immediately before the measurements.

Proline

Proline was measured in the youngest leaves using the method proposed by BATES et al. [14]. Thus, 0.1 g of leaf tissue was pulverized in 2 mL of 3.3% sulfosalicylic acid and was then centrifuged at 4000 rpm for 10 min at 4 °C. Then, 2 mL of ninhydrin reagent and 2 mL of pure glacial acetic acid were added to 2 mL of the resulting extract in separate tubes. The tubes were placed in a bain-marie for 1h. Each tube was then vortexed for 15–20 s after adding 4 mL of toluene. After the formation of two separate phases, the colored upper phase was separated, and the absorbance was measured using a spectrophotometer at a wavelength of 520 nm.

Catalase (CAT)

Activities of catalase (CAT) were measured using the method of CHANCE AND MAEHLY [15] with modification. The CAT reaction solution (3 ml) contained 50 mM phosphate buffer (pH 7.0), 15 Mm H_2O_2 and 0.1 ml enzyme

extract. Reaction was initiated by adding enzyme extract. Changes in absorbance of the reaction solution at 240 nm were read every 20s. One unit CAT activity was defined as an absorbance change of 0.01 unit min-^{1}.

Peroxidase (GPX)

Activities of peroxidase were measured using the method of CHANCE AND MAEHLY [15] with modification. For guaiacol peroxidase acivity assay the reaction mixture (3.0 ml) contained 0.1 M phosphate buffer (pH 6.80), guaiacol (30 mM), H_2O_2 (30 mM) and 0.3 ml enzyme extract. Changes in absorbance of reaction solution at 470 nm were determined every 20s. One unit GPX activity was defined as an absorbance change of 0.01 unit min-^{1}. The activity of each enzyme was expressed on a protein basis.

Statistical analysis was performed using SAS software. Mean comparison was also performed using Duncan's multiple range test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Results obtained from variance analysis indicated that there was a significant difference between changes in chlorophyll content and different seasons. The highest and lowest chlorophyll a contents were observed in summer and winter samples respectively. The difference was significant ($p<0.05$). The highest chlorophyll b content was however recorded in spring which was not meaningfully different from that in autumn and summer samples ($p<0.05$). As with chlorophyll a, the lowest chlorophyll b content was observed in winter which was meaningfully different from others seasons samples (Table 1).

Table 1. The contents of leaf chlorophyll of Aleppo pine in different seasons.

Chlorophyll contents (mg.g-^{1}FW)	Winter	Spring	Summer	Autumn
Chl a	$0.561^{d}\pm0.025$	$0.944^{b}\pm0.040$	$1.564^{a}\pm0.070$	$0.783^{c}\pm0.029$
Chl a	$0.223^{b}\pm0.013$	$0.435^{a}\pm0.012$	$0.422^{a}\pm0.018$	$0.413^{a}\pm0.015$

The chlorophyll content is an important experimental parameter in agronomy and plant biology research [16]. Amount of chlorophyll shows alteration depending on many edaphic and climatic factors such as temperature [17], water stress and fertilizing [18].

Chlorophyll content is also an important factor for photosynthesis capability in plants. This study showed that chlorophyll content in Aleppo pine trees changes with the season so that the highest and lowest contents of chlorophyll a were observed in summer (July) and winter (January). As Table 1 suggests, during the months when samples were collected, July and January had maximum and minimum temperatures respectively. Photosynthesis, one of the most heat sensitive processes, can be completely inhibited by high temperature before other symptoms of the stress are detected [19]. This photosynthesis decrease could result from structural and functional disruptions of chloroplasts and reduction of chlorophyll accumulation under high temperature stress. Chlorophyll a and b contents were lowest in January when the average temperature was at its lowest point. The possibility of low chlorophyll and carotenoid content could be oxidative stress caused by low temperature treatment [20]. Our results have been confirmed by the research of Aghaee et al. [21] that total chlorophyll concentration was reduced under cold treatment. Habibi et al. [22] observed that the low temperature inducted significant decreases in the chlorophyll a and b content as a result of the total chlorophyll content of leaves. As for chlorophyll b, the highest content was recorded in spring although the difference with summer and autumn samples was not meaningful. This is similar to the study on three plant species reported by Aziz [23] who found that chlorophyll synthesis increases during the wet season when the moisture in the soil is high.

The highest leaf soluble sugars contents were observed in summer which was meaningfully different from those in the spring, autumn and winter samples ($p<0.05$). In stems and roots however, the highest and lowest soluble sugars contents were recorded in autumn and winter samples respectively (Table 2). Results also revealed that soluble carbohydrate contents in leaves were higher than those in roots.

Table 2. The soluble sugars contents of Aleppo pine in different seasons.

Sugars contents (µmol g^{-1} DW)	Winter	Spring	Summer	Autumn
Leaf	$402^{d}\pm12.74$	$630^{c}\pm19.18$	$850^{a}\pm21.11$	$760^{b}\pm18.32$
Stem	$306^{c}\pm13.41$	$510^{b}\pm15.13$	$540^{b}\pm6.44$	$645^{a}\pm14.52$
Root	$220^{c}\pm10.42$	$395^{b}\pm13.45$	$420^{b}\pm3.22$	$515^{a}\pm12.45$

The soluble sugars content in the study also varied with the change of season as the highest sugar content in leaves were observed in summer with maximum temperature and minimum rainfall in the region (the dry season). The increase in sugar concentration may be a result of the degradation of starch [24]. From the changes in total soluble

sugar content in roots and shoots of tolerant genotypes, it was observed that soluble sugar content increased at the early drought stage in drought-stressed tissues in both tolerant genotypes. The current hypothesis is that sugars act as osmotica and/or protect specific macromolecules and contribute to the stabilization of membrane structures. In general, soluble sugar content tends to be maintained in the leaves of drought-stressed plants, although rates of carbon assimilation were partially reduced. The maintenance of soluble sugar content may be achieved at the expense of starch, which drastically declines [25]. Studying seasonal variations in carbohydrates contents of some deciduous trees in mild climate, KRAMER and KOZLOWSKI [26] observed that total carbohydrates levels in trunks and branches were at their peak in autumn as the trees were shedding leaves.

On the other hands, the spring reduction in soluble carbohydrates content observed in the present study, agrees with KRAMER and KOZLOWSKI [26] and also SIVACI [27] who worked on the trunks of 3 apple varieties. Why KRAMER and KOZLOWSKI attributed reduction of carbohydrate contents in spring to increased respiration and consumption of these carbohydrates for the growth of new tissues, SIVACI [27] associated this with opening the buds and formation of new leaves.

Increase in soluble sugar contents through inversion of some carbohydrates may contribute to enhanced desiccation tolerance and allows metabolic activity to be maintained. This was in agreement with the results observed in another study [28]. Starch plays an important role in accumulation of soluble sugars in cells. Starch depletion in grapevine leaves was noted by BONHOMME et al. [29] in response to drought stress, too. Increase in concentration of soluble sugars at high osmotic potential was simultaneously paralleled with decrease in the starch concentration. It means that the raised soluble sugar fraction was accompanied by a sharp decrease in the starch fraction as the water potential dropped. This change increased the soluble [sugar/starch] ratio in roots and shoots of both tolerant genotypes.

As for proline, the highest and lowest contents in leaves and roots were observed in summer and autumn, respectively and the differences between the samples of all four seasons were statistically meaningful ($p<0.05$). Additionally, proline content in roots was higher than that in leaves during the 4 seasons of study (Table 3).

Table 3. The proline contents of Aleppo pine in different seasons.

Proline contents (µmol g^{-1} DW)	Winter	Spring	Summer	Autumn
Leaf	3.91^{b}±0.020	2.81^{c}±0.018	4.7^{a}±0.025	1.51^{d}±0.012
Stem	2.90^{b}±0.015	2.60^{b}±0.016	3.5^{a}±0.016	1.8^{c}±0.013
Root	5.71^{b}±0.023	5.25^{b}±0.026	6.4^{a}±0.028	4.3^{c}±0.019

The highest proline content was observed in summer and winter (July) when the temperatures were extreme. One of the ways to deal with adverse effects of heat stress may involve exploring some molecules that have the potential to protect the plants from the harmful effects of high temperature. Proline, an amino acid, which is elevated in response to diverse types of abiotic stresses [30] is one such molecule that has several roles such as turgor generation, storage of carbon and nitrogen, as partial antioxidant, molecular chaperone stabilizing the structure of proteins, maintenance of cytosolic pH, balance of redox status and as part of stress signal influencing adaptive responses. KAUSHAL et al. [31] found that supplementation of proline to the heat-stressed chickpea plants enhanced the proline accumulation to about 63 µmol g^{-1} DW that improved the growth at stressful temperature (45/35 °C) compared to the plants growing without proline at the same temperature. Previous studies have demonstrated that proline application confers protection to the plants growing under different types of abiotic stresses such as osmotic, salt [32] and cold [33] stresses. Our findings demonstrate the protective effects of proline against heat stress not reported so far to the best of our knowledge, at least in case of chickpea plants. The mechanism of proline action in imparting heat stress might involve several cellular sites. For example, we noticed that the proline-treated heat-stressed plants experienced reduction in stress injury measured as decrease in damage to membranes, improvement of chlorophyll content and tissue viability. Additionally, the proline treated plants also maintained greater leaf water content than those growing without it.

Proline plays a vital role in maintaining osmotic balance in plants. The accumulation of proline may function in preventing plants from being damaged by stress. The free proline acts as osmolytes to facilitate osmoregulation, thus protecting plants from dehydration resulting from cold stress by reducing water potential of plant cells [34]. In addition, proline can also function as a molecular chaperone to stabilize the structure of proteins as well as play a role in regulation of the antioxidant system [35]. So increased free proline content protects the plant against the stress. The study found greater accumulation of free proline under cold stress, which may partially account for the higher tolerance of plants to cold stress.

Similarly, antioxidant enzymes activities showed seasonal variations and for peroxidase and catalse enzymes, the highest and lowest activities in leaves were recorded in summer and winter samples. In stems, no meaningful difference was observed between catalase and peroxidase activities in different seasons. In roots, Peroxidase enzyme activities were also not meaningfully different in autumn and summer samples. However, there was a meaningful reduction in peroxidase activities in winter and spring samples. Also, catalase activity in roots showed a meaningful increase from winter to autumn. The highest catalase activities in roots were also observed in summer (Table 4).

Table 4. The enzyme activities of *Pinushalepensis* in different seasons

Seasons Enzyme activity (mg.g-1FW)	Winter	Spring	Summer	Autumn
Catalase leaf	12.53^{d}±1.26	15.66^{c}±139	23.28^{a}±1.91	19.18^{b}±0.89
Catalase Stem	3.9^{a}±0.90	3.6^{a}±0.18	3.78^{a}±0.32	3.19^{a}±0.18
Catalase Root	3.17^{d}±0.48	3.25^{c}±0.54	6.4^{a}±0.56	4.3^{b}±0.31
Peroxidase leaf	12.73^{c}±1.41	17.60^{b}±1.32	20.24^{a}±1.12	13.52^{c}±0.75
Peroxidase stem	2.35^{a}±0.70	2.65^{a}±0.15	2.75^{a}±0.13	2.64^{a}±0.21
Peroxidase root	2.31^{a}±0.42	2.12^{a}±0.13	4.52^{a}±0.45	4.41^{a}±0.41

Antioxidant enzymes activities in this study also showed variations with the change in season. As it was mentioned before, the highest temperature and lowest precipitation in the region under study occur in summer. Accordingly, the findings suggested that antioxidant enzymes activities increased in summer as the region experienced dry conditions. In these circumstances, when there is maximum radiation, closing stomata as a reaction to water or temperature stress reduces CO fixation while photo reaction and electron transportation is still carried out normally.

The exposure of plants to unfavorable environmental conditions increases the production of reactive oxygen species (ROS) such as, singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). The ROS detoxification process in plants is essential for the protection of plant cells and their organelles against the toxic effect of these species [36]. The differences in subcellular localization and biochemical properties of antioxidant enzymes and the distinct responses in gene expression, in addition to the presence of non-enzymatic mechanisms, result in a versatile and flexible antioxidant system able to control the optimum ROS levels [37]. The ROS detoxification systems include enzymatic and non-enzymatic antioxidant components [38]. Ascorbate (AsA) and glutathione (GSH), non-enzymatic antioxidants are crucial for plant defence against oxidative stress, playing a key role as antioxidant buffers [39; 40]. Other non-enzymatic antioxidants involved include flavonoids, phenolic compounds, alkaloids, tocopherol and carotenoids [41].

Subsequently, ROS production contributes to the transduction of the heat signal and expression of heat shock genes [42]. Heat stress results in the misfolding of newly synthesized proteins and the denaturation of existing proteins. Protein thermostability is believed to be provided in part by chaperones, a specific class of proteins capable of assisting other proteins in proper post-translational folding and in maintaining them in a functional state. In standard growth conditions the HSPs control cellular signaling, protein folding, translocation, and degradation, but under heat stress they prevent protein misfolding and aggregation and also act to protect cellular membranes. An increased production of HSPs occurs when plants experience either abrupt or gradual increases in temperature resulting in heat stress [43]. There are considerable variations in the pattern of HSP gene expression in different species and even among genotypes within species. Heat shock factors (HSFs) are the transcriptional activators of HSPs [44]. HSF regulation in *Arabidopsis* was shown to be positively regulated by small HSP and co-chaperones of the HSP90 complex such as ROF1, and negatively affected by an HSF binding protein [45; 46]. The HSP regulation may be achieved by a single “master switch” HSF or by the collective function of several HSFs, depending on the plant species [47].

From the biochemical level, our results show that the action of seasonal change results in variations in proline rates, soluble sugars, chlorophyll and enzymes activities (peroxidase and catalase). In addition, these variations differ according to the relevant organ, probably reflecting different tolerance mechanisms of Aleppo pine for summer (unfavorable season).

REFERENCES

[1] A. Haddad, Lachenal D., Marechal A., janin G., Labiod M. *Cellulose chemistry and technology*,**2009**, 43, 7-8, 287-294
[2] Y.F. Zhou, Zhang L.R., Liu J.Q., Wu G.L., Savolainen O. *Molecular Ecology*,**2014**, 23, 3504-3522.
[3] B.N. Poncet, Herrmann D., Gugerli F., Taberlet P., Holderegger R., Gielly L., Rioux D., Thuiller W., Aubert S. *Molecular Ecology*,**2010**, 19, 2896-2907.
[4] S. Manel, Gugerli F., Thuiller W., Alvarez N., Legendre P., Holderegger R., Gielly L., Taberlet P. *Molecular Ecology*, **2012**, 21, 3729-3738.

[5] I.Y. ruela,*Brazilian Journal of plant physiology*, **2005,** 17, 145-1.
[6] C.A. Jaleel, Manivannan P., Wahid A., Faroog M., Somasundaram R., Pannerselvam R. *International Journal of Agriculture and Biology*, **2009**, 11, 100-105.
[7] J.S. Franks, Hoffmann A. A. *Annual Review of Genetics*, **201**2, 46, 185-208.
[8]A.A. Hoffmann, Sgrò C. M. *Nature*, **2011**,470: 479-85.
[9] R. Mittler. *Trends in Plant Science*, **2002**, 7, 405-410.
[10] D. Prochazkova, Sairam R.K., Srivastava G.C., Singh D.V. *Plant Science*, **2001**, 161, 765-771.
[11] Z., Pakkish, Rahemi M., Baghizadeh A. *World Applied Science Journal*, **2009**, 6, 9, 1193-1199.
[12] H.K. Lichtenthaler. *Methods in Enzymology*, **1987**, 148, 350-382.
[13] M. Dubois, Gilles K.A., Hamilton J.K., Rebers P.A., Smith F.*Anaytical Chemistry*, **1956**,28, 350-356.
[14] L.S. Bates, Waldren R.P., Teare I.D. *Plant and Soil*, **1973**, 39, 205-207.
[15] B. Chance, Maehly S.K. *Methods in Enzymology*, **1955**, 2, 764-775.
[16] J.J. Lamb, Eaton-Rye J.J., Hohmann-Marriott M.F. *Photosynthesis Research*, **2012**, 114: 59-68.
[17] X.Xu, Yang F., Xiao X., Zhang S., Korpelainen H., Li C. *Plant, Cell and Environment*, **2008**, 31, 850-860.
[18] M.M. Tunalı, Çarpıcı E.B., Çelik N. *TarımBilimleriAraştırmaDergisi*, **2012**, 5, 1, 131-133.
[19] D.Camejo, Rodriguez P., Angeles Morales M., Dell' Amico J.M., Torrecillas A., Alarcón J.J. *Journal of Plant Physiology*, **2005**, 162, 281-289.
[20]L.Z. Yadegari, Heidari R., Carapetian J. *Journal of Biological Sciences*, **2007**,7, 436-144.
[21] A. F. Aghaee,Moradi H.,Zare-Maivan F.,Zarinkamar H. SharifiP.*Africain Journal of Biotechnology*,**2011**, 10, 7617-7621.
[22] A. Habibi, Ahmadi M., Pourafshari P., Ayatollahi S.H. *Proceding of the 9th SPE European Formation Damage Conference. Noordwijk, The Netherlands*, **2011**, June 7-10.
[23] I. Aziz.*Pakistan Journal of Botany*,**2007**, 39, 6, 1995-2002.
[24] P. Chantuma, Lacointe A., Kasemsap P., Thanisawanyangkura S., Gohet E., Clement A., Guilliot A., Ameglio T., Thaler P. *Tree Physiology*,**2009**,43, 1-11.
[25] M.C. Caldeira, Fernandéz V., Tomé J., Pereira J.S. *Annals of Forest Science*, **2002**, 59, 99-106.
[26] P.J. Kramer, T.T. Kozlowski. *Academic Press, Orlando, Florida*, 1979, 811 p.
[27] A. Sivaci. *Scientia Horticulturae*, **2006**, 109, 234-237.
[28] D. Epron, Nouvellon Y., Ryan M.G. *Tree Physiology*,**2012**, 32, 639-643.
[29] M. Bonhomme, Peuch M., Ameglio T., Rageau R., Guilliot A., Decourteix M., Alves G., Sakr S., Lacointe A. *Tree Physiology*, **2009**, 30, 89-102.
[30] N.Verbruggen, Hermans C. *Amino Acids*, **2008**, 35, 753-759.
[31]N. Kaushal, Gupta K., Bhandhari K., Kumar S., Thakur P., Nayyar H. *Physiology and Molecular Biology of Plants*,**2011**,17, 203-213.
[32] M.A.Hoque, Okuma E., Banu M.N.A, Nakamura Y., Shimoishi Y., Murata Y. *Journal of Plant Physiology*, **2007a**, 164, 553-561.
[33] M. M. Posmyk, Janas, K. M. *Acta PhysiologiaePlantarum*, **2007**, 29,6, 509-517.
[34] F.Larher, Leport L., Petrivalsky M. Chappart M. *Plant Physiology and Biochemistry*, **1993**, 31, 911-922.
[35] N. Regier, Streb S., Zeeman S.C., Frey B. *Tree Physiology*, **2010**, 30, 979-987.
[36] K. Apel, Hirt H. *Annual Review of Plant Biology*,**2004**, *55*, 373-399.
[37] E. Vranova, Inze D., Van Breusegem F. **2002**, 53, 1227-1236.
[38] J.G. Scandalios. *Brazilian Journal of Medical and Biological Research*, **2005**,38, 995-1014.
[39] C.H. Foyer, Noctor G.*Plant Cell*, **2005**,17, 1866-1875.
[40] R. Mittler, Vanderauwera S., Gollery M., Breusegem F.V. *Trends Plant Science*, **2004**, 9, 10, 490-498.
[41]P.L. Gratão, Polle A., Lea P.J., Azevedo R.A. *Functional Plant Biology*, **2005**,32, 481-494.
[42] H. Königshofer, Tromballa H. W, Löppert H. G. *Plant, Cell & Environment*, **2008**, 31, 1771-1780.
[43] L. Nover, Bharti K., Döring P., Mishra S.K., Ganguli A., Scharf K.D. *Cell stress chaperones*,**2001**, 6, 177-198.
[44] V. Banti, Mafessoni F., Loreti E., Alpi A., Perata P. *Plant Physiology*,**2010**, 152, 1471-1483.
[45] D. Meiri, Tazat K., Cohen-Peer R., Farchi-Pisanty O., Aviezer-Hagai K., Avni A. *Plant Molecular Biology*, **2010**,72, 191-203.
[46] K.D. Scharf, Berberich T., Ebersberger I., Nover L. *Biochimica et BiophysicaActa*,**2011**, 1819: 104-119.
[47] H.C. Liu, Liao H.T., Charng Y.Y. *Plant, Cell & Environment*,**2011**, 34, 738-751.