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Contribution to the study of the adaptation of *Casuarina equisetifolia* to heat stress using biochemical markers

Malika Rached-Kanouni[1], Zahia Kadi[1], Hichem Khammar[1], Rima Bouzobra[2], Fadhila Belghit[2] and Labed Ababsa[1]

[1]Laboratory of Functional Ecology and Environment (F.E.E.L), Department of Life Sciences and Nature, Faculty of Exact Sciences and Life Sciences and Nature, University of Larbi Ben M'hidi, Oum El Bouaghi-Algeria
[2]Department of Biology and Plant Ecology, Faculty of Natural and Life Sciences University of Frères Mentouri Constantine 1, Algeria

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ABSTRACT

The objective of our study is to analyze the effects of heat stress (high temperatures) on *Casuarina equisetifolia* seedlings. In our experiment, variations in protein content, peroxidase (GPX) and catalase (CAT) activities are monitored on the leaves. Heat stress is carried out at temperatures of 36 °C, 38 °C, 40 °C, 42 °C and 44 °C for a period of 3 hours with the aim of estimating the degree of adaptation of seedlings by biochemical markers. The results obtained indicate that protein accumulation is obtained at 44 °C. Catalase and peroxidase activities are significant at 38 °C and 42 °C respectively.

Key words: *Casuarina equisetifolia, High temperatures, Proteins, CAT, GPX.*

Introduction

Forest vegetation physiology and productivity are directly affected by temperature, nutrient availability, water regime and indirectly by intraspecific interaction (Levitt, 1980; Pitzschke *et al.,* 2006). According to several authors, extreme temperature events are the cause of greater problems than those related to a slight change in average temperature, for example, freeze-thaw events in winter, early freeze-ups in fall, late freeze-ups in spring, heat waves; could cause damage to ecosystems. Damage will depend not only on the extreme temperature, but also on the duration of exposure, the characteristics of temperature fluctuations (increase or decrease, rate of change and differences between temperature maxima and minima) and the physiological state of the plant at the time these phenomena occur (Richer *et al*., 2001).

Various abiotic stresses (light, temperature etc.) lead to the overproduction of reactive oxygen species (ROS) in plants, which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA, resulting in so-called "oxidative" stress. In general, oxidative stress is the cellular result of non-oxidative environmental stress. Plants will mobilize antioxidant defense systems: one enzymatic and the other non-enzymatic involving different compounds that have an affinity for free radicals and can be considered antioxidants (Wormuth *et al*., 2007).

In response to heat stress, many species simulate

significant morphological and metabolic changes (Alscher *et al*., 2002). Among these species is *Casuarina equisetifolia*. This study aims to know both the thermal range of this species and its eco-physiological response to heat stress, while trying to understand its adaptive capacity through biochemical markers (antioxidant enzymes).

Materials and Methods

Plant material

The plant material used is one-year-old seedlings of *Casuarina equisetifolia*, supplied by the nursery in El Khroub (Constantine). The plants are raised in natural conditions, placed in plastic bags containing soil from this nursery.

Heat treatments applied

Casuarina equisetifolia seedlings are transferred at high temperatures between 36 °C and 44 °C for 3 hours. For each temperature level, three seedlings are treated.

Biochemical analysis

For each heat treatment, 3 repetitions are carried out.

Determination of soluble protein

Soluble proteins are assayed according to Bradford's (1976) method, based on the principle that in acidic media proteins form complexes with certain organic dyes, most often azodyes with sulphonic acid groups which bind to the proton groups of the side chains of the basic amino acids (Lysine-Arginine-Histidine) and to the α -NH2 free of the polypeptide chain for a given primary structure, the dye used is Coomassie blue G250, by binding to the protein it is converted from the bramble form to the blue form. This complex has an absorption maximum at 595 nm. The staining is very sensitive, can be carried out very quickly and remains stable for 30 minutes. The protein concentration is determined from the regression equation of the calibration curve. The protein concentration is measured in mg /MF = 1/19.556 x Absorbance

Determination of guaiacol peroxidase (GPX)

Guaiacol peroxidase (GPX) activity is determined by spectrophotometry at 470 nm using the technique of Mac Adam (1992). For a final volume of 3mL, the reaction mixture contains: 100 µL enzyme extract, 2700 µL phosphate buffer (100mM, pH 6.5), 100 µL guaiacol (18mM), 100 µL H_2O_2. Calibration of the instrument is performed in the absence of the enzyme extract. The reaction is triggered by the addition of hydrogen peroxide. The GPX activity is expressed in ìmoles oxidized/min/gde MF. It is calculated by the following formula:

Determination of the catalase activity (CAT)

The spectrophotometric determination of catalase activity (CAT) is carried out according to the method of Cakmak and Horst (1991). The decrease in absorbance is recorded for three minutes (Jenway 6300 spectrophotometer) for a wavelength of 240nm; the reaction mixture contains: 100 µL crude enzyme extract, 50 µL 0.3% hydrogen peroxide H_2O_2 and 2850 µL phosphate buffer (50mM, pH= 7.2). Calibration of the instrument is carried out in the absence of the enzyme extract. The reaction is triggered by the addition of hydrogen peroxide. The catalase activity is expressed in µmol/min/mg protein.

Statistical analysis

The results are subjected to an analysis of variance with one or two fixed classification factors, the means are compared using the Newman and Keuls method, based on the smallest significant value, in addition to the correlations established between the different variables, using Excel Stat (2018) software. Results are considered significant when $P \leq 0.05$.

Results

The different stresses applied have effects on protein accumulation. Young plants of *Casuarina equisetifolia* show variable accumulation (Table 1). It is interesting to note that this accumulation is significant at 38 °C. Analysis of variance using two classification criteria shows that there are very significant differences ($p < 0.0001$) between organs and between treatments applied. The effect of high temperatures on protein accumulation in the different organs of the seedlings of this plant shows that protein accumulation is greater in the leaves compared to the stems and roots at 38 °C.

Peroxidases are enzymes synthesized by plants and belong to a heterogeneous family. The assay for peroxidase activity was done in the leaves of young plants subjected to high temperatures. The evolu-

Table 1. Protein content in the different organs of *C. equisetifolia*

	Control	36 °C	38 °C	40 °C	42 °C	44 °C
Leaves	9.36[b]	10.70[a]	11.88[a]	10.84[a]	9.07[b]	9.04[b]
Stems	6.80[c]	6.43[c]	7.68[b]	8.40[a]	6.40[c]	5.20[d]
Roots	5.30[b]	7.20[ab]	8.20[a]	5.70	6.90[ab]	6.30[b]

Table 2. GBX and CAT contents in leaves of *C. equisetifolia*

	Control	36 °C	38 °C	40 °C	42 °C	44 °C
Peroxydases	117.41[c]	183.43[b]	186.01[b]	202.20[a]	211.24[a]	191.30[b]
GBX	12.46[e]	17.80[c]	15.70[d]	19.20[c]	23.30[a]	21.30[b]
Catalases	51.75[f]	95.05[d]	292.70[a]	187.90[b]	131.62[c]	69.12[e]
CAT	5.54[e]	9.32[c]	24.64[a]	18.02[b]	10.83[c]	7.70[d]

tion of peroxidase activity in response to temperature is shown in Table 2. In *C. equisetifolia*, heat stress caused an intensification of peroxidase activity in the leaves. Rates of increase ranged from 56.22% at 36 °C treatment to 79.96% at 42 °C treatment. Analysis of variance for a single classification criterion shows a very significant difference ($P<0.0001$). Newman and Keuls' test shows three homogeneous groupings; group (A) is represented by the 42 °C and 40 °C treatments, which reflects the highest averages. Group (C) is represented by the control with the lowest mean.

The specific activity of the peroxidase (GPX) will not take into account the amount of enzymes present in the medium but rather the intensity of this activity under stress. Under normal conditions (absence of stress), peroxidase activity is practically low. In *C. equisetifolia*, the variation in enzyme intensity compared to the control is respectively (83%, 69.5%, 54.09%, 44.22% and 25.68%), these rates correspond to averages of the treatments (42 °C, 44°C, 40 °C, 36 °C and 38 °C).

Catalase is an enzyme that converts hydrogen peroxide (H_2O_2) into water and oxygen. In *C. equisetifolia*, the rates of variation reached a maximum of 464.59% at 38 °C. Specific activity increased for the majority of treatments compared to the control. This increase was very significant at 38°C, where it reached a maximum of 345%.

Discussion

Plants use oxygen through aerobic metabolism to provide the energy necessary for their growth and development. The reduction of this oxygen is done by cytochromes in the respiratory chain and electron transfer chains in the photosynthetic apparatus, which are capable of producing large quantities of reactive forms of oxygen ROS (Reactive Oxygen Species) by their reactions, these ROS are deleterious to all cellular constituents (DNA, proteins, lipids), and considered as phytotoxic molecules (Parent *et al.*, 2008; Apel and Hirt, 2004). Certain symptoms observed in situations of biotic and abiotic stress in plants, for example; leaf bleaching, chlorosis and necrosis, are consequences of a strong accumulation of oxygenated free radicals and an alteration of cellular homeostasis.

Our results showed that under thermal stress, there is a response of antioxidant enzymes initiated by peroxidases and catalases. This would indicate the installation of a state of oxidative stress induced by high temperatures in the *C. equisetifolia* seedlings tested at leaf level. If we compare the magnitude of oxidative stress induced by heat treatments, we notice that it is greater at 42 °C for peroxidases. Several authors report that the activity of this enzyme is stimulated under conditions of biotic or abiotic stress. This increase in activity reflects the important role played by this enzyme in the detoxification of ROS (Liu *et al*., 2011) and the tolerance of these species to different types of environmental stresses (Arasimowicz and Floryszak-Wieczorek, 2007; Doudech *et al.*, 2008; Ashraf *et al*., 2012).

It is known that peroxidases are involved in many physiological functions. Firstly, peroxidases are involved in the catabolism of auxin and in the control of cell growth (Baccouche, 2001). On the other hand, it has been shown that ionic peroxidases at parietal localization are involved in the lignification and regidification processes of cell walls (Baaziz, 2006). Independently of their role, peroxi-

dases commonly ensure the reduction of hydrogen peroxide to water at the expense of other molecules with high reducing power.

Thus, increasing the activity of peroxidases could be considered as a general response to all stresses. This response is very often associated with the role of peroxidases in the lignification of the walls which leads to the decrease of their plasticity and the limitation of cell elongation (Baccouche, 2001).

Catalase (CAT) is among the enzymes that play an important role in the transformation and elimination of hydrogen peroxide (H_2O_2) into (H_2O). According to our results, the activity of this enzyme was affected by high temperature. The temperature 38°C increased this activity. The catalase and peroxidase systems act in combination to counteract H_2O_2 (Foyer and Noctor, 2005). Catalase detoxifies most of the H_2O_2 produced by photorespiration, while peroxidases neutralize H_2O_2 molecules not destroyed by catalase (Blokhina *et al.,* 2003). The descorbateglutathione ring in the cytosol and chloroplasts detoxifies H_2O_2 from the peroxisome if the catalase is insufficient to cope with its production. GPX and CAT activities increase in response to different types of biotic and abiotic stresses (Khan and Ashraf 2008; Zbadi *et al.,* 2018).

Conclusion

Monitoring the physiological condition of plants under heat stress is essential for improved crop productivity. This work focuses on characterizing the response of the *Casuarina equisetifolia* species to high temperatures. The determination of catalase and peroxidase enzymatic activities is carried out on seedlings of this plant subjected to heat stress of 36 °C, 38 °C, 40 °C, 42 °C, 44 °C. These two enzymatic activities responded to the stressful conditions of high temperatures by increasing their levels. The specific activity of catalase is four times higher at 38 °C than the control, while the enzymatic activity of peroxidase is highly concentrated at 42 °C. At this level of research, it cannot be deduced that this is a consequence of specific defense and tolerance, but nevertheless, it remains an active means.

References

Alscher, R.G., Erturk, N. and Heath, L.S. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *Journal of Experimental Botany.* 53 (372) : 1331-1341.

Apel, K. and Hirt, H. 2004. Reactive oxygen species: Metabolism, Oxidative Stress, and Signal Transduction. *Annual Review of Plant Biology.* 55 (1) : 373-399.

Arasimowicz, M. and Floryszak-Wieczorek, J. 2007. Nitric oxide as a bioactive signalling molecule in plant stress responses. *Plant Science.* 172 (5) : 876-887.

Ashraf, M. and Shahbaza, M. 2012. Growth stag-based modulation in antioxidant defense system and proline accumulation in two hexaploïdes wheat (*Triticum aestivum* L.) cultivars differing in salinity tolerance. *Flora-Morphology, Distribution, Functional Ecology of Plants.* 207 : 388–397.

Khan, A. and Ashraf, M. 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environmental and Experimental Botany*. 63(1-3) : 224-231.

Baccouche, S. 2001. Etude de la contribution des enzymes antioxydantes dans le processus de détoxication de métaux lourds (Ni, Cd) chez le maïs et le tournesol. Thèse Doct. Physiologie végétale. Uni. Oran, Algérie, 55p.

Baaziz, M., Qacif, N., Bendiab, K. and Aouad, A. 2006. Les peroxydases des plantes. Aspect théorique et Applications pratiques. Deuxième Congrès International de Biochimie. *Agadir, Maroc.* 9-12.

Blokhina, O., Virolainen, E. and Fagerstedt, K. V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany.* 91 (2) : 179-194.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry.* 72 (1-2) : 248-254.

Cakmakn, I. and Horst, W.J. 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum.* 83 (3) : 463-468.

Doudech, N., Mhamdi, M., Bettaieb, T. and Denden, M. 2008. Tolérance à la salinité d'une graminée à gazon: *Paspalum notatum* Flüggé. *Tropicultura.* 26 (3) : 182-185.

Foyer, C. H. and Noctor, G. 2005. Oxidant and antioxidant signalling in plants: a reevaluation of the concept of oxidative stress in a physiological context. *Plant, Cell & Environment.* 28 (8) : 1056-1071.

Levitt, J. 1980. Responses of plants to environmental stresses. *Water Radiation, Salt and Others Stresses.* Academic Press, New York, 2 : 365- 406.

Liu C., Liu, Y., Guo, K., Fan, D., Li, G., Zheng, Y. and Yang R. 2011. Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. *Environmental and Experimental Botany.* 71 (2): 174-183.

Mac Adam, J.W., Neleson, C.J. and Sharpe, R.E. 1992.

Peroxydase activity in the leaf elongation zone of tall fescue. *Plant Physiology.* 99 : 872-888.

Parent, C., Capelli, N. and Dat, J. 2008. Formes réactives de l'oxygène, stress et mort cellulaire chez les plantes. *Comptes Rendus Biologies.* 331 (4) : 255-261.

Pitzschke, A., Forzani, C. and Hirt, H. 2006. Reactive oxygen species signaling in plants. *Antioxidants and Redox Signaling.* 8 (9-10) : 1757-1764.

Richer, C., Rioux, J.A., Lamy, M.P. and Daigle, G. 2001. Winter damage on young plants of *Accer saccharinum* L. under different Canadian climatic conditions. *Canadien Journal of Plant Science.* 81 : 145-153.

Wormuth, D., Heiber, I., Shaikali, J., Kandlbinder, A., Baier, M. and Dietz, K.J. 2007. Redox regulation and antioxidative defence in *Arabidopsis* leaves viewed from a systems biology perspective. *Journal of Biotechnology.* 129 (2) : 229-248.

Zbadi, R., Mohti, H. and Moussaoui, F. 2018. Stress oxydatif: évaluation du pouvoir antioxydant de quelques plantes médicinales. *Médecine Thérapeutique.* 24 (2) : 134-141.