Genetics and Plant Physiology – 2017, Volume 7(3–4), pp. 184–194

Available online at http://www.ifrg-bg.com

OPEN ACCESS

Research Article

EFFECT OF LOW TEMPERATURES ON ANTIOXIDANT DEFENSE IN TOLERANT AND NON TOLERANT TO FREEZING STRESS POPULATIONS OF *ARABIS ALPINA*

***Kolaksazov M.*[2]*, K. Ivanova*[1]*, V. Stanev*[1]*, Y. Markovska*[1]*, E. D. Ananiev*[1*]

[1]*Department of Plant Physiology, Faculty of Biology, University of Sofia, Sofia 1674, Bulgaria*

[2]*Department of Physics and Biophysics, Faculty of Pharmacy, MU - Pleven, Pleven 5800, Bulgaria*

Received: *24 August 2017* Accepted: *18 October 2017*

Summary: The paper deals with the effect of low temperatures on nonenzymatic antioxidant defense system in the leaves of *Arabis alpina* plants grown from seeds collected from the French Alps (frost tolerant population, T) and from the mountain of Vercors (non-tolerant to frost, NT). Plants were grown at +22°C under control conditions and after that exposed to chilling stress (+4°C) for 4 days followed by freezing stress (-7°C) for 12h. Recovery was studied after return of the plants to +4°C (4 days) and finally to +22°C. Results showed that chilling stress enhanced MDA, H_2O_2 and flavonoid accumulation only in the leaves of T plants, while freezing stress decreased it. After recovery, the level of MDA and H_2O_2 remained higher only in T plants. The level of reducing sugars did not change only in the plants of T population after both cold treatments. Moreover, the frost stress at -7°C resulted even in stimulation of sugars production (by 30%) as compared to the control. In contrast to T, reducing sugars in NT plants declined after the chilling stress. Based on the changes in nonenzymatic antioxidant stress compounds, it can be concluded that T plants exhibited a much better response to low temperatures than NT.

Keywords: Chilling and frost stress; MDA; H_2O_2; phenols; flavonoids; sugars.

Abbreviations: H_2O_2 – hydrogen peroxide; MDA – malondialdehyde; TCA – trichloroacetic acid.

Citation: Kolaksazov M., K. Ivanova, V. Stanev, Y. Markovska, E. D. Ananiev, 2017. Effect of low temperatures on antioxidant defense in tolerant and non tolerant to freezing stress polulations of *Arabis alpina*. *Genetics and Plant Physiology*, 7(3–4): 184–194.

INTRODUCTION

Chilling stress is a key factor limiting the survival of plants and their geographical distribution. Susceptibility to chilling injury prevents the cultivation of many crops in regions where temperatures can drop much below the optimal growth temperatures (Ercoli et al., 2004). During photosynthesis, low temperatures above zero induce "chilling stress" and exacerbate the imbalance between light absorption and light use by inhibiting Calvin-Benson cycle activity (Logan et al., 2006), whilst temperatures below zero provoke "freezing stress".

*Corresponding author: evgueni_ananiev@yahoo.com

Enhanced photosynthetic electron flux and over-reduction of the electron transport chain results in reactive oxygen species (ROS) formation in the leaves of plants subjected to chilling stress (Hu et al., 2008). Oxidative damage, associated with chilling stress, enhances the production of ROS, slows down metabolism, causes the peroxidation of membrane lipids and results in a significant increase in malondialdehyde (MDA) content (O`Kane et al., 1996). Responses to chilling-induced oxidative stress include alterations in the activities of enzymes of the antioxidant defense system such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate-glutathione cycle (AGC), as well as in the content of low molecular weight antioxidants such as total ascorbate (Asc), glutathione (GSH), carotenoids, tocopherols and phenolics (Arora et al., 2002).

Some cold resistant plants are able to maintain photosynthesis at very low temperatures. They can reduce the amount of ROS and membrane lipid peroxidation by switching on non-photochemical quenching (Jahns and Holzwarth, 2012), state transitions by antenna phosphorylation and dissociation (Nellaepalli et al., 2012), photorespiration, scavenging of ROS by antioxidants with low molecular weight or antioxidant enzymes and cyclic electron transport (Apel and Hirt, 2004; Krieger – Liszkay, 2004).

Arabis alpina L. (Brassicaceae) is a perennial plant distributed in Europe at different altitudes. This plant was chosen as a new model plant in cold stress research and our preliminary investigations showed that tolerant populations from the French Alps and Rila Mountain in Bulgaria possessed strong tolerance to chilling (+4°C) as well as to freezing stress (-7°C) in terms of photosynthetic performance compared with the non-tolerant populations (Kolaksazov et al., 2014).

This paper aimed at establishing the effects of chilling and freezing stress on some parameters of nonenzymatic antioxidant defense system in plants belonging to two different populations of *Arabis alpina* regarding their ability to survive after freezing stress.

MATERIALS AND METHODS

Sampling site and plant materials

Seeds from fertile parts of the plants (siliques) from the French Alps were collected in 2009, 2010 and 2012 by the team of prof. Michel Herzog from the Laboratory of Alpine Ecology, University in Grenoble. The collection was carried out by packing of plants, randomly chosen in the beginning of the flowering period with the aim to prevent the cross-pollination. Ten areals (4 in Vercors and 6 in the Chain of Ecrins, French Alps) were investigated on the basis of a previous study (Poncet et al., 2010), and two of them were chosen by the French scientists. *Arabis alpina* populations from the French Alps (frost tolerant population - T) were collected from the Col du Galibier, near Lautaret alpine station at 2600 m altitude, while the non-tolerant to frost population (NT) was chosen in the mountain of Vercors at 1842 m altitude. Seeds from T and NT plants were led to germinate for 7 days, then seedlings were transferred to a phytostatic growth chamber (phytotron) at a temperature 22°C ± 2°C, relative

humidity 60-70%, photon flux density of 220 μmol $m^{-2}s^{-1}$ and photoperiod 12/12h (day/night). Both tolerant and non-tolerant *A. alpina* plants were subjected consequently to chilling stress (+4°C, 4 days) and freezing stress (-7°C during the 12-h night period of the photoperiod) in a climatic chamber (TK 120, Nüve, Ankara, Turkey). After the frost stress, plants were transferred subsequently to the previous temperature regimes for recovery at the same light conditions. At the end of each temperature treatment middle-aged leaves were frozen and stored in a refrigerator at -28°C until analyses.

Metabolite assays

For determination of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content, 0.15 g FW of leaves were homogenized in a mortar at 4°C with 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged for 20 min at 15 000 g (4°C).

For MDA estimation, 0.5 ml of the supernatant were mixed with 0.5 ml phosphate buffer (pH 7.4) and after the addition of 1 ml 0.5% (w/v) thiobarbituric acid dissolved in 20% trichloroacetic acid, the samples were boiled for 30 min (Dhindsa et al., 1981). After rapid cooling of the samples in ice bath, absorption was measured at 532 and 600 nm using the extinction coefficient 155 mM^{-1} cm^{-1} (Heath and Packer, 1968).

For H_2O_2 assay, 0.5 ml of the supernatant were mixed with 0.5 ml phosphate buffer (pH 7.4) and after the addition of 1 ml of 1 M KI, samples were incubated in the dark for 60 min and absorption was measured at 390 nm. The content was calculated using a standard curve of H_2O_2 in the range of 1 – 100 nmol ml^{-1} of H_2O_2 (Jessup et al., 1994).

Total phenolic content was determined using the Folin-Ciocalteu method, based on the procedure of Pffefer et al. (1999). Fresh plant material (0.025 g) was ground in a mortar with 5 ml 80% hot ethanol. After 30 min the samples were filtered through G4. An aliquot of 0.25 ml of the filtrate was added to 1.5 ml of distilled water and 0.25 ml Folin-Ciocalteu reagent. The mixture was allowed to stay at room temperature for 2 min and then 0.5 ml of 20% sodium carbonate was added to the mixture. The resulting blue complex was measured at 730 nm after 1 h with intermittent shaking. The content of phenolics is expressed as chlorogenic acid equivalents in mg g^{-1} of sample. All values are expressed as mg of chlorogenic acid equivalents per 1 mg FW.

Total flavonoid content was determined according to a modified colorimetric method described previously (Zhishen et al., 1999). Fresh plant material (0.025 g) was ground in a mortar with 10 ml 80% methanol. The homogenous mixture was allowed to stay for 20 min at room temperature, followed by filtration through G4. An aliquot of 0.4 ml of the filtrate was mixed with 0.6 ml distilled water, 5% $NaNO_2$ solution (0.06 ml) and the mixture was allowed to stay for 5 min at room temperature. After 6 min 10% $AlCl_3$ solution (0.06 ml) was added to the mixture. Immediately, 1 N NaOH (0.4 ml) and 0.45 ml distilled water were added to the mixture and allowed to stay for another 30 min. Absorbance of the mixture was measured at 510 nm and (+) catechin was used as a standard compound for the quantification of total flavonoid content. All values are expressed as mg of (+) catechin equivalents per 1 mg FW.

The total content of reducing sugars

was determined in hot 80% ethanol extracts according to the phenol/sulphuric acid method of Dubois et al. (1956). An aliquot of 0.25 ml of the filtrate was added and mixed with 0.75 ml distilled water, 0.5 ml 5% phenol and 2.5 ml 98% H_2SO_4. The resulting orange complex was then measured at 485 nm after 1 h with intermittent shaking. The content of soluble sugars is expressed as glucose equivalents in mg g^{-1} of sample. All values are expressed as mg of glucose equivalents per 1 mg FW.

Statistical data analysis

All data are mean values of at least three to four independent experiments. The mean values ± SE and the exact number of experiments are given in the Figures. The significance of differences between control and each treatment was analyzed by Fisher's LSD test ($P \leq 0.05$) after performing ANOVA multifactor analysis

RESULTS

MDA and H_2O_2 content

Our preliminary results showed that frost stress (-7°C) did not cause visible symptoms of damage in plants, but the injury developed after the recovery period. Initially, chilling stress caused injury at the cellular level and a marked increase in the levels of MDA and H_2O_2 (more than two-fold) was observed. The strong increase in both parameters was detected only in T plants as compared with NT. In NT plants, the level of MDA was twice lower than in T plants, whereas the H_2O_2 content remained approximately the same (Fig. 1). Interestingly, during the period

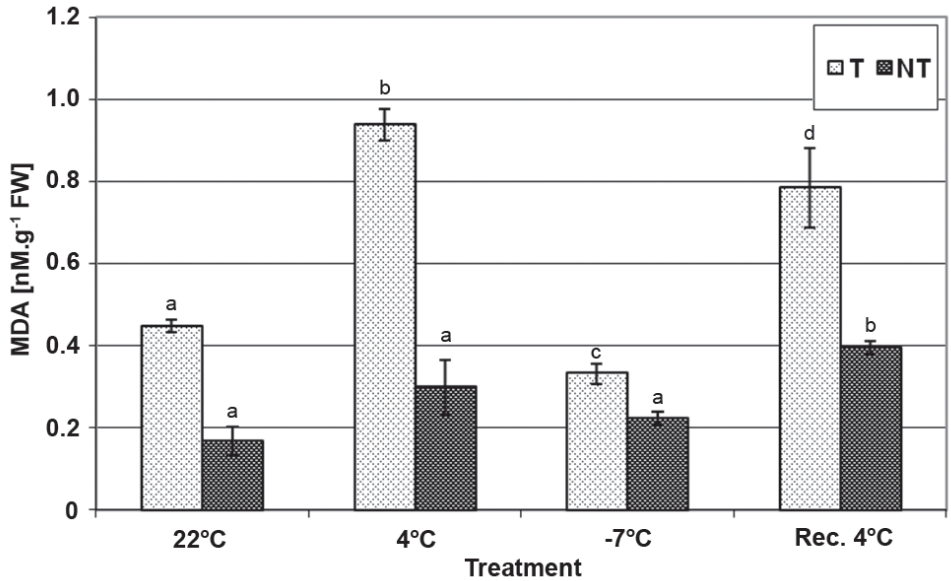

Figure 1. Malondialdehyde (MDA) content in the leaves of *A. alpina* plants grown from seeds collected in the French Alps (frost tolerant population, T) and in the mountain of Vercors (non-tolerant to frost population, NT). Plants were subjected to chilling (+4°C, 4 days) and freezing (-7°C, 12h) stress and subsequently recovered at +4°C for 4 days. Mean values ± SE (n = 4). Different letters indicate statistically significant difference at P≤0.05.

of recovery at +4°C following the frost stress, both MDA and H_2O_2 contents were restored to the values after the chilling treatment only in the leaves of T plants. In NT plants these parameters also remained high, similar to the values after the chilling stress (Fig. 1 and Fig. 2).

Phenolic and flavonoid content

Our results showed that phenolics as well as flavonoids prevailed in the leaves of NT plants (3-fold higher) as compared with T plants. Chilling as well as freezing stress strongly inhibited the phenolics content in both T and NT populations, the decrease in NT plants being much stronger (about 4 times) in comparison with T. In the period of recovery, pnenolics content was restored almost to the control levels at the beginning of the experiment (Fig. 3). Unfortunately, we failed to register the proper recovery changes in T population for the reasons of lost of samples (no data - ND).

In agreement with the enhanced MDA and H_2O_2 content after cold stress, the content of flavonoids increased drastically during chilling (more than 10 times) and freezing stress only in the leaves of T plants (Figs. 1, 2 and 5). Even after the frost stress flavonoids were 5 times more abundant in these plants compared to the control. At the same time flavonoids strongly decreased in the leaves of NT plants. After recovery at +4°C, the flavonoid accumulation in leaves of NT plants was elevated and almost reached the control values at the beginning of the experiment. Summing up, the dynamics of the changes in flavonoid content in control, cold stressed and post-stressed NT plants was similar to that of phenolics in these plants (Fig. 3 and 4).

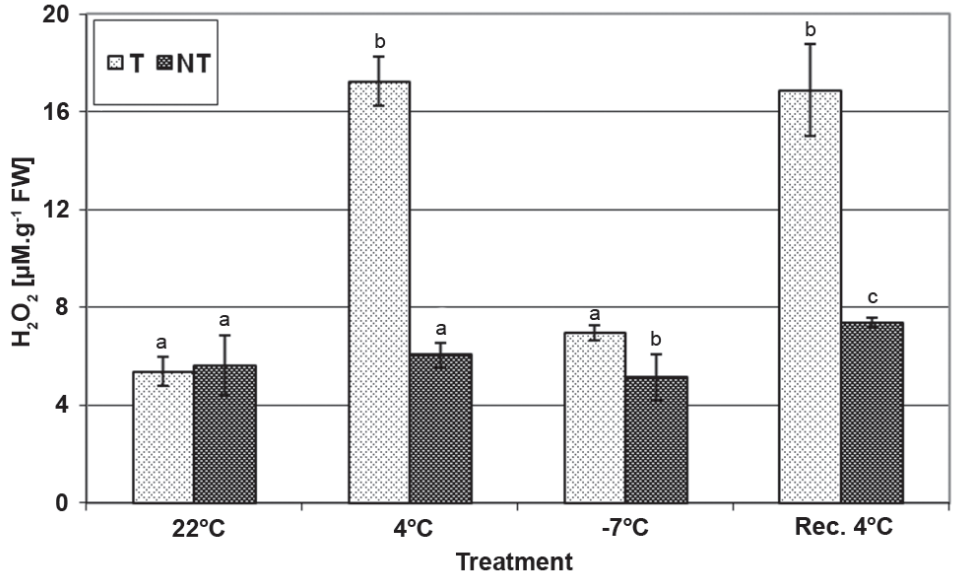

Figure 2. Hydrogen peroxide (H_2O_2) content in the leaves of *A. alpina* plants grown from seeds collected in the French Alps (frost tolerant population, T) and in the mountain of Vercors (non-tolerant to frost population, NT). Plants were subjected to chilling (+4°C, 4 days) and freezing (-7°C, 12h in the dark) stress and subsequently recovered at +4°C for 4 days. Mean values ± SE (n = 4). Different letters indicate statistically significant difference at $P \leq 0.05$.

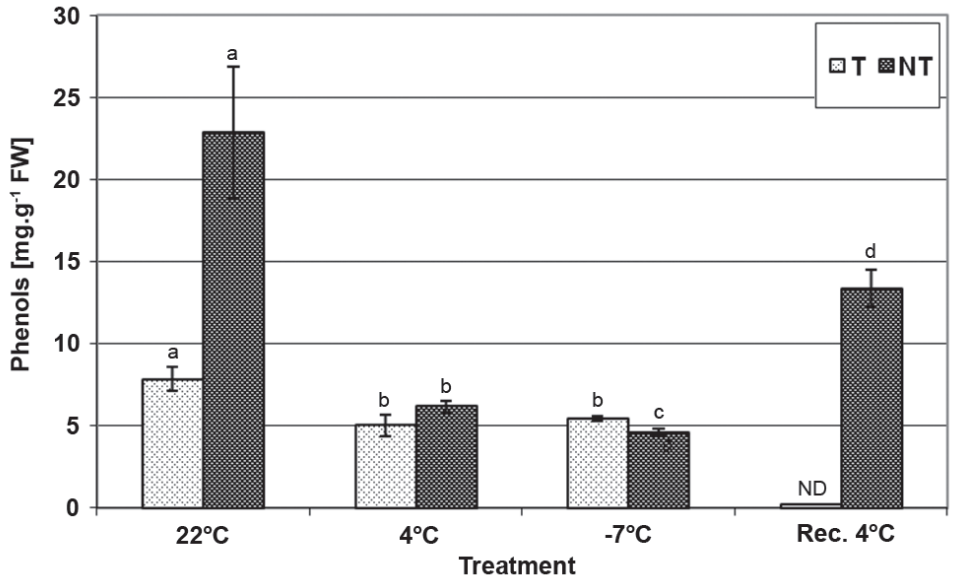

Figure 3. Total phenolic content in the leaves of *A. alpina* plants grown from seeds collected in the French Alps (frost tolerant population, T) and in the mountain of Vercors (non-tolerant to frost population, NT). Plants were subjected to chilling (+4°C, 4 days) and freezing (-7°C, 12h in the dark) stress and subsequently recovered at +4°C for 4 days. ND – data is not available. Mean values ± SE (n = 3). Different letters indicate statistically significant difference at P≤0.05.

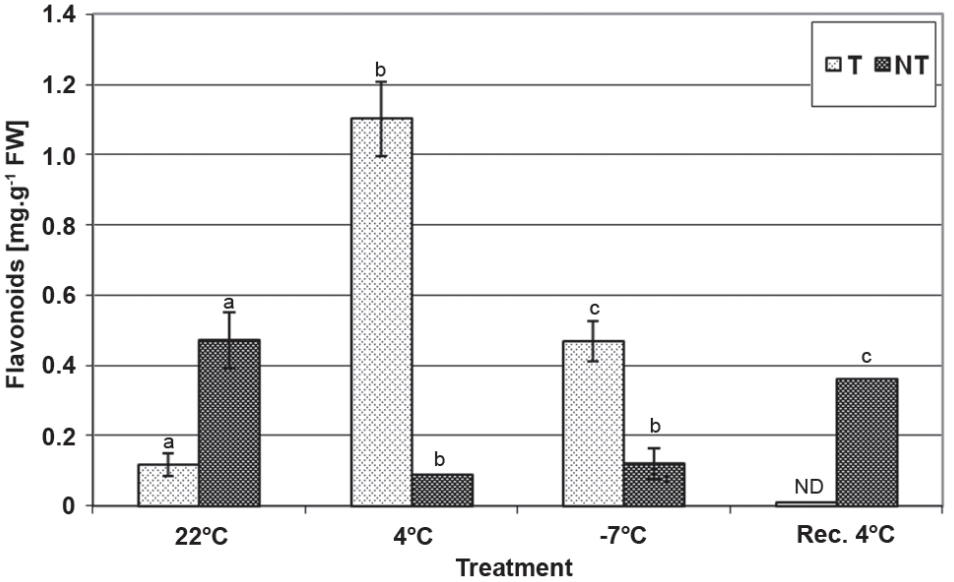

Figure 4. Total flavonoid content in the leaves of *A. alpina* plants grown from seeds collected in the French Alps (frost tolerant population, T) and in the mountain of Vercors (non-tolerant to frost population, NT). Plants were subjected to chilling (+4°C, 4 days) and freezing (-7°C, 12h in the dark) stress and subsequently recovered at +4°C for 4 days. ND – data is not available. Mean values ± SE (n = 3). Different letters indicate statistically significant difference at P≤0.05.

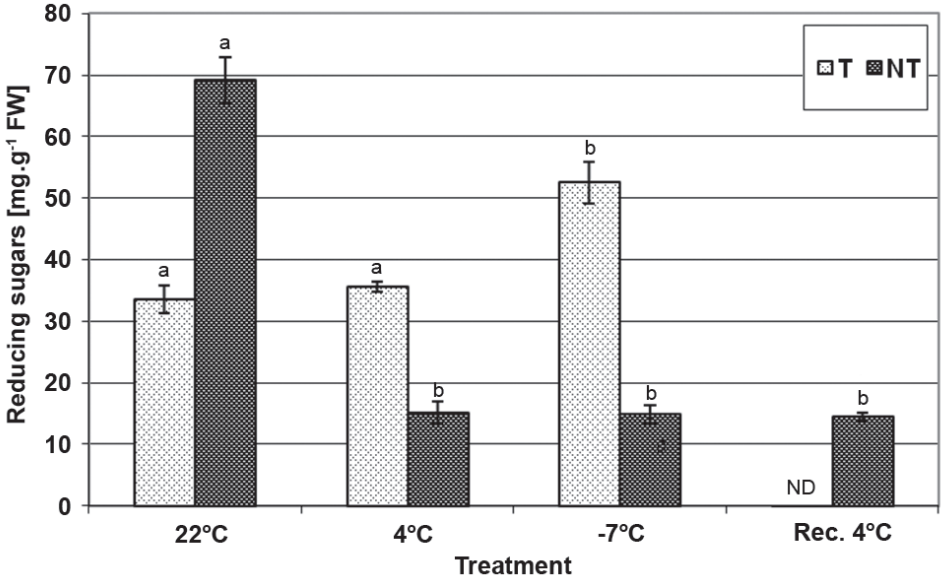

Figure 5. Content of reducing sugars in the leaves of *A. alpina* plants grown from seeds collected in the French Alps (frost tolerant population, T) and in the mountain of Vercors (non-tolerant to frost population, NT). Plants were subjected to chilling (+4°C, 4 days) and freezing (-7°C, 12h in the dark) stress and subsequently recovered at +4°C for 4 days. ND – data is not available. Mean values ± SE (n = 3). Different letters indicate statistically significant difference at P≤0.05.

Sugars content

The content of reducing sugars prevailed in the leaves of control NT plants (two times higher) as compared with T (Fig. 5). Our results showed that the level of reducing sugars did not change only in the plants of T population after both cold treatments at +4°C and the freezing stress at -7°C. Moreover, the frost stress at -7°C resulted even in stimulation of sugars accumulation with registered values about 30% higher as compared with the control at 22°C (Fig. 5). In contrast to T plants, reducing sugars in NT plants declined just after the chilling stress and did not change further.

DISCUSSION

It is well known that under low temperature treatment oxidative stress increases due to the damage caused by ROS to the membrane system of plant cell, and mostly to the sensitive thylakoid membranes in chloroplasts (Tardy and Havaux, 1997; Theocharis et al., 2012). Normally, after cold stress the content of the stress markers MDA and H_2O_2 increases concomitant with the increase in the activities of the antioxidant enzymes SOD, CAT, POX and ascorbate peroxidase (APX) in order to cope with the oxidative stress (Apel and Hirth, 2004; Krieger-Liszkay, 2004).

Our experimental set-up was carried out in such a way, so that the duration of chilling and freezing treatments was not prolonged and both T and NT plants did not show visible symptoms of damage. Nevertheless, or results showed an inhibition of net photosynthetic rate after chilling and freezing treatments by 57%

and 77%, respectively. As CO_2 uptake in *A. alpina* was not ceased even after this strong decrease, we can conclude that the process of photosynthesis in *A. alpina* plants was not completely disturbed, but only temporarily inhibited after the frost treatment (Kolaksazov et al., 2014). Further on, the fast recovery of net photosynthetic rate to almost the same values as in the control plants confirmed this presumption (Kolaksazov et al., 2014).

The contents of sugar as well as proline (data not shown) prevailed in the leaves of control NT plants in comparison with T plants. The changes of leaf sugar content in T plants after chilling and freezing treatments were different (Fig. 5). After both types of stress, the content of sugars in NT plants decreased similarly to the content of phenols and flavonoids, which could be related to the lowered metabolic activity of the plants. We registered a gradual increase in the content of reducing sugars in the leaves of NT plants after the period of recovery at +4°C (Fig. 5), similarly to the observed enhancement of net photosynthetic rate at the same conditions (Kolaksazov et al., 2014). It is well known, that cryoprotective carbohydrates such as sucrose, fructans, and sugar-alcohols can play an important role in achieving tolerance and resistance to cold-induced damage (del Viso et al., 2009). Generally, plants respond to environmental stress with increased content of soluble sugars together with decreased starch content as a consequence of enhanced sugar synthesis. Cold damage results in desiccation due to water removal from the protoplast as a consequence of the growing ice crystals in the apoplast (Loescher and Everard, 2004). Accumulation of compatible solutes, such as sucrose, cyclic or acyclic sugar-alcohols, proline and quaternary amines (like glycine-betaine), could be potentially involved in the regulation of osmotic adjustment and could also provide quickly metabolizable carbohydrates for energy production when carbon is diverted from growth to other functions (Hare et al., 1998).

One of the common physiological responses of higher plants when they are exposed to a number of environmental stresses was found to be the accumulation of proline (Verbruggen and Hermans, 2008). This amino acid is important osmolyte and may protect plant cells against oxidative damage by stabilizing key cellular detoxification mechanisms (Szekely et al., 2008). Its enhanced biosynthesis is suggested to stabilize redox potential and $NAD(P)^+$/NAD(P)H ratios during stress conditions (Hare and Cress, 1997). Our measurements revealed that proline content in leaves of NT plants was 2.8 times higher (1.884 $\mu m.g^{-1}$ FW), as compared with leaves of T plants (0.650 $\mu m.g^{-1}$ FW) (data not shown). In addition, the concentrations of proline and sugars were higher in leaves of control NT *A. alpina* plants as compared with the leaves of T plants grown in a climatic chamber at +22°C.

It is known that different inorganic (K^+, Na^+, Cl^-, and SO_4^{2-}), as well as organic (reducing hexoses) molecules are critical for maintaining osmotic homeostasis in aqueous systems such as the living cells. These compounds are different from compatible solutes, which do not interfere with the *in vitro* enzyme activities and can protect proteins from deleterious effects of salts in shrinking cells (Loescher and Everard, 2004). Accumulation of compatible solutes could help the plant to resist extreme conditions of low temperatures, high salt levels, and water

deficit.

Cold treatment may initially cause injury, but during the recovery period, leaves coordinate and enhance the capacity of the antioxidant system to diminish the potential of ROS. As evidenced by the high MDA and H_2O_2 content after the chilling stress in *A. alpina* leaves, the increased levels of ROS resulted in lipid peroxidation, which could be one of the important damaging factors for plant cell membranes. It is known that jasmonic acid (JA) and its methyl ester (MeJA) as intracellular stress mediators are produced from the oxylipins with lipid hydroperoxides as precursors (Chrispeels et al., 1999). Jasmonates accumulated after stress can inhibit the process of photosynthesis, thus inducing degradation of chlorophyll and activating the synthesis of tocopherol, anthocyans and glutathione, the latter being one of the extremely strong antioxidants (Bell et al., 1995; Szarka et al., 2012). So, the destructive process of lipid peroxidation can be regulated on the feedback principle by means of the jasmonates. However, in case of insufficient antioxidant activity this process can amplify, due to the accumulated ROS that additionally oxidize membrane lipids, leading to damages in the plant cell and, consequently to cell death.

Besides the well-studied non-enzymatic antioxidants like Asc, GSH, tocopherols, our results confirmed the potential role of flavonoids as effective antioxidants. Phenolics, especially flavonoids and phenylpropanoids, are oxidized by peroxidase, and can act in H_2O_2 scavenging as part of the phenolic/Asc/POX system. Their antioxidant action resides mainly in their chemical structure. There is some evidence of the induction of phenolic metabolism in plants in response to multiple stresses (including low temperature) (Michalak, 2006). Our results showed that the content of flavonoids was increased after chilling stress mainly in T plants similarly to the content of MDA and H_2O_2, most probably as a response to the increased production of ROS. In lucerne leaves, after chilling stress in the dark, a significant increase in the level of MDA and H_2O_2 was observed (Bafeel and Ibrahim, 2008). During the recovery period, activities of CAT, APX and glutathione reductase (GR) increased significantly (data not shown), which could possibly restrict the recycling of ROS associated with chilling stress (Bafeel and Ibrahim, 2008). Radyuk et al. (2009) postulate that APX and CAT play an important role in plant cell defense against low temperatures, whereas GR and SOD activities are especially important during the poststress period in barley seedlings.

In conclusion, the results of this study showed that chilling stress enhanced MDA, H_2O_2 and flavonoid production in the leaves of T plants while freezing stress decreased it. After the period of recovery at +4°C, the level of MDA and H_2O_2 remained high only in T plants. T plants exhibited a much better response to low temperatures than NT as evidenced by the changes in MDA, H_2O_2 and flavonoid contents.

ACKNOWLEDGEMENTS

Arabis alpina seeds used in this study were supplied by the team of Prof. Michel Herzog, University of Grenoble, France which is greatly appreciated.

This investigation was financially supported by the bilateral Franco-Bulgarian Project RILA/01/2013 in the frames of NFI, Bulgaria.

REFERENCES

Apel K, H Hirt, 2004. Reactive oxygen species: metabolism, oxidative stress and signal transduction. Ann. Rev. Plant Biol., 55: 373–399.

Arora A, RK Sairam, GC Srivastava, 2002. Oxidative stress and antioxidative system in plants. Curr. Sci., 82: 1227–1238.

Bafeel SO, MM Ibrahim, 2008. Antioxidants and accumulation of Alpha-tocopherol induce chilling tolerance in *Medicago sativa*. Int. Agric. Biol., 10: 593–598.

Bell E, RA Creelman, JE Mullet, 1995. A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in Arabidopsis. Proc. Natl. Acad. Sci. USA, 92: 8675–8679.

Chrispeels MJ, L Holuigue, R Latorre, S Luan, A Orellana , H Pena-Cortes, NV Raikhel, PC Roland, A Trewavas, 1999. Signal transduction networks and the biology of plant cells. Biol. Res., 32: 35–60.

Dhindsa R, T Plumb-Dhindsa, T Thorpe, 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot., 32: 93–101.

Dubois M, KA Gilles, JK Hamilton, PA Rebers, F Smith, 1956. Colorimetric method for the determination of sugars and related substances. Anal. Chem., 28: 350–356.

Ercoli L, M Marioti, A Masoni, I Arduini, 2004. Growth responses of sorghum plants to chilling temperature and duration of exposure. Eur. J. Agron., 21: 93–103.

Hare P, W Cress, 1997. Metabolic implications of stress induced proline accumulation in plants. Plant Growth Regul., 21: 79–102.

Hare PD, WA Cress, JV Staden, 1998. Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ, 21: 535–553.

Heath RL, L Packer, 1968. Photoperoxidation in isolated choroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys., 125: 189–190.

Hu WH, XS Song, K Shi, XJ Xia, YH Zhou, JQ Yu, 2008. Changes in electron transport, superoxide dismutase and ascorbate peroxidase isoenzymes in chloroplasts and mitochondria of cucumber leaves as influenced by chilling. Photosynthetica, 46: 581–588.

Jahns P, AR Holzwarth, 2012. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. Biochim. Biophys. Acta, 1817: 182–193.

Jessup W, RT Dean, JM Gebicki, 1994. Iodometric determination of hydroperoxides in lipids and proteins. Methods Enzymol., 233: 289–303.

Kolaksazov M, F Laporte, V Goltsev, M Herzog, ED Ananiev, 2014. Effect of frost stress on chlorophyll a fluorescence and modulated 820 nm reflection in *Arabis alpina* population from Rila Mountain. Gen. Plant Physiol., SI (Part 1), 4(1-2): 44–56.

Krieger-Liszkay A, 2004. Singlet oxygen in photosynthesis. J. Exp. Bot., 56(441): 337–346.

Logan BA, D Kornyeyev, J Hardison, AS Holaday, 2006. The role of antioxidant

enzymes in photoprotection. Photosynth Res, 88: 119–132.

Loescher W, J Everard, 2004. Regulation of sugar alcohol biosynthesis. In: Photosynthesis: Physiology and Metabolism (RC Leegood, TD Sharkey, S von Caemmerer, eds.), pp 275–299, New York, Kluwer Academic Publishers.

Michalak A, 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Pol. J. Environ. Study, 15: 523–530.

Nellaepalli S, S Kodru, R Subramanyam, 2012. Effect of cold temperature on regulation of state transitions in *Arabidopsis thaliana*. J. Photochem. and Photobiol., 112: 23–30.

O` Kane D., V Gill, P Boyd, R Burdon, 1996. Chilling, oxidative stress and antioxidant responses in *Arabidopsis thaliana* callus. Planta, 198: 371–377.

Pfeffer H, F Dannel, V Römheld, 1998. Are there connection between phenol metabolism, ascorbate metabolism and membrane integrity in leaves of boron-deficient sunflower plants? Physiol. Plant., 104: 479–485.

Radyuk MS, IN Domanskaya, RA Shcherbakov, NV Shalygo, 2009. Effect of low above-zero temperature on the content of low-molecular antioxidants and activities of antioxidant enzymes in green barley leaves. Russ. J. Plant Physiol., 56: 175–180.

Szarka A, B Tomasskovics, G Banhegyi, 2012. The ascorbate-glutathione-α-tocopherol triad in abiotic stress response. Int. J. Mol. Sci., 13: 4458–4483

Szekely G, et al., 2008. Dublicated P5CS genes of *Arabidopsis* play distinct roles in stress regulation and developmental control of proline biosynthesis. Plant J., 53: 11–28.

Tardy F, M Havaux, 1997. Thylakoid membrane fluidity and thermostability during the operation of the xanthophyll cycle in higher plant chloroplasts. Biochim. Biophys. Acta, 1330: 179–193.

Theocharis A, C Clement, EA Barka, 2012. Physiological and molecular changes in plants grown at low temperatures. Planta, 235: 1091–1105.

Verbruggen R, C Hermans, 2008. Proline accumulation in plants: a review. Amino Acids, 35: 753–759.

del Viso F, AF Puebla, CM Fusari et al., 2009. Molecular characterization of a putative sucrose:fructan 6-fructosyltransferase (6-SFT) of the cold-resistant Patagonian grass *Bromus pictus* associated with fructan accumulation under low temperatures. Plant Cell Physiol, 50: 489–503.

Zhishen J, T Mengcheng, W Jianming, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555–559.