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EFFECT OF FROST STRESS ON CHLOROPHYLL *a* FLUORESCENCE AND MODULATED 820 nm REFLECTION IN *ARABIS ALPINA* POPULATION FROM RILA MOUNTAIN

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Summary: Cold stress is one of the most important abiotic factors which in the context of the global climatic changes strongly affects plant productivity. The perrenial plant *Arabis alpina* (Brassicaceae) was used as a model system to study the effect of cold stress on photosynthetic performance as measured by prompt chlorophyll *a* fluorescence (PSII activity), as well as by the modulated 820 nm reflection (PSI activity). Intact wild plants collected from Rila Mountain, and plants grown in laboratory conditions from seeds were used in this study. Plants were grown at 22°C under control conditions and after that they were exposed to chilling stress (+4°C) for 4 days followed by freezing stress (-7°C) for 12h. Recovery from the frost stress was studied after return of the plants to +4°C (4 days) and further to 22°C (4 days). It was found that both the intact wild plants and the plants from seeds were tolerant to the frost stress. Net photosyntetic rate was gradually inhibited during chilling and freezing stresses with CO_2 uptake still active after the frost stress. Photosynthesis was restored during the period of recovery to values close to the control. After frost treatment both intact plants and plants grown in control conditions showed a very slight inhibition of PSII activity as revealed by the quantum yield of the primary photochemical reaction (φ_{Po}), whereas intersystem electron transport (φ_{Eo}), reduction of the end electron acceptors (φ_{Ro}) and the performance index of photosynthesis (PI_{abs}) were inhibited to a greater extent. All chlorophyll *a* fluorescence parameters of PSII were restored during the period of recovery. In contrast to PSII activity, the activity of PSI as probed by the modulated 820 nm reflection was much strongly inhibited after the frost stress especially in its re-reduction phase. Our results indicated that the higher tolerance of *A. alpina* to frost stress was accompanied with higher resistance of the overall photosynthetic performance, including both PSII and PSI.

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Abbreviations: PF – prompt chlorophyll *a* fluorescence; MR – modulated 820 nm light reflection; M-PEA – multifunctional plant efficiency analyser; PSII – photosystem II; PSI – photosystem I; PQ – plastoquinone; PC – plastocyanine; OEC – oxygen evolving complex; ROS – reactive oxygen species.

INTRODUCTION

Cold stress is one of the most important abiotic factors, which limit plant productivity in moderate climatic zones when temperatures fall below zero (Theocharis et al., 2012). Even in the context of the global climatic changes, cold stress can affect agriculture worldwide when temperatures fluctuate unexpectedly (Schwartz et al., 2006). Low temperatures above zero degrees induce "chilling stress" whilst temperatures below zero provoke "freezing stress". The main targets of the cold stress inside the plant cell are the biological membranes, among which most sensitive are thylakoid membranes in chloroplasts (Tardy and Havaux, 1997; Theocharis et al., 2012). Thus, photosynthesis is inhibited by blocking the electron transport between the two systems, which leads to formation of a large amount of ROS. ROS additionally damages the membranes by oxidizing them (Apel and Hirth, 2004; Krieger-Liszkay, 2004).

Some cold resistant plants are able to sustain photosynthesis even at very low temperatures. They can reduce the amount of ROS, as well as membrane lipid oxidation by switching on or off some special features of photosynthesis, like non-photochemical quenching due to PsbS and activation of xanthophyll cycle (Jahns and Holzwarth, 2012), state transitions by antenna phosphorylation and dissociation (Nellaepalli et al., 2012), photorespiration, scavenging or deactivation of ROS (by antioxidants or enzymatically) and cyclic electron transport (Apel and Hirt, 2004; Krieger-Liszkay, 2004).

The main objective of this work was to study how chilling and freezing stresses affect photosynthetic performance in the frost tolerant plant *Arabis alpina* (Brassicaceae) which is a new model plant in cold stress research. It is a perennial plant, widely distributed across Europe at different altitude depending on the latitude. Intact wild plants as well as plants grown from seeds in a climatic chamber, both collected in Rila Mountain (South-West Bulgaria) were investigated. Our results indicated that both plant samples showed strong tolerance to chilling (+4°C) as well as to freezing stress (-7°C), with PSII being more resistant to frost than PSI.

MATERIALS AND METHODS

Wild *Arabis alpina* plants and seeds from the same species were collected in the area of "The Seven Rila Lakes"(between Bliznaka and Trilistnika lakes) in Rila Mountain. Plants were transfered to a phytostatic growth chamber (phytotron) at a temperature of 22°C ±2°C, relative

humidity 60-70%, photon flux density 220 µmol $m^{-2}s^{-1}$ PAR and photoperiod 12h/12h (day/night). After acclimation for 2-3 weeks they were used for cold treatment.

Seeds were planted in soil with 60% peat content and placed in a refrigerator at 8-10°C during one week for stratification. After germination for 7 days, plants were transferred to a phytostatic chamber at the same conditions as the wild plants. Seven-week-old plants were used in the experiments.

Both wild *A. alpina* plants and climatic grown 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 treatment, plants were transferred subsequently to the previous temperature regimes (4°C and 22°C, each for 4 days) at the same light conditions. At the end of each temperature treatment, measurements of the photosynthetic parameters were carried out at room temperature.

Net photosynthetic rate was measured as CO_2 uptake using LCpro+ (ADC BioScientific Ltd., UK) at 500 µmol $m^{-2}s^{-1}$ PAR saturating light. Prompt chlorophyll fluorescence (PF) and modulated light reflection (MR) at 820 nm were measured with M-PEA (Hansatech Instruments Ltd., King's Lynn, Norfolk, UK) after 30-min dark adaptation. Two independent and reproducible experiments were performed, each with three to five different plants and three leaf replicates. For the PF measurements a red light beam (625 nm) was applied for 10 sec at a light intensity of 4000 µmol photons $m^{-2}s^{-1}$. MR measurements were performed applying actinic light at 625 nm and modulated optically filtered pulse at 820 nm. Middle-aged leaves were used in all experiments and the data were statistically analyzed using the program SigmaStat and ANOVA one-way analysis. Statistically significant differences between treatments at $P = 0.05$ are represented by different letters. Standard deviations in each treatment were also shown.

RESULTS AND DISCUSSION

Our results showed that plants did not demonstrate significant visible damages even after frost stress (-7°C) (Fig. 1, C) when only some of the leaves were affected so that damages became clearly

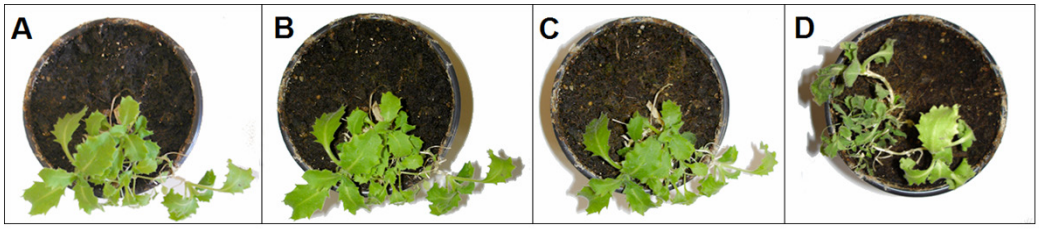

Figure 1. Effects of chilling and freezing stresses on *A. alpina* plants grown in a climatic chamber under different temperature conditions. *A. alpina* plants were grown from seeds collected in Rila Mountain (see Materials and Methods): A) control plants grown at 22°C; B) plants after chilling stress (4°C for 4 days); C) plants after freezing stress (-7°C during the 12-h night period of the photoperiod); D) plants after recovery (4°C for 4 days).

visible after the period of recovery (Fig. 1, D).

The effect of different temperature treatments on net photosynthetic rate in *A. alpina* plants grown in a climatic chamber is presented in Fig. 2. Wild intact plants showed a very similar photosynthetic rate (data not shown). Results indicated a substantial decrease in the net photosynthetic rate even after the first low temperature treatment (chilling stress), when the CO_2 uptake was inhibited by up to 57%. After subsequent exposure to freezing temperature (-7°C), net photosynthesis was further reduced to 77%. It should be noted that CO_2 uptake in *A. aplina* was not ceased even after this strong decrease. Our results clearly showed that plants were able to restore their photosynthetic rate after the period of recovery at 4°C and 22°C, respectively (Fig. 2). In addition, the changes of transpiration rate during the experiment were in agreement with CO_2 uptake (data not shown). The fast recovery of net photosynthetic rate to almost the same values as in the control indicated that the process of photosyntesis in frost stressed *A. alpina* plants was not impaired, but only temporarily inhibited after the frost treatment. Similar recovery of CO_2 uptake was also reported for other types of abiotic stresses, including drought (Human et al., 1990) and salt stress (Delfine et al., 1999), in both cases after rewatering. Photosynthetic inhibition after salt stress was accompanied with a severe decrease in Rubisco activity and stomatal conductance (Delfine et al., 1999). Similarly, CO_2 uptake was significantly decreased in the mountain plant *Geum montanum* (more than the quantum yield of PSII) after a combination of cold stress and strong

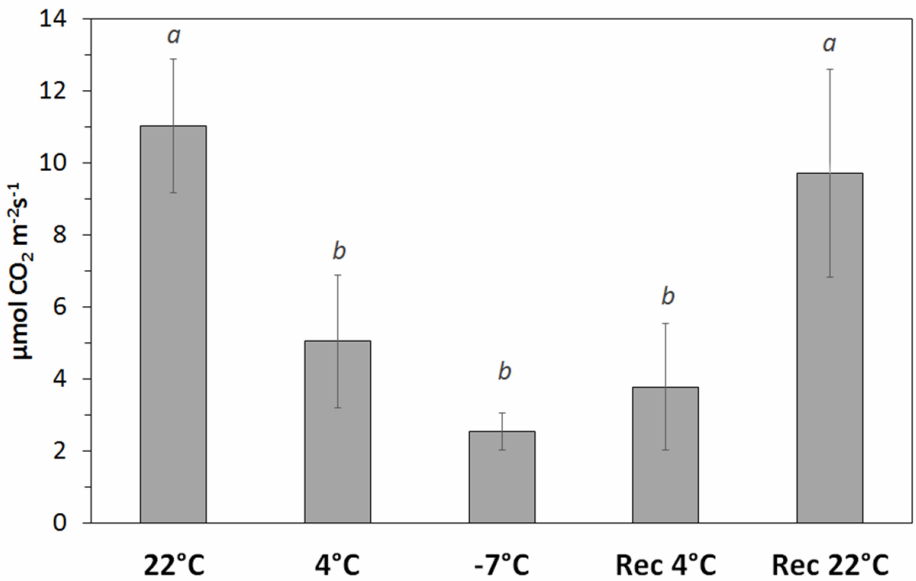

Figure 2. Net photosynthetic rate of *A. alpina* plants grown from seeds collected in Rila Mountain. Plants were subjected to chilling (4°C) and freezing (-7°C) stresses and subsequently recovered at 4°C and 22°C (see Materials and Methods). Different letters indicate statistically significant difference at P = 0.05.

illumination (Manuel et al., 1999). These authors suggested a close relationship between CO_2 fixation and the process of photorespiration as a possible reason for cold tolerance in *G. montanum*.

After that we studied the effect of chilling and freezing stresses on PSII in both plant samples (Fig. 3) as measured by chlorophyll *a* fluorescence (prompt fluorescence, PF) using M-PEA. It is well known, that PF is based mainly on the emission from reaction centers of PSII after illumination with actinic red light (about 625 nm) (Strasser et al., 2004; Stirbert et al., 2013). The rising in PF during an illumination period of about 1 sec is described by the so-called transient "JIP" curve comprised of several characteristic points (O, J, I and P, according to Strasser et al., 1995; 2004), each point representing a quasi-stationary state in the fluorescence emission. At the beginning of the fluorescence measurement (point O), most of P680 are opened and the fluorescence is minimal (F_o), whereas at point P the fluorescence is maximal (F_p) as most of P680 are closed. Points J and I reflect the accumulation of reduced forms of Q_A^- and PQH_2, respectively. The JIP transient curve at control temperature was typical for non-stressed plants, where all JIP characteristic points were clearly visible (Fig. 3). After chilling stress at 4°C, maximal fluorescence (F_M) slightly declined. It is well known that F_M decreases in response to different stress conditions (Strauss et al., 2007; Strasser et al., 2010; Venkatesh et al., 2012) and this decrease is mainly due to denaturation of chlorophyll associated proteins including proteins of LHCII as well as D1 core protein of PSII (Yamane et al., 1997). In *A. alpina* chilling stress resulted in the earlier appearance of O-J phase, accompanied by a slight increase

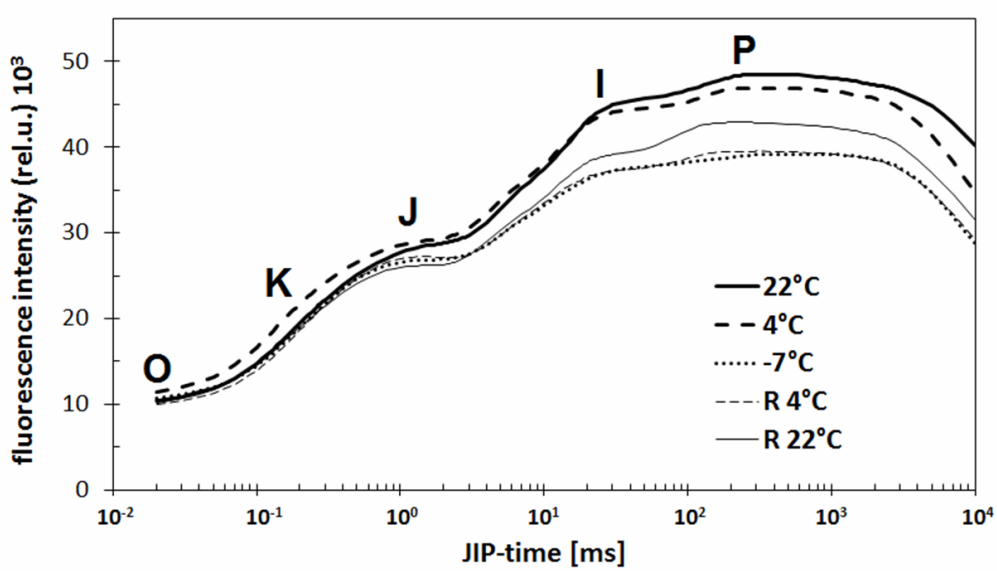

Figure 3. Induction JIP curves of the prompt chlorophyll fluorescence in leaves from *A. alpina* plants, grown in a climatic chamber from seeds collected in Rila Mountain. Plants were exposed to chilling (4°C) and freezing (-7°C) stresses and subsequently recovered at 4°C and 22°C (see Materials and Methods); R – recovery.

in F_o and the J peak (Fig. 3). Such an initial increase of F_o was reported after drought stress in *Haberlea rhodopensis* (Strasser et al., 2010) and cold stress in *A. thaliana* (Mishra et al., 2011; Nellaepalli et al., 2012) and it is mostly due to accumulation of reduced plastoquinone (PQ) (Tóth et al., 2007). Furthermore, the large amount of reduced PQ after chilling stress in *Arabidopsis* was accompanied by partial blocking of the electron flow from PSII to PSI, thus leading to phosphorylation of LHCII (Nellaepalli et al., 2012). LHCII phosphorylation was found after accumulation of reduced PQ in response to abiotic stresses, but without a dependency of light. Phosphorylation of LHCII causes dissociation of LHCII from PSII and presumably protects plants from excessive light, though a migration of LHCII to PSI was not observed after chilling stress in *Arabidopsis* (Nellaepalli et al., 2012). The same author also showed that reduced PQ accumulated after moderate heat stress in *Arabidopsis* facilitated LHCII phosphorylation (Nellaepalli et al., 2011).

The O-J phase can be further characterized by an early rise in the transient fluorescence curve, which is detected at about 300 μs (Strasser et al., 2004). This rise is called K-band and is widely accepted as a sign of dissociation of the oxygen-evolving complex (OEC) (Guissé et al., 1995). Our results also showed that after frost stress at -7°C, a significant decrease in F_M and in the I-P phase occurred (Fig. 3). Moreover, a shift in the P point from 300 ms towards later stages of the transient curve was also observed. Normally, the last part of the JIP-curve represents a reduction of the end electron acceptors from the PSI side (Schreiber et al., 1989; Schansker et al., 2003). Therefore, the observed decrease of I-P phase, which was accompanied with a decrease of F_P could be due to a strong inhibition of the PSI acceptor side as it was earlier observed in pea (Schansker et al., 2005). During the period of recovery at 4°C, the JIP-curve didn't change compared to frost stress, except for the I-P phase and the P peak, which slightly increased (Fig. 3). After recovery at 22°C, the OJIP curve further increased, the F_M being still below the level of the control (Fig. 3).

Modulated 820 nm light reflection (MR) describes the redox state of the reaction centers of PSI (P700) and plastocyanin molecules (PC). When they are in a reduced state (P700, PC), they can reflect more of the absorbed light at 820 nm. After their oxidation ($P700^+$, PC^+), they reflect less of this light (Schansker et al., 2003; Strasser et al., 2010). These changes in PSI light absorption can be detected either as an increase or a decrease in the reflected light, respectively. In addition, M-PEA can measure simultaneously the PF and the MR-curves, so that different phases of the PF-curve correspond to the appropriate phases in the MR-curve, thus giving a detailed picture of the functioning of both photosystems (Strasser et al., 2010). The MR-curves of both wild plants and plants grown in control conditions are presented in Fig. 4. Similar to net photosynthetic activity, the modulated 820 light reflection was investigated mainly in climatic chamber-grown plants (data not shown for the recovery at 22°C). At control temperature, the typical MR-curve was represented by two phases, the first phase of oxidation

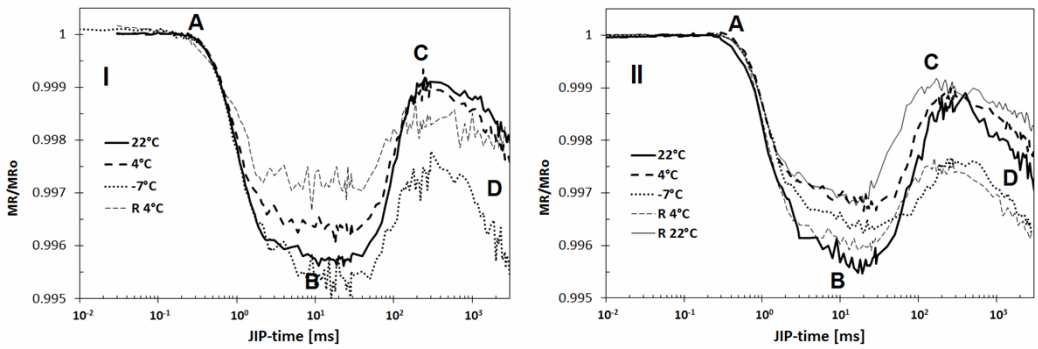

Figure 4. Induction curves of the modulated 820 nm light reflection, measured in *A. alpina* plants collected in Rila Mountain: **I)** leaves of intact wild plants; **II)** leaves of plants grown from seeds. Plants were exposed to chilling (4°C) and freezing (-7°C) stresses and recovered at 4°C and 22°C (see Materials and Methods); R – recovery.

of P700 and PC (downward part of the curve from stage A to B) and the second corresponding to the re-reduction of P700 and PC (upward part of the curve from stage B to C) (Fig. 4, I and II). The oxidation phase of PSI was detected in the range of the first millisecond (100 ms on the logarithmic abscissa axis) from the start of illumination as a decrease in the MR/MRo ratio of 820 nm light reflection (stage A to B). The lowest values of this reflection ratio (stage B) represent the point, at which the oxidation and the reduction of $P700^+$ and PC^+ reach an equilibrium. After that, a re-reduction of PSI reaction centers by electrons from PSII occurred in the next 30 to 100 ms (B - C), reaching a peak around 300 ms from the start, corresponding to the F_P point from the JIP-curve (see Fig. 3). In its final stage, the curve decreases (stage D), which corresponds to ATP and NADPH accumulation and their utilisation in the Calvin-Benson cycle (Strasser et al., 2010). After treatment at 4°C, the lowest part of the curve (B) was higher in both plant samples, thus showing an inhibition of PSI oxidation caused by the chilling stress (Fig. 4, I and II). This inhibition was more pronounced in plants grown in laboratory conditions (see Fig. 4, II). The higher the position of stage B is, the higher is the amount of reduced P700 and PC and vice versa. The increase in stage B of the MR-curve can be related to the accumulation of reduced PQH_2 as presented by the O-J phase of the PF-curve (see Fig. 3) (Oukarroum et al., 2013; Goltsev et al., 2014). The effect of cold stress and the process of recovery of PSI can be studied also by calculating the rate of re-reduction of PSI by the slope of the curve from stage B to C. Fig. 4, I and II clearly showed that the slope in this phase decreased after chilling stress in both plant samples and this slope was further decreased, when applying the frost stress. Using specific inhibitors of the electron transport from PQ to PC it was shown, that the re-reduction phase in the MR-curve from stage B to C disappeared and it coincided with the disappearance of the I-P phase in the transient curve of PF in pea plants (Schansker et al., 2005). Therefore, the decrease of the re-reduction phase of the MR-curve from stage B to C after both types of cold stress was due to an

inhibition of the electron transport from PQ to PC, thus preventing the reduction of PSI from PSII (Schansker et al., 2005). A similar behaviour of the MR-curve in its re-reduction phase was reported in *Haberlea* in response to drought stress (Strasser et al., 2010) and in pea after heat stress (Oukarroum et al., 2013). As compared with the chilling stress, the frost stress in both plant samples resulted in a stronger oxidation of PSI (stages A-B), accompanied by a two-fold decrease in the re-reduction phase of the MR-curves (stage B-C) (Fig. 4, I and II). The changes in the PSI activity may reflect a higher level of oxidized PSI after red light illumination, which was exerted in the presence of inhibited reduction of PSI due to lower electron transport from PSII.

During the period of recovery at 4°C, the MR-curve of the plants from seeds displayed more similar kinetics to the MR-curve at -7°C, which was in contrast to the intact wild plants, where the re-reduction phase of the curve was restored to the level of control (Fig. 4, I and II stage B-C). This observation confirmed that the intact wild plants recovered faster compared to the plants grown in a climatic chamber. After recovery at 22°C, the re-reduction phase of the MR-curve from laboratory grown plants also restored to its normal levels. However, the lowest part of the curve still remained higher than the control of both plant samples (Fig. 4, I and II stage B).

PSII activity was further investigated by calculating the parameters of prompt chlorophyll fluorescence (PF). Both Fig. 5 and Fig. 6 represent different parameters of PF, including the quantum yield of the primary photochemistry reaction (φ_{Po}), the quantum yield of the electron transport between PSII and PSI (φ_{Eo}) and the quantum yield for reduction of the end electron acceptors of PSI (φ_{Ro}). In addition, energy flux dissipation per reaction center DIo/RC and the photosynthetic performance index PI_{abs} were also investigated (Fig. 5 and Fig. 6). It is well known, that each one of these parameters represents the ratio between the energy of absorbed photons by PSII and the energy used in photochemical reactions (Strasser et al., 2004; Goltsev et al., 2014).

Our results showed that cold stress at 4°C did not affect these parameters in both plant samples except for φ_{Ro} and PI_{abs}, the latter being inhibited by 20% and 29% in the wild and the climatic chamber-grown plants, respectively (Figs. 5 and 6). In contrast to chilling stress, frost stress affected strongly all quantum yield parameters, except for φ_{Po}, which was less sensitive. In both plant samples, φ_{Po} which represents the quantum yield of the primary photochemical reacton in PSII, was inhibited by less than 20%. In this respect, our results are in agreement with the finding that PSII was highly resistant to photoinhibition after cold stress in three other alpine plants (*Ranunculus glacialis, Homogyne alpina* and *Soldanela alpina*) (Streb et al., 1997). Briefly, φ_{Po} can be considered as one of the most stable photosynthetic parameters and for that reason it is widely used in monitoring the effect of different environmental stresses on photosynthetic behavior in plants (Strasser et al., 2004). φ_{Po} is related to the deactivation or full degradation of PSII reaction centers and for that reason the relatively small inhibition of this parameter after frost

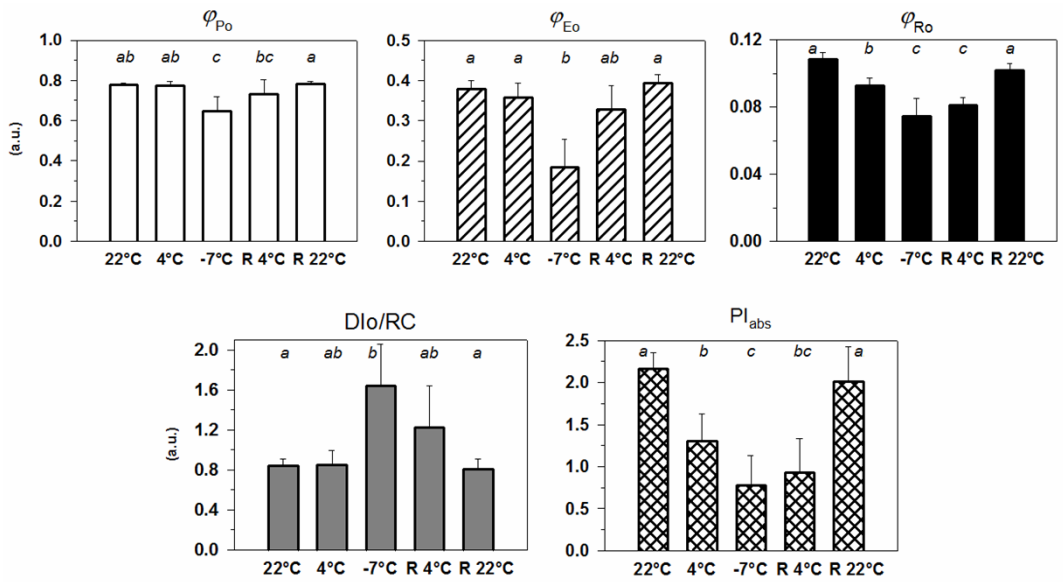

Figure 5. Parameters of the prompt chlorophyll fluorescence – PF (quantum yield, energy dissipation and performance index), measured in intact *A. alpina* plants collected in Rila Mountain. Plants were exposed to chilling (4˚C) and freezing (-7°C) stresses and recovered at 4°C and 22°C (see Material and Methods); R – recovery. Different letters indicate statistically significant difference at P = 0.05.

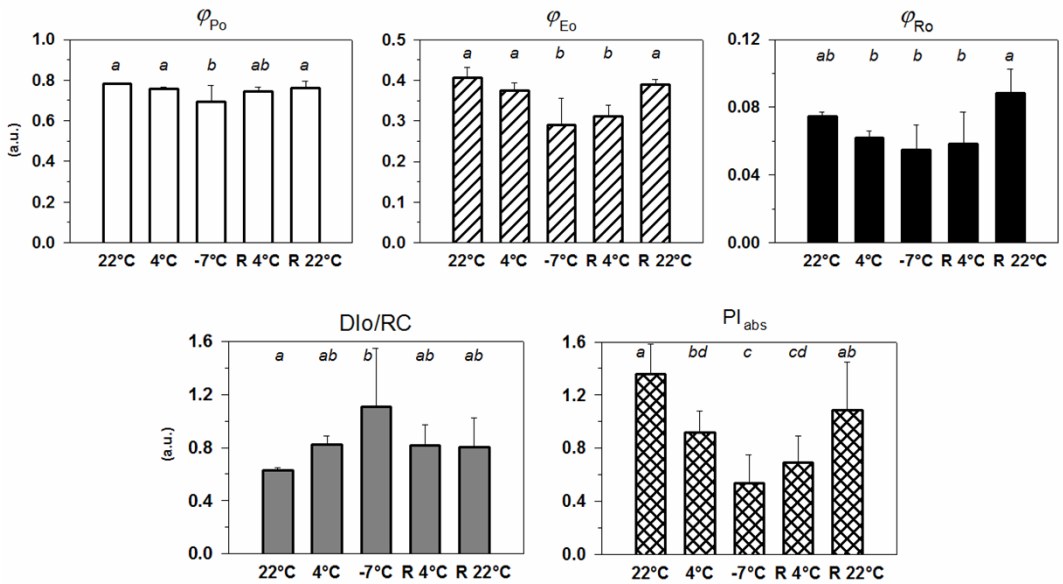

Figure 6. Parameters of the prompt chlorophyll fluorescence (quantum yield, energy dissipation and performance index), measured in *A. alpina* plants grown from seeds, collected in Rila Mountain. Plants were exposed to chilling (4°C) and freezing (-7°C) stresses and recovered at 4°C and 22°C (see Materials and Methods); R – recovery. Different letters indicate statistically significant difference at P = 0.05.

stress indicated that PSII was not strongly affected by the frost. Compared to φ_{Po}, much stronger was the effect of frost stress on φ_{Eo} and φ_{Ro}, where inhibition of about 30% and 40- 50% was observed in climatic chamber- grown plants and wild intact plants, respectively. Thus, our results confirm the accumulated body of evidence that the parameters φ_{Eo} and φ_{Ro}, which reflect processes in the photosynthetic electron chain closer to PSI are more sensitive to frost stress compared to φ_{Po} (Venkatesh et al., 2012). Concerning the energy dissipation flux per reaction center of PSII (DIo/RC), which represents the amount of energy, which the plant is not able to utilize in phytochemical reactions, our results presented in Figs. 5 and 6 showed that this parameter gradually increased along with the decrease in temperature, DIo/RC being mostly stimulated during the frost stress (2-fold). The last measured parameter of PF was the performance index of photosynthesis on absorption basis PI_{abs}. PI_{abs} is considered as the most sensitive among all parameters concerning the photosynthetic activity of PSII and the efficiency of the electron transport reactions between PSII and PSI. In both *A. aplina* samples PI_{abs} strongly decreased after both chilling and freezing stresses, the frost stress being much more pronounced (60-65% inhibition). As it was previously shown (van Heerden et al., 2007), in *A. alpine,* PI_{abs} also closely correlated with the CO_2 uptake.

Furthermore, we studied the recovery of the photosynthetic parameters after plants were transferred to previous temperature regimes. As shown in Figs. 5 and 6 the wild plants from Rila Mountain recovered faster from the frost stress. After the recovery at 4°C, φ_{Eo} reached inhibition values of 8% and 25% of the control in wild and laboratory-grown plants, respectively, while φ_{Ro} did not significantly increase. The process of recovery continued to values close to the control after 22°C. The same trend of stepwise recovery at 4°C and 22°C was observed also for the other parameters studied, including the energy dissipation (DIo/RC) and PI_{abs}.

Several conclusions could be drawn from these results:

- Both chilling (4°C) and freezing (-7°C) stresses inhibited net photosynthetic rate in *A. alpina* plants from Rila Mountain, but photosynthesis continued even 12 h after frost treatment. The inhibition of CO_2 uptake was most probably a result of decreased stomatal conductance.
- The performance index (PI_{abs}), which represents the overall activity of PSII and the intersystem electron flow, was strongly inhibited after both chilling and freezing stresses and correlated with their effect on CO_2 uptake. Both PI_{abs} and CO_2 uptake recovered after ceasing the cold stress.
- The quantum yield of the primary photochemical reaction (φ_{Po}) of PSII displayed the lowest inhibition amongst all PF parameters even after frost treatment, thus indicating higher tolerance of PSII to freezing stress in *A. alpina* plants.
- The electron transport between PSII and PSI as revealed by the quantum yield φ_{Eo} and the modulated 820 nm reflection (MR) of PSI was strongly inhibited after freezing stress. The inhibited electron transport between PSII and PSI was restored to control

values in the period of recovery at 22°C.

- The activity of PSI as measured by its oxidation and re-reduction phase of the MR curves gradually decreased after chilling and freezing stresses. Frost mediated inhibition of PSI was characterized by a decrease in the rate of PSI re-reduction accompanied with an increased rate of oxidation, thus indicating that frost mediated suppression of PSI was due to the inhibited electron transport from PSII to PSI.
- We can assume that after chilling stress in *A. alpina* plants, enhanced amount of reduced PQ was accumulated, which led to lower rate of the electron transport to PSI, the latter resulting in decreased PSI activity. Obviously, this negative effect was further developed after freezing stress. All these changes in the intermediate electron transport system and especially in the function of PSI can be considered as a protection mechanism against accumulation of ROS in the photosynthetic apparatus during cold stress.

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REFERENCES

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

Delfine S, A Alvino, MC Villani, F Loreto, 1999. Restrictions to Carbon Dioxide Conductance and Photosynthesis in Spinach Leaves Recovering from Salt Stress. Plant Physiology, 119: 1101–1106.

Goltsev V, H Kalaji, M Kouzmanova, S Allakhverdiev, 2014. Variable and Delayed Chlorophyll a Fluorescence. Basics and Application in Plant Sciences. (Eds. VZ Pashchenko, TE Krendeleva) Moscow–Izshevsk: Institute of Computer Sciences, pp. 220.

Guissé B, A Srivastava, RJ Strasser, 1995. The polyphasic rise of the chlorophyll a fluorescence (O-K-J-I-P) in heat stressed leaves. Arch. Sci., 48: 147–160.

Human JJ, WJ Roux, LP de Bruyn, 1990. The effect of leaf water potential on the carbon-dioxide uptake rate during different growth stages of wheat (Triticum aestivum L.). South African Journal of Plant and Soil, 7(2): 136–141.

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

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

Manuel N, G Cornic, S Aubert, P Choler, R Bligny, U Heber, 1999. Protection against photoinhibition in the alpine plant Geum montanum. Oecologia, 119: 149–158.

Mishra A, KB Mishra, II Höermiller, AG Heyer, L Nedbal, 2011. Chlorophyll fluorescence emission as a reporter

on cold tolerance in Arabidopsis thaliana accessions. Plant Signaling & Behavior, 6(2): 301–310.

Nellaepalli S, NR Mekala, O Zsiros, P Mohanty, R Subramanyam, 2011. Moderate heat stress induces state transitions in *Arabidopsis thaliana*. Biochim. Biophys. Acta, 1807 (9): 1177-1184.

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.

Oukarroum A, V Goltsev, RJ Strasser, 2013. Temperature effects on pea plants probed by simultaneous measurements of the kinetics of prompt fluorescence, delayed fluorescence and modulate 820 nm reflection. PLoS ONE, 8(3): e59433.

Streb P, J Feierabend, R Bligny, 1997. Resistance to photoinhibition of photosystem II and catalase and antioxidative protection in high mountain plants. Plant, Cell and Environment, 20: 1030–1040.

Schansker G, A Srivastava, Govindjee, RJ Strasser, 2003. Characterization of the 820-nm transmission signal paralleling the chlorophyll a fluorescence rise (OJIP) in pea leaves. Functional Plant Biology, 30(7): 785–796.

Schansker G, SZ Toth, RJ Strasser, 2005. Methylviologen and dibromo-thymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl a fluorescence rise OJIP. Biochim. et Biophys. Acta, 1706: 250–261.

Schreiber U, C Neubauer, C Klughammer, 1989. Devices and Methods for Room-Temperature Fluorescence Analysis. Phil. Trans. R. Soc. Lond., 323(1216): 241–251.

Schwartz M, R Ahas, A Aasa, 2006. Onset of spring starting earlier across the Northern Hemisphere. Global Change Biol., 12: 343–351.

Stirbert A, GYu Riznichenko, AB Rubin, Govindjee, 2013. Modeling chlorophyll a fluorescence transient: relation to photosynthesis. Biochemistry (Moscow), 79: 291–323.

Strasser RJ, A Srivastava, Govindjee, 1995. Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. Photochem. Photobiol., 61: 32–42.

Strasser RJ, M Tsimilli-Michael, S Qiang, V Goltsev, 2010. Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant Haberlea Rhodopensis. Biochimica et Biophysica Acta, 1797(6-7): 1313–1326.

Strasser RJ, M Tsimilli-Michael, A Srivastava, 2004. Analysis of the chlorophyll a fluorescence transient. In: Papageorgiou G. C., Govindjee (Eds.), Chlorophyll a fluorescence: A signature of photosynthesis. Advances in photosynthesis and respiration. Kluwer Academic Publishers, the Netherlands, 19: 321–362.

Strauss JA, GHJ Krüger, RJ Strasser, PDR van Heerden, 2007. The role of low soil temperature in the inhibition of growth and PSII function during dark chilling in soybean genotypes of contrasting tolerance. Physiologia Plantarum, 131: 89–105.

Tardy F, M Havaux, 1997. Thylakoid membrane fluidity and thermostability

during the operation of the xanthophyll cycle in higher-plant chloroplasts. Biochim. et Biophys. Acta, 1330: 179–193.

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

Tóth SZ, G Schansker, RJ Strasser, 2007. A non-invasive assay of the plastoquinone pool redox state based on the OJIP-transient. Photosynth. Res., 93: 193–203.

van Heerden PDR, JW Swanepoel, GHJ Krüger, 2007. Modulation of photosynthesis by drought in two desert scrub species exhibiting C3-mode CO_2 assimilation. Environ. Exp. Bot., 61(2): 124–136.

Venkatesh J, CP Upadhyaya, JW Yu, A Hemavathi, DH Kim, RJ Strasser, SW Park, 2012. Chlorophyll a fluorescence transient analysis of transgenic potato overexpressing D-galactouronic acid reductase gene for salinity stress tolerance. Hort. Environ. Biotechnol., 53(4): 320–328.

Yamane Y, Y Kashino, H Koike, K Satoh, 1997. Increases in the fluorescence Fo level and reversible inhibition of Photosystem II reaction center by high-temperature treatments in higher plants. Photosynthesis Research, 52(1): 57–64.