Annals of R.S.C.B., Vol. XXI, Issue 3, 2017, pp. 43 - 51
Received 11 March 2018; accepted 30 May 2018.
doi: 10.ANN/RSCB-2018-0002:RSCB

Effects of sucrose on structure and functioning of photosynthetic apparatus of *Galanthus nivalis* l. leaves exposed to chilling stress

O. M. FEDIUK, N. O. BILYAVSKA*, O. K. ZOLOTAREVA
Department of Phytochemistry and Membranology, Institute of Botany, NAS of Ukraine, 2 Tereschenkivska Str., Kyiv 01030, Ukraine
**Corresponding autor*
Bilyavska Ninel Department of Phytochemistry and Membranology, Institute of Botany, NAS of Ukraine, 2 Tereschenkivska Str., Kyiv 01030, Ukraine
Phone: +38(095)1193304,
e-mail: nbel2@ukr.net

Key words: *Galanthus nivalis* L., leaf, ultrastructure, chloroplast, grana, thylakoid, chlorophyll a fluorescence

Summary

The effects of sucrose (Suc) on the ultrastructure of photosynthetic apparatus (PSA) and its functional characteristics were investigated in chilling-tolerant Snowdrop (Galanthus nivalis L.). On the first stage of the experiments, the plants were acclimated at +5 °C and then their detached leaves were subjected to low temperatures (–5 or –15 °C) in the presence of Suc (0.02 or 0.1 M). The electron microscopic analysis showed that Suc treatment at +5 °C induced granal thylakoid elongation and reduction of number of thylakoids. At negative temperatures, the number of thylakoids per granum and the height of grana increased after Suc treatment. The data obtained by method of slow induction chlorophyll fluorescence showed that sharp drop in functional parameters (Fv/Fm, qP, and ETR) with decrease in temperature from +5 to –15 °C was prevented in part by treating of leaves with 0.02 M Suc and, to a large extent, after exposure of leaves in 0.1 M Suc solution. Non-photochemical quenching of fluorescence increased with decrease in the temperature to –15 °C and stabilized with Suc-treatment. The effect of Suc on G. nivalis PSA ultrastructure and functioning suggests that their chloroplasts are capable of osmotic adjustment in response to cold stress

Introduction

Plants are constantly exposed to a variety of environmental stresses, including freezing or extremely low temperature that constitute a key factor influencing plant growth, development and productivity (Miura & Furumoto, 2013). Plant response to cold is a highly complex process, which involves a broad range of morphological, physiological and biochemical modifications (Kratsch & Wise, 2000; Baxter, 2014). Water-soluble carbohydrates, which have a variety of functions, play an important role in such adaptations of plants. As is known, carbohydrates are the main substrates for cellular respiration, for the synthesis of stress proteins and lipids as well as for the repair of these macromolecules after low-temperature stress, and act as cryoprotectors and osmoregulators (Miura & Furumoto, 2013). There is an evidence of signaling and antioxidant function of sugars (Heidarvand & Amiri, 2010). One of the obligatory conditions for the development of cold resistance is the accumulation of sugars in plant tissues (Ruelland et al., 2009). Under low-temperature stress, both intracellular and extracellular sugars are involved in the protection of membranes (Koleva et al., 2012). Soluble sugars can reduce the temperature of the solution, in which ice crystallization takes place, to stabilize the phospholipid membrane bilayer, interacting

doi: 10.ANN/RSCB-2018-0002:RSCB
Received 11 March 2018; accepted 30 May 2018.

with polar heads of phospholipids and preserving (at dehydration) the liquidcrystalline state of lipids (Wisniewski et al., 2014). Consequently, the role of endogenous sugars in increasing resistance to low temperatures is uncertain, but there is an interesting approach to exogenous Suc enrichment with the aim of disclosing mechanisms, whose regulation depends on the carbohydrate status and contributes to increasing cold resistance (Deryabin et al., 2011). The adaptive nature of leaf variability has been demonstrated by comparative structural and functional studies of foliar traits driven by environmental characteristics (Wyka et al., 2007; Niinemets 2015; Jankowski et al., 2017). Under natural conditions, low temperatures of atmospheric air and the surface layer of the soil dominate in the early spring. For most plants with a long summer period of development, such conditions are unfavorable. Some molecular and physiological processes activated in response to low temperature occur in the chloroplast, which acts as sensor of the environmental changes triggering specific signaling pathways (Pinas Fernandez and Strand, 2008). However, early-spring ephemeroids had formed some adaptive properties during the long evolution, which ensure their development under short hypothermal conditions. The object of research in this work is the leaves of ephemeroid G. nivalis, which are adapted in the early stages of development to low temperatures. Previously, we have found that in natural conditions, at low atmospheric air temperatures, the ultrastructure of mitochondria and chloroplasts in mesophyll cells of G. nivalis leaves has distinctive features (Fediuk & Bilyavska, 2015; Fediuk et al., 2017). These features, obviously, cause the adaptation to the hypothermal conditions of the functioning of the mesophyll cell systems in the leaves. The study of fluorescence of chlorophyll as a cold-resistant screening method, which has already been used in experiments on maize genotypes differing in sensitivity to this factor (Fracheboud et al., 1999), has been used by us.

We assumed that the treatment of G. nivalis hardened leaves with the solution of exogenous Suc would make it possible to detect the effect of Suc on the cold resistance of chloroplasts, so the purpose of the work was to find out the effect of changes in the ultrastructure of granal thylakoids on the photosynthetic characteristics of the leaves of G. nivalis and the role of Suc in providing their cold resistance.

Materials and methods

Plant material and experimental set-up G. nivalis, known as Snowdrop, is perennial bulbous petaloid monocot, belonging to Amaryllidaceae, tribe Galantheae Parl. It is one of the earliest flowering ephemeroid in Europe. In Ukraine, Snowdrops occupy territory from Transcarpatian lowland in the west to Pridnieprovien lowland and spurs of Middle-Russian Upland in the east, from Polesian lowland in the north to forest-steppe zone in the south (Melnyk & Didenko, 2013). Snowdrop plants were grown from bulbs in growth pots filled with a soil substrate, in which the content of nitrogen was 120 mg/l, phosphorus – 160 mg/l, potassium – 190 mg/l, pH 5.5-5.8. The ambient air temperature was maintained within 22-23 °C, and the soil relative humidity reached 80-85%. Following 17 days of the bulbs germination in dark and the appearance of leaves on Snowdrop plants, a cultivation continued under an 8-h light/16-h dark cycle at moderate light intensity (180 μmol of photons • m-2 • s-1).

Low-Temperature Treatments Prior to the experiments, the plants were exposed to low temperatures (+5 °C in the daytime and +3°C nightly) for 14 days with an 8-h photoperiod with artificial light (80 μmol of photons • m-2 • s-1). A low light density was used to prevent the photoinhibition within hardening period. The leaves were detached from the plants and placed in Petri dishes. For the chilling treatment, control (untreated) and experimental (treated with Suc) leaves were gradually cooled to reach a temperature values of –5 or –15 °C during 1 h. Then, they were gradually heated to a temperature of +5 °C. Sucrose treatment To find out the

doi: 10.ANN/RSCB-2018-0002:RSCB
Received 11 March 2018; accepted 30 May 2018.

influence of Suc on the adaptation of Snowdrop leaves to the hypothermic conditions, cold-hardened plants were used, in which the leaves were cut and placed on wet filter paper in Petri dishes. One set of hardened leaves placed in Petri dishes was treated with a 0.02 M solution of Suc, and other – with 0.1 M Suc solution for 1 h. Before the experiments, control hardened leaves were placed in Petri dishes in a humidified medium at a temperature of +5 °C, without adding Suc solutions.

Electron Microscopy For ultrastructural analysis of leaf chloroplasts, 2 × 2-mm sections were cut from the middle part of the leaves. A prefixation of the material was carried out at room temperature (18–22 °C) by vacuum infiltration with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 8 h at 4 °C. Then, the samples were buffer-washed (2 ×20 min) and postfixed in 1% OsO4 in 0.1 M cacodylate buffer, pH 7.2, overnight at 4 °C. The preparations were dehydrated in a graded ethanol series and infiltrated via a propylene oxide series. Then, samples were soaked in epoxy resin mixtures and propylene oxide, embedded in Epon-araldite resin and transferred to a thermostat for the polymerization at 37 and 60°C. Ultra-thin sections were made with a LKB-V ultramicrotome (LKB, Sweden) and stained with Pb citrate. The preparations were examined in a JEM-1300 transmission electron microscope (JEOL, Japan) with magnification of 100 000×.

Analysis of data The images of 30 chloroplasts were analyzed for each variant. Morphometric image analysis of the chloroplast thylakoid and determination of transverse dimension of the grana were carried out using an ImageJ computer program (NIH, USA). In Fig. 1 J, distances between the negative peaks (a) were considered as the width of the thylakoid, and lumen width was determined by the width of the positive peaks (b). The dimensions of 10 granal thylakoids were determined for each grana image using statistical methods of data processing. The significance of differences between mean values of experimental and control variants was calculated using a Student's t-test. Differences were considered statistically significant at 5% ($P < 0.05$).

Determination of functional parameters of the photosynthetic apparatus by the chlorophyll induction fluorescence method The chlorophyll fluorescence measurements on Snowdrop intact leaves were performed using XE-PAM fluorimeter (Walz, Germany) at an average temperature of +23 ºC according to the procedure described earlier (Topchiy et al., 2005). Minimal fluorescence (F0) of dark-adapted leaves was determined at low photon flux density (PFD) near 0.2 μmol quanta m-2 • s-1. Maximal fluorescence (Fm) of dark-adapted and light-adapted (F'm) leaves was detected at saturating irradiance (1 s) of halogen lamps (3 000 μmol quanta m-2 • s-1. "Actinic lights" used were 500 μmol quantum m-2 • s-1 PFD. The following parameters of chlorophyll fluorescence induction curve were calculated: Fv/Fm – the maximum quantum yield of photochemical reactions of PSII in adapted to darkness status (van Kooten and Snel, 1990), qP – photochemical quenching of chlorophyll fluorescence (Schreiber et. al., 1986), qN – nonphotochemical quenching of chlorophyll fluorescence (qN) (Schreiber et al., 1986), and ETR – the electron transfer rate according to the formula: $ETR = \varphi PSII \bullet PFD \bullet 0.5 \bullet 0.84$, with using values of effective quantum yield of electronic transport (φPSII) (Genty et al., 1989), photon flux density (PFD), of absorption coefficient 0.84 and assumption uniformity of distribution photosynthetic active radiation between PSI and PSII (Schreiber et al., 1995). Measurements were conducted on the adaxial surface of the leaf lamina at different atmospheric air temperatures. Average data obtained in three to five experiments, each of which was investigated in at least three replications, are presented.

Results and discussions

The ultrastructure of the chloroplast grana Under conditions of G. nivalis experimental cultivation, the growth of leaves and the development of chloroplasts took place without noticeable negative changes in the

doi: 10.ANN/RSCB-2018-0002:RSCB
Received 11 March 2018; accepted 30 May 2018.

ultrastructure of granal thylakoids in the early stage of the vegetative period, at the appearance of leaves above the soil surface at +5 °C. Following by 14 days of cold hardening of plants at a temperature of +5 °C, on average, 8 thylakoids per grana were found, the width of lumen was uneven in the chloroplast grana of the control hardened leaves and the thylakoids, which had signs of swelling and wryness, were placed not tightly in grana (Fig. 1 A, Table). Earlier in similar studies, it has already been noted that swelling and distortion of thylakoids in chloroplasts are early symptoms of cold stress in a plant cell (Kratsch & Wise, 2000). Cold stress can cause membrane damage, denaturation of proteins, accumulation of toxic substances, which may ultimately lead to even death (Bowers, 1994). When the Snowdrop leaves were treated with Suc (0.02 or 0.1 M), a number of the thylakoids per granum decreased by 23 and 26 %, respectively, in comparison to control (0 M Suc), a length of granal thylakoids increased by 16 and 74 %, thickness of the thylakoids rose by 12 and 22 %, a width of granal lumen enhanced by 11 and 14 %, while a height of granum dropped by 15 and 13 %. The area of grans practically didn't change at 0.02 M Suc but it highly increased at 0.1 M Suc (up to 52 %) mainly due to a significant increase (by 74%) of the length thylakoids (Table). Thus, after the treatments, the thylakoids had no signs of swelling and distortion and were more densely packed compared to ones in control plants, indicating optimization of their structure under the influence of Suc solutions, at a given temperature (Fig. 1 B, C). Following by gradual cooling to a temperature of –5 °C, there were observed abrupt swelling the grana in control (untreated with Suc) chloroplasts, which decreased in leaves subjected to Suc treatments (Fig. 1 DF). Such treatments resulted in an increase in a number of the thylakoids per granum at 0.02 M Suc and particularly at 0.1 M Suc (by 17 and 119 %, respectively), whereas a length of granal thylakoids decreased by 33 and 22 %, as well as a thickness of the thylakoids (by 3 and 22 %). The width of granal lumen by 0.02 M Suc decreased by 2%, and by 0.1 M Suc remained unchanged (Table). An increase in the number thylakoids of grans by 119% (by 2 times) at 0.1 M Suc, resulted in an increase by 53% areas of grans. As the temperature dropped to –15 °C, we did not find lethal injuries in the chloroplasts of the control leaves, but there were observed fragmentation and swelling the grana, which thylakoids had a wavy shape (Fig. 1 G). The disturbances in the structure of the granal thylakoid resulted in the unevenness of the lumen width that obviously negatively reflected the effectiveness of the functioning PSA. At frosty temperature (–15 °C), a degree of swelling and of heterogeneity the grans did not increased in the leaves treated once with 0.02 M Suc, compared to control (Fig. 1 H). It was found that a number of thylakoids per granum, a thickness of the thylakoids змінювались changed insignificantly ($P \geq 0.05$) in these chloroplasts, however, a length of granal thylakoids and an area of granum section redoubled in comparison to respective control data (Table). In leaves that were pre-treated with a 0.1 M Suc solution at the same temperature, numerous thylakoids had slight signs of swelling, but were located tightly in grana (Fig. 1 I). A number of thylakoids per grana increased to 11, which corresponded to a double increase compared to control, while their length and thickness showed no changes; at once a width of granal lumen decreased by 25 %. Nevertheless, an area of granum section rose by 82 % in comparison to control (0 M Suc).

Thus, at a temperature of -15 °C compared to control in leaves treated with 0.02 and 0.1 M Suc, the thickness of thylakoids decreased slightly (correspondingly to 4 and 3%), and the thickness of lumens by 25 % at 0.1 M Suc. In spite of a decrease in the thickness of thylakoids and lumens, there Identified a tendency to increase the area of grana. At the 0.02 M Suc, of the magnitude increased by 108 % due to the increase in the length of thylakoids (by 105 %), and at 0.1 M Suc due to the increase in the number of thylakoids by 113 % (2 times).

Annals of R.S.C.B., Vol. XXI, Issue 3, 2017, pp. 43 - 51
Received 11 March 2018; accepted 30 May 2018.
doi: 10.ANN/RSCB-2018-0002:RSCB

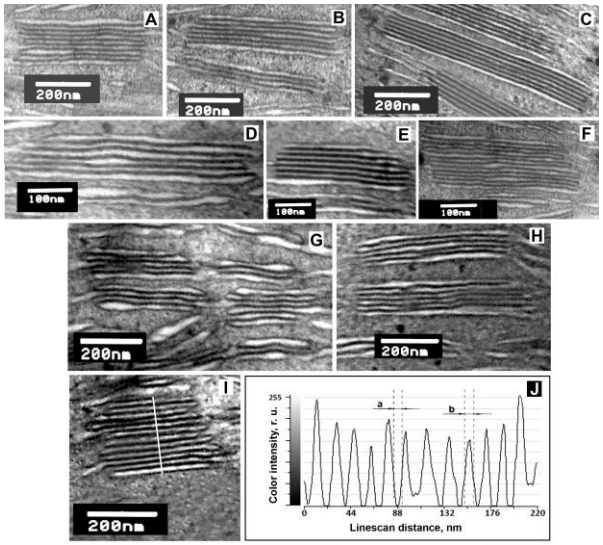

Fig. 1. Ultrastructure of granal thylakoids in chloroplasts of Galanthus nivalis L. leaves at irradiance of 80 μmoles photons m-2 • s-1: (A–C) after 1-h exposition at +5 °C, A – without exogenous sucrose, B – with exogenous 0.02 M sucrose, C – with exogenous 0.1 M sucrose; (D–F) after 1-h exposition at –5 °C, D – without exogenous sucrose, E – with exogenous 0.02 M sucrose, F – with exogenous 0.1 M sucrose; (G–J) after 1-h exposition at –15 °C, G – without exogenous sucrose, H – with exogenous 0.02 M sucrose, I, J – with exogenous 0.1 M sucrose; J – histogram of linear scan profile shown the color intensity in related units (the pixels), where a – thylakoid thickness; b – lumen width.

Compared with our data obtained at +5 ° C, the amount of thylakoids at (-15 ° C) 0.02 M Suc decreased by 10% to an average value of 5 and increased at 0.1 M Suc by 88%. Thylakoid thickness was greater at 0.02 M Suc compared to 0.1 M Suc and increased on average by 18% and 10 % respectively. The area of the thylakoids at 0.02 M Suc increased by 31 % due to the lengthening of the thylakoids by 16 %, and to increasing their thickness by 18 %. At the 0.1 M Suc, mainly due to a decrease in the length of thylakoids by 62% and a width of thylakoids by 9 %, their area of decreased by 26 % (Table 1).

Table. Dimensions of gran and their components in chloroplasts of leaves *Galanthus nivalis* L. at the conditions of influence low temperatures and exogenous sucrose

T, °C	[Suc], M	Number of thylakoids per granum	Length of granal thylakoid, nm	Thickness of granal thylakoid, nm	Width of granal lumen, nm	Height of granum, nm	Area of granum section, nm^2
+5	0	8.1±0.78^b	447.29±11.23^c	6.8±0.30^a	7.3±0.70^a	114.21±5.78^b	51084.99±302.45^c
+5	0.02	6.2±0.66^a	519.23±14.06^d	7.6±0.62^{ab}	8.1±0.34^a	97.34±6.13^a	50541.85±591.39^c
+5	0.1	6.0±0.60^a	778.00±10.45	8.3±0.52^{ab}	8.3±0.38^{ab}	99.60±8.09^{ab}	77488.80±439.75^f
–5	0	5.2±0.53^a	542.23±12.46^d	9.0±0.43^{ab}	9.5±0.19^b	96.23±3.83^a	52162.53±996.14^c
–5	0.02	6.1±0.60^a	361.30±11.18^b	8.7±0.57^{ab}	9.3±0.44^{ab}	109.81±3.34^b	39670.74±517.58^b
–5	0.1	11.4±0.62^c	423.11±10.44^c	7.0±0.22^a	9.5±0.17^b	188.18±2.48^c	79586.99±754.98^g
–15	0	5.3±0.51^a	294.32±8.50^a	9.4±0.11^b	10.9±0.81^c	107.59±5.48^b	31665.89±908.55^a
–15	0.02	5.6±0.53^a	605.01±12.29^e	9.0±0.21^{ab}	10.5±0.17^c	109.24±2.19^b	66067.09±921.49^e
–15	0.1	11.3±0.71^c	295.12±9.12^a	9.1±0.34^{ab}	8.2±0.88^{ab}	195.49±9.62^c	57693.01±260.83^d

Presented of mean values 20-30 measurement (± SE). For data presented in each column, significant differences are denoted by different superscript letters ($P \leq 0.05$)

The importance of the Suc in the adaptation of plants to hypothermal conditions is noted in the studies of some authors (Uemura et al., 2003; Li et al., 2006; Koleva et al., 2012), who came to the conclusion that the use of exogenous Suc can lead to an increase in cold resistance of plants. In similar studies in laboratory experiments, other authors proved the feasibility of cold adaptation for plant cells and associated it with increased sugar content in plants (Koster & Lynch, 1992; Sasaki et al., 1996; Shao et al., 2007). In leaves treated with 0.1 M Suc solution, the ultrastructure of the grana has undergone significant changes at –15 °C (Fig. 1 I). The presence of high grana formed from tightly packed, relatively short thylakoids and the absence of deformations of thylakoids may indicate the positive effect of this concentration of Suc solution on the adaptability of the granal structure to frosty temperatures. Under natural conditions, cold resistance of plants is formed, when the gradual influence of low positive temperatures occurs, this process is known as cold acclimation (Thomashow, 1999). During cold acclimation, mechanisms for protecting cells from cold stress and freezing, which cause cell expression in genes and changes in the structure of membrane complexes, are activated (Orvar et al., 2000; Shao et al., 2008), the concentration of Suc increases (Ristic et al., 2003; Wanner & Junttila, 1999). It is believed that in the adaptation of plants to hypothermal conditions, Suc performs not only the osmoprotective role, but also due to the interaction with the lipid bilayer of membranes, protects them from dehydration and freezing.

Annals of R.S.C.B., Vol. XXI, Issue 3, 2017, pp. 43 - 51
Received 11 March 2018; accepted 30 May 2018.
doi: 10.ANN/RSCB-2018-0002:RSCB

Functional characteristics of the photosynthetic apparatus The changes in the thylakoid ultrastructure in the grana of the chloroplasts can correlate with changes in photochemical activity (Ma & Zhang, 2009). The level of photochemical activity of chloroplasts is determined
predominantly by the state of the photosystem II (PSII), a key transmembrane pigmentprotein complex in chloroplasts, which provides absorption and transformation of light energy, and also plays a leading role in protecting photosynthesis from photoinhibition (Krall & Edwards, 1992). In our study, the effect of Suc on the functional state of thylakoids in G. nivalis
chloroplasts at hypothermal conditions was determined by the method of slow induction fluorescence kinetics of chlorophyll a The results showed that the maximum quantum yield of photochemical energy transformation (Fv/Fm) in adapted to dark cold-hardened leaves sharply dropped (from 0.79 to 0.38) with a decrease in temperature from +5 to –15 °C (Fig. 2 A).

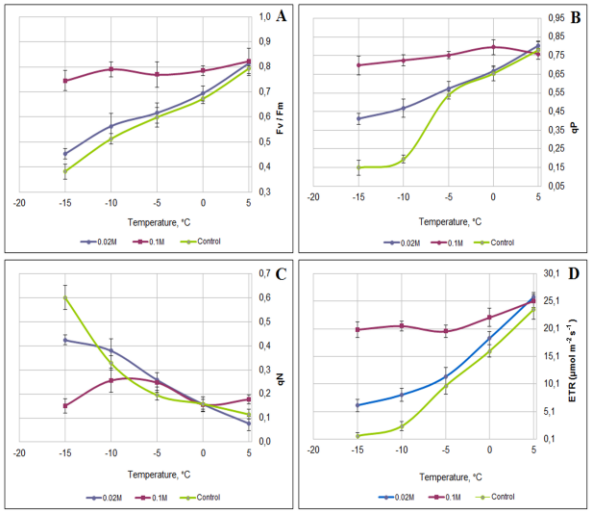

Fig. 2. Responses of chlorophyll fluorescence to low temperature and Sucrose (0.02 and 0.1 M) treatments. Panels show (A) the dark-adapted potential maximum quantum yield of PSII photochemistry (Fv/Fm); (B) photochemical quenching (qP); (C) nonphotochemical quenching (qN); and (D), ETR - the electron transfer rate (Schreiber et al., 1995; Maxwell and Johnson, 2000).

The Fv/Fm value about of 0.82 is characteristic for healthy unstressed plants (Björkman & Demmig, 1987). In our study, a severe decrease in Fv/Fm in cold-treated control leaves at negative temperatures indicated
significant reduction of photosynthetic efficiency of G. nivalis PSA (Fig. 2 A). The same effect of negative temperatures was observed for photochemical quenching of fluorescence (qP) which is an
approximate grade of oxidation of the primary acceptors of QA, that is, the part of the open reaction centers of the PSII (Fig. 2 B) and electron transfer rate (Fig. 2 D). These parameters also decreased at negative temperatures. After the exposition in Suc solutions, the functional parameters of PSA of detached cold-hardened leaves were restored to the values recorded at positive temperature (+5°C) (Fig. 2 A, B, D). The data indicate stabilizing effect of Suc on PSA functional state in G. nivalis leaves at negative temperatures (Fig. 2 A, B, D). So, it should be noted that ultrastructural changes in the granal thylakoids of Suctreated leaves (see Fig. 2) are correlated with the relative stability of the functioning of the PSII in the chloroplasts (see Fig. 2 A). The pattern of the dependence of nonchemical quenching of chlorophyll fluorescence (qN) on temperature and Suctreatment (Fig. 2C) differed from the behavior other functional parameters in G. nivalis leaves under these conditions (Fig. 2 A, B, D). The coefficient qN characterizes changes in thermal dissipation in PSII complexes and provides a mechanism of defence from photoinhibition (Maxwell & Johnson, 2000). According data from Fig. 2 C, the Suctreatment reduced the involvement of this mechanism in the adaptation of the G. nivalis plant to negative temperatures. The boundaries of optimum temperatures for the PSA activity in G. nivalis leaves were determined by coefficients of the photochemical (qP) and non-photochemical (qN) chlorophyll fluorescence quenching values. The photochemical quenching coefficient qP is assumed to exceed 0.5 at the optimum temperatures (Korneev, 2002). In the control leaves of G. nivalis, the temperature limits from +5 to –5 °C, are optimal for the functioning of the PSA, as indicated by the coefficients qP = 0.53 (Fig. 2 B) and qN = 0.19 (Fig. 2 C). The same limits

doi: 10.ANN/RSCB-2018-0002:RSCB

of the optimal temperatures for the functioning of the PSA with qP = 0.57 and qN = 0.25 coefficients were measured in the experimental leaves that were in the exogenous solution of 0.02 M Suc.

Significant expansion of the boundaries of optimal temperatures for the functioning of the PSA was recorded in leaves that were treated with a more concentrated solution of Suc (0.1 M). In particular, for them the optimum temperature limits range from +5 to –15 °C, with coefficients qP = 0.69 and qN = 0.15. Following by a gradual decrease of the temperature to –5 °C, in all leaves, the rateof electron transport also decreased, but in leaves treated with 0.1 M exogenous Suc, this decrease was slower compared with the leaves checked and treated with 0.02 M Suc (Fig. 2 A). With a further drop of the temperature from –5 to –15 °C, in the leaves treated with a 0.1 M solution of exogenous Suc, the electron transport rate stopped decreasing and tended to stabilize at a value of 20.1. Under these conditions, in the leaves treated with a 0.02 M Suc solution, the rate of electron transport was not stabilized, but continued to decrease slower compared to the control leaves that is probably due to the insufficient effect of the low concentration of the exogenous Suc solution. In the leaves of cold-resistant plants under hypothermal conditions, there are simultaneous interruptions both in the ultrastructure and in the functioning of the photosynthetic apparatus (Mamushina et al., 2002, 2011; Tu et al., 2012; Recchia et al., 2017). Similar ultrastructural changes were found in the control leaves of G. nivalis, however, the treatment with the solution of 0.1 M exogenous Suc not only prevented following destruction of chloroplasts under cold stress, but also reduced the deformation of the granal thylakoids that correlated with the effective functioning of the PSA. The data obtained allow suggesting that in G. nivalis under hypothermal conditions, Suc plays an important role in the photochemical and structural changes in the granal thylakoids aimed at ensuring their effective functioning. Such structural changes thylakoids of grana the ephemeroids, apparently, are adaptive sign of PSA, which develop under hypothermal conditions. The observed effects of Suc in G. nivalis PSA ultrastructure and functioning suggests that chloroplasts of leaves are able to osmotic adjustment of response to cold stress.

Conclusion

Results obtained on hardened leaves of G. nivalis plants are evidence of structural reorganization and functional modification in their photosynthetic apparatus under cold conditions. After treatments with Suc, the granal components of the chloroplasts reach optimal structure under cooling and freezing. A partial compensative effect of Suc on PSA functioning in leaf mesophyll cells of G. nivalis was revealed under cool stress. The compatible solution was very effective at high concentration (0.1 M), but less effect occurs at less concentration of Suc (0.02 M). We believe that this is the first demonstration of such adaptive pattern induced by Suc in PSA leaves of G. nivalis in relation to low temperature.

References

Baxter, B., Plant acclimation and adaptation to cold environments. In: Temperature and Plant Development, vol.2, pp. 19-48, 2014. Edited by K. A. Franklin and Ph.A. Wigge, John Wiley & Sons, New York

Björkman, O., Demmig, B.: Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta, 170, 4, 489–504, 1987

Bowers, M.C.: Environmental effects of cold on plants. In: Wilkinson, R.E. (Ed.). Plant-Environment Interactions. 391–411. 1994

Deryabin, A.N., Sabelnikova, E.P., Burakhanova E.A.: Dependence of the formation of cold resistance in plants in vitro from sucrose concentrations in the medium of expression. Bulletin of the Mordovian University., 4, 200–205, 2011

Fediuk, O.M., Bilyavska, N.O. Ultrastructural changes in Galanthus nivalis L. foliar mitochondria at vegetation in hypothermal conditions. Visn. Kharkiv. nats. agrar. untu, Ser. Biology., 2, 35, 58–63, 2015

Fediuk, O.M., Bilyavska, N.O., Zolotareva, O.K.: Ultrastructural peculiarities and state of the photosynthetic apparatus in leaves of Galanthus nivalis (Amaryllidaceae) in its spring stage of ontogenesis. Ukr. Bot. J., 74, 5, 475–487, 2017

doi: 10.ANN/RSCB-2018-0002:RSCB

Fracheboud, Y., Haldimann, P., Leipner J., Stamp P.: Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (Zea mays L.). J. Exp. Bot., 50, 338, 1533–1540, 1999

Genty, B., Briantais, J.M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta., 990, 87–90, 1989

Heidarvand, L., Amiri, R.M.: What happens in plant molecular responses to cold stress? Acta Physiol. Plant. 32, 419–431, 2010

Jankowski, A., Wyka, T., Żytkowiak, R., Nihlgård, B., Reich, P.B., Oleksyn, J., Cold adaptation drives variability in needle structure and anatomy in Pinus sylvestris L. along a 1,900 km temperateboreal transect. Funct. Ecol., 31, 12, 2212–2223, 2017

Koleva, D., Ganeva, T., Stefanova, M. Effect of cryoprotectants sucrose and ABA on chloroplasts structure in regenerated after cryopreservation Orthosiphon stamineus Benth. plants. J. Pharm. Res.: 5, 8, 4172 – 4174, 2012

Korneev, D.Yu.: Photochemical and nonphotochemical quenching of chlorophyll fluorescence. In: Information Capabilities of Chlorophyll Fluorescence Induction Method, pp. 60-64, 2002. Edited by L.A. Sirenko and T.M. Shadchina "AlterPres", Kyiv, Ukraine (In Russian) Koster,

K.L., Lynch, D.V.: Solute accumulation and compartmentation during the cold acclimation of puma rye. Plant Physiol., 98, 108–113, 1992

Krall, J.P., Edwards, G.E.: Relationship between photosystem II activity and CO2 fixation in leaves. Physiol. Plant., 86, 1, 180–187, 1992

Kratsch, H.A., Wise, R.R.: The ultrastructure of chilling stress. Plant, Cell & Environment., 23, 4, 337–350, 2000

Li, Y.H., Lee, K.K., Walsh, S., Smith, C., Hadingham, S., Sorefan, K., Cawley, G., Michael, W.: Establishing glucose- and ABA-regulated transcription networks in Arabidopsis by microarray analysis and promoter classification using a Relevance Vector Machine. Genome Res.,16, 414– 427, 2006

Mamushina, N.S., Voznesenskaya, E.V., Zubkova, E.K., Maslova, T. G., Miroslavov, E.A.: Structural and functional changes in mesophyll cells during leaf growth in two species of spring ephemers. Russ. J. plant physiol., 49, 2, 171–178, 2002

Mamushina, N.S., Zubkova, E.K., Bubolo, L.S., Tyutereva, E.V.: Structuralfunctional characteristic of ephemeroid of the boreal zone. Bot. J., 96, 7, 906–916, 2011

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence – a practical guide. J. Exp. Bot., 51, 659–666, 2000

Melnyk, V. I., Didenko, S. Ja., Growth conditions of G. nivalis in Ukraine. In: The Species of the Genus Galanthus L. (Amaryllidaceae) in Ukraine, pp.68-75, 2013. Edited by A.V. Yena, S. Yu. Popovich and I. I. Chorney, National Botanical Garden of the National Academy of Sciences of Ukraine and "VIPOL", Kyiv (in Ukrainian)

Miura, K; Furumoto, T.: Cold Signaling and Cold Response in Plants. Int. J. Mol. Sci., 14, 3, 5312–5337, 2013 Niinemets, Ü. Is there a species spectrum within the world-wide leaf economics spectrum? Major variations in leaf functional traits in the Mediterranean sclerophyll Quercus ilex. New Phytol., 205(1), 79-96, 2015

Orvar, B.L., Sangwan, V., Omann, F., Dhindsa, R.S.: Early steps in cold sensing by plant cells: the role of actin cytoskeleton and membrane fluidity. Plant J., 23, 785–794, 2000

Pinas Fernandez, A., Strand, A.: Retrograde signaling and plant stress: plastid signals initiate cellular stress responses. Curr. Opin. Plant Biol., 11, 5, 509–513, 2008

Recchia, I., Sparla, F., Pupillo P.: Photosynthetic properties of spring geophytes assessed by chlorophyll fluorescence analysis. Plant Physiol. Biochem., 118, 510–518, 2017

Ristic, L.A., Ashworth, E.N.: Changes in leaf ultrastructure and carbohydrates in Arabidopsis thaliana L. (Heyn) cv. Columbia during rapid cold acclimation. Protoplasma., 172, 111–123, 1993

Ruelland, E., Vaultier, M.N., Zachowski A., Hurry V.: Cold signaling and cold acclimation in plants. Adv. Bot. Res., 49, 35–150, 2009

Sasaki, H., Ichimura, K., Oda, M.: Changes in sugar content during cold acclimation and deacclimation of cabbage seedlings. Ann. Bot., 78, 365–369, 1996

Schreiber, U., Bilger, W., Neubauer, C.: Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Ecophysiology of Photosynthesis, 100, 49–70, 1995

Schreiber, U., Schliwa, U., Bilger, W.: Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth. Res., 10, 1–2, 51–62, 1986

Shao, H.B., Chu, L.Y., Shao, M.A., Zhao, C.X.: Advances in functional regulation mechanisms of plant aquaporins: Their diversity, gene expression, localization, structure and roles in plant soil-water relations (Review). Mol. Membr. Biol., 25, 1–12, 2008

Shao, H.B., Guo, Q.J., Chu, L.Y., Zhao, X.N., Su, Z.L., Hu, Y.C., Cheng, J.F.: Understanding molecular mechanism of higher plant plasticity under abiotic stress. Biointer., 54, 37–45, 2007 Thomashow, M.F.: Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Ann. Rev. Plant Physiol. Plant Mol. Biol.,50, 571–599, 1999

Topchiy, N.M., Sytnik, S.K., Syvash, O.O., Zolotareva, O.K.: The effect of additional red irradiation on the photosynthetic apparatus of Pisum sativum. Photosynthetica, 43, 451–456, 2005

Tu, W.F., Li, Y., Zhang, Y.M., Zhang, L., Liu, H.Y., Liu, C., Yang, C.: Diminished photoinhibition is involved in high photosynthetic capacities in spring ephemeral Berteroa incana under strong light conditions. J. Plant Physiol., 169, 1463–1470, 2012

Uemura, M., Steponkus, P.L.: Modification of the intracellular sugar content alters the incident of

doi: 10.ANN/RSCB-2018-0002:RSCB

freeze-induced membrane lesions of protoplasts isolated from Arabidopsis thaliana leaves. Plant Cell Environ., 26, 1083–1096, 2003

Van Kooten, O., Snel, J.F.H.: The Use of Chlorophyll Fluorescence Nomenclature in Plant Stress Physiology. Photosynthesis Research, 25, 147–150, 1990

Wanner, L.A., Junttila, O.: Cold-induced freezing tolerance in Arabidopsis. Plant Physiol., 120, 391–400, 1999 Wisniewski, M., Gusta, L., Neuner, G.: Adaptive mechanisms of freeze avoidance in plants: A brief update. Env. Exp. Bot., 99, 133–140, 2014

Wyka, T., Robakowski, P., Żytkowiak, R.: Acclimation of leaves to contrasting irradiance in juvenile trees differing in shade tolerance. Tree Physiol., 27, 1293–1306, 2007.