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Mini Review

Chloroplasts of cold-tolerant plants

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Abstract

Cold is one of the main stress factors affecting plant growth and development. The structure and function of chloroplasts is most vulnerable to cold. This brief review summarizes the influence of low temperature on both chloroplasts' structure and functioning across cold-tolerant plant species. One of the features of the chloroplast structure is the presence of stromules. We attempted to define a core set of such changes for plants with different habitats. Some changes might be consistent across all species, which were studied; however, some other characteristics were species- or family-specific. Elucidating the interrelation between the mechanisms controlling photosynthesis during cold stress will facilitate the development of strategies to enhance plant tolerance to low-temperature environmental conditions.

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Introduction

Despite the increase in the average annual global temperature in recent decades, the urgency of cold and frost resistance of plants does not only decrease but also increases. In particular, there is a large amplitude of temperatures in the winter and spring periods from high to low (from –15 to +13 ºC) in Ukraine (1). In this regard, the study of the mechanisms of plant adaptation to low temperatures is an important scientific aspect to be studied in the area.

Low temperature is one of the main environmental stresses that inhibit plant growth (2). At the cellular level, the most susceptible to cold organelle is the chloroplast, which ultrastructural changes induced by chilling include the swelling of chloroplasts, deformation of thylakoid membranes, and decrease in number or size of starch granules; the mechanism for supporting of photosynthetic activity during cooling is maintaining membranes in a fluid state that allow them to resist subzero temperatures (3).

The study of plants constantly growing in arctic conditions helps to identify mechanisms of adaptation to low temperatures. Numerous studies of plants originating from the Arctic and Antarctica have shown a higher level of metabolic activity in their cells in comparison with plants from regions with temperate climate; plants from extremely cold environments use a larger span of temperatures,

where photosynthesis, respiration and most enzymes work (4). Among the 2200 species of vascular plants found in the Arctic (5), only two species could naturally colonize the Antarctic coastal areas, namely *Deschampsia antarctica* Desv. (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl. (*Caryophyllaceae*) (6). Studies have shown that Antarctic plants really differ from other cold-tolerant plants with the ability to exist at low temperatures throughout the year, since they should grow and reproduce at constant low temperatures (from 5 to 7ºC), although the photosynthesis of their leaves is active, even at zero temperatures (7).

Ultrastructural changes

In *D. antarctica* plants grown in the coastal areas of the Antarctic, a crowded layer of abundant tightly packed chloroplasts was observed along the cell wall of mesophyll cells (8). The chloroplasts, which had round form, dense stroma and well-developed granal thylakoids, were specific for them. A system of numerous stromal thylakoids linked granal stacks. There were small osmiophilic plastoglobuli occurred between thylakoids in stroma. In some cases, chloroplasts in the foliar mesophyll cells had irregular forms with protrusions and pockets or invaginations within organelles that lead to an increase in the surface area of chloroplasts and the volume of substances exchanged between cytoplasm and chloroplasts or other organelles (9). Deformed surfaces of chloroplasts were observed in ultrastructural research of the mesophyll cells of *C. quitensis* plants collected in Antarctica. The deformations had the form of invaginations closely surrounded by single mitochondria, or long stromal protuberants called stromules. Chloroplasts of *C. quitensis* also contained small starch grains and numerous plastoglobuli (10).

Similar spaces in chloroplasts included only stroma (stromules) were observed in several species of plants, namely, *Geum montanum* L., *Geum reptans* L., *Oxyria digyna* (L.) Hill. and *Ranunculus glacialis* L., which grew in polar and alpine regions (4, 11, 12). In the analyzed species, various specific protrusions, which had equal width or were wider at the terminal zones, were formed in chloroplasts (Fig. 1A). In *C. alpinum* and *C. quitensis*, narrow long protrusions included thylakoids and membranes were also noticed. Lutz and Engel (12) studied in the details cytological characteristics of foliar cells in six high-alpine plants. The species included plants of subalpine/alpine *Geum montanum*, alpine *Geum reptans*, *Poa alpina* var. *vivipara*, *Oxyria digyna*, and nival *Cerastium uniflorum* and *Ranunculus glacialis*. Numerous protrusions in chloroplasts were found in *O. digyna* and, to a less extent, in *G. reptans*; however, the others demonstrated only an infrequent formation of such chloroplast substructures. In leaves of *G. montanum* and *O. digyna* plants, which grew at about 50 m above sea level, such a pattern of structural changes was rare. In *O. digyna* chloroplasts of foliar cells, it was shown that the protrusions could be formed as broad long outgrowths of chloroplast envelope, which had form of pocket-like structures, where microbodies and mitochondria were spatially close to the chloroplast and/or to each other. The authors suggested that formation of such chloroplast outgrowths in some high-alpine plants may ensure fast exchange of molecules between plastid and cytoplasm as an adaptation mechanism allowing to survive and to develop during short vegetation period in the Alps, although other species may have formed another patterns of changes, which are not expressed in the plastids at the ultrastructural level (12).

Significant heterogeneity and dynamism of qualitative and quantitative changes in the structure of chloroplasts were detected in foliar mesophyll cells of ephemeroid *Galanthus nivalis* L. at different stages of their early-spring development (13). When leaves appear from soil, their chloroplasts had an elongated shape, sometimes with outgrowths (stromules). At subzero temperatures, contacts between
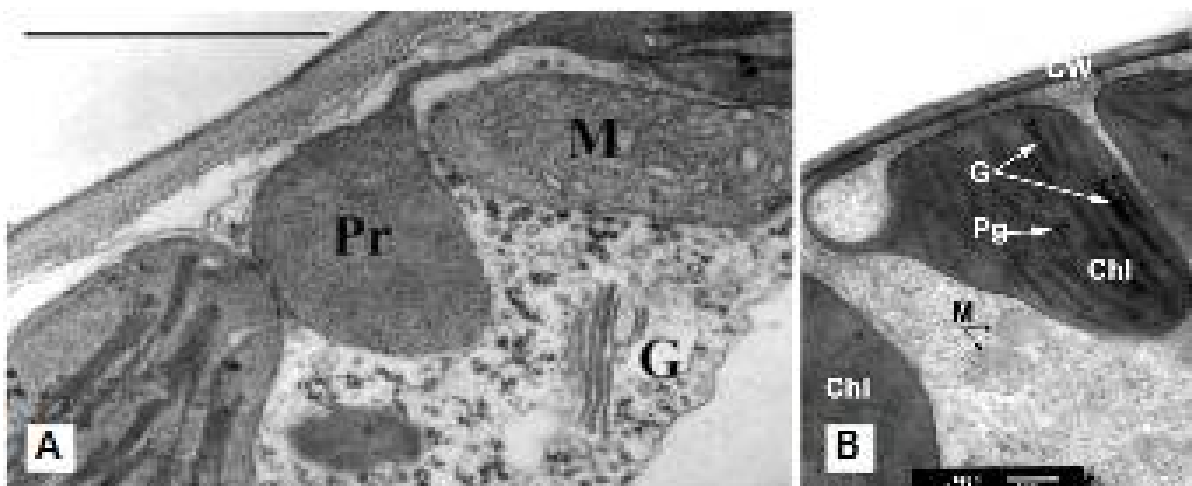

Fig. 1. Chloroplast protrusion in *Ranunculus glacialis* (**A**), Lütz and Engel (12); chloroplast stromule in *Galanthus nivalis* L. (**B**), Fediuk et al., (13). Cell Wall (CW), Chloroplast (Chl), Grana (G), Mitochondrion (M), Protrusion (Pr), Plastoglobule (Pg)

chloroplasts, the endoplasmic reticulum and mitochondria were detected. In foliar starchless chloroplasts, low grana were present, local swelling of granal thylakoids and stromal thylakoids were noted. In the electron-dense chloroplast stroma, there were low grana with several thylakoids, stromal thylakoids and a few plastoglobuli that was confirmed by the lowest parameters of the granum and thylakoid section areas and indicated the initial stage of the photosynthetic apparatus formation. At the early vegetative stage, the presence of numerous amoeboid plastids with stromules in *G. nivalis* leaves can obviously be explained by their response to a low temperature (Fig. 1B). During the vegetative stage, the section areas of chloroplasts, grana and thylakoids gradually increased and reached the maximum values; chloroplasts took a classical ellipsoid form; the height of the grana increased due to the increase in the number of thylakoids, along the envelope of the organelles appeared vesicles of the peripheral plastid reticulum. It is believed that the last could represent adaptive mechanism for intense transport into/out of the plastid by enhancing a surface area of the chloroplast inner membrane of envelope, which determinates exchange of metabolites between the organelle and the cytoplasm (14). At the generative stage, there was a gradual decrease in all the above-mentioned parameters of the structure of *G. nivalis* chloroplasts, indicating a decrease in the level of photosynthesis in the leaves under the conditions of development of generative organs of the plant.

The functions of the stromules were the subject of several assumptions and debates (15-19). Hypotheses for functions of stromules are: 1) increase in surface area of the plastid envelope for import and export of molecules; 2) channels through which molecules may move from the main plastid body to other intracellular locations; 3) interactions with other organelles, such as mitochondria, nucleus and plasma membrane, to reduce diffusion distance for exchange of molecules and biochemical intermediates; 4) production of plastid-derived vesicles for signalling of environmental stimuli or for recycling of chloroplast contents during nutrient stress; 5) placement of plastid enzymes and plastid contents in additional regions of the cells to facilitate import and export of molecules; 6) interactions with endoplasmic reticulum through hemifusion for exchange of non-polar substrates; 7) through close proximity, transmission of proteins and reactive oxygen species to signal plastid redox stress; 8) storage of imported outer envelope proteins; 9) anchoring of plastids to locations within the cell; 10) dispersion of toxic molecules such as reactive oxygen species through stromule formation and breakage into vesicles (19). Most alpine and polar grasses develop stromules during photosynthetic activity (11, 12) and use them to enhance energy metabolism (4).

In our opinion, the most convincing hypothesis explains the formation of chloroplast stromules by the need for rapid exchange of molecules by increasing the surface area of the plastid, although the participation of the mechanisms described by other hypotheses cannot be ruled out.

Biochemical and photochemical changes

During the day when plants actively photosynthesize, low temperatures can have a significant effect on energy balance since the absorbed energy may be greater than that used or dispersed to induce photooxidative stress (20). In laboratory experiments, Antarctic species showed a high ability to withstand photoinhibition (21-24). A number of studies have shown that the effect of low temperatures is a major factor in inducing some photoprotective mechanisms (22, 24-26). Cold acclimation also contributes to the defence from photoinhibition, probably due to mechanisms for restoring the structure of the photosynthetic apparatus (24).

Several biochemical mechanisms in these plants determine their resistance to freezing during the vegetation period. Nevertheless, most of the studies were carried out on *D. antarctica*, but not yet on *C. quitensis* (23-26). It was shown that *D. antarctica* leaves contain usual contents in polar lipids and in the degree of unsaturation of fatty acids in most lipid fractions compared to other Gramineae (27), high activity of antifreeze proteins in apoplast (28), and other stress-induced proteins such as dehydrin (29), as well as high concentrations of various nonstructural carbohydrates (30-32), which may take part in the defense of photosynthetic apparatus from the damaging effects of cold (33). Both Antarctic species showed somewhat different photoprotective strategies. At the same time, *C. quitensis* can regulate its pathways of electron transport, which avoids O_2 depletion, while *D. antarctica* uses oxygen as an alternative to electrons to protect PSII from excessive light (26, 34). Both plants enhance non-photochemical quenching (NPQ), when light intensity increases at cold temperatures (4 °C); nevertheless, only *C. quitensis* showed a significantly higher level of the heat generation (maximal NPQ) at cold temperatures (21). Indeed, the thermal dissipation in plants of *C. quitensis* is chiefly owing to dynamic and reversible processes (namely, xanthophyll cycle), pointing out the tolerance of this species to an altering light and temperature factors of environment (22).

Studies of photosynthesis on leaves of *G. nivalis* showed that the influence of low temperatures induced a high level of effective quantum yield of photochemical energy conversion in leaves; this is obviously due to the peculiarities of the thylakoid structure of chloroplasts that prevent the destruction of the PSII (13). It has been shown that, like other spring

ephemeroids, *G. nivalis* is characterized by high activity of photosynthetic electron transport at low level of non-photochemical quenching of fluorescence, which indicates the adaptation of the photosynthetic apparatus of leaves for development under full sunlight and low above-zero temperatures in early spring (13).

Conclusions and future perspectives

The chloroplasts of cold-tolerant plants are characterized by high level of plasticity. In all species, chloroplasts are forming stromules, which can enlarge surface of contacts between neighbouring cellular organelles or the cytoplasm. Such characteristics of the organelles can point out tight cooperation and intense metabolic processes, which may be defining for development of cold-tolerant plants in severe conditions of environment. It correlates with a high level of effective quantum yield of photochemical energy conversion, which indicates the existence of a link between structural changes in chloroplasts and the peculiarities of the photosynthetic apparatus functioning.

Many aspects of mechanisms of cold tolerance associated with chloroplasts still await characterization. The precise mechanism(s) underlying particular molecular changes in cold-tolerant plants remains much less understood. The identification of new components involved in signalling and response to low temperatures in cold-tolerant plants is challenging and will require different approaches relative to a gene expression and signal networks. These regulatory components are likely to represent critical steps where cold signals are integrated in unified responses. Elucidating the interrelation between the mechanisms controlling signalling during cold stress will facilitate the development of strategies to enhance plant tolerance to low-temperature environmental conditions.

Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

All authors jointly collected of data, wrote, viewed and revised the manuscript.

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