

INFLUENCE OF ENVIRONMENTAL CHANGES ON PHYSIOLOGY AND DEVELOPMENT OF POLAR VASCULAR PLANTS

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ABSTRACT: Polar vascular plants native to the Arctic and the Antarctic geobotanical zone have been growing and reproducing effectively under difficult environmental conditions, colonizing frozen ground areas formerly covered by ice.

Our macroscopic observations and microscopic studies conducted by means of a light microscope (LM) and transmission electron microscope (TEM) concerning the anatomical and ultrastructural observations of vegetative and generative tissue in *Cerastium arcticum*, *Colobanthus quitensis*, *Silene involucrata*, plants from Caryophyllaceae and *Deschampsia antarctica*, *Poa annua* and *Poa arctica*, from Poaceae family. In the studies, special attention was paid to plants coming from diversity habitats where stress factors operated with clearly different intensity. In all examinations plants, differences in anatomy were considerable. In *Deschampsia antarctica* the adaxial epidermis of hairgrass leaves from a humid microhabitat, bulliform cells differentiated. Mesophyll was composed of cells of irregular shapes and resembled aerenchyma. The ultrastructural observations of mesophyll in all plants showed tight adherence of chloroplasts, mitochondria and peroxisomes, surface deformations of these organelles and formation of characteristic outgrowths and pocket concavities filled with cytoplasm with vesicles and organelles by chloroplasts. In reproduction biology of examined Caryophyllaceae and Poaceae plants growing in natural conditions, in the Arctic and in the Antarctic, and in a greenhouse in Olsztyn showed that this plant develops two types

of bisexual flowers. Almost all ovules developed and formed seeds with a completely differentiated embryo both under natural conditions in the Arctic and the Antarctic and in a greenhouse in Olsztyn.

KEY WORDS: Polar vascular plants, Caryophyllaceae, Poaceae, morphology, ultrastructure, organelles, stress reactions, seed development, Arctic, Antarctic.

INTRODUCTION

Arctic and Antarctic vascular plants, like other plants occupying exposed areas of the polar geobotanical zone after the ice-cover has melted, are in danger of being affected by various unfavourable environmental factors, both biotic and abiotic. According to Levitt (1980), each environmental factor potentially unfavourable to a living organism means stress. Larcher (1987) claims that stress consists of both destructive and constructive elements, and is a selection factor contributing to greater resistance and faster adaptative evolution.

The existence of vascular plants in exceedingly difficult (extreme) Arctic and Antarctic conditions, an individual's growth success and production of offspring prove that these plants are adapted to unfavourable environmental conditions, that is they are stress-tolerant.

Cerastium arcticum, *Colobanthus quitensis*, *Silene involucrata* and *Deschampsia antarctica*, *Poa annua* and *Poa arctica* developed a number of anatomical and physiological features in order to successfully fight the effect of stress (Pirożnikow 1996, Romero *et al.* 1999, Mantovani and Vieira 2000, Lewis Smith 2003, Giełwanowska and Szczuka 2005, Piotrowicz-Cieślak *et al.* 2005).

Variability of plants tissues under the influence of habitat factors

An analysis of the anatomy of vegetative organs of polar vascular plants growing in different habitat conditions showed considerable variability caused mainly by water, its lack or surplus. Like all vascular plants growing in other regions of the Earth.

Many authors emphasise the xerophytic features in the Arctic and Antarctic species of vascular plants, *Cerastium arcticum*, *Colobanthus quitensis*, *Poa arctica* and *Deschampsia antarctica* (Pirożnikow 1996, Romero *et al.* 1999, Mantovani and Vieira 2000, Barcikowski *et al.* 2001, Lewis Smith 2003, Giełwanowska *et al.* 2005, Giełwanowska and Szczuka 2005).

On the basis of anatomical studies, conducted with a light and transmission electron microscope while this work was in progress, it can be concluded that individual tissues of Arctic and Antarctic Caryophyllaceae and Poaceae were closely related to microhabitat conditions.

Deschampsia antarctica plants which grew beyond the reach of sea waves in the dry, exposed microhabitat had leaves covered with epidermis without bulliform cells, and small groups of sclerenchymatic cells under the abaxial side. However, they did not form sclerenchymatic fibres on the adaxial side. All the leaves, both on vegetative and generative shoots, were stiff, hard, and had xerophytic features such as small blade area and thick-walled epidermal cells. Epidermal cell walls were cutinized and additionally covered with a cuticular layer. Size and shape variation of epidermal cells in Antarctic hairgrass was described by Romero *et al.* (1999).

The author found a distinct concentration of cells on the surface of leaves growing in the Antarctic in comparison with the leaves of Antarctic hairgrass cultivated in a greenhouse.

The author's own observations confirm the greater variation of cells in the adaxial epidermis described by Romero *et al.* (1999). The xerophytic features visible in the structure of Antarctic hairgrass and induced mainly by low temperature, but also other stress factors, occur for example in plants from other colder regions of the world (Körner and Larcher 1988, Lewis Smith 2003, Gielwanowska and Szczuka 2005).

An even greater variation of epidermal cells, mainly on the adaxial side, was visible in plants growing near the sea, within the reach of sea waves. Features opposed to the xerophytic ones could be observed here. In the adaxial epidermis, big, thin-walled bulliform cells, which cause the unfolding of the leaf blade and keep it flat, were found. A significant role in the reaction to salinity stress is assigned to bulliform cells, since they store big amounts of water in large vacuoles (Levitt 1980). Under the influence of salinity stress, osmoprotectants, mainly pinitol accumulate in bulliform cells of *Mesembryanthemum crystallinum*. Pinitol, the same as proline, is an important osmoprotective substance which can be stored in greater amounts by bulliform cells than by mesophyll cells (Paul and Cockburn 1989).

A strong effect of stress factors on the differentiation of tissues was also observed in the structure of vascular bundles. Vascular streaks of *Deschampsia antarctica* were surrounded with two bundle sheaths (Mantovani and Vieira 2000, Alberdi *et al.* 2002, Gielwanowska *et al.* 2005). The outer sheath was built of parenchyma cells with chloroplasts, and the inner, mestome one, small cells with thick, lignified walls (Romero *et al.* 1999). In big cells of the parenchymatic bundle sheath one could see chloroplasts, as it was in the paper presented by Romero *et al.* (1999).

The parenchyma cells of the bundle sheath in plants growing in the coastal habitat, that is frequently flooded with salty water, were very irregular in shape and size. Deformed shapes were also observed within the mestome sheath. These cells were of varied shape and had distinctly thinner cell walls compared with mestome sheath cells of plants from the remaining microhabitats. However, bundle sheaths in plants growing in greenhouse conditions were best-organized. Thus it can be concluded that stress factors disturb the formation of both outer and inner bundle sheath (Gielwanowska *et al.* 2005).

Authors describing disturbances in cell differentiation and growth under the influence of various stress factors emphasise changes in the balance of hormones in tissues. These changes consist in a reduction of the concentrations of growth stimulation hormones and the simultaneous increase of the concentrations of ethylene, jasmonic acid and abscysic acid, that is hormones which slow down growth, disturb lignification of cell walls and accelerate tissue maturation (Levitt 1980, Yamamoto *et al.* 1987).

Cytological and molecular responses of polar vascular plants to environmental factors

Difficult environmental conditions of the Arctic and the Maritime Antarctic are shaped not only by low air temperature, but also by cyclic freezing and melting, humidifying and drying, high intensity of solar radiation in summer, including UV-B, lack of light in winter, salinity and strong winds (Alberdi *et al.* 2002). Studies by Bravo *et al.* (2001) showed that *Deschampsia antarctica* is highly frost-resistant even in summer, and the mechanism of this resistance consists in the tolerance of freezing. This strategy of frost-tolerance is not, as it seems, connected with increased fluidity of cytoplasmic membranes, as the content of unsaturated lipids in membranes of *Deschampsia antarctica* is comparable to other Poaceae (Zúñiga *et al.* 1994). According to Zúñiga *et al.* (1996), a higher content of soluble carbohydrates in tissues than in other grasses is the cause of this species' great frost-tolerance. These sugars can play the role of cryoprotectants. Studies by Piotrowicz-Cieślak *et al.* (2005) show that fructans do not occur here in as great amounts as in other grasses, but mostly sucrose and raffinose abound. A high content of starch was also recorded. Starch, situated primarily in chloroplasts, is an osmotically neutral store of assimilates and, in difficult, stressful situations for the plant, most probably constitutes a reserve of easily accessible energy and is a material for the synthesis of cryoprotectants. *Deschampsia antarctica* has a relatively high photosynthetic index on cold days, which is in line with observations by Xiong *et al.* (1999).

Colobanthus quitensis plants growing in varied microhabitat conditions looked very similar. All of them had succulent properties. The plants growing in the Antarctic had, compared with the ones from the greenhouse, short, distinctly thickened, pillow-like leaves. They were covered with epidermis built of thick-walled cells. The radial epidermal cell walls of *Colobanthus quitensis* were strongly undulated, and outer tangents three times as thick as the remaining ones, all of them being distinctly cutinized. The parts of epidermal cell walls which had contact with air, that is the outer and inner ones bordering with subepidermal intercellular spaces, were most intensely saturated with lipid compounds (most osmophilic, our unpublished data). The fibrillar material in cell walls was very regularly arranged, and between fibrils there were no areas of amorphous material described, for example, in epidermal cell walls of *Brassica napus* L. var. *oleifera* L. under the influence of low temperature (Stefanowska *et al.* 1999).

The epidermis of *Colobanthus quitensis* shoots was covered with a homogeneous cuticular layer as in all vascular plants and some bryophytes. Properties, structure and numerous functions of such epidermal cuticle have been described in detail (Frey-Wyssling and Mühlethaller 1959).

Cutinized cell walls and the layer of cuticle on the surface of epidermis in Antarctic plants separate plant tissues from their environment and give the shoot surface effective protection against transpiration and other factors, including mechanical ones or salty aerosol.

In the nuclei of mesophyll cells of *Colobanthus quitensis*, symmetrically arranged, crystalline protein structures were observed. They were most often located in the equatorial region of the nucleus, close to the inner surface of the nuclear envelope (Gielwanowska 2005). These are most probably stress proteins. It is not known whether they were formed in the nucleus or imported from the cytoplasm. In the extensive literature on the emergence of special proteins in cells under the influence of stress factors, proteins which stabilise chromatin were also described (Velazquez and Linquist 1984). Employing the method of differential centrifugation linked with the use of special mono- and polyclonal antibodies, the role of HSP proteins (heat-shock proteins) was determined (Baszczyński 1986, Porankiewicz and Gwóźdź 1993). HSP68, HSP70 and HSP22-28 were found in the nuclear fraction by means of this method (Brodł 1991). These proteins are very resistant to salt extraction and nuclease treatment. This suggests that they may play some role as structural proteins in the nucleus or beyond it, as nuclear elements of the cytoskeleton (Brodł 1991). The presence of HSP70 and the low molecule HSP were also recorded in the cell nuclei of soya beans (Lin *et al.* 1984) and maize (Cooper and Ho 1987).

Today it is known that the mechanisms controlling resistance to and tolerance of various stress factors, such as drought, salinity, low temperature, atmospheric pollution and invasion of pathogens, are very similar. It is often observed that for instance plants with increased resistance to drought find it also easier to tolerate salinity stress (Levitt 1980).

Environmental conditions and the ultrastructure of organelles

Different stresses exert an effect not only on the modifications of metabolic pathways in plants (Levitt 1980, Ratajczak *et al.* 1994), changes in the activity of many enzymes and the emergence of new molecular forms of these enzymes (Levitt 1980, Ratajczak *et al.* 1994), but also the appearance of differentiating and mature organelles, mostly chloroplasts (Mostowska and Gwóźdź 1995). The above-mentioned authors emphasise that the effect of such environmental factors as high light intensity, UV radiation, herbicides and water or salinity stress induce oxidative stress, which causes, in effect, similar damage to the structure of chloroplasts and their dysfunctions, irrespective of the original environmental factor. Structural

damage and disturbances of functions are similar to the symptoms of chloroplast senescence and are visible mainly in the progressing degradation of thylakoids, excessive accumulation of plastoglobules and starch, photodestruction of pigments and inhibition of photosynthesis (Mostowska and Gwóźdź 1995).

Ultrastructural studies of leaf mesophyll in *Colobanthus quitensis* and *Deschampsia antarctica* showed that their chloroplasts often had an irregular surface. They formed small vesicles, long protrusions and characteristic pockets or invaginations with other organelles inside. The invaginations filled with cytoplasm with numerous small vesicles were described by Giełwanowska and Szczuka (2005). The analysis of electronograms showed that the vesicles formed as a result of convexities of the outer chloroplast membrane.

Pockets in the chloroplasts of *Silene*, *Colobanthus*, *Deschampsia* and *Poa* formed in a similar way, that is after the outer and inner membrane concaved. In the pockets, apart from the surrounding cytoplasm with ribosomes or vesicles, mitochondria were visible. However, no peroxisomes were observed in them. In the chloroplasts of mesophyll cells of pearlwort, numerous, sometimes joining together, pocket-like invaginations could be seen. The greatest number of pocket-like concavities, protrusions and vesicles were observed in the chloroplasts of mesophyll cells of the Antarctic pearlwort from the coastal habitat, which was often flooded with sea water. In the mesophyll of these plants, individual chloroplasts formed a few structures at the same time: long protrusions, concavities and pocket-like invaginations. The transfer of one organelle's products as a substrate to another one could in this way take place, as it seems, at the expense of a smaller energy input connected with transport, since the distance between organelles was maximally reduced. In the plants growing in harsh climatic conditions of the Arctic and the Antarctic, where the energy balance is fragile, such economy can be of considerable significance.

The ultrastructural observations of mesophyll cells of *Cerastium arcticum*, *Colobanthus quitensis*, *Silene involucreta* and *Deschampsia antarctica* showed that mitochondria, vesicles, small vacuoles and peroxisomes tightly adhered to the chloroplast membrane (our unpublished data). Such close contact between organoids, and the special property of chloroplasts consisting in the ability to form uncharacteristic structures (invaginations, vesicles and outgrowths) or pockets retaining mitochondria is most probably an adaptation to the specific Antarctic climatic conditions (Giełwanowska *et al.* 2005, Giełwanowska and Szczuka 2005). These difficult conditions undoubtedly cause *Deschampsia antarctica* and *Colobanthus quitensis* to both use metabolites economically and develop very quickly (Lewis Smith 2003, Romero *et al.* 1999).

On the other hand, however, the close contact between cytoplasmic organelles is known in all plants, not only the ones growing in the Antarctic conditions. The metabolic cooperation of organoids, which takes place for example in the process of photorespiration, is well known, described and discussed in plant

physiology (Köhler *et al.* 1997, Ratajczak *et al.* 1994). The detailed observations of the ultrastructure variation of chloroplasts, mitochondria, peroxisomes and the remaining components of the protoplast of *Cerastium arcticum*, *Colobanthus quitensis*, *Deschampsia antarctica* and *Poa arctica* confirm the close cooperation between these organelles and the intensity of metabolic processes in these plants.

Mitochondria observed in the mesophyll cells of Antarctic hairgrass and pearlwort were often characterized by a greatly expanded system of inner cristae and, at the same time, a large surface area of membranes, where the enzymes of the respiration cycle are located. Apart from such mitochondria, mitochondria with a poorly organized system of inner membranes were observed.

The observed changes in the cytoplasmic organelles were analyzed as responses to abiotic factors of the Arctic and the Antarctic environment. Like all other plants growing in the Arctic and in the Antarctic, plants from Caryophyllaceae and Poaceae, are exposed to various abiotic and biotic stresses, and their reactions are the outcome of their responses to all kinds of environmental factors.

CONCLUSIONS

All specimens of Arctic and Antarctic plants from Caryophyllaceae and Poaceae family show xerophytic features. Histological, cytological and embryological examinations of plants growing under widely differentiated microhabitat conditions on the King George Island (South Shetlands, Maritime Antarctic), on the Spitsbergen and greenhouse conditions indicated high morphological plasticity of *Colobanthus quitensis*, *Cerastium arcticum*, *Silene involucrate*, *Deschampsia antarctica*, *Poa annua* and *Poa arctica*. The size and habitus of plants, as well as the number and size of organs produced over a short vegetation season, showed a close correlation with habitat conditions, such as: air temperature, access to water and nutrients, salinity, exposure to wind and blowing sand.

Histological differentiation, which is an anatomic response to stress factors, was visible within the epidermis, mesophyll, sclerenchyma and bundle sheaths.

Cytoplasmic organelles, such as chloroplasts, mitochondria and peroxisomes in mesophyll cells adhered very tightly to one another in all plant species. The chloroplasts of polar vascular plants had wrinkled surfaces. They formed vesicles surrounded by a single membrane, long chloroplast outgrowths or invaginations with double membranes, filled with cytoplasm with organoids, primarily mitochondria.

Complete chasmogamic and cleistogamic flowers developed in all species. Cleistogamy was induced by low temperatures, high air humidity and strong winds. A small number of microspores differentiated in the microsporangia of the plants examined, which is typical of cleistogamic species. A monospore embryo sac of the Polygonum type differentiated in the tenuinucellar ovule of *Dechampsia antarctica* and *Poa arctica*. A monospore embryo sac of the Polygonum type developed in

the crassinucellar ovule of *Cerastium arcticum*, *Colobanthus quitensis* and *Silene involucrata*. During the differentiation of the embryo sac, there developed nucellar tissue which accumulated reserve substances. Fertile seeds differentiated in all examined species, endospermic in Poaceae and perispermic in Caryophyllaceae.

ACKNOWLEDGEMENT

We are grateful to Dorota Górniak and Maja Lisowska for delivery plants from Spitsbergen.

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