Cell Organelle Structure and Function in Alpine and Polar Plants are Influenced by Growth Conditions and Climate

5

Cornelius Lütz, Paul Bergweiler, Lavinia Di Piazza, and Andreas Holzinger

Abbreviations

ARP   Anti-radical power
AWI   Alfred Wegener institute for polar research
CP   Chloroplast protrusion
Fv/Fm   Photosynthetic optimum quantum yield
LM   Light microscopy
PAR   Photosynthetic active radiation
PS II   Photo system II
SEM   Scanning electron microscopy
TEM   Transmission electron microscopy

5.1 Introduction

The alpine environment requires high flexibility in morphology, anatomy, cell structures and physiology for all biological life forms that grow and propagate there. These demands also characterize most of the polar environments.

Alpine and polar plants have been studied in several ecophysiological and physiological aspects, especially to describe the light- or temperature adaptations of their unique growth environments (Amils et al.

2007; Billings 1974; Crawford 2008; Körner 2003; Larcher 2001; Larcher et al. 2010; Lütz 2010). In comparison to lowland plants or crops, many biochemical processes were found changed, such as intensified metabolic activities or higher antioxidant amounts as a result of the pressure of the local climate and growth conditions – and the short time span for physiological processes, growth and propagation. Cell membranes as places of vital electron transport chains, of biosyntheses and controlling metabolite exchanges between compartments should also show signs of special adaptations. But cellular functions can only be understood when the physiological and structural (mostly membrane) properties of the cells/tissues are known and interpreted in a common view. A combination of modern TEM/SEM analyses, advanced light microscopy (e.g. confocal) and cell metabolic studies therefore provides best chances to approach an understanding of high alpine and polar plant life on a cellular level.

When investigating higher plants from the Arctic, a detailed description of occurring species and plant associations serves as a good basis (e.g. Möller et al. 2001). Other arctic field studies combine systematics, ecology or geobotany (Eurola 1968; Crawford and Balfour 1983; Hadac 1989; Heide 2005; Wielgolaski and Karlsen 2007). Some ecophysiological pieces of research addressed the cold adaptation of plants (Kappen 1983; Wüthrich et al. 1999; Robinson et al. 1999). The fragility of arctic plant covers and examples of their survival strategies have been described by Crawford (1997, 2008) and Oerbaeck et al. (2007). Several of the investigated species are also found in the High Alps, for which numerous

C. Lütz (✉) • L. Di Piazza • A. Holzinger
Institute of Botany, University of Innsbruck, Innsbruck, Austria
e-mail: Cornelius.luetz@uibk.ac.at

P. Bergweiler
German Aerospace Center Project Management Agency, Bonn, Germany

publications offer a great deal of physiological and ultrastructural data.

A short comparison of ecophysiological data collected for alpine vs. arctic plants is presented in Körner (2003) and in Crawford (2008).

Plant biologists working with higher plant stress- or ecophysiology have only limited sources of plants in the Antarctic. In coastal regions, mostly around and on the Antarctic Peninsula and surrounding islands, only two vascular plants have survived for thousands of years: *Deschampsia antarctica* (Gramineae) and *Colobanthus quitensis* (Caryophyllaceae) (Lewis Smith 2003; Mosyakin et al. 2007). Both plant species can also be found in the High Andes, while other high mountain plants have not been able to establish continuous growth in the maritime Antarctic. The two antarctic species obviously reach their limits of distribution when air humidity becomes too low and water access is too limited because of frozen soil, which happens around the polar circle and southwards. In comparison to the Arctic, the latitude of the distribution limit is much lower here, which is determined by the extension of the cold Antarctic continent. Overviews on stress physiology of biota from Antarctica mostly include marine or microbial systems and human impacts on the environment; plants are respected, but limited (Bargagli 2008; Beyer and Bölter 2002; Huiskes et al. 2003). Several plant physiological studies will be mentioned in Paragraphs 3 and 4.

It is a challenge for modern plant (stress-) physiology or ecophysiology to study the cellular and functional properties of plants from both polar regions and to compare them with the considerably well documented plant adaptations in the European Alps. Physiological and cellular studies on high mountain plants from other mountains of the world are limited, yet some sources can be found in Akhalkatsi and Wagner (1997), Crawford (2008), Körner (2003), Schulze et al. (2005), Nagy and Grabherr (2009).

Alpine plant stress research has clearly demonstrated that physiological or cell biological observations made with plants taken from the field have to be correlated with the ambient micro- and macroclimate as well as with soil and general weather conditions. This is addressed in Chaps. 1–3 in this book, or by Larcher and Wagner (2009), Körner (2003), Nagy and Grabherr (2009).

All these environmental influences exaggerate short- or long-term answers or adaptations in the plant body. This makes it more complex and difficult to compare physiological or cell structural results, especially with low land plants or lab experiments. The human experience that high mountain or polar expeditions can be stressful has often influenced the interpretation of data collected for plants from extreme environments as indications of "stress". However, even the growth form of such plants is mostly a stress avoidance mechanism (Crawford 2008; Körner 2003; Larcher 2001), and the physiological adaptation often does not underpin real stress. Therefore, the term "stress" found in interpretations of metabolic data must be used carefully and in the sense of the stress definitions by Larcher (1987) and Lichtenthaler (1996), as discussed in Lütz (2010).

This chapter preferentially describes ultrastructural and cell physiological aspects of extreme environment adaptations mainly in alpine plants, because they are much easier to study than polar plants – from a logistical point of view and due to a well documented history of plant research. Plants from both polar regions are described and compared under cellular and physiological aspects, with a focus on high Arctic species, which allow for greater flexibility in research objects than the two higher plants in Antarctica. We will not go into any detail with scenarios of future effects of global change; merely the influences of short-wave solar irradiation on plants from extreme environments are discussed and compared by Lütz and Seidlitz in Chap. 4 of this book.

5.2 Cell Organelle Structural Adaptations in Alpine and Polar Plants

5.2.1 European Alpine Plants

For many decades the variability of plastid shapes has been described in the literature. In developing tissues, the plastids have often not yet reached their final differentiation and appear amoeboid with great plasticity of the envelope membranes. Newcomb (1967) shows variable shapes of protein storing plastids in bean root tips, and mitochondria located in pockets of young plastids have been described by Devidé and Ljubešić (1989) for onion bulbs. The enormous flexibility of the envelopes was shown in a 3-D reconstruction of leukoplasts in pine secretory cells forming

protrusion-like outgrowths even larger than observed in alpine plant chloroplasts (see below) (Charon et al. 1987). *Funaria* haustorium cells contain long, thin plastid protrusions (Wiencke and Schulz 1975, 1977). The observed variability of plastid outer membranes seems to be similar to the de-differentiating young chloroplasts to leukoplasts in tissue cultures (Sjolund and Weier 1971). Spencer and Wildman (1962) describe a "mobile jacket" surrounding the chloroplasts, which became visible by LM.

Permanent thylakoid-free regions of the active chloroplast were described for the first time in high alpine plants by Lütz and Moser (1977) and Lütz (1987), earlier referred to as "proliferations", but later as "chloroplast protrusions" (CP). Most herbaceous alpine and polar plants develop CP during photosynthetic activity (Holzinger et al. 2007b; Buchner et al. 2007a; Lütz and Engel 2007). A list of over 30 plant species studied by TEM for CP occurrence is given in Lütz (2010). Their ultrastructural appearance together with the respective plant species growing in the Alps or the High Arctic is given in Figs. 5.1, 5.2, 5.5, 5.9 and 5.10.

Plastids can be artificially induced to form similar protrusion-like structures in different ways, as reported for most of the alpine/polar plants: after tobacco mosaic virus infection (Shalla 1964); under osmotic stress after isolation (Spencer and Unt 1965); manganese deficiency (Vesk et al. 1965); water stress in wheat (Freeman and Duysen 1975), high temperature (32°C) stress in rye (Schäfers and Feierabend 1976); exposure of *Funaria* protonemata to lead (4 µM PbCl$_2$, Krzesłowska and Woźny 2002).

Some reports suggest that plastid structural changes can be seen as an adaptation or stress effect to low temperatures. Musser et al. (1984) decreased the temperature from 25°C to 10°C in soybean and described long protrusion-like plastid outgrowths, more similar to stromules. These TEM observations, however, were made after extremely extended glutaraldehyde fixation (48 h!). However, perturbations in chlorophyll fluorescence because of strong pigment decomposition indicate artificial membrane formation. Similarly, under low temperature plus high light, photooxidation may induce some protrusion-like plastid formations in pea and in cucumber plants combined with strong decreases in CO$_2$ fixation (Wise and Naylor 1987). This is a typical response of non-acclimated systems, different from alpine plants. More comparable with alpine and polar plants are observations that lowland plants undergoing cold acclimation do not develop cell organelle structural changes, but increase metabolic activities (Ciamporova and Trginova 1999). However, an important difference to high alpine plants comes from their way of using longer vegetation periods for their metabolism. Kratsch and Wise (2000) compared chilling resistant vs. chilling sensitive species: only the latter group showed several different injuries in the membrane system of the cells.

In response to the environmental parameters, it has been proven that temperature is a critical factor in CP formation (Buchner et al. 2007a, b), and light intensity contributes only marginally. To investigate the effect of temperature on chloroplasts, a special temperature-controlled chamber for the light- and confocal laser scanning microscope was constructed (LM-TCC; Buchner et al. 2007b). During observation, it controls object temperature in a range of −10°C to + 95°C with an accuracy of ±0.1°C in the stationary phase, therefore preventing uncontrolled overheating of the
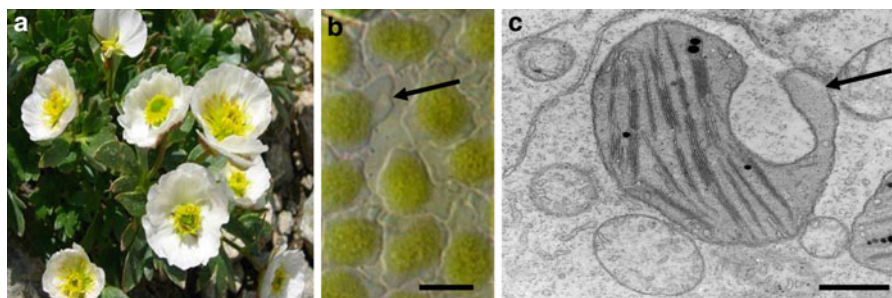
Fig. 5.1 (**a**) *Ranunculus glacialis* (flowering plants). (**b**) DIC image of leaf mesophyll cell showing chloroplasts with protrusions (*arrow*). (**c**) TEM image of chloroplast with protrusion (*arrow*). DIC images were generated by a Zeiss Axiovert 200 light microscope. *Bars*: (**b**) 5 µm, (**c**) 1 µm
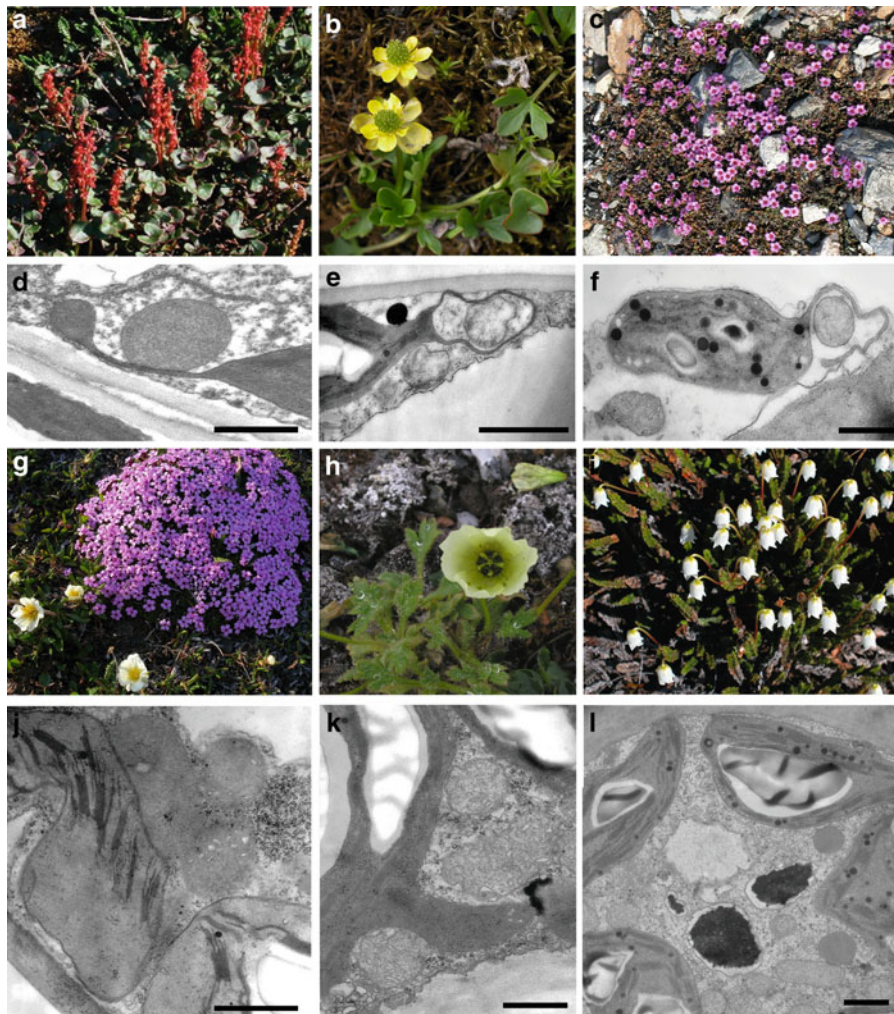
Fig. 5.2 Flowering plants (**a–c**, **g–i**) and corresponding details of leaf cell ultrastructure (**d–f**, **j–l**) of plants from the arctic Ny Alesund. (**a**, **d**) *Oxyria digyna*; (**b**, **e**) *Ranunculus pygmaeus*; (**c**, **f**) *Saxifraga oppositifolia*; (**g**, **j**) *Silene acaulis*; (**h**, **k**) *Papaver dahlianum*; (**i**, **l**) *Cassiope tetragona*. For all images of the ultrastructure: samples were fixed at the field research site in glutaraldehyde, buffered by sodium cacodylate, postfixed in OsO_4 in the same buffer. After dehydration specimens were embedded in epoxy resin. Ultrathin sections were examined under a Zeiss 902 or Libra 120 Transmission electron microscope. *Bars*: 1 μm

samples during microscopy. Using this technique, we were able to follow the formation and shaping of CP such as in *Ranunculus glacialis* and compare this with the long-known TEM appearance (Figs. 5.1 and 5.2, Buchner et al. 2007a). The observed structures are clearly distinct from the thin tubular emergences of the chloroplast termed stromules by Köhler et al. 1997 (for a review, see Kwok and Hanson 2004a).

These stroma-filled, highly dynamic structures may underpin a specific advantage for plants under climatic pressure in that they facilitate metabolite exchange and chloroplast-organelle or chloroplast–chloroplast interaction, as will be explained in the following sections. When considering all of these observations, it is not surprising that plastids are capable of changing the surface and stroma volume according to the physiological demands either of a developmental program or induced by secondary factors. Many alpine and polar plants growing under harsh climate conditions in a limited time of productivity use these properties to ameliorate their energy metabolism.

5.2.1.1 Comparison with the Model Plant *Arabidopsis thaliana*

Stable transformants of *Arabidopsis thaliana*, where the stroma is brightly stained by GFP, gave details of the structural aspects of stromules and chloroplast protrusions and their relation to different temperature scenarios (Holzinger et al. 2007b). Although *A. thaliana* is not an alpine plant, the findings are important in order to understand stromule and CP dynamics. In leaf mesophyll cells of *A. thaliana* plants, stromules with a diameter of about 400–600 nm and a length of up to 20 µm were predominantly observed in cells with spaces between the chloroplasts. They appeared extremely dynamic, occasionally branched or polymorphic. The occurrence of stromules in *A. thaliana* has also been investigated against the background of *arc* mutation (accumulation and replication of chloroplast, Holzinger et al. 2008).

With the above mentioned LM-TCC, a temperature-dependent appearance of chloroplast protrusions was found in *A. thaliana* mesophyll cells (Holzinger et al. 2007b). These structures had a considerably smaller length to diameter ratio than typical stromules and reached lengths of 3–5 µm. At 5–15°C (low temperatures), almost no chloroplast protrusions were observed, but they developed with increasing temperatures. At 35–45°C (high temperatures), numerous chloroplast protrusions with a beak-like appearance extended from a single chloroplast. One can assume that the temperature threshold for CP formation in alpine plants is lower because of the prevailing environmental temperature. Studies on chloroplasts of the cold-adapted plant *R. glacialis* showed a tendency to form stroma-filled protrusions, depending on the exposure temperature in the LM-TCC (Buchner et al. 2007b). The relative number of chloroplasts with protrusions decreased at 5°C when compared to the number at 25°C. This effect was reversible.

The occurrence of chloroplast protrusions may be interpreted as an adaptation to environmental strain, such as cold plus high light values, and the physiological demand for coping with a short vegetation period (Lütz 2010; Lütz and Engel 2007). This is of particular interest, as these plants are generally regarded as being adapted to cold temperatures.

5.2.1.2 Function of the Cytoskeleton in Chloroplast Protrusion Formation: Inhibitor Studies with Alpine *Oxyria digyna*

Most alpine species are characterized by chloroplast structures like CPs, but important questions about their formation remain still open. Are microtubules or microfilaments involved in the development of CPs?

To address how these structures are generated, first detailed investigations of microtubules and actin filaments were undertaken in *O. digyna* (Holzinger et al. 2007a). The aim of this study was to elucidate if chloroplast protrusions are directly dependent on the activity of cytoskeleton components. Leaves from *O. digyna* collected in the Arctic at Svalbard (79°N) and in the Austrian Alps (47°N) were compared at cellular and ultrastructural levels. *O. digyna* plants collected in Svalbard had significantly thicker leaves than the samples collected in the Austrian Alps. This difference was generated by increased thickness of the palisade and spongy mesophyll layers in the arctic plants, while the size of the epidermal cells did not significantly differ in the two habitats.

Arctic-alpine as well as cultivated samples contained CP, 2–5 µm broad and up to 5 µm long. They were positioned in the cells in close spatial contact with other organelles including mitochondria and microbodies (Lütz and Moser 1977; Lütz and Engel 2007), but membrane fusion was never observed. Mitochondria were also present in invaginations of the chloroplasts. A dense network of cortical MTs was found in the mesophyll cells (Fig. 5.3, in addition for *Papaver dahlianum*). However, no direct interactions between MTs and chloroplasts were observed, and disruption of the MT arrays with the anti-MT agent oryzalin at 5–10 µM did not alter the appearance or dynamics of chloroplast protrusions (Holzinger et al. 2007a). These observations suggested that, in contrast to studies on stromule formation in *Nicotiana* (Kwok and Hanson 2004b), MTs were not involved in the formation and morphology of chloroplast protrusions in *Oxyria digyna*.

To address a possible role of actin, the microfibril (MF)-disrupting drug latrunculin B (5–10 µM for 2 h) arrested cytoplasmic streaming and altered the cytoplasmic integrity of mesophyll cells in *O. digyna*.
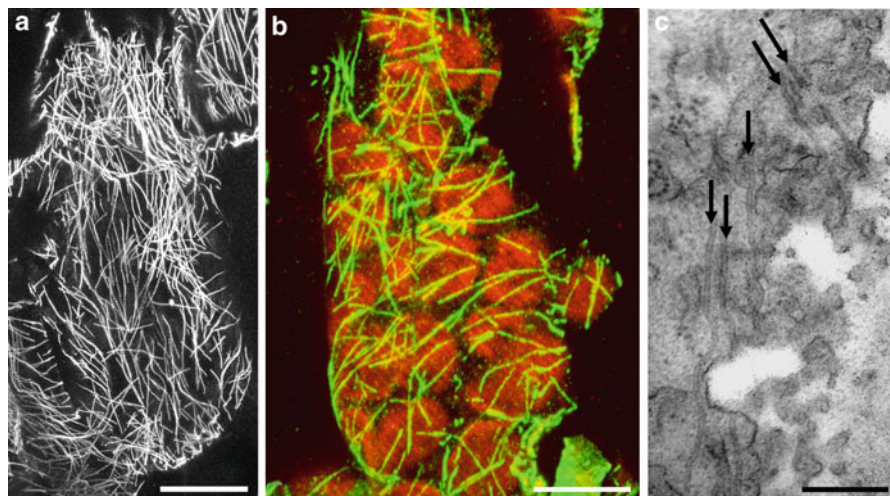
Fig. 5.3 (**a**, **b**) Visualization of microtubules in alpine *Oxyria digyna*; visualized by immunofluorescence with anti-microtu-bule antibody, viewed under a Zeiss confocal microscope with 505–550 nm bandpass filter for the *green channel* and 560 nm longpass filter for the *red channel*. (**c**) TEM image of a mesophyll cell cortical section of high Arctic *Papaver dahlianum* showing numerous microtubules (*arrows*). *Bars*: (**a**) 20 μm, (**b**) 10 μm, (**c**) 200 nm

However, at the ultrastructural level, stroma-containing, thylakoid-free areas (CP) were still visible, mostly at the concave sides of the chloroplasts.

5.2.1.3 Effects of Ethephon

Ethephon is a pesticide often used in agriculture to make fruits and vegetables mature faster, as it regulates the plant growth by releasing ethylene (Worthing 1983; Thomson 1992). Ethephon changes the disassembly of proteins and causes reorientation of microtubules. There are several studies about the effects of herbicides or ethylene on chloroplasts. Shimokawa et al. (1978), Stoynova et al. (1997), Pechová et al. (2003) as well as Paramonova et al. (2004) demonstrate the occurrence of "finger-like protuberances", "protrusions" and "pockets filled with cytoplasm". This means, the shaping of the plastid and envelope structural adaptations were not hindered or even induced in these (non-alpine) samples.

A different approach to follow possible connections of microtubules and CP formation was taken with the application of ethephon to some alpine plants under field conditions. The following species were studied in their natural habitats in alpine regions: *Cerastium uniflorum*, *Homogyne alpina*, *O. digyna*, *Poa alpina*, *R. glacialis*. Three different concentrations of ethephon were applied as intense spraying on the leaves (0.8 mM, 4 mM, 40 mM), while controls were sprayed with water only. On the 5th and on the 14th day, the plants treated with 40 mM ethephon solution were sampled. Leaf sections were viewed with a Zeiss Axiovert 200 microscope. For each sample, ten cells were chosen randomly and the number of chloroplasts counted under DIC conditions. Then the occurrence of CPs in these cells was recorded. In parallel, leaf samples were fixed for TEM.

Both observations, made under the light micro-scope and the TEM, confirmed the presence of CPs in all five species (Fig. 5.4). *R. glacialis* showed the highest percentage (up to 70%) of CPs, while *P. alpina* showed the lowest (approximately 10%). The experi-ment was repeated in intervals throughout the summer, and it was noticed that the number of CPs was higher in early summer then in late summer. Their formation can therefore be modulated by seasonal climatic conditions. Investigation of the ultrastructure showed the typical close association between CPs and other organelles, like mitochondria (Fig. 5.4), even under ethephon treatment.

The observations made in the above cited five plant species support the results of the previous inhibitor
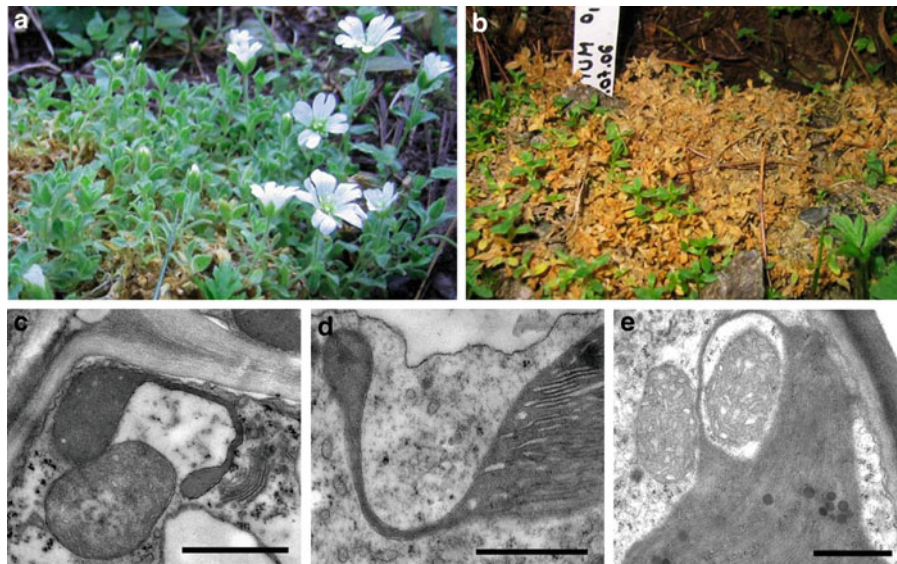
Fig. 5.4 Ethephon treatments of alpine plants. (**a**) *Cerastium uniflorum* control plants. (**b**) Two weeks after 40 mM ethephone treatment in the field. (**c–e**) Ultrastructure of 40 mM ethephone treated plants: (**c**) *C. uniflorum*, (**d**) *H. alpina*, (**e**) *R. glacialis*. *Bars*: 1 μm

studies. It was observed how ethephon damaged the plants (yellow–brown leaves); this is the proof that ethephon was absorbed by the plants. In the case of *O. digyna*, newly formed leaves under higher herbicide treatment were smaller, but still contained plastids with many CP. In green leaves of all treated plants and the control (water spray), the chloroplasts did not cease CP formation. This indicates that microtubules are not involved in their formation.

Finally, we would like to draw attention to a different system of stress adaptation/avoidance as has been found in plants inhabiting tropical high mountain regions, such as at Mt. Kenya or the Andes (Schulze et al. 2005) in altitudes of about 4,000 m a.s.l.. "Summer every day but winter every night" characterizes these climates. Freezing is avoided by delayed supercooling of the plant body, until sunrise provides higher temperatures. Results of photosynthesis and internal plant temperature measurements as well as light microscopy of the leaves have been reported by Beck (1994), but ultrastructural studies are still missing to our knowledge. It would be a challenging task to try leaf sample fixation for TEM studies at the growth sites, comparing possible effects of different in vivo temperatures on cell ultrastructure.

5.2.2 Cell Ultrastructure of Plants from the High Arctic and the Maritime Antarctic

5.2.2.1 Examples from High Arctic Plants

The arctic international research site around Ny Ålesund in North–West Spitsbergen (79°N, Norway) enables a plant stress physiologist or ecophysiologist to perform a broad range of studies and lab experiments close to the growth sites. A variety of climatic and environmental parameters are available for this research settlement (e.g. Wiencke 2004). The arctic environment is characterized by lower irradiation in comparison to the European Alps, but a 24 h daytime. Additionally, rather low temperatures in summer (0°C to ca. 10°C) and air humidity (about 30%) prevail, according to Elberling (2007).

Even UV light does not seem to stress plants in the High Arctic. Detailed studies on vascular plants from Svalbard did not support an impact of increased UV irradiation, because sufficient UV-screening mechanisms are already present in the plants (Caldwell et al. 1998; Nybakken et al. 2004). UV-B, applied additionally in the field for years (experimental setup: C. Gehrke and L.O. Björn, in: Caldwell et al. 1995), did

not drastically change the ecosystem. Apart from some single, but minor effects, mainly litter decomposition and biomass changes in mosses have been described as being influenced. Similarly, Phoenix et al. (2000, 2001) described that this heathland vegetation showed some growth responses under increased UV-B, but that these effects disappeared if interactions with the increased summer precipitation were taken into consideration.

An even stronger impact on arctic biota during the next decades may come from the increase in temperature caused by global warming and increases in CO_2 and methane; an overview relating to Svalbard can be found in Oerbaek et al. (2004) and Elberling (2007). The possible effects of global warming on arctic ecosystems are discussed by Wüthrich et al. (1999) and Robinson et al. (1997) for Svalbard and Abisko. The latter authors clearly point out that ecophysiological studies in the arctic should consider the different climate and soil conditions prevailing in high arctic (Ny-Alesund) and low arctic (Abisko) regions (for the latter site many experiments are published as "arctic" in a general meaning). Arctic ecosystems in particular have a considerable efflux in CO_2 output from the soil (for Svalbard: Thannheiser et al. 1998; Wüthrich et al. 1999; Lloyd 2001), even under the snow, which can be used by small plants such as mosses or several angiosperms, such as *Saxifraga* or *D. octopetala* species. Such important studies on soil–atmosphere connections have been made in different places in the Arctic, including Svalbard (Laurila et al. 2001; Möller et al. 2001). Robberecht and Junttila (1992) studied the cold tolerance mechanisms of cushion plants from Ny Alesund and Tromsoe. Barsig et al. (1998) present one of the rare cytological studies on arctic plants, in this case mosses.

A further good basis for comparisons with alpine plants are the investigations of *D. octopetala* and *B. vivipara* responding to simulated global change carried out by Wookey et al. (1995), and Robinson et al. (1997). A short comparative summary of ecophysiological data collected for alpine vs. arctic plants is given in Körner (2003). However, little remains known about the physiology and ultrastructure of plants from high latitudes.

A comparison of the climate adaptation mechanisms and their amplitude in high arctic and high alpine plants on a cellular level is of scientific interest *per se*: knowledge about the usually occurring range of adaptations to these "extreme" environments is the basis for discussing possible effects of man-made stress on vegetation, like UV-B irradiation or high CO_2.

In this part, we describe some ultrastructural features of higher plants growing in the Kongsfjord area near Ny-Ålesund (Figs. 5.2 and 5.5). Specimens of *Cerastium arcticum*, *Saxifraga oppositifolia*, *P. dahlianum*, *O. digyna*, *D. octopetala*, *Silene acaulis*, *Ranunculus pygmaeus*, *Bistorta vivipara* were collected during several field campaigns (2002–2008) for comparison with alpine species. From the investigated species, seven species showed marked chloroplast protrusions (compare also the list of plants in Lütz 2010, and Fig. 5.2), *R. pygmaeus* shows CPs, occasionally ring-like structures are observed, together with high amounts of starch grains. In *S. acaulis* large and broad chloroplast protrusions have been observed, and the cellular structures are similar to the alpine species. *O. digyna* developed numerous CP, like in plants from alpine origins, frequently seen in association with other organelles like mitochondria. *S. oppositifolia* again
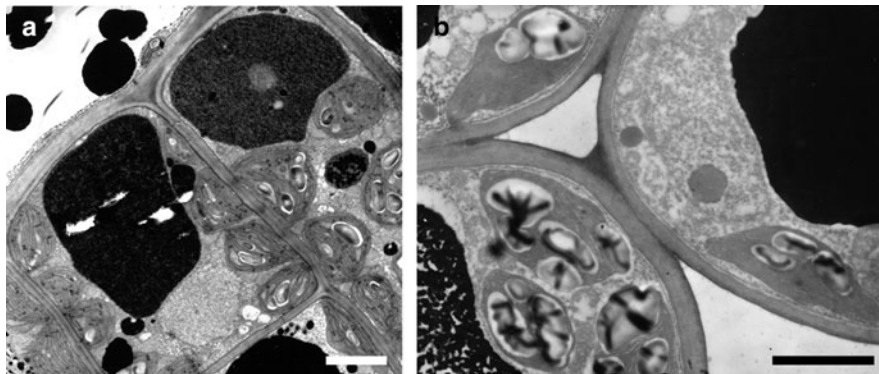
Fig. 5.5 (**a**) Details of the leaf ultrastructure of *Dryas octopetala* collected in Ny Alesund, Spitsbergen. (**b**) Plants collected at 2,200 m a.s.l. in the Northern Limestone Alps. *Bars*: 2.5 μm

possesses ring-shaped chloroplast protrusions. In *C. arcticum* the chloroplast protrusions appear plain and contain small membranous structures, apparently not thylakoids. When sectioned transversally, those membranes are especially obvious. In this species, again close spatial contact with mitochondria is observed. In *P. dahlianum* the amount of microbodies appears to be higher than in the other species. This species is the highest climbing angiosperm in the Svalbard archipelago (up to 1,200 m a.s.l, Rønning 1996). It would be interesting to investigate the special climate adaptation of this species. In *B. vivipara* both ring-shaped and plain CP have been observed, mostly in close proximity to mitochondria. Among the investigated species *D. octopetala* is the only one not containing chloroplast protrusions. In *Dryas*, especially vacuoles appear heavily stained, as in the alpine species from 2,200 m altitude with very similar cell structures (Fig. 5.5). This observation is of interest because *D. octopetala* leaves remain green during winter and as a permanent defence screen have large amounts of anti-radical power, more than herbs with annual leaves (see below, and Lütz 2010; Oppeneiger 2008). This holds also for *C. tetragona* from Svalbard. Remarkable stability shows the thylakoid and grana formation between those growth sites, despite the fact that at 79°N plants are exposed to 24 h of light vs.

the diurnal conditions in the Alps – with much higher average light intensities. All in all there are no clear differences in ultrastructure between plants from the High Arctic, harvested at about sea level, and European alpine plants taken from 2,200 to 3,200 m a.s.l..

5.2.2.2 Cell Structures of Antarctic Phanerogams

Only in some parts of the maritime Antarctic two higher plants survived after isolation of this continent from South America about 30 million years ago (Huiskes et al. 2003; Parnikoza et al. 2007), when living conditions became harder and harder: *Deschampsia antarctica* (Gramineae) and *Colobanthus quitensis* (Caryophyllaceae). Their physiology is described better (see next paragraph) than their cytology. An introduction into ecophysiology of both plants is given by Alberdi et al. (2002), with a characterization of their environment by Beyer and Bölter (2002), Bergstrom et al. (2006) and by Wiencke et al. (2008).

The first ultrastructural studies (TEM) on *D. antarctica* were performed by Gielwanowska and Szczuka (2005), Piotrowicz-Cieślak et al. (2005) and Giełwanowska et al. (2005), and recently for this plant and for *C. quitensis* by Lütz (2010). The general feature of leaf ultrastructure for both plants (Fig. 5.6)
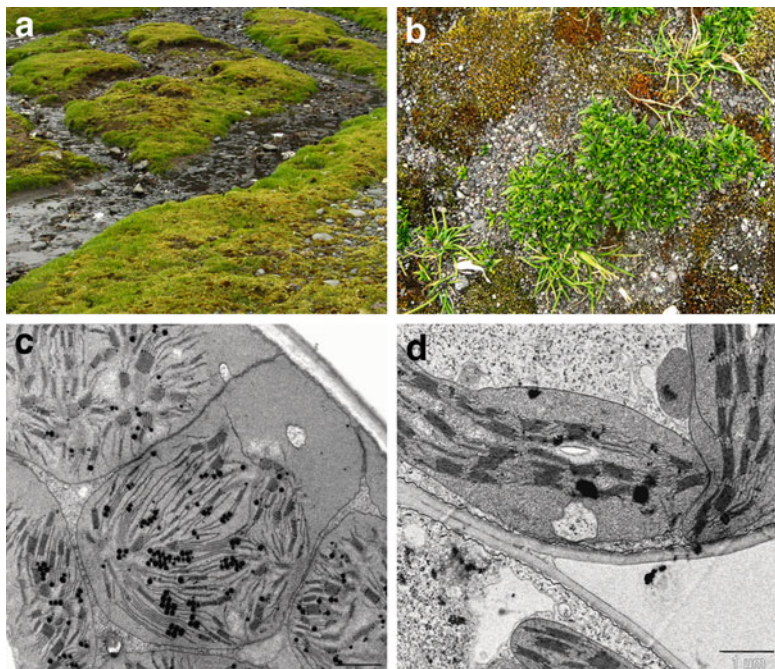
Fig. 5.6 (**a**) Growth site of *Deschampsia antarctica* and *Colobanthus quitensis* near melting water in the coastal tundra of King George Island, maritime Antarctic. (**b**) Colonisation of tundra soil by both plants. (**c**) Ultrastructure of a leaf cell of *Deschampsia*. (**d**) Ultrastructure of leaf cells of *Colobanthus*. *Bars*: 1 μm

points to an active metabolism in the mesophyll cells, but screening of many tissue areas did not indicate any specific visible ultrastructural adaptation to the environment of the maritime Antarctic. The only exception is that plastids in both plants form CP in considerable amounts, as has been described in the previous paragraph or, in summary, by Lütz (2010) for many arctic and high alpine species. Cell organelles and internal cell walls appear very similar to other plants, e.g. to *S. acaulis* (Fig. 5.2), which may be taken instead of *C. quitensis,* as a similar Caryophyllaceae from alpine and arctic sites with a similar cushion growth form.

In *D. antarctica* the occurrence of CP was found to depend on growth conditions: plants stored in an artificial climate chamber environment for several weeks did not form CP in the newly developed leaf tissue (Lütz and Seidlitz, Chap. 4 of this book). This observation corroborates the observation described by Lütz and Engel (2007) with *O. digyna*; this plant did not form CP under moderate temperatures and the longer vegetation period of valley conditions in a botanical garden.

Climatic growth conditions in the maritime Antarctic seem to affect plant metabolism (see below) and cellular ultrastructure in a similar way as do the conditions in the High Alps or the High Arctic.

5.3 Chloroplast Functional Adaptations

Plants growing at the margins of life (Crawford 2008) may have developed extraordinary functional adaptations in the cell organelles, especially in the chloroplast. For many years it has been well described

that high alpine plants are able to run photosynthesis below zero, individual species at down to $-7°C$ (Körner 2003; Larcher and Wagner 1976; Larcher 1977; Larcher et al. 2010; Moser 1970; Moser et al. 1977). Furthermore, a broad amplitude in light intensity usage has been reported for species such as *R. glacialis* and *C. alpinum* (Bergweiler 1987; Moser et al. 1977). These range from no photoinhibition in full sunlight at 3,180 m altitude to survival for months under a snow cover or clouds, with energy uptake remaining active all the same, as was shown for snow covered *S. alpina*.

Similarly, several arctic species efficiently photosynthesize even at lower average temperatures (Jones and Demmers-Derks 1999) compared to their alpine counterparts, where leaf temperatures in the field can easily reach 30°C or more (Larcher and Wagner 1976, Larcher, Chap. 3 of this book; Neuner and Buchner, Chap. 6 of this book).

Our own assays of photosynthetic oxygen production (polarographic method, as is described in Bergweiler (1987), Lehner and Lütz (2003), Lütz (1996)) was performed in plants taken from the field to the research station or the institute.

Figure 5.7 compares the photosynthetic oxygen production (net photosynthesis) as dependent on light intensity for several high arctic plants and the two antarctic species. It is remarkable that no species became photoinhibited over the period of about 1 h required to complete the whole run from dark adaptation to the highest intensities. The antarctic species are within a similar range as the arctic plants. *D. octopetala* shows less activity, which is not a matter of relation to fresh weight, but remains similar if leaf

Fig. 5.7 Light-dependent net photosynthesis per fresh weight of plants from Svalbard (*Bistorta vivipara, Oxyria digyna, Dryas octopetala, Ranunculus pygmäus, Cerastium arcticum*) and from the maritime Antarctic (*Colobanthus quitensis, Deschampsia antarctica*). Measuring temperature: 15°C. Samples were taken directly from field-grown plants
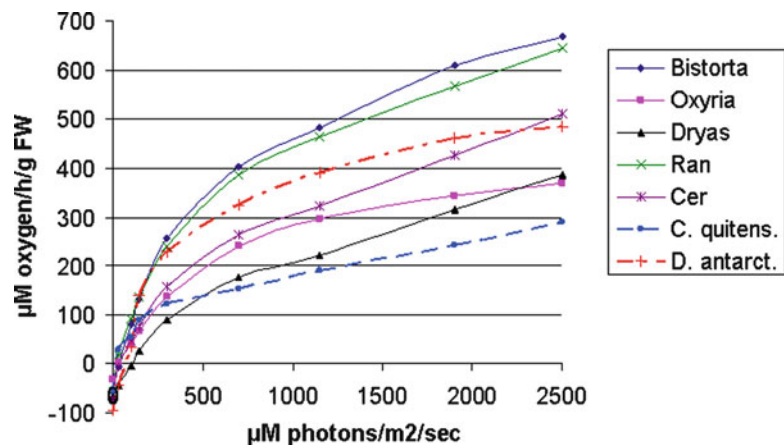
surface is taken as reference. *D. octopetala* is the only shrub in this collection, and it has leaves that remain green during winter. In general, plants with long-living leaves show somewhat slower photosynthetic activities, but can keep up this process much longer than most herbaceous plants (own observations). In contrast to our observations, Jones and Demmers-Derks (1999) measured down-regulation of photosynthesis at higher light intensities in *D. octopetala* and *B. vivipara* sampled near Ny-Alesund, Svalbard. The results, however, were generated only by leaf fluorescence measurements. This downregulation should avoid damage to photosynthesis.

The resistance of activity even under much higher light intensities than usually experienced at their growth sites (approx. 800 µMol photons at 79°N, NW-Svalbard; and approx. 1,000 µMol photons at the maritime Antarctic, 62°S, own measurements and Wiencke et al. (2008)) points to genetically preserved photosynthetic adaptation to withstand light stress. Both antarctic plants, *D. antarctica* and *C. quitensis*, still grow in the High Andes, as ecotypes adapted to strong irradiation (Casanova-Katny et al. 2006), much stronger also in the UV region of the spectrum. The arctic species either grow in the high Alps, in the form of same species, or are closely related to alpine species (like *R. pygmaeus* and *C. arcticum*). The measured activities are in the same range as Moser (1970), Moser et al. (1977) and Bergweiler (1987) reported for several high alpine plants, not inhibited until 2,200 µM photons. Obviously evolution has prepared

plants growing in "extreme" environments with a broad plasticity of photosynthetic fitness, which guarantees survival.

The measurements do not provide a difference between the plants growing under permanent light for months (high Arctic) or long-day conditions like on King George Island (maritime Antarctic).

For insight into whether arctic plants keep a diurnal rhythm under continuous daylight in photosynthesis, fluorescence measurements of photosystem II were been performed from 9 a.m. in the morning until about 3 p.m. at "night" (Fig. 5.8a). The recorded Fv/Fm values are all in the positive activity range between 0.7 and 0.8, when determined in the leaves under outdoor conditions. This observation is different from European lowland species, where a noon depression of the values is found under high light values, which recovers during the night. These data point to a perfect acclimation of photosynthesis to the cold environment. The photosynthetic pigments of some alpine and arctic plants have been compared by Lütz and Holzinger (2004). Despite the differences in the light regimes during the vegetation period, the main pigments of the thylakoids show similar amounts, only accumulation of the xanthophyll cycle pigments was slightly higher in the alpine species – an influence of the higher PAR exposure above timberline compared to near sea level growth sites in the High Arctic.

This long-term physiological stability may also be based on a good radical defence power (ARP), because all electron-bound processes, especially in
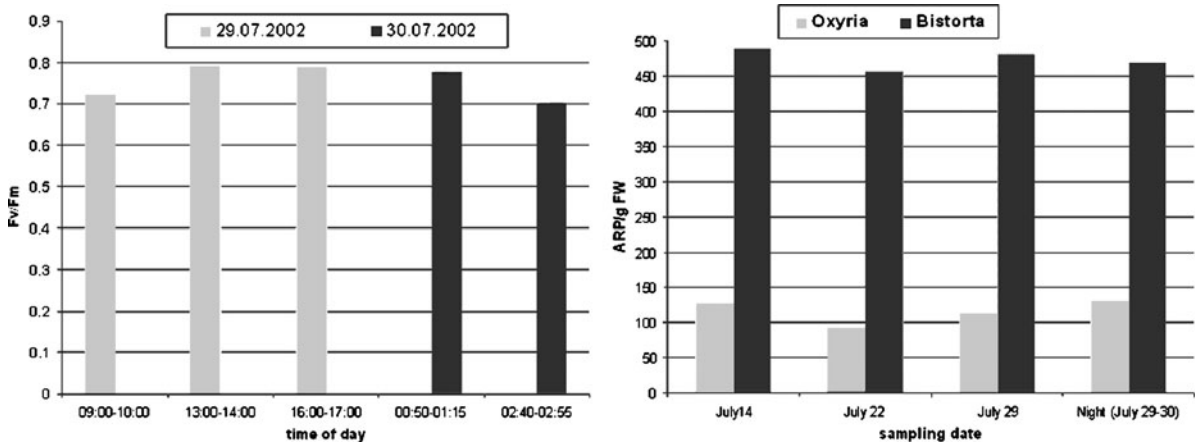
Fig. 5.8 (**a**) Photosystem II activity in leaves of *Dryas octopetala* from Svalbard. The Fv/Fm data were recorded for field-grown leaves, also during the "sunny midnight" conditions.

Means of ten assays per time. (**b**) Status of ARP in *Oxyria digyna* and in *Bistorta vivipara* over 2 weeks and during a polar "night". Daytime samples were taken between 10 a.m. and 12 o'clock

photosynthesis, can form radicals. The main ARP-active compounds in higher plants are flavonoids, ascorbic acid, glutathione, ß-carotene and tocopherols (Wildi and Lütz 1996). A survey of ARP values from plants in polar and alpine regions is given by Lütz (2010). The highest values were measured in plants with overwintering leaves, such as *D. octopetala* or *C. tetragona*, and the lowest in *O. digyna*. A more detailed view of two high arctic plants' ARP, sampled according to the timetable as for the Fv/Fm measurements, is shown in Fig. 5.8b. While *O. digyna* contains drastically less ARP activity compared with *B. vivipara*, there is no remarkable change over a period of 2 weeks, and also none in the polar "night". The small depression at "July 22" is due to rainy weather during sampling, when plants did not require much ARP.

The stability of photosynthesis under conditions of high light and cold values can additionally be explained by the activity of plastid-located alternative oxidase (PTOX) (Streb et al. 2005). *R. glacialis* contains this protein in large amounts. It dissipates excess electrons, if photorespiration is blocked or disturbed. The positive role of photorespiration as a valve in energy metabolism under extreme climate conditions has been discussed in more detail by Lütz and Engel (2007) and by Lütz (2010).

Several experiments addressed the photosynthetic performance of both antarctic phanerogams. Chlorophyll fluorescence as a measure for PS II activity and CO_2 fixation experiments were conducted by Xiong et al. (1999) in the field for both plants; they showed a broad range of activity from about $-3°C$ as low temperature compensation point to about $26°C$ as high temperature compensation point. Other authors studied photosynthesis with plants collected in the maritime Antarctic after transferring them to their home laboratory (Bravo et al. 2007; Edwards and Smith 1988; Montiel et al. 1999; Perez-Torres et al. 2007). These data match measurements for alpine plants (see above) and can be explained by the High Andean origin of the species. The low temperature resistance (Bravo et al. 2001, 2007; Bravo and Griffiths 2005) may indicate well developed cellular protection mechanisms like dehydrin formation (Olave-Concha et al. 2005) together with carbohydrate accumulation (Piotrowicz-Cieslak et al. 2005; Zuniga-Feest et al. 2005). The high light photostability, as shown by Peres-Torres et al. (2004a, b, 2007), can also be explained by the genetic history of the plants, which

maintained this means of protection under the much lower light intensities present at about sea level and higher latitude of the growth sites compared to Andean growth sites. Therefore, we can conclude that polar plants with their counterparts in high mountain regions (see Crawford 2008; Körner 2003 and Larcher 2001 for an overview), have developed a broad range of stress adaptation to high light values, as is demonstrated in the comparison shown in Fig. 5.7, and also to short-wave irradiation, as is discussed in Chap. 4 of this book.

5.4 Developmental Aspects

Spring time is a critical growth period for herbaceous plants. Young, developing tissues need protection against high PAR and UV irradiation; the young meristematic plant parts contain relatively more water, which, if not adapted, may freeze during frequent cold events.

The development of chloroplasts is retarded until average tissue temperatures allow for enzyme and transport activities without blockage. Otherwise the biophysical processes of light energy being transformed into chemical energy, which are largely temperature-independent, will continuously produce electrons which accumulate and soon exceed the antioxidant capacity of the cells. Bleaching and destruction follows, as has been described for sudden summer snow situations in alpine plants (Lütz 1996). Most alpine plants therefore retard greening until growth conditions allow for more or less undisturbed metabolism (Körner and Larcher 1988), because the last enzyme in chlorophyll synthesis, the chlorophyll synthetase complex, is inhibited at temperatures lower than approx. $10°C$ (Blank-Huber 1986). Hence, young light-exposed leaves of alpine, but also of spring vegetation at lower altitudes remain yellow-greenish until temperatures rise. Deep yellow leaves of young developing *Taraxacum alpinum* from sites at 2,500 m contain etioplasts when taken out from an approx. 20 cm snow cover, which allows for penetration of sufficient light for greening under normal temperatures (Fig. 5.9 and Bergweiler 1987), but the inner structures are still small prolamellar bodies (as typical for dark grown plants, Lütz 1981, 1986). Near to the snow, and fully sun-exposed, etio-chloroplasts indicate an arrested intermediate stage, with
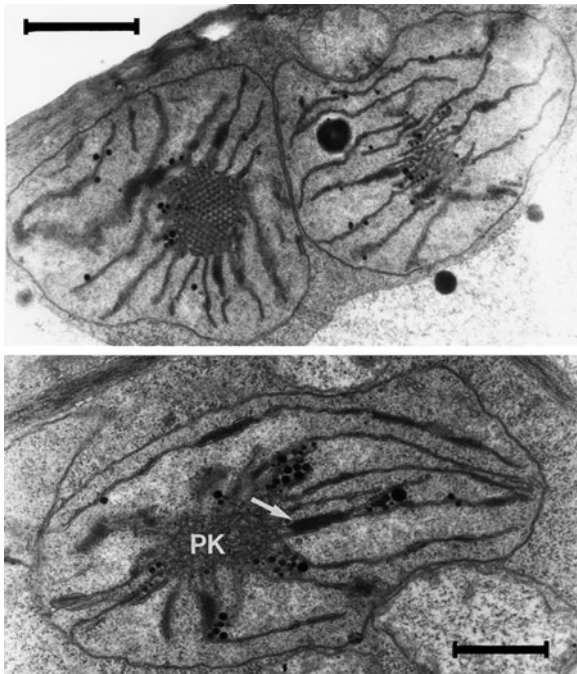
Fig. 5.9 Ultrastructure of plastids from yellow leaves of *Taraxacum alpinum* (2,500 m altitude) taken directly out of a 20 cm snow cover (*top*), and from yellow-greenish leaves exposed to the sun near a snowfield for min. 2 days (*bottom*). *Arrow*: first small grana formation, *PK* prolamellar body, disintegrating. *Bar*: 1 μm


Fig. 5.10 Chloroplasts from *Soldanella alpina* leaves, which were snow-free for about 2 weeks before sampling (*top*), and taken out of a 30 cm snow cover of the last winter (*bottom*). *Bar*: 1 μm

disintegrating prolamellar bodies as well as first thylakoids, occasionally with small grana (Fig. 5.9).

Lütz (1996) reported a similar development for the alpine *Eriophorum angustifolium*, including pigment assays and photosynthesis comparisons between the yellow, greening and green parts of the leaves. Some herbaceous plants (not shrubs) keep their green leaves over winter, like *S. alpina*. Bergweiler (1987) compared the ultrastructure of green leaves taken out from ca. 30 cm of snow in springtime with those exposed to full sunlight for several days (Fig. 5.10). No obvious difference can be found despite the snow cover being from the last winter.

The light penetration of the snow (data in Lütz (1996)) seems enough for these leaves to maintain full structural competence, the chlorophyll contents are similar (Bergweiler 1987). In the same study, the chlorophyll protein complex composition found in alpine plants was described for the first time (Fig. 5.11a). As expected from TEM, there is no striking difference between the composition of

subunits resolved from both photosystems or the light harvesting complex subunits. Merely light harvesting complex I seems to be enriched – an indication of the lower light intensity under snow. This analysis was extended to other high alpine species (light-exposed), and again the samples from *R. glacialis, O. digyna, T. alpinum* and *P. alpina* (Fig. 5.11b) developed very similar chlorophyll protein complex compositions in the leaves. Isolated thylakoids of *S. alpina* were measured for electron transport rates, and the rates measured at 21°C did not differ markedly. On the other hand, measurements of oxygen development in *S. alpina* intact leaves revealed a four times higher activity at 20°C in the sun-exposed samples vs. the samples taken from the snow cover (Bergweiler 1987). This points to a physiological rather than a membrane-structural adaptation in the leaf cells: the complex architecture of a membrane seems to be sufficiently prepared for a range of temperature/light changes.
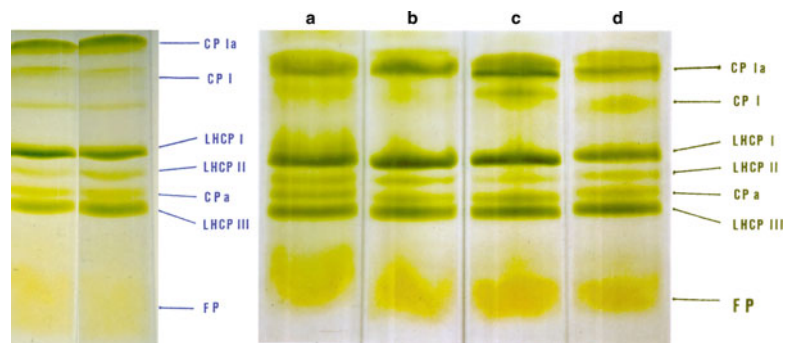
Fig. 5.11 (**a**) Chlorophyll protein complex separation of leaves from *S. alpina* samples. *Left*: leaves sun-exposed for about 2 weeks, *right*: leaves taken out of 30 cm of old snow cover. (**b**) Chlorophyll protein complex separations of leaves from (**a**) *Ranunculus glacialis,* (**b**) *Oxyria digyna,* (**c**) *Tanacetum alpinum,* (**d**) *Poa alpina. CP I, CP Ia* complexes derived from photosystem I, *LHCP I, II, III* subunits of the light harvesting complex, *CP a* reaction center of photosystem II, *FP* free pigment. Separation methods followed Anderson et al. (1978) and Argyroudi-Akoyunoglou and Akoyunoglou (1979) with a mild detergent mixture according to Sarvari et al. (1984)

Spring aspects of alpine plants, which overwinter with green leaves, have further been discussed by Lehner and Lütz (2003) and Lütz et al. (2005).

Therefore, we can assume that the photosynthetic apparatus is constructed relatively conservatively, in a way that, irrespective of the species, best adaptation is guaranteed by more or less identical principles of membrane construction and metabolic performance.

5.5 Concluding Remarks

The large number of different "stressors" in alpine environments has resulted in a broad spectrum of different life forms. There is no "dominant type" of acclimation as a general solution, but a surprisingly large variety of strategies to cope with the environmental pressure and the short vegetation period. This generalization also characterizes the polar higher plants.

In comparison to the well-documented lowland plants of temperate regions, plants from extremely cold environments use a larger span of temperatures, where photosynthesis, respiration and most enzymes work, and adaptation to low as well as to high light intensities. An additional strategy in many alpine/polar species is a result of enlargement of the plastid outer membrane surface for faster and more efficient molecule transport combined with an increase in stroma volume, visible as chloroplast protrusions described herein and in related articles (Lütz 2010). The plastids are often found close to mitochondria and

peroxisomes, indicating photorespiration as a valve for energy surplus under light/cold stress conditions. On the other hand, when comparing leaf cell ultrastructures of herbs and trees from lowland, high mountains or polar sites, all other cell organelles and membranes are indistinguishable between the plants. Evolution has obviously constructed mesophyll cells in a way to cope with most environmental pressures without the need for complex reconstruction of cellular architectures when climate conditions change. Cell organelle structures seem to be more conservative than functional processes.

Alpine and polar climates may induce a spectrum of radicals in the cells, but measurements have shown that the plants are well equipped with anti-radical activities. These protective mechanisms work on different physiological levels in the cells and tissues; plants with perennial leaves have by far the highest ARP-activities. Measurements of such plants taken from the snow showed that their high ARP may also protect them against biotic attack during the winter. Winter-green leaves are ready to start their metabolism even under a shallow snow cover: this elegant adaptation leads to an extension of the short vegetation period.

Cell physiology and metabolic activities have been found to be more intense in plants from extreme ecosystems than in those growing under temperate conditions. Therefore, alpine and polar plants can be used as excellent study objects to understand the adaptation to multiple so-called stressors on plants such as high and low (UV-) light intensities, contrasting

temperatures, retarded mineral turnover in the soil, mechanical inputs from storm and ice, pathogen attacks under long snow cover etc.. This opportunity should be taken particularly by cell physiology, plant molecular biology and "metabolomics". A long tradition of research in ecophysiology, geobotany, systematics, soil ecology, climate research and in the growing field of plant stress research has laid a useful and indispensable basis for these modern research fields. And one should not forget:

Organisms in alpine and polar climates are not designed to maximize any kind of yield, but to stabilize at least the minimum life functions for continuous survival of a species (Larcher 1987).

References

Akhalkatsi M, Wagner J (1997) Comparative embryology of three *Gentianaceae* species from the Central Caucasus and the European Alps. Plant Syst Evol 204:39–48

Alberdi M, Bravo LA, Gutiérrez A, Gidekel M, Corcuera LJ (2002) Ecophysiology of Antarctic vascular plants. Physiol Plant 115:479–486

Amils R, Ellis-Evans C, Hinghofer-Szalkay H (eds) (2007) Life in extreme environments. Springer, Dordrecht

Anderson JM, Waldron JC, Thorne SW (1978) Chlorophyll protein compexes of spinach and barley thylakoids. Spectral characteristics of six complexes resolved by an improved electrophoretic procedure. FEBS Lett 92(2):227–233

Argyroudi-Akoyunoglou JH, Akoyunoglou G (1979) The chlorophyll protein complexes of the thylakoids in greening plastids of *Phaseolus vulgaris*. FEBS Lett 104(1):78–84

Bargagli R (2008) Antarctic ecosystems. Environmental contamination, climate change, and human impact, vol 175, Ecological studies. Springer, Berlin

Barsig M, Gehrke C, Schneider K (1998) Effects of ultraviolet-B radiation on leaf ultrastructure, carbohydrates and pigmentation in the moss *Polytrichum commune* in the subarctic. Bryologist 101:357–365

Beck E (1994) Cold tolerance in tropical alpine plants. In: Rundel PW, Smith AP, Meinzer FC (eds) Tropical alpine environments. Plant form and function. Cambridge University Press, Cambridge, pp 77–110

Bergstrom D, Convey P, Huiskes A (2006) Trends in Antarctic terrestrial and limnetic ecosystems. Springer, Dordrecht

Bergweiler P (1987) Charakterisierung von Bau und Funktion der Photosynthese-Membranen ausgewählter Pflanzen unter den Extrembedingungen des Hochgebirges. Ph.D. thesis, University of Köln

Beyer L, Bölter M (2002) Geoecology of Antarctic ice-free coastal landscapes, vol 154, Ecological studies. Springer, Berlin/Heidelberg

Billings WD (1974) Adaptations and origins of alpine plants. Arct Alp Res 6(2):129–142

Blank-Huber M (1986) Untersuchungen zur Chlorophyll Biosynthese. Solubilisierung und Eigenschaften der Chlorophyll-Synthetase. Ph.D. thesis, University of Munich

Bravo LA, Griffith M (2005) Characterization of antifreeze activity in Antarctic plants. J Exp Bot 56(414):1189–1196

Bravo LA, Ulloa N, Zuñiga GE, Casanova A, Corcuera LJ, Alberdi M (2001) Cold resistance in Antarctic angiosperms. Physiol Plant 111:55–65

Bravo LA, Saavedra-Mella FA, Vera F, Guerra A, Cavieres LA, Ivanov AG, Huner NPA, Corcuera LJ (2007) Effect of cold acclimation on the photosynthetic performance of two ecotypes of *Colobanthus quitensis* (Kunth.) Bartl. J Exp Bot 58(13):3581–3590

Buchner O, Holzinger A, Lütz C (2007a) Effects of temperature and light on the formation of chloroplast protrusions in leaf mesophyll cells of high alpine plants. Plant Cell Environ 30:1347–1356

Buchner O, Lütz C, Holzinger A (2007b) Design and construction of a new temperature-controlled chamber for light and confocal microscopy under monitored conditions: biological applications for plant samples. J Microsc 225 (2):183–191

Caldwell MM, Teramura AH, Tevini M, Bornman JF, Björn LO, Kulandaivelu G (1995) Effects of increased solar ultraviolet radiation on terrestrial plants. Ambio 24:166–173

Caldwell MM, Björn LO, Bornman JF, Flint SD, Kulandaivelu G, Teramura AH, Tevini M (1998) Effects of increased solar ultraviolet radiation on terrestrial ecosystems. J Photochem Photobiol 46(1–3):40–52

Casanova-Katny MA, Bravo LA, Molina-Montenegro M, Corcuera LJ, Cavieres LA (2006) Photosynthetic performance of *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) in a high-elevation site of the Andes of central Chile. Rev Chil Hist Nat 79:41–53

Charon J, Launay J, Carde J-P (1987) Spatial organization and volume density of leucoplasts in pine secretory cells. Protoplasma 138:45–53

Ciamporova M, Trginova I (1999) Modifications of plant cell ultrastructure accompanying metabolic responses to low temperatures. Biol Bratisl 54(4):349–360

Crawford RMM (1997) Habitat fragility as an aid to long-term survival in arctic vegetation. In: Woodin SJ, Marquiss M (eds) Ecology of Arctic environments. Blackwell, Oxford, pp 113–136. ISBN 0-632-04218-4

Crawford RMM (2008) Plants at the margin. Ecological limits and climate change. Cambridge University Press, Cambridge

Crawford RMM, Balfour J (1983) Female predominant sex ratios and physiological differentiation in arctic willows. J Ecol 71:149–160

Devidé Z, Ljubešić N (1989) Plastid transformation in greening scales of the onion bulb (*Allium cepa, Alliaceae*). Plant Syst Evol 165:85–89

Edwards JA, Lewis Smith RI (1988) Photosynthesis and respiration of *Colobanthus quitensis* and *Deschampsia antarctica* from the maritime Antarctic. Br Antarct Surv Bull 81:43–63

Elberling B (2007) Annual soil CO_2 effluxes in the High Arctic: the role of snow thickness and vegetation type. Soil Biol Biochem 39:646–654

Eurola S (1968) Über die Feldheidevegetation in den Gebirgen von Isfjorden und Hornsund in Westspitzbergen. Aquilo. Botanica 7:1–56

Freeman TP, Duysen ME (1975) The effect of imposed water stress on the development and ultrastructure of wheat chloroplasts. Protoplasma 83:131–145

Gielwanowska I, Szczuka E (2005) New ultrastructural features of organelles in leaf cells of *Deschampsia antarctica* Desv. Polar Biol 28:951–955

Giełwanowska I, Szczuka E, Bednara J, Górecki R (2005) Anatomical features and ultrastructure of *Deschampsia antarctica* (Poaceae) leaves from different growing habitats. Ann Bot 96:1109–1119

Hadac E (1989) Notes on plant communities of Spitsbergen. Folia Geobot Phytotax 24:131–169

Heide OM (2005) Ecotypic variation among European arctic and alpine populations of *Oxyria digyna*. Arct Antarct Alp Res 37(2):233–238

Holzinger A, Wasteneys G, Lütz C (2007a) Investigating cytoskeletal function in chloroplast protrusion formation in the arctic-alpine plant *Oxyria digyna*. Plant Biol 9:400–410

Holzinger A, Buchner O, Lütz C, Hanson MR (2007b) Temperature-sensitive formation of chloroplast protrusions and stromules in mesophyll cells of *Arabidopsis thaliana*. Protoplasma 230:23–30

Holzinger A, Kwok EY, Hanson MR (2008) Effects of *arc3*, *arc5* and *arc6* mutations on plastid morphology and stromule formation in green and nongreen tissues of *Arabidopsis thaliana*. Photochem Photobiol 84:1324–1335

Huiskes AHL, Gieskes WWC, Rozema J, Schorno RML, van der Vies SM, Wolff WJ (2003) Antarctic biology in a global context. Backhuys, Leiden. ISBN 90-5782-079-X

Jones HG, Demmers-Derks HHWM (1999) Photoinhibition as a factor in altitudinal for latitudinal limits of species. Phyton 39(4):91–98

Kappen L (1983) Anpassungen von Pflanzen an kalte Extremstandorte. Ber Deutsch Bot Ges 96:87–101

Köhler RH, Cao J, Zipfel W, Webb WW, Hanson MR (1997) Exchange of protein molecules through connections between higher plant plastids. Science 276:2039–2042

Körner C (2003) Alpine plant life, 2nd edn. Springer, Berlin

Körner C, Larcher W (1988) Plant life in cold climates. Symp Soc Exp Biol 42:25–57

Kratsch HA, Wise RR (2000) The ultrastructure of chilling stress. Plant Cell Environ 23:337–350

Krzesłowska M, Woźny A (2002) Why chloroplasts in apical cell of *Funaria hygrometrica* protonemata treated with lead are distributed in different way than in control. Biol Plant 45 (1):99–104

Kwok EY, Hanson MR (2004a) Stromules and the dynamic nature of plastid morphology. J Microsc 214:124–137

Kwok EY, Hanson MR (2004b) In vivo analysis of interactions between GFP-labeled microfilaments and plastid stromules. BMC Plant Biol 10:2

Larcher W (1977) Ergebnisse des IBP-Projektes "Zwergstrauchheide Patscherkofel"Sitzungsber. Österr. Akad. Wiss. Abt I, Vol 186. Springer Berlin

Larcher W (1987) Streß bei Pflanzen. Naturwissenschaften 74:158–167

Larcher W (2001) Ökophysiologie der Pflanzen, 6th edn. Ulmer, Stuttgart

Larcher W, Wagner J (1976) Temperaturgrenzen der CO_2-Aufnahme und Temperaturresistenz der Blätter von Gebirgspflanzen im vegetationsaktiven Zustand. Oecol Plant 11:361–374

Larcher W, Wagner J (2009) High mountain bioclimate: temperatures near the ground recorded from the timberline to the nival zone in the Central Alps. Contrib Nat Hist 12:857–874

Larcher W, Kainmüller C, Wagner J (2010) Survival types of high mountain plants under extreme temperatures. Flora 205:3–18

Laurila T, Soegaard H, Lloyd CR, Aurela M, Tuovinen JP, Nordstroem C (2001) Seasonal variations of net CO_2 exchange in European Arctic ecosystems. Theor Appl Climatol 70:183–201

Lehner G, Lütz C (2003) Photosynthetic functions of cembran pines and dwarf pines during winter at timberline as regulated by different temperatures, snowcover and light. J Plant Physiol 160:153–166

Lewis Smith R, Lewis Smith RI (2003) The enigma of *Colobanthus quitensis* and *Deschampsia antarctica* in Antarctica. In: Huiskes AHL, Gieskes WWC, Rozema J, Schorno RML, van der Vies SM, Wolff WJ (eds) Antarctic biology in a global context. Backhuys, Leiden. ISBN 90-5782-079-X

Lichtenthaler HK (1996) Vegetation stress: an introduction to the stress concept in plants. J Plant Physiol 148:4–14

Lloyd CR (2001) On the physical controls of the carbon dioxide balance at a high arctic site in Svalbard. Theor Appl Climatol 70:167–182

Lütz C (1981) On the significance of prolamellar bodies in membrane development of etioplasts. Protoplasma 108:99–115

Lütz C (1986) Prolamellar bodies. Review article in: "photosynthetic membranes," section lipids. In: Arntzen C, Staehelin A (eds) Encyclopedia of plant physiology, vol 19. Springer, Berlin, pp 683–692

Lütz C (1987) Cytology of high alpine plants II. Microbody activity in leaves of *Ranunculus glacialis* L. Cytologia 52:679–686

Lütz C (1996) Avoidance of photoinhibition and examples of photodestruction in high alpine *Eriophorum*. J Plant Physiol 148:120–128

Lütz C (2010) Cell physiology of plants growing in cold environments. Protoplasma 244:53–73 (Review)

Lütz C, Engel L (2007) Changes in chloroplast ultrastructure in some high alpine plants: adaptation to metabolic demands and climate? Protoplasma 231:183–192

Lütz C, Holzinger A (2004) A comparative analysis of photosynthetic pigments and tocopherol of some arctic-alpine plants from the Kongsfjord area, Spitzbergen, Norway. In: Wiencke Ch (ed) Reports on polar research, vol 492. AWI, Bremerhaven, pp 114–122, 1618-3193

Lütz C, Moser W (1977) Beiträge zur Cytologie hochalpiner Pflanzen. I. Untersuchungen zur Ultrastruktur von Ranunculus glacialis L. Flora 166:21–34

Lütz C, Schönauer E, Neuner G (2005) Physiological adaptation before and after snow melt in green overwintering leaves of some alpine plants. Phyton 45:139–156

Möller I, Wüthrich Ch, Thannheiser D (2001) Changes of plant community patterns, phytomass and carbon balance in a high arctic tundra ecosystem under a climate of increasing cloudiness. Biomonitoring 35:225–242

Montiel P, Smith A, Keiller D (1999) Photosynthetic responses of selected Antarctic plants to solar radiation in the southern maritime Antarctic. Polar Res 18(2):229–235

Moser W (1970) Ökophysiologische Unersuchungen an Nivalpflanzen. Mittl Ostalp-din. Ges f Vegetkde 11:121–134

Moser W, Brzoska W, Zachhuber K, Larcher W (1977) Ergebnisse des IBP-Projekts "Hoher Nebelkogel 3184 m". Sitzungsber Österr Akad Wiss. Math-naturw Kl Abt 1 186:387–419

Mosyakin SL, Bezusko LG, Mosyakin AS (2007) Origins of native vascular plants of Antarctica: comments from a historical phytogeography viewpoint. Cytol Genet 41(5):308–316

Musser RL, Thomas SA, Wise RR, Peeler TC, Naylor AW (1984) Chloroplast ultrastructure, chlorophyll fluorescence, and pigment composition in chilling-stressed soybeans. Plant Physiol 74:749–754

Nagy L, Grabherr G (2009) The biology of Alpine habitats. Oxford University Press, Oxford

Newcomb EH (1967) Fine structure of protein-storing plastids in bean root tips. J Cell Biol 33:143–163

Nybakken L, Bilger W, Johanson U, Björn LO, Zielke M, Solheim B (2004) Epidermal UV-screening in vascular plants from Svalbard (Norwegian Arctic). Polar Biol 27:383–390

Oerbaeck JB, Kallenborn R, Tombre I, Hegseth EN, Falk-Petersen S, Hoel AH (eds) (2007) Arctic Alpine ecosystems and people in a changing environment. Springer, Berlin

Oerbaek J, Tombre I, Kallenborn R (2004) Challenges in Arctic–Alpine environmental research. Arct Antarct Alp Res 36:281–283

Olave-Concha N, Bravo LA, Ruiz-Lara S, Corcuera LJ (2005) Differential accumulation of dehydrin-like proteins by abiotic stresses in Deschampsia antarctica Desv. Polar Biol 28:506–513

Oppeneiger C (2008) Einfluss von klimatischen Faktoren auf den Primär- und Sekundärstoffwechsel von Dryas octopetala L. Ph.D. thesis, University of Innsbruck

Paramonova NV, Shevyakova NI, VlV K (2004) Ultrastructure of chloroplasts and their storage inclusions in the primary leaves of Mesembryanthemum crystallinum affected by putrescine and NaCl. Russ J Plant Physiol 51(1):86–96

Parnikoza IY, Maidanuk DN, Kozeretska IA (2007) Are Deschampsia antarctica Desv. and Colobanthus quitensis (Kunth) Bartl. migratory relicts? Cytol Genet 41:226–229

Pechová R, Kutík J, Holá D, Kocová M, Haisel D, Vicánková A (2003) The ultrastructure of chloroplasts, content of photosynthetic pigments and photochemical activity of maize (Zea mays L.) as influenced by different concentrations of the herbicide amitrole. Photosynthetica 41(1):127–136

Pérez-Torres E, García A, Dinamarca J, Alberdi M, Gutiérrez A, Gidekel M, Ivanov AG, Hüner NPA, Corcuera LJ, Bravo LA (2004a) The role of photochemical quenching and antioxidants in photoprotection of Deschampsia antarctica. Funct Plant Biol 31(7):731–741

Pérez-Torres E, Dinamarca J, Bravo LA, Corcuera LJ (2004b) Responses of Colobanthus quitensis (Kunth) Bartl. to high light and low temperature. Polar Biol 27:183–189

Pérez-Torres E, Bravo LA, Corcuera LJ, Johson GN (2007) Is electron transport to oxygen an important mechanism in photoprotection? Contrasting responses from Antarctic vascular plants. Physiol Plant 130:185–194

Phoenix GK, Gwynn-Jones D, Lee JA, Callaghan TV (2000) The impacts of UV-B radiation on the regeneration of a subarctic heath community. Plant Ecol 146:67–75

Phoenix GK, Gwynn-Jones D, Callaghan TV, Sleep D, Lee JA (2001) Effects of global change on a sub-arctic heath: effects of enhanced UV-B radiation and increased summer precipitation. J Ecol 89:256–267

Piotrowicz-Cieślak AI, Gielwanowska I, Bochenek A, Loro P, Górecki RJ (2005) Carbohydrates in Colobanthus quitensis and Deschampsia antarctica. Acta Soc Bot Pol 74(3):209–217

Robberecht R, Junttila O (1992) The freezing response of an arctic cushion plant, Saxifraga caespitose L.: acclimation, freezing tolerance and ice nucleation. Ann Bot 70:129–135

Robinson CH, Michelsen A, Lee JA, Whitehead SJ, Callaghan TV, Press MC, Jonasson S (1997) Elevated atmospheric CO₂ affects decomposition of Festuca vivipara litter and roots in experiments simulating environmental change in two contrasting arctic ecosystems. Glob Change Biol 3:37–49

Robinson CH, Kirkham JB, Littlewood R (1999) Decomposition of root mixtures from high arctic plants: a microcosm study. Soil Biol Biochem 31:1101–1108

Rønning OI (1996) Svalbards flora, 3rd edn. Norsk Polarinstitutt, Oslo. ISBN 82-7666-101-7

Sarvari E, Nyitrai P, Gyöve K (1984) Chlorophyll protein derivative of the peripheral light-harvesting antenna of photosystem I. Photobiophy 8:229–237

Schäfers H-A, Feierabend J (1976) Ultrastructural differentiation of plastids and other organelles in rye leaves with a high-temperature-induced deficiency of plastid ribosomes. Cytobiol/Eur J Cell Biol 14:75–90

Schulze ED, Beck E, Müller-Hohenstein K (eds) (2005) Plant ecology. Springer, Berlin

Shalla TA (1964) Assembly and aggregation of tobacco mosaic virus in tomato leaflets. J Cell Biol 21:253–264

Shimokawa K, Sakanoshita A, Horiba K (1978) Ethylene-induced changes of chloroplast structure in Satsuma mandarin (Citrus unshiu Marc.). Plant Cell Physiol 19:229–236

Sjolund RD, Weier TE (1971) An ultrastructural study of chloroplast structure and dedifferentiation in tissue cultures of Streptanthus tortosus (Cruciferae). Am J Bot 58:172–181

Spencer D, Unt H (1965) Biochemical and structural correlations in isolated spinach chloroplasts under isotonic and hypotonic conditions. Aust J Biol Sci 18:197–210

Spencer D, Wildman SG (1962) Observations on the structure of grana-containing chloroplasts and a proposed model of chloroplast structure. Aust J Biol Sci 15:599–610

Stoynova E, Petrov P, Semerdjieva S (1997) Some effects of chlorsulfuron on the ultrastructure of root and leaf cells in pea plants. J Plant Growth Regul 16:1–5

Streb P, Josse E-M, Gallouët E, Baptist F, Kuntz M, Cornic G (2005) Evidence for alternative electron sinks to photosynthetic carbon assimilation in the high mountain species *Ranunculus glacialis*. Plant Cell Environ 28:1123–1135

Thannheiser D, Möller I, Wüthrich Ch (1998) A case study of the vegetation, the carbon budget and possible consequences of climatic changes in western Spitsbergen. Verh Ges Ökologie 28:475–484

Thomson W (1992) Agricultural chemicals. Book II: herbicides. Thomson, Fresno

Vesk M, Mercer FV, Possingham JV (1965) Observations on the origin of chloroplasts and mitochondria in the leaf cells of higher plants. Aust J Bot 13:161–169

Wielgolaski FE, Karlsen SR (2007) Some views on plants in polar and alpine regions. Rev Environ Sci Biotechnol 6:33–45

Wiencke Ch (2004) Reports on polar and marine research, vol 492. AWI, Bremerhaven, 1618-3193

Wiencke C, Schulz D (1975) Sporophyte development of *Funaria hygrometrica* Sibith. I. Structural data of water and nutrient uptake in the haustorium. Protoplasma 86:107–117

Wiencke C, Schulz D (1977) The development of transfer cells in the haustorium of the *Funaria hygrometrica* sporophyte. Bryophytorum Bibliotheca 13:147–167

Wiencke Ch, Ferreyra GA, Abele D, Marenssi S (2008) Reports on polar and marine research, vol 571. AWI, Bremerhaven, 1618-3193

Wildi B, Lütz C (1996) Antioxidant composition of selected high alpine plant species from different altitudes. Plant Cell Environ 19:138–146

Wise RR, Naylor AW (1987) Chilling-enhanced photooxidation. The peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. Plant Physiol 83:272–277

Wookey PA, Robinson CH, Parsons AM, Welker JM, Press MC, Callaghan TV, Lee JA (1995) Environmental constraints on the growth, photosynthesis and reproductive development of *Dryas octopetala* at a high arctic polar semi-desert, Svalbard. Oecologia 102:478–489

Worthing CR (1983) The Pesticide manual: a world compendium, 7th edn. The British Crop Protection Council, Champaign, USA

Wüthrich CH, Möller I, Thannheiser D (1999) CO_2 fluxes in different plant communities of a high-arctic tundra watershed (Western Spitsbergen). J Vegetat Sci 10:413–420

Xiong FS, Ruhland CT, Day TA (1999) Photosynthetic temperature response of the Antarctic vascular plants *Colobanthus quitensis* and *Deschampsia antarctica*. Physiol Plant 106:276–286

Zuñiga-Feest A, Ort DR, Gutiérrez A, Gidekel M, Bravo LA, Corcuera LJ (2005) Light regulation of sucrose-phosphate synthase activity in the freezing-tolerant grass *Deschampsia antarctica*. Photosynth Res 83:75–86