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Review

Physiological and Biochemical Background of Deacclimation in Plants, with Special Attention Being Paid to Crops: A Minireview

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Abstract: Global climate change, which is connected to global warming and changes in weather patterns, affects various parts of the environment, including the growth/development of plants. Generally, a number of plant species are capable of acquiring tolerance to frost after exposure to cold (in the cold-acclimation/cold-hardening process). In the last few decades, there have been more and more frequent periods of higher temperatures—warm periods that, e.g., break down the process of cold acclimation. This generates deacclimation, which could stimulate growth and lower frost tolerance in plants. Generally, deacclimation causes the reversal of changes induced by cold acclimation (i.e., in concentration of sugars, accumulation of protective proteins, or hormonal homeostasis). Unlike cold acclimation, the phenomenon of deacclimation has been less studied. The aim of this article was (1) to briefly describe the problem of deacclimation, with more attention being paid to its significance for economically important winter crop species, (2) to review and characterize the physiological-biochemical changes that are induced in plants by deacclimation, and (3) to discuss the possibilities of detecting deacclimation earlier in order to counteract its effects on crops.

Keywords: cold acclimation; deacclimation; frost tolerance; climate change; crop plants; plant metabolism

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1. Introduction—Significance of Cold Acclimation and Deacclimation for Crops

Global climate change (regardless of its causes) is accompanied by global warming. In the first two decades of the 21st century, the global surface temperature has increased by 0.99 °C when compared with the years 1850–1900 [1]. In countries located in the temperate climate zone, the average air temperature has changed in the past sixty years. In Poland, the last decade was exceptionally warm compared to previous periods [2]. The average annual temperature reached as high as 9.1 °C. For comparison, in the reference period 1961–1990, it was 7.5 °C. Global climate change has a variety of effects on nature and the environment, including on the growth of plants. This may be important, especially for cultivated species, for example, from the group of winter plants. Because the temperature in the world is generally rising, the episodes of higher temperatures in autumn and winter are happening more frequently, i.e., in countries where winter cultivars of crops are cultivated. To winter cultivars belong many economically important species, such as oilseed rape or cereals. Normally, winter cultivars require exposure to cold in order to complete their growth cycle and induce generative development (in the vernalization process) [3]. Simultaneously, growth in cold conditions results in the acclimation of plants to lower temperatures (below zero), which increases their frost tolerance. Cold acclimation, for example in eastern EU, takes place in late autumn at a temperature usually ranging from between +2 to +4 °C, 3–6 weeks. Well-cold-acclimated (cold-hardened) crops in autumn can survive freezing temperatures that reach approximately −20 °C [4]. Cold acclimation is accompanied by, among other things, the following more important biochemical and physiological changes: (1) an increased accumulation of sugars, which thicken the cell sap and decrease sap freezing temperature [5]; (2) an increase in membrane fluidity due to an increased amount of unsaturated fatty acids, which improves membrane functioning under cold temperatures [6,7]; (3) an increased concentration of stress hormones, e.g., abscisic acid (ABA), which regulates, e.g., water management [8]; (4) an increased accumulation of the heat shock proteins (HSP), which play protective functions for other proteins [9,10]; (5) an accumulation of the cold-responsive proteins (COR) [11]; and (6) a lowered intensity of photosynthesis [12,13]. Cold generally affects all parts of a cell as well as its signaling pathways. For example, microtubules, which are generally considered to be the scaffolds of cells, are involved in sensing physical stimuli such as cold and they disassemble as a result of cold exposure [14]. On a molecular level, cold acclimation generally induces the expression of the cold-induced genes that function against freezing injuries ([15] and literature cited there).

Winter cultivars (cereals, oilseed rape) are threatened by more frequent episodes of higher temperatures, e.g., 9–16 °C or more in late autumn or early winter, because warm periods might stimulate growth and lower their frost tolerance (Figures 1 and 2). Periods of higher temperatures induce deacclimation, which is accompanied by the reversal of the metabolic 'adaptations' that occur during cold acclimation. In such cases, a sudden frost after the deacclimation period significantly increases the risk of frost injuries because deacclimated plants are more susceptible to frost [13,16,17]. In winter crops such as oilseed rape or the cereals that are cultivated in regions of the EU, deacclimation can occur after warm periods, e.g., 9–16 °C or more in autumn (in late November and December), in winter (in warm periods in January and February), and even in early spring. The process of deacclimation in winter crops can usually be reversed when the temperature decreases once again after warm periods (this is called the reacclimation/rehardening process).

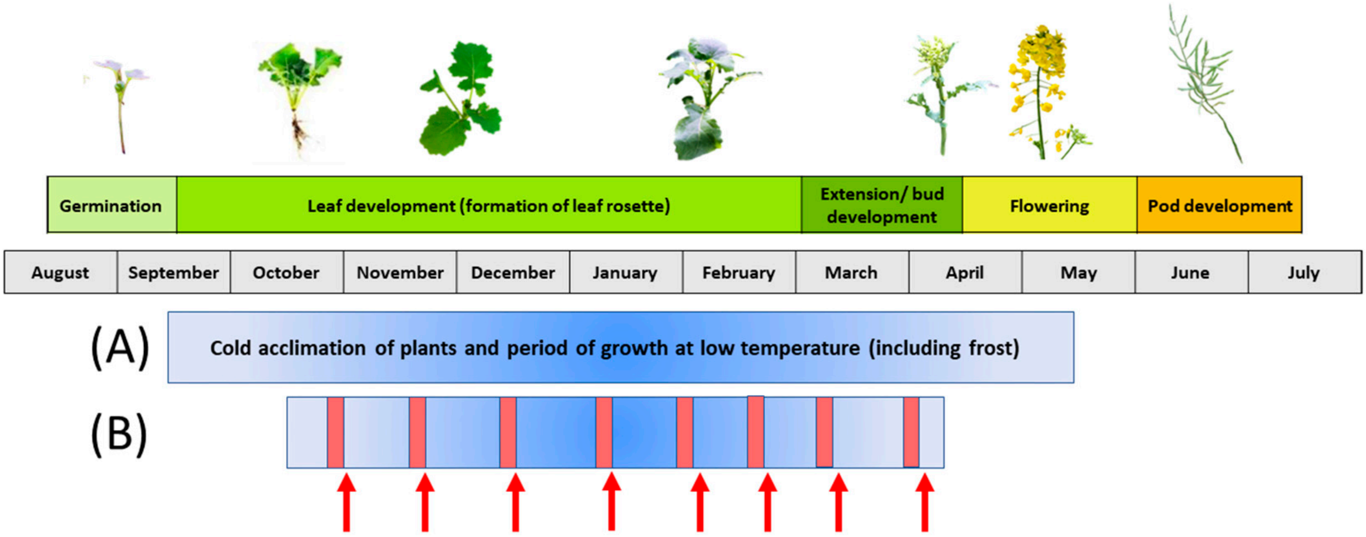

Figure 1. Simplified growth cycle of winter oilseed rape. The times of the year for particular stages of development are characteristic, for example, for regions of central/eastern EU. Panel (**A**) shows the approximated time frames for the period during which cold acclimation occurs and the next period of growth of plants at a low temperature (including frost episodes). Panel (**B**) shows exemplary occurrences of warm breaks (dark pink rectangle) that deacclimate plants, thus increasing the risk of frost injuries in the event of a sudden frost (red arrows).

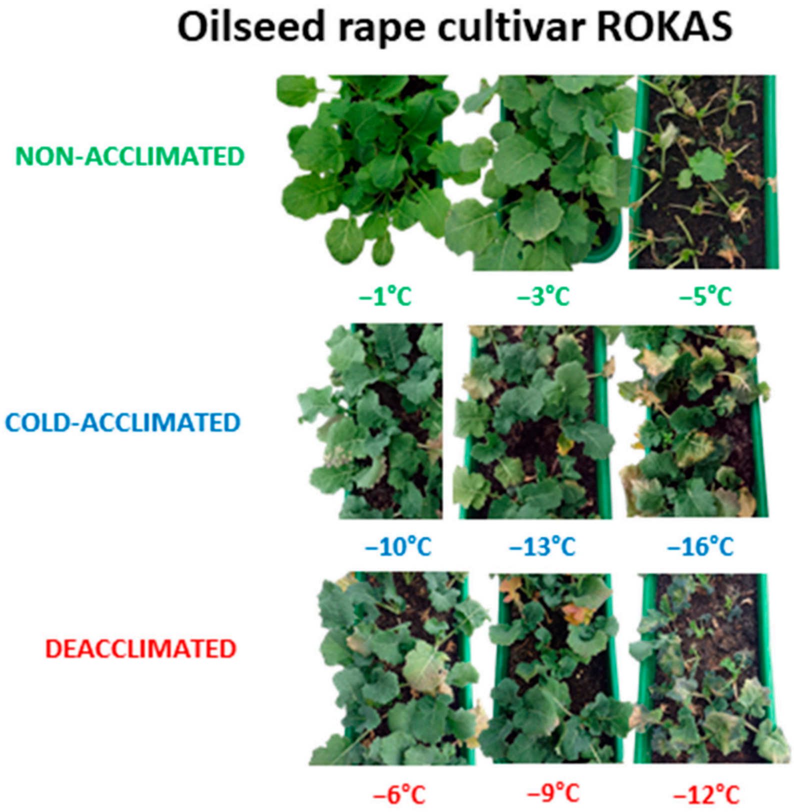

Figure 2. Changes in the frost tolerance of the non-acclimated, cold-acclimated, and deacclimated winter oilseed rape cultivar Rokas. Frost tests were performed at temperatures ranging between −1 °C and −16 °C. The estimation of frost injuries was based on observations of the regrowth of plants (previously exposed to frost) after growing for two weeks at 12 °C (for detailed data, see [13]). Photographs—J. Stachurska.

The phenomenon of cold acclimation and deacclimation is therefore important from the point of view of the cultivation of winter crops. However, it also concerns other species that grow in more extreme temperature conditions, including some trees (e.g., birch, mulberry, pine trees), grasses (e.g., Antarctic hair grass, creeping bentgrass), tea, etc. [18–22]. However, for each of these species the temperature conditions and the periods needed for cold acclimation/deacclimation or reacclimation can differ. Generally, however, deacclimation requires a shorter time frame than cold acclimation. At the transcriptome level, the effects of deacclimation are very rapid—changes can be observed within 24 h [23,24]. Physiological/metabolomic changes (and a decrease in frost tolerance as a result) are observed later (usually in a matter of days), although there can be differences between species. In oilseed rape, a significant reverse of the metabolic changes that are induced by cold acclimation was observed after seven days of a higher temperature (16 °C/9 °C d/n), while cold acclimation in this case took three weeks [13,25]. On the other hand, in *Arabidopsis thaliana* L. the deacclimation-induced changes (in the plasma membrane proteins) were observed even faster—after two days of a warm temperature [26]. In the studies of *Solanum commersoni* (Poir.) it was shown that a few hours (2–3 h) at 20 °C was enough to initiate the deacclimation process [27]. One day was sufficient to deacclimate the tested plants while, in this case, the cold acclimation took 15 days at 2 °C [27].

Contrary to the well-studied process of the acclimation of plants to low temperatures [28], especially the metabolic changes that occur during deacclimation, are still less known [17]. In the presented article, we aim to focus on summarizing and updating the knowledge concerning the characteristics of the physiological-biochemical changes that are induced in plants by deacclimation, with special attention being paid to crop plants. We also discuss the possibilities of the earlier detection of deacclimation in crops in order to counteract its effects in winter crops.

2. Factors That Influence the Phenomenon of Deacclimation

The kinetics of deacclimation depend on a few factors. Among them, the most important are temperature and the length of the warm period. Usually, higher temperatures and longer periods of warmth result in more effective deacclimation. Cold-acclimated cabbage (*Brassica oleracea* (L.)) seedlings that had been exposed to deacclimation at different temperatures (15 °C, 20 °C, and 25 °C) showed that the higher the deacclimation temperature was, the more accelerated was the loss of freezing tolerance [29]. Studies on oilseed rape revealed that deacclimation was more effective at 20 °C than at 12 °C [30]. Furthermore, when deacclimation lasted no longer than five days, reacclimated oilseed rape plants were able to recover their tolerance to a low temperature at a level that was close to (or even higher) than before the deacclimation process [31]. Moreover, in this species, the changes that are induced by deacclimation might be reversible when it is not accompanied by elongational growth [30] and when there are temperature conditions that allow plants to reacclimate once again. The deacclimation temperature can differ between day and night, but a higher temperature during the night is also important for this process. Studies conducted on Antarctic hair grass (*Deschampsia antarctica* (E. Desv.)) revealed that the deacclimation process had a greater effect in nocturnal-warmed plants [21].

In addition to the temperature and the length of the warm period, another important factor is also the species/cultivars. Specific species, and even cultivars, can be more or less susceptible to deacclimation. In this context, we can use the term “tolerance for deacclimation”. Tolerance to deacclimation can be understood as the capacity of a plant to maintain the highest possible frost tolerance despite deacclimation [13]. A comparison of two species of *Solanum* (*S. multidissectum* and *S. megistacrolobum* subsp. *toralapanum*) that had been exposed to deacclimation (at 18 °C for 12 h) showed that the rate of deacclimation depends on the cultivar [32]. In oilseed rape, the tolerance for deacclimation also differs depending on the cultivar [13]. According to Pagter et al. [33], hardier species (which was defined based on their winter freezing tolerance) of *Hydrangea*, *H. paniculata* (Siebold) deacclimated more quickly than *H. macrophylla* (Thunb.), a less hardy species. In *H. macrophylla*, the deacclimation kinetics showed a sigmoid course with a short lag-phase that was followed by a rapid deacclimation rate.

Tolerance to deacclimation can also depend on the genotype of a plant. Pociecha et al. [34] tested the frost tolerance of the deacclimated barley cultivar Bowman and its near isogenic lines with disturbances in the biosynthesis and the signaling of brassinosteroids (plant steroid hormones). The authors found that both lines tolerated frost better after deacclimation compared to the wild-type cultivar (Bowman). Wójcik-Jagła and Rapacz [35] also demonstrated that some lines of barley were characterized as being more tolerant to deacclimation than others. According to the authors, the results indicated that the freezing tolerance and the tolerance to deacclimation might be independent traits, whereas cold-acclimated plants with a high freezing tolerance can be sensitive to deacclimation [35].

Finally, Takeuchi and Kasuga [18] discovered an interesting phenomenon that different tissues of one plant might deacclimate in a different manner. In birch (*Betula platyphylla* var. *japonica* (Hara)), bark cells required much higher temperature (10–20 °C) to decrease their freezing tolerance than xylem cells (deacclimated at subfreezing temperature −2 °C).

The physiological and biochemical basics of the tolerance to deacclimation require further studies. Based on the current knowledge, we can suspect that the decreases in frost tolerance after deacclimation might be lower in those plants/cultivars/lines (or even parts of plants) in which the metabolic changes that are induced by cold acclimation are sufficiently maintained after deacclimation.

3. The Physiological-Biochemical Changes That Occur in Plants during Deacclimation

As mentioned, compared to the process of the acclimation of plants to low temperatures, the metabolic changes that occur during deacclimation are still less well known and have been under more detailed research relatively recently, partly due to the intensification of studies that have been devoted to global climate change. The metabolic changes that accompany the process of deacclimation have been studied in woody plants [36], herbaceous plants [4], and grasses [22]. Much deacclimation research has been done in the model plant *Arabidopsis thaliana* ((L.) Heynh), among others, [24,37–39]. In the group of crop plants, the deacclimation process has been studied in oilseed rape [13,25,30,40], barley [34], rice [41], wheat [42], and triticale [43], among others.

3.1. The Cell Walls and Cell Membranes

The structural and compositional changes in the cell wall during the process of cold acclimation and deacclimation are important for the acquisition or loss of freezing tolerance as well as in the growth response [39]. In earlier studies, Solecka et al. [44] observed that the deacclimation of oilseed rape resulted in decreased content of pectin to a level similar to control plants. During the deacclimation of *Arabidopsis* (a plant of the same family as oilseed rape), the genes encoding cell-wall-related proteins such as xyloglucan endotransglycosylase, xylosidase, xylose isomerase, pectinesterase, and the arabinogalactan proteins were up-regulated [23]. The components of the cell wall of *Arabidopsis*, such as the arabinogalactan proteins and pectic galactan, changed along with the changes in frost tolerance and growth during cold acclimation and deacclimation [39]. Interestingly, although some reversible tendencies were induced in the cell wall by deacclimation (versus cold acclimation), arabinan and xyloglucan did not return to the level observed in the non-acclimated control. According to Kutsuno et al. [39], deacclimation rather initiates a specific novel composition of the cell wall. Cell wall polysaccharides could probably work to achieve the regulation that is necessary to balance the trade-off between freezing tolerance and growth in plants, and also prepare for, for example, reacclimation.

The changes in the lipid part of cell membranes that are induced by cold acclimation are well known and they usually go in the direction of an increase in membrane fluidity, which improves membrane functioning at low temperatures [6,7]. The fluidity of the cell membrane is significantly associated with larger amounts of the unsaturated fatty acids that are incorporated into membranes. It can be additionally modified via incorporation into the membranes, including the chloroplast membranes, of compounds such as sterols or tocopherols and carotenoids [45,46].

Deacclimation can modify lipid composition, which was observed in trees, mulberry bark (*Morus bombysis* (Koidz.)), and Scots pine roots (*P. sylvestris* (L.)) [19,47]. The ratio of unsaturated to saturated fatty acids of the phospholipids decreased, and that was a reversal effect compared to the changes that were induced by cold acclimation. These findings are in agreement with the results of our last studies, which were conducted on lipids that had been isolated from the chloroplasts of four cultivars of non-acclimated, cold-acclimated and deacclimated oilseed rape. Deacclimation (7 days, 9°/16 °C d/n) changed the molar percentage of the fatty acids of lipids; however, not in all of the fatty acids [48]. The reversal effect of deacclimation compared to cold acclimation was visible the best after the calculation of the ratio of the two most unsaturated fatty acids 18:3/18:2 (linoleic [18:2] and α-linolenic [18:3]) in the fractions of the monogalactolipids and phospholipids. The ratio increased after cold, while after deacclimation it decreased again. In some cultivars, the ratio decreased even to the level that had been observed in non-acclimated plants.

Under cold conditions, simultaneously with the alterations of the membrane lipid composition, there are changes in the content of low-molecular antioxidants such as the tocopherols and carotenoids [46]. α-Tocopherol, the tocopherol that occurs most often [49], is involved in the scavenging of ROS [50]. The carotenoid (β-carotene) also acts as a ROS scavenger [51]. Therefore, β-carotene and α-tocopherol might limit the membrane lipid peroxidation. β-Carotene and α-tocopherol, after incorporation into the membranes, also modify their physicochemical properties [52,53]. β-Carotene acts as membrane stabilizer [53]. The studies of Hincha [52] showed the influence of the tocopherols on the stability and lipid dynamics of model membranes (mimicking the lipid composition of the plant chloroplast membranes) in cold conditions. Based on model systems that were mainly built from the phospholipids, the specific action of α-tocopherol on the physicochemical properties of the membranes, such as modifying the phase behavior and lipid dynamics and decreasing the motional freedom of the lipid fatty acyl chains, was confirmed. These responses were particularly observed at a low temperature. Regarding deacclimation, our studies on oilseed rape revealed that the content of tocopherols after deacclimation generally decreased compared to the cold-acclimated plants [48]. α-Tocopherol and γ-tocopherol were the most abandoned in the chloroplast membranes [48]. In some of the tested cultivars, the content of α-tocopherol in the chloroplasts after deacclimation even reached the level that had been observed in the non-acclimated control. In the case of β-carotene, the changes after deacclimation were more cultivar-dependent.

The reverse effect of deacclimation on membrane components such as the fatty acids or the content of low-molecular antioxidants might be one of the more important reasons for the deacclimation-induced lower tolerance of plants to frost. The cold-acclimated (7 days) and then frost-exposed seedlings of cabbage (*B. oleracea* (L.)) were characterized by decreased electrolyte leakage (less membrane injuries) than the seedlings that had been exposed to frost after only one day of deacclimation [5]. Electrolyte leakage (membrane permeability), which was measured in *Arabidopsis* leaves, was clearly lower in the cold-acclimated and frost-exposed plants compared to non-acclimated plants or deacclimated plants that were then exposed to frost [26]. Lower electrolyte leakage (membrane permeability) in cold-acclimated/frost-exposed plants informs about the lower injuries of membranes. Higher values of electrolyte leakage in non-acclimated, deacclimated/frost-exposed plants informs about the more severe injuries of membranes. One of the causes of membrane injuries as a result of exposition to low temperature (frost) may be lipid peroxidation. It is usually measured by MDA (malondialdehyde) content [54]. MDA is considered to be the final product of lipid peroxidation in the plant cell membrane. Finally, it is also worth mentioning that the cold-acclimated winter wheat cultivars that had accumulated a higher amount of tocopherols and β-carotene had a higher frost tolerance than the cultivars with a lower amount of these compounds in the leaves [55].

3.2. Soluble Sugar Concentration and Water Management

Cold acclimation generally increases the accumulation of sugars, which thickens the cell sap and lowers its freezing temperature [5]. It is usually accompanied by decreased osmotic potential and an increase in frost tolerance. Studies on many species have confirmed a reverse tendency in sugar accumulation after deacclimation compared to cold acclimation. In cabbage seedlings (*B. oleracea* L.), after only one day of deacclimation, the amounts of sugars such as sucrose, glucose, and fructose decreased significantly [5]. A decrease in the content of soluble sugars was also observed in deacclimated cultivars of white clover stolons (*Trifolium repens* L.) [56], the stems of *Hydrangea* plants (*H. macrophylla* ssp. *macrophylla* Thunb.) [33], seedlings of Aleppo pine (*Pinus halepensis* Mill.) and radiata pine (*Pinus radiata* D. Don) [20], crowns of grasses (*Agrostis stolonifera* L. and *Poa annua* L.) [22], and the shoot tissues of peach (*Prunus persica* L.) [57]. In deacclimated (at temperature 16/11 °C d/n, 2 weeks) blackcurrant (*Ribes nigrum* L.), although the concentration of a primary soluble carbohydrate (sucrose) decreased significantly in the buds of all of the tested plants, the rate of the decrease depended on the cultivar [58]. Additionally, the concentration of raffinose decreased in these species as a result of deacclimation [58]. Rys et al. [25] described a lower accumulation of soluble sugars in the leaves of deacclimated (7 days, 9°/16 °C d/n) oilseed rape. The content of these sugars decreased in this species to a level that was observed in the non-acclimated control. The authors also noted that cold acclimation reduced the osmotic potential of oilseed rape (by approximately 20–25%), while deacclimation increased it again by approximately 23–45%. The changes were accompanied by a decrease in the frost tolerance of the plants [25]. Finally, a deacclimation-induced lower content of sugars was also described by Kutsuno et al. [39] in *A. thaliana.*

In addition to lowered content of sugars in plant tissues, deacclimation causes the up-regulation of some genes associated with carbohydrate metabolism, such as β-galactosidase, sucrose synthase, and β-fructosidase [23]. β-Galactosidase is involved in lactose catabolism, while sucrose synthase and β-fructosidase are involved in sucrose metabolism [23].

A high content of water in tissues is unfavorable for freezing tolerance, because temperatures below 0 may lead, e.g., to membrane injuries by forming ice outside or inside a cell. Deacclimation generally results in an increase in the osmotic potential and rehydration of tissues [25,58]. Ögren [59], in experiments made in field conditions on *Vaccinium myrtillus*, observed a significant dependency between sugar content and rehydration of tissues and susceptibility of deacclimated plants to frost. In the buds of deacclimated blackcurrant (*Ribes nigrum* (L.)), a higher water content was observed (calculated as [Fresh Weight-Dry Weight]/Dry Weight) [58]. In oilseed rape (cultivar Kuga and Thure), cold acclimation generally decreased the value of the relative water content (RWC). The RWC increased significantly in the deacclimated plants of one cultivar [25].

The dehydration of plant tissues is controlled by proteins such as aquaporins, which are involved in water transport in cells [60]. In cold-acclimated oilseed rape cultivars, the accumulation of protein BnPIP1 (plasma membrane intrinsic protein) was generally higher, probably for the purpose of better dehydrating the cells [25]. Deacclimation decreased BnPIP1 to a level that was also observed in non-acclimated plants, or even lower. Interestingly, the cold acclimation of oilseed rape drastically reduced the accumulation of the transcript aquaporin *BnPIP1*, but its level remained unchanged after deacclimation [25]. It did not correlate with changes in the protein accumulation, which could suggest a role of some of the posttranscriptional mechanisms in controlling aquaporin production differently in cold-acclimated and deacclimated plants; however, this requires further studies.

To conclude, the reverse effect of deacclimation (compared to cold acclimation) on the sugar metabolism and water management is surely one of the more important factors that are responsible for lowering the tolerance of plants to frost.

3.3. Accumulation of Selected Proteins

Generally, proteomic analyses that have investigated the influence of deacclimation on plasma membrane proteins have shown that an increase or a decrease in particular proteins during cold acclimation has the opposite tendency during deacclimation [26]. In deacclimated *A. thaliana*, the majority of the membrane proteins whose accumulation is stimulated by cold returned after deacclimation, even to a level that was similar to the non-acclimated plants [26]. In deacclimated tea (*Camellia sinensis* (L.) Kuntze), comparative proteomic studies also revealed differences in the accumulation of many proteins from the groups that are involved in, among other things, the cell wall, photosynthesis, protein synthesis, antioxidation, or sugar metabolism when compared with cold-acclimated plants [61]. In deacclimated plants compared to cold-acclimated plants, 115 up-accumulated and 136 down-accumulated proteins were detected. Compared to the non-acclimated plants, 477 differentially accumulated proteins, including 253 up-accumulated proteins and 224 down-accumulated proteins, were observed in deacclimated plants [61].

Based on the aforementioned works, it can be said that, similar to the research of the cell wall conducted by Kutsuno et al. [39], deacclimation initiates a novel composition of many proteins from different tested groups. However, focusing our review on three groups of proteins with particular significance for cold acclimation (COR, heat shock proteins (HSP), and dehydrins (DHN)), it can be seen that most often deacclimation has the reverse effect on them compared to cold acclimation. For example, in deacclimated wheat and oilseed rape, the COR78 protein decreased to an undetectable level, which was similarly observed in non-acclimated plants compared to the increased accumulation in cold-acclimated plants [62]. Our studies on oilseed rape showed that during cold acclimation the accumulation of the protective proteins from a group of HSP is enhanced [63]. The main role of HSP is to act as molecular chaperones. They are responsible for regulating protein folding as well as its accumulation, location, translocation, and degradation [64,65]. After the deacclimation of oilseed rape, the amount of cytoplasmic HSP70 and HSP90 most frequently decreased compared to cold-acclimated plants [63]. Deacclimation also results in a decrease in the accumulation of DHN in Scots Pine (*Pinus sylvestris*) [66]. DHN are protective proteins that are produced in dehydrated tissues [67], which is observed in cold [68]. The role of DHN is to protect the other proteins and membranes against negative structural changes that are induced by the dehydration of tissues [67]. In deacclimated wheat and oilseed rape, the accumulation of dehydrin (47-kD) decreased to a level that was observed in the non-acclimated plants [62]. The relative expression of a gene that is related to dehydrins (*PpDhn1*) revealed a decrease in its amount during the deacclimation of peach (*Prunus persica* (L.) Batsch) [57].

To summarize, in deacclimated plants there is usually a decreased accumulation of stress-related and protective proteins [62,63], along with a simultaneous increase in the number of proteins associated with the metabolic processes [26,61]. This seems to be an important reason for the deacclimation-induced lowering of frost tolerance.

3.4. Hormonal Balance

Plant hormones and the interactions between them play an important role in a plant's stress responses, including low-temperature stress. The interplay between growth-promoting hormones such as gibberellins, auxins, and cytokinins and stress hormones such as abscisic acid (ABA) seem to be particularly important in the context of the balance between growth and the frost tolerance of cold-acclimated plants. The deacclimation process can disturb this balance. The research that was conducted on deacclimated *A. thaliana* revealed an increased expression of the genes that are associated with growth-promoting hormones biosynthesis, for example, gibberellins and auxins [24]. In deacclimated *Vitis* plants, the downregulation of ABA synthesis might play an important role in the loss of cold hardiness and budbreak [69]. In deacclimated barley (*Hordeum vulgare* L.) plants, there was an increase in the growth-promoting hormones, including indole-3-acid (IAA), some gibberellins, and cytokinins [34]. Similar phenomena were observed in deacclimated bermudagrasses (*Cynodon* spp.)—the amount of cytokinin was higher while the amount of ABA was lower [70]. Stachurska et al. [63] conducted detailed analysis of the hormonal homeostasis in cold-acclimated and deacclimated oilseed rape (four cultivars). In the tested cultivars, the concentration of ABA increased (in a cultivar-independent manner) in the cold-acclimated plants, while it decreased significantly as a result of seven days of deacclimation [63]. Changes in the growth-promoting hormones (their precursors and inactivated forms) were more cultivar-dependent, but the general tendency was a decrease in their concentrations, i.e., cytokinin: cis-zeatin, active auxin (IAA) and its precursors or some gibberellins (GA_7) during cold acclimation that was followed by an increase after deacclimation. Studies allowed the preparation of a model of hormonal changes characterizing the winter and spring cultivars of cold-acclimated and deacclimated oilseed rape [63].

Another group of hormones that have recently been studied in the aspect of deacclimation are brassinosteroids (BR). BR are plant steroids that are involved in plant growth and stress response. Pagter et al. [24] found that, in deacclimated *A. thaliana*, the expression of the genes related to brassinosteroid biosynthesis was higher. Our studies on cereals [71] and oilseed rape [72] showed that endogenous BR generally increased in leaves, but only after cold acclimation. In the case of oilseed rape, there was, however, a dependency on the cultivar and on the type of analyzed BR. Deacclimation (in oilseed rape) rather decreased some of the BR [72]. In the winter wheat cultivar Grana, long periods of cold acclimation (43 days) finally resulted in a high accumulation of brassinosteroid (28-homocastasterone) in crowns (Figure 3). Deacclimation (7 days) reversed this effect (Figure 3).

Interestingly, in contrast to the BR level, the accumulation of the BR receptor (BRI1) clearly decreased in cold but increased after deacclimation [72]. This higher accumulation of the BR receptor protein (despite the fluctuations in the BR levels in deacclimated plants) could mean that there is a strong signal transduction towards the resumption of growth that is induced by these growth-promoting steroids, which can be unfavorable from the point of view of frost tolerance.

Shifting the hormonal balance (of which a model is presented in the work [63]) in the direction of increasing the concentrations of the growth-promoting hormones and decreasing the amount of the stress hormone (ABA) might be one of the possible causes of the decrease in plant frost tolerance after deacclimation in oilseed rape and other plants. On the other hand, the example of brassinosteroids shows that not only the concentration of hormones may be important. Attention should also be paid to the elements of signal transduction, including the appearance/concentration of receptor proteins that receive hormonal signals. These issues, however, will require further research.

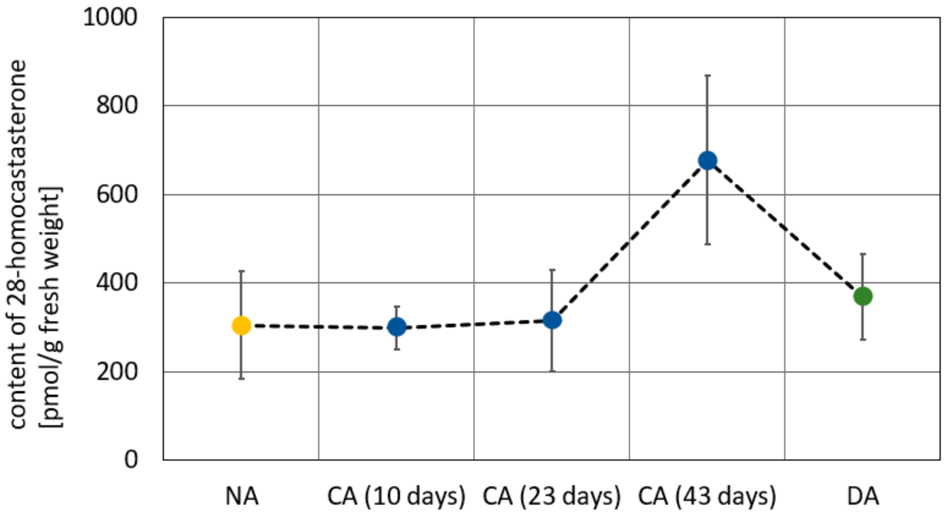

Figure 3. Changes of content of brassinosteroid (28-homocastasterone) in the crowns of winter wheat cultivar Grana in NA, CA, and DA plants. NA—control plants (non-acclimated plants) growing at 20 °C (11 days), CA—cold-acclimated plants (10, 23, 43 days at 5 °C), DA—deacclimated plants (after cold acclimation exposed to 20 °C (7 days)). Data represent mean values ± SE (n = 3), [73].

3.5. Photosynthesis

The process of photosynthesis is a marker of the different stresses that affect plants, including low-temperature stress. Despite its importance for plants, little is known about its changes in deacclimated plants. Regarding the efficiency of the light reactions during photosynthesis, Pukacki et al. [74] observed that after the deacclimation of the needles (and during release of deep rest) in *Picea schrenkiana* there was an increase in the intensity of the delayed fluorescence emissions. The effect was stronger in the frost-susceptible *P. schrenkiana*, which reacted to the deacclimation more strongly. Research on wheat revealed that the differences between cold-acclimated and deacclimated plants were changes in the values of the parameters that described the phenomenological and specific energy fluxes (expressed, among other things, by the parameters ABS, TRo, Dio, or ψo [16]). Studies on oilseed rape also revealed that, after deacclimation, the values of the Fv/Fm parameter, which is the maximum quantum yield of PSII photochemistry, was significantly higher compared to the values that were observed in the cold-acclimated plants, even compared to the values that are characteristic for non-acclimated plants [25]. Similarly, later studies by Stachurska et al. [13] showed that deacclimation reversed the cold-induced changes in the photosystem efficiency and resulted in an intensification of the light reactions of photosynthesis in oilseed rape. The changes in PSII efficiency appeared to be correlated with changes in the chlorophyll content. According to Rys et al. [25], the cold acclimation of oilseed rape decreased the chlorophyll content, while deacclimation increased it. In Scots pine (*Pinus sylvestris* L.), cold acclimation and deacclimation were associated with the reduction and recovery of Amax (light-saturated assimilation rate) and α (quantum yield) [75]. Further, regarding the dark reactions of photosynthesis, studies by Rapacz and Hura [76] showed that deacclimated oilseed rape plants had, conversely to cold-acclimated plants, a decreased activity of Rubisco and sucrose-phosphate synthase (SPS), which is in agreement with the later studies of Rys et al. [25], where the authors showed that, after deacclimation, the intensity of photosynthesis (net photosynthesis, parameter P_N) was lower in both of the tested cultivars of oilseed rape (Kuga and Thure). On the other hand, this seems to be a slight contradiction to our last observations [77] of two cultivars of oilseed rape (Pantheon and President), where the deacclimated plants were characterized by an increased intensity of the CO_2 assimilation. In our opinion, an increase in P_N is more likely after deacclimation because it is accompanied by a further resumption of growth. This is why the issue may require more studies, especially because P_N is sensitive to the soil water content, and therefore even a slight deficit of water in a controlled pot culture is mirrored in photosynthetic activity due to the effect on the stomata closure.

As a result of the deacclimation of *A. thaliana*, the following genes that are connected to photosynthesis, chlorophyll a/b-binding protein; ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit; photosystem I reaction center subunit II, and photosystem II 5 KD protein, were up-regulated [23]. Other studies on *A. thaliana* revealed that, although they were suppressed during cold acclimation, the genes associated with photosynthesis, which encode the D2 subunits of the photosystem II complex, were reactivated during deacclimation [37]. Research on the gene expression of cold-acclimated and deacclimated *B. napus* revealed differences in the genes that are responsible for, among other things, photosynthesis and light-regulated diurnal responses, whose expression was reversed within one week of deacclimation [78].

3.6. Other Changes

Antioxidants. Research conducted on the needles of Norway spruce (*Picea abies* L.) revealed that the concentration of the $O^{\cdot -}{}_2$ radical and H_2O_2 did not vary significantly between the tree populations during the deacclimation period [79]. In contrast, the concentrations of the low-molecular-weight antioxidants such as flavonoids, ascorbic acid, and glutathione were lower, while similarly a decrease was also observed in the activity of superoxide dismutase (SOD) and guaiacol peroxidase (PO). The activity of catalase (CAT) did not change between the tree populations [79]. In winter wheat plants, the concentrations of antioxidants such as ascorbate and glutathione generally tended to decrease after deacclimation, while they were higher in the cold-acclimated plants [80]. In olive leaves (*Olea europea* L.), antioxidant activity such as ascorbate peroxidase, CAT, peroxidase, and SOD increased during cold acclimation and decreased during deacclimation [81]. It is known that low-temperature stress is accompanied by oxidative stress (increasing the accumulation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyradicals). A too-high concentration of ROS is harmful for cellular structures and macromolecules. Plants developed a defense system against oxidative stress, which is among others connected with increased activity of the aforementioned ROS-scavenging enzymes. By lowering the activity of ROS-scavenging enzymes or lower accumulations of other antioxidative compounds, deacclimation weakens plant antioxidative capacities, thus making them more susceptible to frost-induced damages.

Under abiotic stress such as low temperature, plants accumulate an increasing amount of anthocyanins [82], and this phenomenon was observed in many plant species, e.g., maize, grape, apple [83–85]. The expression of the genes responsible for the biosynthesis of anthocyanins was enhanced under low-temperature treatment [86]. In oilseed rape, cold acclimation significantly increased the content of anthocyanins, while deacclimation caused the decrease in anthocyanin content [25].

Amino acids (proline). Proline is accumulated by plants under stress and is involved in, among other things, antioxidative protection [87]. In *A. thaliana*, the concentration of proline decreased gradually during the three days of deacclimation, compared to the high levels of this amino acid in cold-acclimated plants [38]. In oilseed rape, the level of proline decreased significantly after the exposure of plants to elevated temperature (18/16 °C) [88]. A lower concentration of proline and amino acids was also detected in deacclimated winter wheat when compared with cold-acclimated plants [80].

General metabolic profile. The FT-Raman technique, which provides general information about the changes in the metabolic profile (the content and composition of carotenoids, chlorophylls, flavonoids, fatty acids, or polysaccharides, etc.), has been implemented to compare non-acclimated, cold-acclimated, and deacclimated oilseed rape [25]. A cluster analysis (chemometric method) revealed clear differences in the FT-Raman spectra. The non-acclimated and deacclimated plants were in one group, while the cold-acclimated plants were in another group. This indicates that seven days of the deacclimation of oilseed rape is generally the period that significantly reverses the cold-induced metabolic changes, thus making the plant metabolism more similar rather to non-acclimated plants than to cold-acclimated plants.

Physiological and biochemical changes that occur in plants during cold acclimation and deacclimation have been summarized in Table 1, where we compared two species from the same family, Brassicaceae, a model plant *Arabidopsis thaliana*, and a crop plant oilseed rape.

Table 1. The effects of cold acclimation and deacclimation on the physiological, biochemical, and genetical changes in two plants of the *Brassicaceae* family—the model plant *A. thaliana* and the crop plant oilseed rape. Cold-acclimated plants were compared to non-acclimated plants. Deacclimated plants were compared to cold-acclimated plants.

	Arabidopsis thaliana		**Oilseed Rape**	
	Cold-Acclimation	**Deacclimation**	**Cold-Acclimation**	**Deacclimation**
Cell walls	Down-regulation of the genes encoding cell-wall-related proteins such as putative xyloglucan endotransglycosylase, xylosidase arabinogalactan protein, and xylosidase [23]	Up-regulation of the genes encoding cell-wall-related proteins such as putative xyloglucan endotransglycosylase, xylosidase, xylose isomerase, pectinesterase, and arabinogalactan protein [23]	The content of pectin in the cell walls increased [44] Higher levels of non-covalently bound pectins and an increased content of galactose, arabinose, and glucose in the pectins and of galactose and arabinose in the hemicelluloses [89]	The content of pectin in the cell walls decreased to a level similar to that in the control plants [44]
	Suppression of the cell wall-related genes [37]	Reactivation of the cell-wall-related genes [37]		
	Increase in the arabinogalactan protein content [39]	Decrease in the arabinogalactan protein content [39]		
Cell membranes	Lower electrolyte leakage (membrane permeability) after the frost test [26]	Higher electrolyte leakage (membrane permeability) after the frost tests [26]	Increased ratio of the unsaturated to saturated fatty acids [48]	Decreased ratio in the unsaturated to saturated fatty acids [48]
	Increase in membrane fluidity [6]			
Sugars	Increased sugar content [38]	Decreased sugar content [38]	Increased sugar content [25]	Decreased sugar content [25]
	Increased starch content [39]	Decreased starch content [39]		
	Downregulation of the genes associated with carbohydrate metabolism (e.g., β-galactosidase, sucrose synthase) [23]	Upregulation of the genes associated with carbohydrate metabolism (e.g., β-galactosidase, sucrose synthase) [23]		
Water management	Down-regulation of the genes encoding the water channel proteins (such as tonoplast intrinsic protein gamma (TIP)) [23]	Up-regulation of the genes encoding water channel proteins (such as tonoplast intrinsic protein gamma (TIP)) [23]	Decreased osmotic potential, decreased relative water content (RWC), increased accumulation of aquaporin (BnPIP1) [25]	Increased osmotic potential, increased relative water content (RWC) in one cultivar, decreased accumulation of aquaporin BnPIP1 [25]
Proteins	Increased *COR* expression [12]	Reduced amount of *COR* transcripts [38]	Increased accumulation of heat shock proteins (HSP) [63]	Decreased accumulation in the heat shock proteins (HSP) [63]
	Increased accumulation of the cell structure-related proteins and decrease in protein synthesis, destination and storage-related proteins [26]	Reversal of the changes induced by cold in the majority of the protein accumulations (e.g., in the protein synthesis, destination and storage-related proteins, decrease in cell structure-related proteins [26]	Increased accumulation of dehydrins (DHNs) [62]	Decreased accumulation in dehydrins (DHNs) [62]
			Increased accumulation of the cold-regulated protein (COR78) [62]	Decreased accumulation in cold-regulated protein (COR78) [62]

Table 1. *Cont.*

	Arabidopsis thaliana		Oilseed Rape	
	Cold-Acclimation	**Deacclimation**	**Cold-Acclimation**	**Deacclimation**
Photosynthesis	Suppression of the photosynthesis-related genes encoding the D2 subunit of the PSII complex [37]	Reactivation of the photosynthesis-related genes encoding the D2 subunit of the PSII complex [37]	Decreased maximum quantum yield of the PSII photochemistry (Fv/Fm) [25]	Increased maximum quantum yield of the PSII photochemistry (Fv/Fm) [25]
	Inhibition of photosynthesis (reactions of the dark phase) and increase in sucrose-phosphate synthase (SPS) activity [12]	Increased expression of the genes involved in the light reactions of photosynthesis [37]	Limited intensity of the light reactions of photosynthesis [13]	Intensification of the light reactions of photosynthesis [13]
	Down-regulation of the photosynthesis-related protein genes, e.g., encoding a small subunit of RuBisCO [23]	Up-regulation of the photosynthesis-related protein genes, e.g., encoding a small subunit of RuBisCO [23]	Increased activity of RuBPCO and SPS [76]	Decreased activity of RuBPCO and SPS [76]
			Unchanged or decreased intensity of CO_2 assimilation (P_N) (cultivar-dependency) [25,77]	Decreased or increased intensity of photosynthesis (CO_2 assimilation, P_N) (probably dependent by additional factors) [25,77]
Hormonal homeostasis	Down-regulation of the genes involved in auxin and gibberellin metabolism, down-regulation of the BR biosynthesis pathway [90]	Increased expression of the genes associated with growth-promoting hormones (auxins and gibberellins) and an increased expression of the genes associated with BRs [24]	Increased ABA content and a decrease in growth-promoting hormones [63]	Decreased ABA content and an increase in growth-promoting hormones [63]
			Tendency to increase the content of brassinosteroids (dependent on the cultivar and on the type of analyzed steroid) [13]	Tendency to decrease the content of brassinosteroids (dependent on the cultivar and on the type of analyzed steroid) [13]
			Decrease in the accumulation of the brassinosteroid receptor (BRI1) [72]	Increase in the accumulation of the brassinosteroid receptor (BRI1) [72]
Other effects	Increased level of proline [38]	Decreased level of proline [38]	Increased level of proline [88]	Decreased level of proline [88]
			Increased anthocyanin content [25]	Decreased anthocyanin content [25]

4. How Could the Results of Deacclimation Studies Be Used in Practice?

Summarizing the results presented in this work, it should be emphasized that the degree of the reversal of the cold-induced changes via deacclimation is dependent on the species, cultivar, and above all, on the duration and the temperature of the deacclimation. Moreover, some physiological or biochemical parameters return to the values that are observed before cold acclimation more easily, while others are more stable. The research on the physiological-biochemical changes that accompany deacclimation will require further research. Nevertheless, the described physiological and biochemical changes that are induced by deacclimation can help to explain the basics of a lower frost tolerance in deacclimated plants. Deacclimation is a problem, especially in the case of winter crop plants. This is because the frost tolerance decreased by the deacclimation generates specific economic problems, as it may be connected to the loss of yields.

Today, we are dealing with the problem of global climate change and the challenge of feeding an increasing number of people in the world. Because the problem of deacclimation is occurring more frequently, we will have to consider the following: (1) cultivating crops that are characterized by a higher frost tolerance after deacclimation (deacclimation tolerant), (2) choosing cultivars that are characterized by a slower/later deacclimation process [91], or even (3) applying special preparations to protect deacclimated plants from frost injuries. It is also important to extend the current knowledge about the timeframes of deacclimation in order to predict more precisely how fast particular changes occur (in given species/cultivars).

Tracking the metabolic changes induced by deacclimation in field conditions is complicated because it is difficult to evaluate those changes visually, while complex laboratory analyses require the invasive collection of samples and are time-consuming. Fortunately, there are a few non-invasive measurements, such as measuring the chlorophyll *a* fluorescence, whose effectiveness has been proven for detecting deacclimation-induced changes in crop plants [13,16,25]. Measurements of the efficiency of photosystems based on chlorophyll *a* fluorescence can be used as a sensitive tool for monitoring the deacclimation process. What is even more important is that this method could also be used on large-scale cultivations in fields using drones or satellites to detect any changes in the fluorescence. Another method that could be used to evaluate the rate of deacclimation could be measuring the spectral properties of leaves (leaf reflectance measurements). Selected parameters such as ARI1 and ARI2, which define the level of anthocyanins, correspond well with the changes that are induced by deacclimation [25,77].

Another technique that could be used in assessing plant freezing tolerance during deacclimation could be electrical impedance tomography (EIT). Even though this technique is not yet popular in plant science, it could be used to examine the tolerance of plants to various stressors [92]. EIT is a rapid, nondestructive measurement, and the values of EIT images can be useful in a quantitative evaluation of changes induced during deacclimation and estimation of frost tolerance [92].

The results of laboratory research on deacclimation and non-invasive measurements taken together indicate that the deacclimation process (as mentioned above) generally partly or fully reverses the changes that are induced by acclimation in cold. However, many of the results were obtained in a controlled environment (plants grown in growth chambers with controlled light, temperature, humidity, etc.). It seems reasonable to conduct further similar studies in field conditions, which are more unpredictable. Already, in 1976, Gusta and Fowler [93] conducted research on winter wheat crowns artificially cold-acclimated and field-cold-acclimated. The results clearly indicated that the field-cold-acclimated crowns deacclimated faster and had a higher water content than the artificially acclimated plants [93].

Finally, it is worth emphasizing once again that the phenomenon of deacclimation is dangerous (e.g., for winter crops) when frost occurs after a period of warming. Deacclimated plants have the ability to reacclimate again, which may even result in acquiring higher frost resistance [31]. The mechanisms of this phenomenon are also worth deeper studies.

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