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Review

Deacclimation and reacclimation of cold-hardy plants: Current understanding and emerging concepts

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Abstract

The abilities of cold-hardy plants to resist deacclimation during transient warm spells and to reacclimate when cold temperatures return are significant for winter survival. Yet compared to the volume of research on the biology of cold acclimation, relatively little is known about how plants maintain and/or reacquire cold hardiness in late winter and spring. This review summarizes the past 40 years of research into deacclimation and reacclimation in herbaceous and woody plants and suggests questions that should be addressed with multi-disciplinary approaches to more comprehensively understand the biology of winter-survival in plants. Deacclimation and reacclimation are highly dependent on exogenous and endogenous factors such as the ambient temperatures, water availability, photoperiod, energy budget and metabolism, growth and development, and the dormancy status of plants. Putative mechanisms of these hardiness transitions are discussed based on the published accounts of changes in carbohydrates (e.g., compatible solutes), membrane lipids, proteins (e.g., dehydrins), antioxidants, photosynthesis, and gene expression. In conclusion, the relationships between environmental determinants, gene expression and regulation, cellular and organismal structure and function, and the consequent cold hardiness transitions in plants are discussed and debated.

Keywords: Chilling requirement; Dehardening; Dormancy; Freezing tolerance; Phenology; Rehardening

Contents

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1. Introduction

Survival of plants at freezing temperatures is dependent on their ability to cold acclimate in response to environmental stimuli such as short-days and low temperatures [1,2]. Plant species in cold climates have evolved adaptations such as dormancy, rapid acclimation, and maintainence of high cold hardiness throughout winter singly or in combination [3–5]. Although researchers have intensively studied various aspects of acclimation, the processes of cold deacclimation and reacclimation remain less understood. As will be described in this review, deacclimation resistance and reacclimation capacity play a significant role in determining plant hardiness during late winter and early spring when plants are particularly vulnerable to cold-injury due to emergence from dormancy.

Some terms should be defined at the outset. *Cold acclimation*, also known as *cold hardening*, is an increase in tolerance over time to cold temperatures and cellular desiccation in response to inductive conditions such as cold temeperature, short photoperiods, mild drought, etc., and that results from changes in gene expression and physiology [1,2,5]. Dormancy was defined by Lang et al. as a ''temporary suspension of visible growth of any plant structure containing a meristem'' [6]. When the dormancy-inducing, environmental or endogenous signals (e.g., low temperature, short photoperiod, hormones, etc.) are specifically perceived *within* (i.e., ''endo'') the affected meristem, it is called *endodormancy* which is regulated by physiological factors originating inside the affected structure. This is different from *paradormancy* that involves a dormancy-inducing signal originating in a structure *other than* (i.e., ''para'') the affected structure. *Ecodormancy* includes all those cases of growth suspension that result from unsuitable environmental (i.e., ''eco'') factors (e.g., hot or cold temperatures, dehydration, nutrient deficiencies, etc.) which have a non-targeted effect on all aspects of development and physiology including those of the dormant organ [6].

The term *deacclimation* has often been defined as a reduction in those levels of hardiness that were originally attained through an earlier acclimation process. However, deacclimation may also refer to mechanisms that mediate reduced hardiness rather than simply the loss of hardiness per se. Additionally, the term deacclimation can be used to describe losses in hardiness due to such diverse factors as environmental stimuli (i.e., warm temperatures), phenological changes, and reactivation of growth. Furthermore, deacclimation may be either reversible by subsequent re-exposure to low temperatures or result in a largely irreversible loss of hardiness.

In this paper, the term *deacclimation* will refer to a loss of acclimated cold hardiness measured at the cellular, tissue, or whole-plant level, irrespective of the stimulus that initiated deacclimation or the mechanism by which it occurred. Changes in structure, physiology, or gene expression associated with the loss of hardiness represent putative mechanisms that could account for deacclimation. If a deacclimated plant is subsequently exposed to cold temperatures, it may regain some or most of the lost hardiness in a process called *reacclimation*. Similar considerations apply to the definition of reacclimation as have been stated for deacclimation. The terms deacclimation and reacclimation are used in this paper in preference to the synonymous terms *dehardening* and *rehardening* often found in the agronomic, horticultural, and forestry literature.

This review is divided into three subject areas. In Section 2, the general characteristics of deacclimation and reacclimation are introduced with an emphasis on the role of temperature. Section 3 illustrates how growth and development affect deacclimation and reacclimation and how the influence of growth is modulated by photoperiod and dormancy. Section 4 summarizes information on biochemistry, molecular genetics, and physiology associated with deacclimation. Emphasis is placed throughout this review, but particularly in the conclusion, on identifying gaps in our current understanding and suggesting possibilities for future research.

2. Description of deacclimation and reacclimation

2.1. Deacclimation kinetics

Deacclimation occurs more rapidly (days to weeks) than acclimation (weeks to months) in both natural and controlled environments. Cold acclimated *Solanum commersonii* leaves exposed to 20 °C began to deacclimate within 2–3 h and all acquired hardiness was lost after 1 day [7]. In comparison, 15 days at 2 °C were needed for maximum acclimation. 'Grasslands Paroa' annual ryegrass (*Lolium multiflorum*) lost 4 °C in hardiness after 7 days at 12 °C, whereas an acclimation of 4 °C required 22 days of exposure to 2 °C [8]. Autumnal acclimation of peach (*Prunus persica*) bark and xylem tissues was significantly slower than their deacclimation [9]. In addition, deacclimation was more rapid than reacclimation in apple (*Pyrus malus*) bark, in which 1 day of deacclimation (loss of 15 °C in hardiness) required 3 cold days to reverse [10].

Differences in acclimation and deacclimation kinetics may be related to divergent energy requirements. Acclimation involves changes in structure and function, necessitating large amounts of energy [11]. Deacclimation, however, may be a relatively less energy-intensive process, in which more downregulation of gene expression and biosynthesis occurs

than upregulation. Moreover, deacclimation could perhaps be fueled by the catabolism of metabolites (e.g., compatible solutes, stress proteins, etc.) that often are synthesized or accumulate during cold acclimation. Since acclimation is an energy-intensive and lengthy process it is curious that reacclimation is typically rapid [12]. However, assuming that only partial deacclimation occurs, the amount of de novo gene expression and biosynthesis required during reacclimation to regain maximal hardiness should be less than the amount required by acclimation. Additional research on energy availability and consumption during transitions in hardiness is needed.

Woody perennials exhibit two forms of deacclimation that we term ''active'' and ''passive''. ''Active deacclimation'' occurs in response to substantial increases in ambient temperature, progresses rapidly, and is associated with wide-ranging structural and functional changes associated with resumption of growth. Although typically occurring in the spring, active deacclimation may occur prematurely during winter in response to transient warm spells. In contrast, ''passive deacclimation'' results from the exposure of fully acclimated plants in mid-winter to small to moderate elevations (∼5 °C or less) in temperature for extended durations of time. Large-scale changes in gene expression have not been observed during passive deacclimation that is largely associated with depletion of carbohydrate reserves due to enhanced metabolism. Artificial elevation of winter temperatures by only 2–3 °C resulted in deaccclimation and premature budbreak in bilberry (*Vaccinium myrtillus*) [13]. In a field study, Ögren [14] also observed passive deacclimation in bilberry during a mild winter (+5 °C). He believed it was a consequence of increased use of stored, soluble sugars to support higher rates of respiration that were not offset by CO_2 fixation. Passive deacclimation can leave plants more susceptible to subsequent active deacclimation. Needles of Scots pine (*Pinus sylvestris*) subjected to elevated temperatures (+5 to +20 °C) lost hardiness in spring up to 46 days earlier than needles of saplings kept in constant cold [15].

Although several groups have studied the potential impact of global warming on passive deacclimation of boreal plants [13–17], mechanistic studies are needed to increase our knowledge of how slight elevations in winter temperatures cause decreases in both the extent and duration of freezing tolerance. Due to the emphasis on global warming in some experiments [13–16], plants were exposed to warm temperatures through all or part of the autumn to simulate a long-term increase in ambient temperatures. However, such an experimental design makes it difficult to study passive deacclimation if the temperature regimes used in these experiments did not allow maximum acclimation to occur in the first place. Consequently, it may be difficult to separate the effects of elevated temperatures on incomplete acclimation from actual passive deacclimation.

2.2. Effects of temperature (degree and duration) on deacclimation

Cold hardiness is strongly affected by the recent history of ambient temperatures to which the plant has been exposed [10]. This relationship is partly quantitative; as ambient temperatures increase both the rate and the extent of deacclimation increase commensurately. Deacclimation of *S. commersonii* leaves was greater at 20 °C than at 10 °C [7] and crowns of winter wheat (*Triticum aestivum*) and winter rye (*Secale cereale*) deacclimated more rapidly at 20 °C than at 10 or 15 °C [18]. Larch (*Larix xeurolepis*) seedlings deacclimated about 4–5 weeks earlier during an atypically moderate winter than during a colder winter [19]. Moreover, the history of temperature exposure affects acclimation and deacclimation in complex ways, in that, an identical temperature regime could either cause acclimation or deacclimation depending on the previous conditions to which plant was exposed. 'Optima' annual ryegrass grown at 15 °C and acclimated at 2 °C, when deacclimated at 6, 8, or 10 °C, was on average 1 °C hardier than plants grown at 15 °C but acclimated at 6, 8, or 10 °C without subsequent deacclimation [8]. A divergence in the final hardiness after acclimation or deacclimation under the same temperature indicates that a given temperature may regulate these processes differently [8]. Thus, the prediction of hardiness solely from current temperatures is inadequate, as it appears to be influenced by the environmental and physiological history of the plant.

The extent and rate of deacclimation depends not only on the magnitude of the increase in temperature but also on the duration of exposure. While the level of deacclimation increases with duration of exposure, the rate of deacclimation decreases as deacclimation progresses. In winter faba-bean (*Vicia faba*) foliage exposed to 15 °C, the rate of deacclimation decreased 13-fold over a period of 6 days [20], and the deacclimation was more rapid in plants exposed to higher deacclimating temperatures. The relationship of warm-temperature duration to the rate of deacclimation, however, is not always linear. Whereas some plants may rapidly deacclimate immediately upon exposure to warm temperatures and then exhibit gradually decreasing rates [12,20], others deacclimate at more stable and moderate rates for relatively longer durations. Exposure of potatos (*Solanum*) to 18 °C for 12 h induced 63% and 20% deacclimation in *S. multidissectum* and *S. megistacrolobum* subsp. *toralapanum*, respectively, yet a longer exposure (24 h, 18 °C) caused up to 65% and ∼100% deacclimation in the former and the latter, respectively [21].

Since the response to deacclimating temperatures is not instantaneous, there may be a 'lag phase' during which exposure to warm temperatures does not result in deacclimation. Depending on the species, the lag phase may vary from a few minutes to hours. There is little information about the early stages of deacclimation in the literature. Future research should be directed at monitoring changes in gene expression and physiology during the first minutes to hours of increased temperatures. These data may clarify how the temperature signal is transduced and deacclimation initiated.

2.3. Reacclimation

Although reacclimation is common in many overwintering plants, a return to pre-deacclimation levels of hardiness is not

always possible (see Ref. [22]). The capacity for reacclimation becomes more limited as the degree or duration of warm-temperature exposure increases and deacclimation advances. Apple bark tissues could reacclimate only to the hardiness level exhibited on the day previous to the final day of deacclimation [10]. Furthermore, Gusta and Weiser [23] exposed Korean boxwood (*Buxus microphylla* var. *koreana*) to alternating temperature cycles (25 °C followed by −10 °C) and found that although deacclimation was fully reversible after one such cycle, reacclimation capacity declined with subsequent cycles. Whether the loss of reacclimation capacity is due simply to a lack of energy-producing substrates (required for acclimation) or irreversible developmental changes following deacclimation has not been determined [1,24,25].

'Reacclimation potential' decreases with increasing deacclimation duration. However, since the difference between the hardiness of a cold acclimated and a deacclimated plant increases as deacclimation progresses, the amount of reacclimation required to re-establish a fully acclimated status should increase with lengthening deacclimation duration, provided the reacclimation potential is not impaired. The question is: how do these two seemingly opposing effects of deacclimation duration on *actual* reacclimation relate to one another and at what point during the course of deacclimation is the reacclimation highest? Conceivably, actual reacclimation described as a function of deacclimation duration might be a parabolic response, whereby the greatest reacclimation occurs partway through the deacclimation time-course.

Temperature fluctuations, such as day/night cycles, impact hardiness differently than do constant temperatures because of their effect on deacclimation–reacclimation cycles. The hardiness resulting from alternating exposures to warm and cold often depends on the magnitude and duration of each temperature. Perennial ryegrass (*Lolium perenne*) exposed to combinations of high and low temperatures exhibited levels of hardiness intermediate to those attained under each temperature individually [26]. Acclimated *Forsythia* x*intermedia* stems exposed to 21 °C (18–24 h) and to 4 °C (0–6 h) lost more hardiness as the duration of exposure to 21 °C increased [27]. Cold night-time temperatures can promote reacclimation as long as the day-time temperatures are not too warm and long. Constant 6 °C temperatures were associated with steady deacclimation of Scots pine needles whereas fluctuating temperatures that averaged a 6 °C exposure (11–1 °C) were associated with deacclimation and reacclimation; needles reacclimating over 10 °C during the colder phase of the treatment [28]. These data suggest that alternating temperatures should be included in investigations of deacclimation physiology in order to mimic natural conditions.

2.4. Deacclimation and reacclimation: influence of genetics and environment

Both the magnitude and kinetics of deacclimation may differ between closely related species, varieties, or ecotypes and most likely reflect the evolutionary pressures exerted by the environment within the habitat of origin. For example, northern ecotypes of mountain birch (*Betula pubescens*) deacclimated to a lesser degree than those from more southerly latitudes [29]. In white clover (*Trifolium repens*) stolons the Norwegian variety 'Bodø' exhibited greater resistance to deacclimation at 6 °C than the British variety 'Aberherald' [30]. These studies indicate that deacclimation is regulated by the environment through interaction with the genetics of the plant.

Importantly, field and controlled studies on the same species can result in different responses to temperature. Compared to artificially acclimated winter wheat crowns, field-acclimated crowns deacclimated faster and had higher water content [18]. The reacclimation capacities of artificially acclimated crowns were not dependent on deacclimation temperature, but reacclimation of naturally acclimated crowns was larger when they were deacclimated at colder temperatures (10 or 15 °C versus 20 °C). It appears that the environmental context under which initial acclimation occurs can affect deacclimation and reacclimation as much as the genetic background.

2.5. Mathematical modelling

Acclimation and deacclimation data have been used to develop mathematical models that predict how these processes are affected by changing physiological and environmental parameters. Gay and Eagles [8] modeled acclimation and deacclimation in ryegrass based on variables such as cultivar, initial hardiness, duration of warm-temperature exposure, and the temperature-dependent maximum change in hardiness. Jönsson et al. [31] used a computer-based global warming simulation to estimate cold-injury for Norway spruce (*Picea abies*). Variables in that study included the timing of deacclimation and bud burst, initial hardiness, and the severity of cold temperatures after unseasonable warm spells. In a study by Lecomte et al. [32], the deacclimation rate of winter wheat was associated with the difference between the hardiness of non-acclimated and fully acclimated plants. However, variables such as genotype, developmental stage, and the plant's history of hardiness were not considered in these studies.

Although models use simplistic assumptions, the findings from deacclimation models generally are consistent with empirical data. For example, acclimation and deacclimation rates in ryegrass were proportional to temperature within a limited range [8], and the deacclimation rate in winter wheat increased linearly as the temperature rose above 0 °C [32]. Hardiness of ryegrass during acclimation and deacclimation was described by an exponential curve that plateaued at a final temperature-dependent value [8]. Models are most valuable when they simulate conditions that occur over large spatial or temporal scales that are difficult to replicate in a field study.

2.6. Deacclimation as related to mid-winter hardiness and acclimation rate

It seems reasonable to assume that overwintering plants with high mid-winter hardiness would also exhibit a high degree of resistance to deacclimation, and that therefore the mechanisms

responsible for these processes might somehow be linked. Studies on a variety of species, however, indicate that this hypothesis is overly simplistic, and that high deacclimation resistance and high mid-winter hardiness represent two different attributes that are inherited independently. Among potato [21] and filbert (*Corylus* spp.) [33] varieties, high deacclimation resistance was not always associated with large acclimation capacities. Although the 'Concord' grape (*Vitis labrusca*) had higher mid-winter hardiness than 'Cabernet Sauvignon' (*Vitis vinifera*), the former deacclimated more rapidly [34]. The maximum hardiness of *Rhododendron kiusianum* floral buds did not differ between fall- and spring-bloom clones but deacclimation resistance was higher in the latter [35]. In blueberry (*Vaccinium*) cultivars, maximal hardiness was closely related to deacclimation resistance in *V. ashei* 'Tifblue' and *V. corymbosum* 'Bluecrop' but this relationship could not be applied to all the cultivars [36].

Published data also indicate that high mid-winter hardiness and high deacclimation resistance are not necessarily present together in plants that have evolved in cold climates. Although two Japanese *Rhododendron* species, *R. kiusianum* and *R. scabrum*, exhibit similar deacclimation kinetics, the former is a high altitude (1000 m) species whereas the latter is native to subtropical islands [37]. The deacclimation resistance of floral buds from nine genotypes of American deciduous azaleas (*Rhododendron*) was often correlated with high maximal hardiness and a geographical origin in cold climates. However, the cold-hardy *R. canadense* (−28.0 °C), native to the north-Atlantic states, deacclimated faster than the Georgian species *R. prunifolium* (−24.6 °C) [12]. Clearly, the deacclimation kinetics of a plant cannot be simply predicted from its mid-winter hardiness or the mean winter temperature or elevation of its habitat.

Furthermore, the ability to rapidly acclimate is also not always associated with a high cold acclimation capacity or high deacclimation resistance. *S. commersonii* attained higher levels of hardiness (−9.3 °C) than five other potato species but acclimated more slowly than some species and deacclimated faster than others [21]. Similar examples of a lack of correlation between the ability to rapidly acclimate, achieve high maximum levels of hardiness, and resist deacclimation have been observed in Chinese elm (*Ulmus parvifolia*) [38] and filberts [33].

2.7. Possible explanations for deacclimation resistance

If deacclimation resistance is not highly correlated with the timing and speed of acclimation, maximal hardiness, or climate of origin, the question arises what other environmental or biological factors impact deacclimation resistance more consistently? One possibility is that deacclimation resistance is a function of the degree of temperature fluctuations (frequency and magnitude) to which plants are exposed in their native habitats rather than low temperatures per se. Plants growing under relatively stable conditions would experience little evolutionary pressure to develop deacclimation resistance to transient increases in temperature [12]; this hypothesis is supported by the following example. Although exposure of lodgepole pine (*Pinus contorta* var. *latifolia*) to elevated temperatures resulted in reduced hardiness and sugar levels, this response was not observed for similarly treated Norway spruce and Scots pine [16]. These Scandinavian conifers evolved under winter temperatures that are more variable compared to those typical of the central Canadian habitat of lodgepole pine. On the other hand, there are also published reports that do not support this hypothesis [12,29].

However, a closer examination of this hypothesis may be warranted. Conceivably, temperature variability would only be significant for the evolution of deacclimation resistance if temperatures regularly rose or fell over ranges that would *actually* impact hardiness [12]. Therefore, studies of the effects of temperature fluctuations on deacclimation must establish the threshold temperatures that impact deacclimation and describe the effect of different temperature magnitudes and durations. When such data is analyzed in the context of climatic conditions, it should be possible to determine if plants exposed more frequently to transient increases in physiologically relevant temperatures indeed exhibit higher deacclimation resistance [12].

Alternatively, high deacclimation resistance could be prevalent in plants with delayed spring development or deeper ecodormancy. To illustrate, the flower development and anthesis of *R. prunifolium* is slower than in other deciduous azaleas [39] and this species exhibits high deacclimation resistance under controlled conditions [12]. Finally, it is important to note that low deacclimation resistance may not always be deleterious to winter survival, especially if sufficient reacclimation can quickly occur. Therefore, both deacclimation resistance and reacclimation capacity need to be examined when determining the overall response to low temperatures by overwintering species.

3. Relationship of deacclimation and reacclimation to growth and development

3.1. Direct and indirect effects of growth and development on hardiness

As previously discussed, warm temperatures can induce plants to deacclimate. To a greater or lesser extent this deacclimation is reversible, i.e., it does not preclude reacclimation. Warm temperatures, however, can also promote the resumption of growth in non-endodormant plants, which can lead directly or indirectly to deacclimation which is not reversible [28]. High growth rates in oilseed rape (*Brassica napus* var. *oleifera*) were associated with enhanced deacclimation and reduced or eliminated the capacity for subsequent reacclimation [25,40]. Similar reductions of reacclimation capacity were reported by Repo [22] in Scots pine. Deacclimation and development can also have additive effects on the loss of cold hardiness. For example, the hardiness of *Salix dasyclados* cambial cells declined by 70 °C during outdoor deacclimation in early spring and further declined by 7–12 °C after budburst in May [41].

The mechanism by which ontogenetic development modulates hardiness has been the subject of some speculation but relatively little research. Growth and development could have a negative effect on hardiness by altering sub-cellular structure. For example, the increased cellular water and reduced cytosol to vacuole ratios that accompany cell expansion can cause reduced hardiness [42,43]. High water content renders the plant more susceptible to mechanical damage from extracellular ice and promotes intracellular freezing which is invariably lethal [1]. Alternatively, active growth interferes with deacclimation resistance and reacclimation capacity by competing for energy resources [1,25,40]. Research indicates that vegetative growth and cold acclimation in winter rye were dependent on both the total amount of available energy and how these resources were allocated to these two processes [44]. When photosynthetic rates were high, energy was available for both growth and acclimation, but as photosynthesis declined both processes were impacted negatively. Moreover, when vegetative growth was inhibited by short days, plants required less light exposure (i.e., less production of photosynthates) to achieve the same degree of hardiness as those plants that grew at faster rates.

Growth initiation involves developmental reprogramming that requires de novo gene expression that can have a deleterious influence on hardiness [24,25,40,45]. The relationship of phenology to hardiness in winter wheat suggested that the *vrn* and *ppd* genes determined hardiness during deacclimation and reacclimation through effects on development [24,45]. Rapacz [40] favored the developmental reprogramming hypothesis over the resource competition model because hardiness was independent of osmotic potential (soluble sugars) during deacclimation and reacclimation in oilseed rape.

3.2. Role of photoperiod in deacclimation and growth

Evidence shows that long-days can enhance deacclimation and diminish reacclimation capacity at specific developmental stages. Long-day exposure (16 h) enhanced stem elongation, increased deacclimation rates, and reduced reacclimation capacities for a spring cultivar (no vernalization requirement) of oilseed rape [25]. When exposed to long-days (17 h), Scots pine saplings deacclimated more rapidly than those exposed to natural photoperiods only after (but not before) initiation of shoot-elongation [28]. In addition, growth rates of pine were greater under long-days and were associated with increased deacclimation. Long-days appear to decrease hardiness by stimulating development [25,28], possibly by altering photoperiod-dependent gene expression. Long-days could also enhance growth by promoting photosynthesis simply due to more light [28] but, as will be discussed later, evidence for this interpretation is lacking.

The work of Mahfoozi et al. [24] with fully vernalized winter wheat demonstrates the effect of photoperiod during acclimation on subsequent deacclimation and reacclimation as well as the influence of photoperiod on deacclimation-associated growth. For example, the plants cold-acclimated under short-days (56 days, 4 °C; 8 h) did not show vegetative to reproductive phase transition when deacclimated (14 days, 20 °C) also under short days. In contrast, plants acclimated under long days (20 h) made this transition successfully when deacclimated under short days. Furthermore, short-day acclimated plants also underwent this developmental transition when deacclimated under long-days. Finally, plants that were originally acclimated under long-days had smaller reacclimation capacities (−7.0 to −3.5 °C) following deacclimation than those acclimated under short-days (−13.7 to −11.5 °C).

Nevertheless, long-day photoperiods do not always promote development and subsequent deacclimation and their relationship to dormancy is not always clear. Three latitudinal ecotypes of mountain birch with different critical day-lengths did not exhibit ecotype-specific responses to changing photoperiods with regards to dormancy breaking, rehydration, or spring deacclimation of buds [46]. Thus deacclimation and dormancy transitions in mountain birch were modulated mainly by temperature and not photoperiod [46]. Discrepancies regarding the effect of photoperiod on hardiness and development might be clarified by identifying the genes and signal transduction pathways involved in these processes. Is the communication between the signaling and response pathways in those plants that lack photoperiodic regulation of deacclimation and development impaired? Why regulation of deacclimation by photoperiod exists in some plants but not in others remains an important ecological question.

3.3. Relationships among dormancy, deacclimation, and growth

Dormancy often inhibits or prevents resumption of growth and accompanying deacclimation. Deacclimation resistance was high when grape buds were endodormant (deacclimation required 16 days at 23 °C) but deacclimation occurred more readily as spring approached [34]. Tart cherry (*Prunus cerasus*) floral buds resisted deacclimation during dormancy, such that buds closer to emerging from endodormancy were less resistant to deacclimation [47]. Exposure of red-osier dogwood (*Cornus sericea*) to 24/18 °C (D/N) did not induce significant deacclimation of endodormant stems, but once dormancy was broken the deacclimation was rapid [48]. Although dormant *Viburnum plicatum* ssp. *tomentosum* deacclimated by less than 4–6 °C (7 days, 21 °C), non-dormant plants lost 11–12 °C of hardiness under identical conditions [49]. The amount of deacclimation in *F.* x*intermedia* stems exposed to 6 days at 21 °C increased from 3 to 13 °C as the depth of dormancy diminished between December and March [27]. Also, the length of low-temperature (4 °C) exposure within a daily temperature cycle required to prevent deacclimation of *F.* x*intermedia* increased as dormancy weakened, a response indicative of decreased deacclimation resistance. Deacclimation kinetics of mountain birch ecotypes were associated with chilling requirements predicted from altitude, latitude, and distance from the coast [29]. Winter-collected floral buds from *R. kiusianum* fall-bloom clones deacclimated after 4 weeks at 17 °C but buds of spring-bloom (dormant) clones did not [35]. Disappearance of the *ppdhn1* dehydrin transcript/protein in late winter and spring was more rapid in an evergreen, non-dormant

genotype of peach than in an endodormant deciduous sibling [50]; it is noteworthy that dehydrin accumulation was quantitatively associated with hardiness in these peaches.

The intensity of dormancy may also modulate the reacclimation capacity of overwintering plants. Whereas dormant *Viburnum* exhibited a reacclimation capacity of 6–11 °C in mid-winter, non-dormant plants could only reacclimate by 7 °C or less [49]. The potency of photoperiodic and temperature signals to promote reacclimation and prevent deacclimation decreased following the completion of dormancy in Scots pine seedlings [28]. Artificial regulation of dormancy has also been shown to have the same effects on deacclimation and growth as natural dormancy transitions; treatments have included ABA [49], hot water (47–50 °C) [34,51], and hydrogen cyanamide [51]. However, it is noteworthy that stresses such as heat or toxins may often lead to global effects on physiology that may not be discernible from their specific effects. Research also indicates that dormancy or reduced growth may not always be required to prevent deacclimation. *Rhododendron* x*akebono* exhibited greater resistance to deacclimation than *R. kiusianum* despite its earlier development and flowering in the spring [37]. Also, no association was found between chilling requirements and deacclimation resistance in blueberry cultivars in either controlled environments [36] or field studies [52]. Further research should allow us to better understand the role of dormancy in deacclimation.

4. Physiology, biochemistry, and molecular biology of deacclimation and reacclimation

4.1. Water content and distribution

Deacclimation and renewed growth are associated with tissue/cellular rehydration. If cold weather returns, this high water content may result in mechanical damage due to extracellular freezing and can increase the rate of ice propagation through tissues [53]. Research indicates that the hardiness in deacclimating oilseed rape [25] and perennial ryegrass [54] was negatively associated with increasing water content and growth. Also, the moisture content of winter wheat and rye crowns increased throughout deacclimation and was usually correlated with hardiness [18,55]. However, the tissue hydration did not correlate as strongly with the hardiness in wheat and rye following reacclimation [55].

During acclimation of dormant floral buds of certain species water moves from frost-sensitive immature flowers and peduncles to frost-tolerant tissues such as bud scales and axes [56–58], where it freezes. This ‘‘extra-organ freezing’’ can be maintained throughout winter by low temperatures and dehydration even after endodormancy is complete [37]. Deacclimation of deciduous azaleas [56,59] was associated with water flux from the scales and lower bud axis into the flowers, peduncles, and upper bud axis, a reversal of what happens during cold acclimation. Bud reacclimation in *Rhododendron japonicum* [56] and *Prunus avium* [60] was associated with a redistribution of water analagous to that observed during acclimation. However, not all studies unequivocally support the importance of water content [37] or water distribution [61] in bud hardiness.

Tissue moisture can also impact hardiness transitions through mechanisms distinct from its effect on extracellular freezing. Tissue dehydration provides cross-protection against the cold because of universal adaptations to dehydrative stress at the molecular level. In addition, cellular dehydration reduces metabolic activity [14], growth, and energy consumption. For example, dehydration of bilberries undergoing passive deacclimation at elevated temperatures (+5 °C) improved stem hardiness by 5–10 °C relative to plants growing in more moist conditions [14].

4.2. Carbohydrates

Carbohydrate metabolism is altered during both passive and active deacclimation. Soluble sugars probably decrease during passive deacclimation as carbon losses surpass carbon gains at slightly elevated winter temperatures. This follows because low temperatures reduce mitochondrial respiration less than they do photosynthesis [14,17]; respiration is also more resistant to cellular dehydration and freeze–thaw stress [62]. Research shows that passive deacclimation of bilberry shoots was associated with reduction in osmolytes, including soluble sugars [14]. Also, a linear correlation emerged between soluble sugar levels and hardiness of Scots pine needles after exposure to various warm temperatures that reduced sugars by up to 54% [17]. Similar reductions have been observed also during active deacclimation. Controlled deacclimation of aleppo pine (*Pinus halepensis*) and radiata pine (*Pinus radiata*) was associated with declining soluble sugars [63]. Deacclimation of both reed canary grass (*Phalaris arundinacea*) (14 days, 12–20 °C) and cabbage (*Brassica oleracea*) (1 day, 20/15 °C D/N) was associated with decreasing levels of fructose, glucose, and sucrose [64,65]. Deacclimation (12 or 18 °C) reduced proline in white clover stolons; proline is often correlated with enhanced hardiness during acclimation and is suggested to be a compatible solute [30].

Qualitative changes in specific carbohydrates may occur during deacclimation even when there is no clear quantitative relationship between total sugars and hardiness. In floral buds of *F.* x*intermedia* and *Forsythia suspensa* total sugar levels decreased during early deacclimation but subsequently increased in late spring even as hardiness continued to decline [66]. However, concentrations of raffinose-family oligosaccharides (RFOs) (stachyose, raffinose) and a precursor (galactose) were associated with hardiness throughout deacclimation. Importantly, a possible function of RFOs is to protect membranes from freeze-induced desiccation stress [67].

The concentrations or activities of enzymes involved in carbohydrate metabolism may also change during deacclimation. Deacclimation of *Arabidopsis thaliana* was associated with the expression of a putative myo-inositol oxygenase that produces D-glucuronate [68]; D-glucuronate can be used to synthesize matrix polysaccharides or be catabolized during respiration. Acclimation and deacclimation in timothy (*Phleum*

pretense) and reed canary grasses were related to increases and decreases, respectively, in invertase activity [64]. Invertase, a sucrose-hydrolyzing enzyme which normally resides within the cell-wall or vacuole [69], may aid incorporation of translocated sucrose into acclimated sink-tissues [64] and also provide higher osmoticum to cold acclimated cells. Downregulation of cell-wall invertase during deacclimation may be conducive for subsequent growth. Translocated sucrose is converted by sucrose synthase to UDP-glucose which can then be used by cellulose synthase to produce cellulose fibers in an expanding cell [69]. Thus, absence of invertase downregulation could result in inhibition of sucrose synthase due to competition for sucrose and negatively regulate growth.

A few studies have implicated α-galactosidase in deacclimation. In timothy and reed canary grasses α-galactosidase activity remained unchanged and increased during acclimation and deacclimation, respectively [64]. During deacclimation of petunia (*Petunia* x*hybrida*) α-galactosidase degrades raffinose-family oligosaccharides (RFO) (i.e., raffinose, stachyose, and mellobiose) which accumulated during acclimation [67]. In petunia *PhGAL* (α-galactosidase gene) transcription increased after 1 h of deacclimation and then decreased to pre-deacclimation levels after another hour. Elevated *PhGAL* expression was associated with increased α-galactosidase activity and decreased raffinose levels. It follows from these examples that temperature-dependent expression of *PhGAL* could regulate RFO degradation in the spring and mediate deacclimation [67]. Future experiments such as above, incorporating both molecular genetics and physiology, would likely produce testable mechanisms for deacclimation.

Changes in starch content have also been implicated in deacclimation. Deacclimation in cabbage [65] and roots of Scots pine [17] were associated with starch degradation. However, others have noted increases in starch during deacclimation or both increases and decreases at different stages of the process. Passive deacclimation of bilberries occurring from October to May (at 2–3 °C elevated winter temperatures) reduced glucose, fructose, and sucrose while increasing starch reserves [13]. Although deacclimation of white clover stolons at 18 °C was initially accompanied by starch accumulation, levels subsequently declined to pre-deacclimation amounts [30]. Starch content in *Salix dasyclados* cambium was high during October, low throughout winter, and then increased during spring deacclimation [41]. These observations imply that there is no general relationship between starch reserves and hardiness. Hence it is probable that fluctuating amounts of starch, presumably resulting from net carbon gain or loss, do not *directly* contribute to the underlying mechanisms of deacclimation and reacclimation, as was suggested by Levitt [1]. Measurements of carbon input and output during deacclimation and reacclimation, via employing radioactive tracers or gas exchange measurements, would help to resolve this question. Comparison of starch levels in mutants with enhanced or depressed rates of photosynthesis or respiration during deacclimation would also be enlightening.

Descriptive physiology has a limited ability to unravel *cause and effect* relationships; decreasing concentrations of soluble sugars during deacclimation may either cause plants to lose hardiness or may themselves be a consequence of lost hardiness. In some cases changing levels of carbohydrates are merely associated contingently with hardiness transitions and do not represent mechanisms of deacclimation or reacclimation (e.g., changing starch levels). Since enzymatic reactions are temperature-sensitive, changing size of carbohydrate pools may follow from the effects of warm temperatures per se rather than regulation of hardiness. For example, after deacclimation at high day and low night temperature combinations, carbohydrate levels in the perennial ryegrass 'Premo' were not correlated with hardiness [26]. Moreover, decreasing the night temperature or increasing the day temperature to 10 °C both increased carbohydrate levels during deacclimation. In the above examples, changing carbohydrate levels probably resulted from a temperature-induced imbalance of carbon gain to carbon loss rather than from adaptations for hardiness [26]. The use of molecular genetic tools, coupled with proteomics and metabolomics of cold hardiness transitions (to document more variables than traditional physiology) could aid in separating mechanisms from fortuitous correlations.

4.3. Photosynthesis

Despite the significance of photosynthesis, relatively few studies have examined its role in deacclimation and reacclimation. A seasonal study showed that cold acclimation and deacclimation in Scots pine were associated with a decrease and an increase, respectively, in both the light-saturated assimilation rate and apparent quantum yield; recovery of photosynthesis during spring was faster than the loss of hardiness [70]. Gene expression and protein activity related to photochemistry and CO_2 fixation can respond to changing temperatures in overwintering green tissues. Transcript levels for a chlorophyll *a*/*b*-binding protein, the rubisco subunits, ferredoxin-thioredoxin reductase, and a transketolase declined during deacclimation of winter rye leaves; transcription was higher in acclimated than in non-acclimated tissues [71]. Research also shows that cytosolic fructose-1,6-bisphosphatase transcription decreased during deacclimation, although transcript levels were lower in acclimated than in non-acclimated leaves.

In theory, two diametrically opposite outcomes of the inability to produce photosynthate during deacclimation may be conceived. On one hand, insufficient carbohydrate might negatively regulate growth and developmental gene expression, resulting in hardiness being maintained. On the other hand, carbohydrate scarcity would render deacclimation resistance or reacclimation more difficult due to their high energy cost, resulting in enhanced deacclimation. However, little experimental data exist to address this paradox. In one study, acclimated winter wheat and barley (*Hordeum vulgare*) both lost the same amount of hardiness irrespective of deacclimation occurring in light or dark [72]. In addition, reacclimation of wheat and barley was still possible in dark. Although interpretation of these experiments is difficult because carbon-exchange rates were not measured, apparently the

additional energy provided by photosynthesis did not strongly affect deacclimation or reacclimation [72].

Renewed growth can induce changes in gene expression and/or enzyme activity otherwise uncharacteristic of deacclimation and impair subsequent reacclimation of the photosynthetic apparatus. Deacclimation in oilseed rape was associated with decreasing activities of rubisco and sucrose-phosphate synthase (SPS) [73]. SPS activity subsequently increased during reacclimation. Upregulation of these enzymes is needed during acclimation for photosynthesis to be more efficient at low temperatures. However, enhanced growth during deacclimation was related to sustained and transitory increases in SPS and rubisco activities, respectively. Furthermore, failure to sufficiently reduce the growth rate during reacclimation inhibited maintenance or recovery of photosynthetic capacity. These observations indicate that photosynthesis cannot be studied in isolation vis-à-vis cold hardiness transitions, as it influences and is itself influenced by carbohydrate pools, growth rates, and the antioxidant capacity.

4.4. Antioxidants

Green tissues in winter often absorb more solar energy than can be processed by photosynthesis due to the inhibition of CO_2 fixation by cold [74]. If this extra energy is not quenched (by photochemical or non-photochemical means), the photosystems become excessively reduced resulting in the production of reactive oxygen species that are injurious to macromolecules [74]. Some studies suggest that deacclimation is associated with reduced tolerance of plants to oxidative stress. For example, malate, which comprised 22% of total non-structural carbon in acclimated leaves of *Ranunculus glacialis*, decreased substantially during deacclimation [75]. Malate could exchange reducing equivalents from chloroplasts to sites of oxidation in mitochondria via malate shuttle systems [69]. It is thus possible that enhanced malate shuttling prevents excessive excitation of photosynthetic electron transport in cold-hardy *Ranunculus glacialis* and that in deacclimated plants this system is compromised resulting in oxidative stress [75]. Research also indicates that transcript and protein levels of peptide methionine sulfoxide reductase (PMSR), an antioxidant enzyme, declined during deacclimation of winter rye leaves; PMSR upregulation was probably due to photooxidative stress resulting from exposure to light at low temperatures [71].

However, not all research supports a connection between deacclimation and reduced protection against oxidative stress. Passive deacclimation of bilberry resulted in minor reductions in glutathione and did not affect its redox state [13]. Evidence also indicates that loss of particular antioxidants does not necessarily result in oxidative damage. Deacclimation of *Ranunculus glacialis* was associated with an increase in some antioxidants (carotenoids and α-tocopherol) without affecting ascorbate and glutathione [75]. In both *Ranunculus glacialis* and *Soldanella alpina*, exposure to 22 °C was associated with decreased xanthophyll cycle pigments and zeaxanthin synthesis, but not with diminished protection against excessive light excitation [75].

4.5. Lipids and membranes

Membrane lipid composition has been linked to cold tolerance in plants. It has been suggested that hardiness is a function of membrane fluidity or the transition temperatures of solid and gel phases [1]. It is therefore surprising that only four studies known to the reviewers have examined the role of lipids in deacclimation. Deacclimation of *Salix dasyclados* phloem parenchyma was associated with unidentified metabolic changes in lipid bodies [41]. In both mulberry (*Morus bombysis*) bark [76] and Scots pine roots [77] deacclimation is associated with decreased ratios of unsaturated to saturated phospholipids, phospholipids to proteins, and phospholipids to sterols. In general, membrane lipid changes are largely a reversal of those observed during autumnal acclimation [76,77]. The changing composition of cellular membranes during deacclimation reduces their ability to survive subsequent freezing stress. Whereas the site of freeze–thaw injury in acclimated protoplasts of Jerusalem artichoke (*Helianthus tuberosus*) was the plasmalemma, deacclimated protoplasts most often suffered injury to both the plasmalemma and tonoplast [78]. Tonoplast injury occurred concurrently with cytoplasmic acidification and disappearance of transvacuolar strands. Thus deacclimation not only increased the risk of low-temperature stress but also changed the site at which injury occurred. Regulation of genes important in lipid metabolism and the interactions between lipids and other macromolecules in the deacclimating cell remain potential areas of research.

4.6. Proteins and gene expression

Little research has been done to investigate qualitative and quantitative protein changes during deacclimation. It is generally assumed that gene expression during deacclimation reflects simply a reversal of what occured during acclimation. But, examples show that downregulation of certain genes during deacclimation does not necessarily mean that they were originally upregulated during acclimation relative to a non-hardy state. It is also noteworthy that transcription patterns related to hardiness probably cannot be segregated into two distinct categories, *acclimated* and *non-acclimated*, wherein expression patterns *during* acclimation and deacclimation are expected to fall between the two extremes. Gene expression in winter rye leaves related to RNA and protein metabolism (RNA-binding protein, UMP synthase, and a transcription elongation factor) was lower after deacclimation than following acclimation [71]. In contrast, genes encoding a plasma-membrane H^+-ATPase, a disulfide isomerase, and lethal leaf spot-1 (*Lls1*) were all up-regulated during deacclimation. However, expression of all these genes was similar in acclimated and in non-acclimated leaves. Genes (as in the above example) may be temporarily down- or upregulated during deacclimation and could return to base-line levels once hardiness stabilizes. Alternatively, although gene expression may change during deacclimation, such expression may actually be more closely related to developmental transitions.

Of particular significance to deacclimation and reacclimation is the expression and regulation of hydrophilic members of the late embrygenesis abundant (LEA) gene family known as group-2 LEA/dehydrins. Although dehydrins are believed to form complexes with other macromolecules and protect them from freeze-induced desiccation and consequent loss of functional structure, persuasive evidence regarding their in vivo role is lacking [79,80]. Nevertheless, seasonal changes in dehydrin transcripts and proteins have been extensively documented among woody perennials in a variety of tissues and the circumstantial evidence for their role in cold hardiness is overwhelming [36,50,81]. Transcript abundance of the *BpuDhn1* and *BpuDhn2* dehydrins in buds of the mountain birch reached a maximum following acclimation and then progressively declined during spring deacclimation [46]. The degree to which dehydrin accumulation is quantitatively related to hardiness is dehydrin- and species-specific. The disappearance of a 14-kDa dehydrin was associated with deacclimation of blueberry buds more closely than were levels of 65- and 60-kDa dehydrins [36]. Expression of non-dehydrin LEA genes also changes during deacclimation; transcript abundance of a group-3 LEA gene (*HVA1*) in winter barley increased during acclimation and disappeared after 2 h of deacclimation [82].

Use of evergreen and deciduous sibling peach genotypes allowed researchers to study protein changes specifically associated with hardiness or dormancy transitions. Deciduous genotypes undergo endodormancy in autumn whereas evergreen genotypes do not, but they both exhibit autumnal acclimation and spring deacclimation. Spring deacclimation of these genotypes occurred in concert with decreasing levels of a 60, 19, and 16 kDa polypeptides which had accumulated during acclimation [9]. The 60 kDa protein was found to be a dehydrin [83] encoded by the *ppdhn1* gene [50]. Transcript and protein levels of *ppdhn1* decreased more rapidly during spring-deacclimation in the evergreen peach than in the more cold-hardy deciduous sibling, and quantitatively mirrored the relative hardiness of both genotypes [50]. The endodormancy in the deciduous genotype apparently increased the acclimation ability and slowed deacclimation with the associated decline in dehydrins. The 19-kDa polypeptide showed homology to allergens, pathogenesis-related proteins, and ABA-responsive proteins whereas the 16-kDa polypeptide was characterized as a ‘‘bark-storage protein’’ [84].

Dormancy could conceivably determine dehydrin levels directly or indirectly through its well-established effect on hardiness. Deacclimation of blueberry floral buds was quantitatively associated with progressively decreasing levels of 65-, 60-, and 14-kDa dehydrins [36,81]. However, the deacclimation treatment (warm-temperatures) used by Arora et al. [81] did not affect chill-unit accumulation by buds and therefore was dormancy-neutral. The authors concluded that accumulation patterns of dehydrins were in general more closely related to deacclimation than to the changes in dormancy status [81]. However, in other species development may have greater control over spring dehydrin levels than does deacclimation. In Scots pine needles, a 60-kD dehydrin decreased in abundance upon resumption of spring growth, yet there was no definite relationship between dehydrin levels and needle hardiness in this study [85].

5. Conclusions and future directions

The picture of deacclimation at a cellular level that emerges from the past 40 years of research is complicated but relatively consistent (Fig. 1). Environmental signals such as photoperiod, light intensity, water availability, and temperature impinge on the cell and modulate gene expression that regulates endodormancy, growth and development, and cold hardiness. These environmental conditions also can directly affect cellular energy balance and metabolic reaction rates (not shown in Fig. 1). Transcriptional and/or post-transcriptional regulation control the mechanisms that maintain winter hardiness and modulate deacclimation, reacclimation (if any), or growth during the spring. Fig. 1 presents the linear flow of information from the environment to physiological responses and does not include possible alternative movements of biological information. For example, physiological conditions within the acclimated cell (as indicated in green in Fig. 1) could regulate expression of genes related to cold tolerance or dormancy transitions (indicated in blue) via positive or negative feedback. It is also probable that the expression of developmental and dormancy-related genes could modulate the expression of stress-responsive genes (*COR*/dehydrative stress) and vice-versa. However, there is need for more cross-disciplinary research to better understand the biology of deacclimation. Carefully designed experiments using molecular tools (mutants, genomics, proteomics, and metabolomics) are needed to separate causal factors of deacclimation from mere correlative relationships. Sophisticated models and long-term field work are required to untangle the evolutionary history and ecological significance of deacclimation. Some of the themes that should be addressed in the future are:

- *Signal transduction*. The patterns of informational flow from the environment demonstrated in Fig. 1 are based largely on associations between ambient conditions and biological responses and not on biochemical or molecular research. Data showing to what extent and at what times variables such as light intensity, photoperiod, water availability, and temperature influence hardiness are needed.
- *Gene expression*. Data regarding patterns of gene expression during deacclimation and reacclimation are limited in the literature, although research on global transcription patterns is ongoing in several labs [86,87]. As for signal transduction, Fig. 1 outlines probable routes of hereditary information within the cell based on known associations between ambient conditions and responses at the physiological level. It is not clear how independent the sets of genes shown in this figure (i.e., genes related to dehydrative, mechanical, cold, and oxidative stresses, endodormancy, and growth and development) really are from one another. Are the effects of these different genes merely additive or are there interactions between them that make the effect of any one gene dependent on the cellular environment and/or expression of other genes?

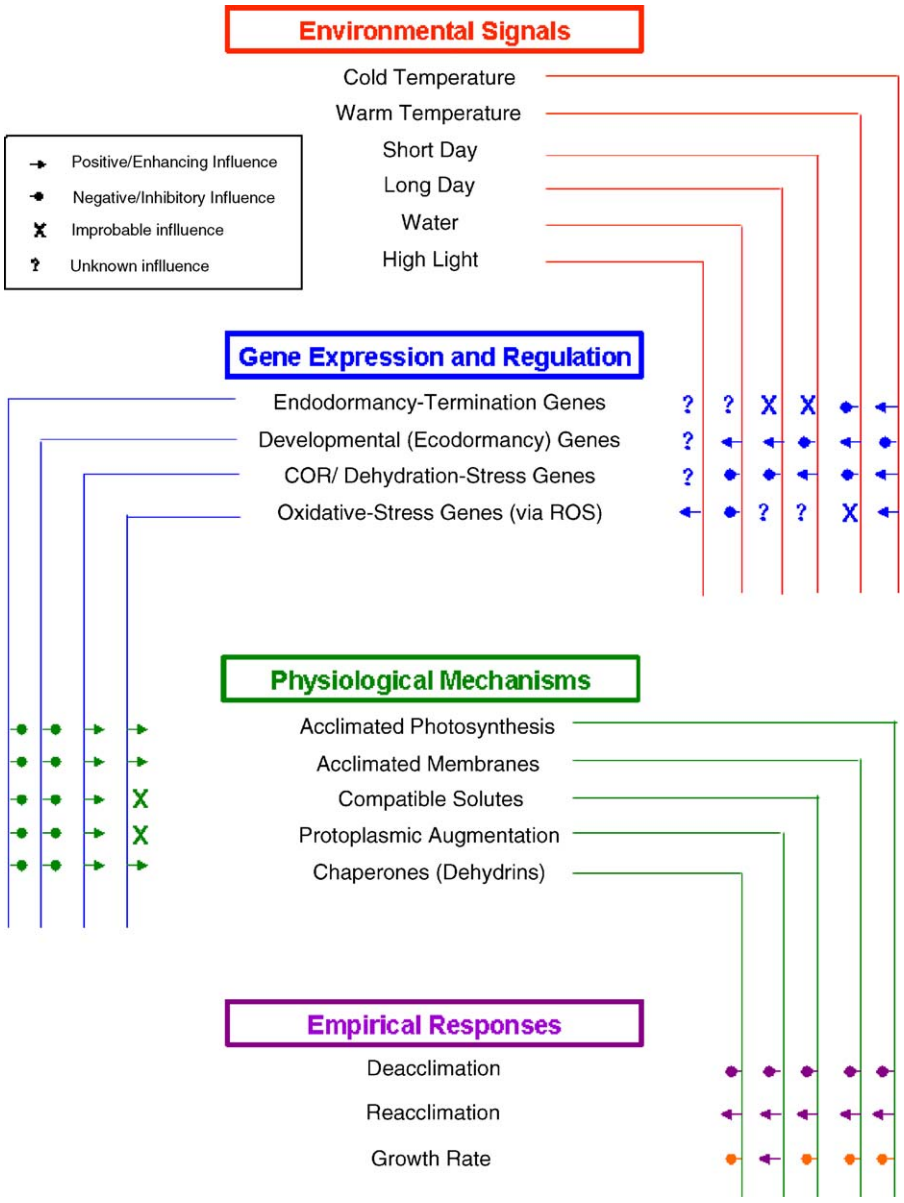

Fig. 1. Possible sequence of events underlying transitions in cold hardiness and associated regulatory networks involving four hierarchical levels of control/response, namely environmental signals (red); gene expression and regulation (blue); physiological mechanisms (green); empirical responses (violet). Environmental signals regulate the transcription of genes related to dormancy, development, and stress-resistance. Gene expression leads to quantitative and qualitative changes in proteins, lipids, and metabolites that determine the extent of deacclimation, reacclimation, and growth. The informational flow among the controls/responses is represented by lines and symbols. The factors which comprise each level of control/response are described in a positive sense (e.g., "endodormancy-termination genes" designates these genes as being expressed). A line extends from each factor (within a higher level) towards all the responses (within a lower level) which may or may not be regulated by this effector. Symbols such as pointed arrows (→), lollypops (–●), question marks (?), and cross marks (×), emanating from the perpendicular lines and pointing towards a particular response indicate a potential cause and effect relationship. Pointed arrows indicate a positive/enhancing influence; lollypops indicate a negative/inhibitory influence; question marks specify that available information is inadequate to propose a relationship; ×-marks designate associations that authors believe to be improbable. The line is the color of the causal factor and the symbol is the color of the potential effect. Orange symbols indicate an indirect effect on plant growth (by causal factors) via competition for energy reserves rather than direct regulation. Additional relationships between factors not indicated in the figure are discussed in Section 5.

Is deacclimation best regarded as the absence of hardiness adaptations or are there specific genes expressed solely during deacclimation itself? Are the same sets of cold hardiness genes expressed during autumnal acclimation, winter/spring reacclimation, and when hardiness is maintained in mid-winter?

- *Mechanism of adaptation*. How do proteins and metabolites that appear or disappear during deacclimation protect the plant from cold temperatures or prepare the plant for future development? If plants lack certain molecular mechanisms for cold acclimation (e.g., knock-out mutants) do they also deacclimate more readily or are there redundancies of adaptation that can assume some of the functions of the lost mechanisms?
- *Evolutionary origins*. Which abiotic environments promote the development of deacclimation resistance, reacclimation

capacity, and endodormancy? What are the costs to survival due to these adaptations in environments where they are not necessary? Does population or community ecology (e.g., competition, symbioses) affect the evolution of deacclimation and reacclimation strategies?

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