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EXPERT VIEW

Deacclimation after cold acclimation—a crucial, but widely neglected part of plant winter survival

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Abstract

Temperate and boreal plants show natural low temperature acclimation during autumn. This cold acclimation process results in increased freezing tolerance. Global climate change is leading to increasing spring and autumn temperatures that can trigger deacclimation and loss of freezing tolerance, making plants susceptible to both late-autumn and late-spring freezing events. In particular, spring frosts can have devastating effects on whole ecosystems and can significantly reduce the yield of crop plants. Although the timing and speed of deacclimation are clearly of crucial importance for plant winter survival, the molecular basis of this process is still largely unknown. The regulation of deacclimation is, however, not only related to freezing tolerance, but also to the termination of dormancy, and the initiation of growth and development. In this paper, we provide an overview of what is known about deacclimation in both woody and herbaceous plants. We use publicly available transcriptome data to identify a core set of deacclimation-related genes in *Arabidopsis thaliana* that highlight physiological determinants of deacclimation, and suggest important directions for future research in this area.

Keywords: *Arabidopsis thaliana*, cold acclimation, cold memory, deacclimation, dormancy, transcriptome analysis, winter survival, woody plants.

Introduction

Plant deacclimation after cold acclimation: why should we care?

Low temperature is a major ecological and evolutionary driver that limits the geographical distribution of plant species (Weiser, 1970; Kreyling *et al.*, 2015). To overcome the constraints of low temperature, plants native to temperate and boreal climates show natural low temperature acclimation during autumn in preparation for winter frost. This process is termed cold acclimation and leads to increased freezing tolerance (Levitt, 1980).

Maximum freezing tolerance is generally reached mid-winter, and upon exposure to warmer temperatures in spring plants lose the freezing tolerance acquired during acclimation by the process of deacclimation, while they resume growth and development (Xin and Browse, 2000). However, deacclimated plants may regain lost freezing tolerance in a process called reacclimation when temperatures drop again (Byun *et al.*, 2014; Kovi *et al.*, 2016). Several interchangeable terms have been used in the literature concerning low-temperature responses of plants. As indicated above, the terms 'cold acclimation',

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'deacclimation', and 'reacclimation' will be used here, in preference to the synonymous terms 'cold hardening', 'dehardening', and 'rehardening', which are often found in the horticultural, agronomic, and forestry literature.

While considerable efforts have been directed toward understanding how plants cold acclimate and adapt to low temperature at the physiological and molecular level, research on deacclimation is still limited, although there is increasing evidence that the low-temperature range limits of many plant species are not set by the absolute minimum temperature in winter. Rather, the autumn and spring temperatures that determine cold acclimation and deacclimation, respectively, may be decisive in shaping the cold-range limits (Vitasse *et al.*, 2014; Rapacz *et al.*, 2017; Vitra *et al.*, 2017). The rate and timing of deacclimation are therefore key determinants of survival, in particular during early spring when plants undergo the transition to growth and development. If this transition is made too late, plants lose valuable time during the growth season. A premature transition, on the other hand, involves the danger of freezing damage during a late-season cold spell, unless the plants have the ability to reacclimate rapidly.

Since deacclimation is mainly driven by temperature, the process is strongly influenced by the effects of global climate change (Pagter and Arora, 2013). Global climate models predict an increase in the mean surface air temperature and in the frequency and severity of erratic temperature events (IPCC, 2014). Hence, winters in temperate regions are becoming progressively shorter and milder. For example, maximum spring temperatures increased twice as much from 1975 to 2016 as minimum winter temperatures (Gu *et al.*, 2008; Augspurger, 2009; Hufkens *et al.*, 2012; Menzel *et al.*, 2015; Vitasse *et al.*, 2018), contributing to an increase in the frequency of unseasonable warm spells in spring, leading to more frequent acclimation and deacclimation cycles (Pagter and Arora, 2013; Vitasse *et al.*, 2014). In addition, shifting phenological patterns, such as earlier flowering caused by an earlier start of the growth season (Fitter and Fitter, 2002; Karlsen *et al.*, 2007), increase the risk of tissue damage by subsequent frost. These changing climate patterns have wide-ranging consequences for global ecosystems and crop yield, and erratic weather events are expected to increase in frequency and severity in the future (Gu *et al.*, 2008; Hufkens *et al.*, 2012; Augspurger, 2013; Smith and Katz, 2013; Menzel *et al.*, 2015). Furthermore, different species show different deacclimation responses. For example, some subarctic evergreen dwarf shrubs and tree seedlings show much higher mortality during simulated extreme winter warming events than deciduous birch seedlings and grasses (Bokhorst *et al.*, 2018), which could lead to massive shifts in ecosystem composition with global warming.

In view of current climate change predictions, deacclimation has received increased attention in recent years. Key topics addressed in the literature in the last two years include metabolic, proteomic, and transcriptomic responses in model species such as *Arabidopsis thaliana* during deacclimation under controlled conditions and in woody species or crops during seasonal warming in the field, modelling of factors determining freezing damage in trees, and the consequences of deacclimation on plant ecosystems (Box 1). In the following sections, we review recent advances in our understanding of deacclimation mechanisms at the physiological and molecular level in woody and herbaceous plants, and highlight possible future research directions.

Deacclimation in woody plants

Woody plants in temperate and boreal zones have to adapt to multiple cycles of cold acclimation and deacclimation throughout their lifetime. The process of deacclimation in woody plants was first mentioned as dehardening in a study on black locust trees (*Robinia pseudoacacia*) (Siminovitch and Briggs, 1953). Unlike studies on herbaceous plants that are mostly conducted under controlled conditions, most studies of woody plants have investigated deacclimation under natural conditions.

In temperate tree species, bud break occurs in spring and depends on the transition of the buds from an endodormant to an ecodormant state (see Cooke *et al.*, 2012, for a review). Regulation of the initiation and progression of bud break is highly complex and depends on both internal and external factors (Vitasse *et al.*, 2014). Many species require a period of cold temperatures, known as the chilling requirement, to transition from endo- to ecodormancy (Dhuli *et al.*, 2014; Andersen *et al.*, 2017; Vitasse *et al.*, 2018) and thereby become competent to react to warm temperatures and increasing day length with bud break. Similarly, release from endodormancy is often a prerequisite for woody perennials to deacclimate and lose freezing tolerance in response to warm temperatures (Poirier *et al.*, 2010; Pagter *et al.*, 2015; Shin *et al.*, 2015; Liu *et al.*, 2017; Vitra *et al.*, 2017; Kuprian *et al.*, 2018).

The rate of deacclimation in woody plants depends on multiple factors. Both increasing day length and ambient temperature in spring lead to faster deacclimation (Poirier *et al.*, 2010; Jönsson and Bärring, 2011; Basler and Körner, 2014; Takeuchi and Kasuga, 2018). In addition, genotype and the type of organ have an effect on the deacclimation kinetics. Species- and genotype-specific responses may be related to differences in timing of dormancy release or temperature/day length requirements. Broader studies will be necessary to properly define these differences. In addition, different tissues in woody species deacclimate at different temperatures and rates. For example, the xylem of birch twigs loses its freezing tolerance at lower temperatures and at higher rates than the bark (Takeuchi and Kasuga, 2018). Similarly, the tissue of the freezing-sensitive grapevine cultivar Chardonnay that responds most strongly to deacclimation is the internode xylem, followed by the phloem and the bud, whereas the more tolerant cultivar Merlot shows no significant differences between tissues (Antivilo *et al.*, 2017).

A metabolite analysis of buds and needles of two coniferous tree species during forced bud break has indicated that major metabolic changes occur faster in buds than needles (Dhuli *et al.*, 2014). Bud break is also associated with a remodelling of the metabolome in blackcurrant, where the content of several amino acids and organic acids is increased (Andersen *et al.*, 2017). In general, the concentration of soluble sugars in different tissues of woody plants rises during

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Box 1. Key developments in the investigation of deacclimation in woody and herbaceous plants

- **Transcriptional and metabolic regulation of deacclimation in *Arabidopsis thaliana***

  Pagter *et al.* (2017) reported the first combined transcriptomic and metabolomic analysis of the initial phase of deacclimation. A tight regulation was shown to control the underlying processes, namely the loss of freezing tolerance, activation of growth, and re-activation of the circadian clock.

- **Dynamic models for assessing frost damage in trees**

  Charrier *et al.* (2018a) used data for three walnut genotypes with contrasting tolerance from 5 years of freezing tolerance monitoring at two locations of different altitude for a simulation of freezing tolerance that considered temperature and photoperiod in interaction with developmental stage. A better performance of the models was reached with a photothermal versus a thermal model and a strong correlation of predicted freezing damage with the minimum winter temperature was shown.

- **Deacclimation in an ecosystem of different sub-Arctic plants under field and laboratory conditions**

  Bokhorst *et al.* (2018) showed that evergreen shrubs and tree seedlings were more affected by extreme winter warming than deciduous birch tree seedlings and grasses in the sub-Arctic. Climate change may in the future result in changes of sub-Arctic plant communities by favoring grasses and deciduous trees.

- **The influence of global increasing temperatures on deacclimation make it a crucial factor for winter survival of cereals**

  Rapacz *et al.* (2017) performed field studies at 11 sites during three consecutive years and found that the rate of deacclimation was independent of cold acclimation ability. Instead, deacclimation under natural conditions appeared to be a crucial determinant for winter survival.

- **Metabolic and transcriptional responses to seasonal warming in buds differ between two cultivars of a woody perennial with different chilling requirements**

  Andersen *et al.* (2017) compared comprehensive metabolite and transcript analyses of buds of differently freezing-tolerant cultivars of blackcurrant under natural winter conditions, and under the same conditions but with artificially elevated temperatures. Remodeling of the metabolome was observed during bud break with differences in seasonal regulation between the cultivars.

- **Temporal proteomics of Arabidopsis plasma membrane during cold acclimation and deacclimation**

  Miki *et al.* (2019) used proteomic approaches for the first time to analyse the composition of plasma membrane proteins of Arabidopsis leaves during cold acclimation and deacclimation. Most of the cold acclimation-responsive proteins returned to non-acclimated levels during deacclimation, but several stress-related proteins showed a higher abundance after deacclimation compared to the non-acclimated control state. This may be a strategy to prepare the plants for a sudden freezing event.

autumn/cold acclimation and decreases during spring/deacclimation, while starch content shows the opposite behavior (Pagter *et al.*, 2008, 2015; Poirier *et al.*, 2010; Lee *et al.*, 2012; Dhuli *et al.*, 2014; Shin *et al.*, 2015; Andersen *et al.*, 2017; Liu *et al.*, 2017), indicating the mobilization of soluble sugars from storage carbohydrates to achieve maximum freezing tolerance and re-synthesis of carbohydrate reserves to support flushing buds in spring. According to a study focusing on proteomic changes in bark and xylem of *Hydrangea paniculata*, deacclimation is characterized by a distinct decrease in the abundance of stress- or defence-related proteins, most of which are known to be associated with increased freezing tolerance, and an increasing abundance of proteins related to renewed growth (Pagter *et al.* 2014).

The molecular mechanisms and the regulation of deacclimation in woody plants are still only poorly understood. Differential analysis of cDNA libraries of blueberry during cold acclimation and bud break has indicated that genes belonging to the 'metabolic process' category are more highly expressed during deacclimation/bud break than during cold acclimation, in agreement with a reactivation of metabolism at this developmental stage (Rowland *et al.*, 2012). On the other hand, genes encoding dehydrins, a class of proteins associated with plant freezing tolerance, show increased expression during winter and decreased expression in spring in blackcurrant (Andersen *et al.*, 2017). It has also been suggested that the interaction of *CBF* and *RGL* genes, which code for transcription factors important for cold acclimation and for DELLA proteins that negatively regulate plant growth, respectively, may be important for the balance between deacclimation and growth (Wisniewski *et al.*, 2015).

Although cold acclimation and deacclimation have been described as reversible processes, reacclimation in response to low temperatures in woody plants seems to be limited. Studies in

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temperate trees have shown that reacclimation shortly after the beginning of deacclimation is possible and restores full freezing tolerance. However, repeated cycles of deacclimation followed by reacclimation result in decreased freezing tolerance after reacclimation (Shin *et al.*, 2015). In addition, reacclimation of blackcurrant flower buds is no longer possible in late winter, pointing to a critical role of seasonal timing in the capacity to reacclimate (Kjaer *et al.*, 2019).

Modelling approaches are increasingly being used to predict the effects of climate change on dormancy, cold acclimation, deacclimation, and freezing tolerance. Although there are still several problems associated with, for example, dormancy modelling (Blümel and Chmielewski, 2012; Chmielewski and Götz, 2016), developmental responses to air temperature have been identified as critical traits determining the risk of frost damage during warm spells in winter and early spring in boreal forest trees (Hänninen, 2006). In addition, a robust model has been developed based on freezing-tolerance data for dormant buds from autumn to spring of three grapevine genotypes over 22 years (Ferguson *et al.*, 2011), and indicates that deacclimation rates are dependent on the cultivar and dormancy state. Recent dynamic models accurately predict the freezing tolerance of dormant walnut trees based on climatic data and also taking carbohydrate dynamics into account (Charrier *et al.*, 2018*a*, *b*). This indicates that including metabolomic data may lead to more accurate models to predict the effects of climate change on winter survival and freezing tolerance of woody plants.

Molecular responses during deacclimation in herbaceous plants

The timing and extent of deacclimation in herbaceous plants depend on factors such as temperature, genotype, and photoperiod (Pagter and Arora, 2013). Deacclimation may also be linked to vernalization, as shown for two *Festuca pratensis* populations with high and low vernalization requirements (Ergon *et al.*, 2016). The rate of deacclimation may depend on the degree of cold-acclimated freezing tolerance, as shown for different accessions of Arabidopsis (Zuther *et al.*, 2015) and annual bluegrass (Hoffman *et al.*, 2014), while such a dependence is not found in cereals (Rapacz *et al.*, 2017). In Arabidopsis, natural variation in deacclimation rate is linked to the plastid antioxidant system, where a lower expression of the corresponding genes under cold conditions in freezing-sensitive accessions results in an extended maintenance of H_2O_2 levels during deacclimation. This has been suggested as an adaptive strategy to prevent rapid reversion of cold-acclimation responses (Juszczak *et al.*, 2016).

The majority of studies on deacclimation in herbaceous plants have focused on physiological responses and have only investigated small numbers of genes, proteins, or metabolites, and no genetic studies (QTL mapping, GWAS, mutant screens) to elucidate the molecular basis of deacclimation have been published yet. We therefore focus this review on studies using transcriptomic, proteomic, and metabolomic methods to elucidate the molecular basis of deacclimation, mainly in Arabidopsis. However, it should be stressed that it is often difficult to directly compare the results of different studies, as they differ widely in their experimental conditions for both cold acclimation and deacclimation. For example, for both treatments, times ranging from a few hours to several days have been used (Table 1). It has been shown that the kinetics of deacclimation are very rapid, with most changes already taking place during the first 24 h, with the transcriptome responding faster to the increase in temperature than the metabolome (Pagter *et al.*, 2017). After 24 h, the levels of several primary metabolites are still significantly different from the pre-acclimation state (Kaplan *et al.*, 2004; Pagter *et al.*, 2017) and these higher metabolite levels partially persist for up to three (Zuther *et al.*, 2015) to seven days (Zuther *et al.*, 2019), making comparisons across time-points difficult. In addition, freezing-sensitive accessions of Arabidopsis show a faster reduction of sugar levels than more freezing-tolerant accessions (Zuther *et al.*, 2015). The reduced levels of primary metabolites may not only be related to freezing tolerance, but also to the increased need for carbon sources due to the resumption of growth and development at the elevated temperature, including increased respiration (Pagter and Arora, 2013). This is in agreement with an increased expression of

Table 1. *Gene expression studies on cold acclimation and deacclimation in* Arabidopsis thaliana*, showing the different experimental conditions used.*

Citation	Plant age (weeks)	Growth medium	Temperature (°C)				Time			
			C	Acc	Deacc	Reacc	Acc	Deacc	Reacc	Method
Oono *et al.* (2006) **(O)**	3	MS plates, 2% sucrose	22	4	22		24 h, **7 d**	1 h, 2 h, 5 h, 10 h, **24 h**		MA
Byun *et al.* (2014)	3	Soil	23	0	23	0	24 h	3 d	24 h	MA
Nakaminami *et al.* (2014)	2	MS plates, 1% sucrose	22	2	22		7d	6h, 12 h, 24 h		MA
Firtzlaff *et al.* (2016) **(F)**	7	Soil	20	4	20	4	**5 d**	**24 h**	2d	MA
Pagter *et al.* (2017) **(P)**	4	Soil	20	4	20		**3 d**	2h, 4h, 6h, 12h, **24 h**		MA
Zuther *et al.* (2019)	4	Soil	20	4	20	4	3 d	7 d	3 d	RNA-seq

C, control; Acc, cold acclimation; Deacc, deacclimation; Reacc, reacclimation; MA, microarray; RNA-seq, RNA sequencing. The data sets highlighted in bold were used for a meta-analysis to identify a core set of transcripts with changed abundance after 24 h of deacclimation compared to cold acclimation (see Box 2 and Table 2).

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development-related genes such as *PIF4* and several genes related to hormone metabolism during the first 24 h of deacclimation (Pagter *et al.*, 2017).

There are currently only two published proteomic studies that investigate the effects of deacclimation. One is focused on plasma membrane proteins and it shows that proteins that increase or decrease during cold acclimation generally show the opposite behavior during deacclimation. In particular, abiotic stress-responsive proteins and protein kinases/phosphatases decrease in abundance during deacclimation, while proteins related to metabolic processes increase (Miki *et al.*, 2019), in agreement with the onset of growth and development considered above. A combined analysis of mRNA and protein abundances during cold acclimation and deacclimation in Arabidopsis revealed sets of mRNAs that are transcribed under cold conditions, but are stored and masked to be translated later upon deacclimation. These mainly ribosomal proteins are rapidly accumulated during deacclimation as they do not require transcription, thereby ensuring a rapid resumption of growth and development (Nakaminami *et al.*, 2014).

There are currently six published studies that report transcriptomic analyses of the deacclimation process in Arabidopsis (Table 1). Five of these studies have employed different forms of microarrays, while the most recent used an RNA-seq approach. Here, we use these published data to search for common transcriptional deacclimation responses in Arabidopsis. As noted above, such a meta-analysis is hampered by the widely diverging experimental protocols used in the different studies. To allow for a meaningful comparison, we therefore selected the three studies that employed the same acclimation temperature (4 °C) and a common deacclimation time-point of 24 h (Oono *et al.*, 2006; Firtzlaff *et al.*, 2016; Pagter *et al.*, 2017). From these studies we extracted lists of genes identified as changed in expression after 24 h of deacclimation compared to cold-acclimated samples. The number of such genes varies from 612 selected from 7k cDNA microarrays (Oono *et al.*, 2006) to 2335 identified from Affymetrix whole-genome microarrays (Pagter *et al.*, 2017), and 5732 identified from Agilent whole-genome microarrays (Firtzlaff *et al.*, 2016). There is an overlap of 25 up-regulated and 23 down-regulated genes among the three studies (Box 2), and these are listed in Table 2. We consider these 48 genes to be a core set that is regulated during deacclimation independently of experimental conditions and array technology. Of course, we acknowledge that this is only a momentary snapshot and that with more transcriptome data becoming available, in particular more RNA-seq data, this set of core genes will probably expand.

Core genes down-regulated during deacclimation include many that are cold-induced, such as genes from the CBF regulon and genes encoding enzymes involved in the accumulation of compatible solutes such as sugars (Pagter *et al.*, 2017). Several genes in our set (e.g. *sucrose synthase 1*, *galactinol synthase 3*, *COR47*, *COR15a*, *COR15b*, *KIN1*, *KIN2*) are in this group and the encoded proteins are either known (COR15a, COR15b; Thalhammer *et al.*, 2014) or assumed to play a functional role in freezing tolerance.

Genes up-regulated during deacclimation include those encoding transcription factors regulating development and growth. Likewise, genes related to the metabolism of auxin, gibberellins, brassinosteroids, jasmonate, and ethylene are

Box 2. Overlap of genes with changed expression after 24 h of deacclimation compared to cold-acclimated conditions identified in three publicly available data sets

The data sets used are Pagter *et al.* (2017) (P), Firtzlaff *et al.* (2016) (F), and Oono *et al.* (2006) (O). For experimental conditions see Table 1. The numbers of up-regulated genes are shown in (A) and the numbers of down-regulated genes are shown in (B).

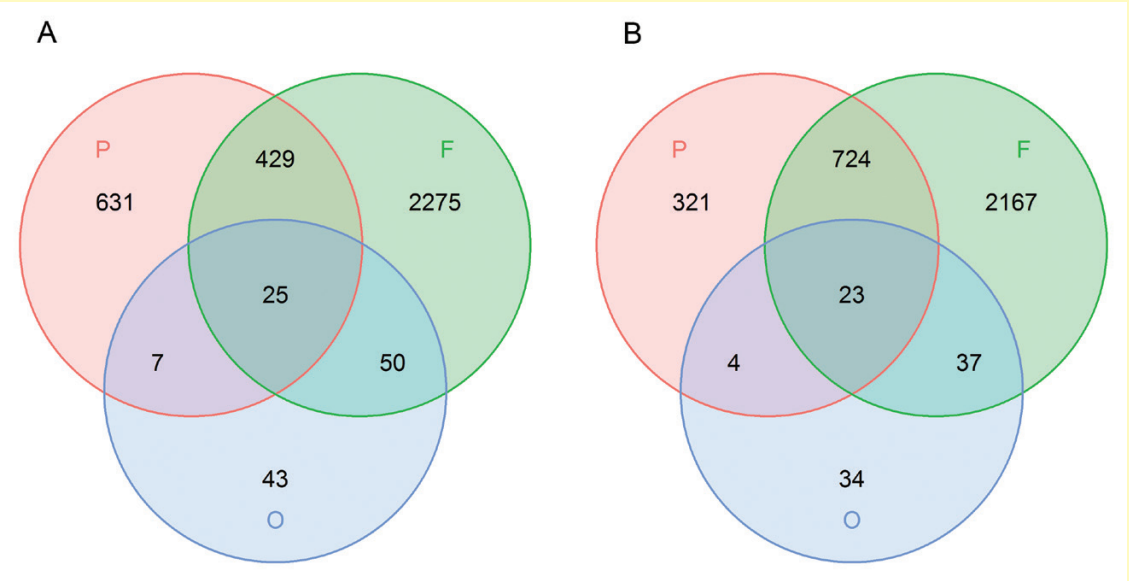

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Table 2. *Differentially regulated genes after 24 h deacclimation compared to cold acclimated conditions.*

	ID	Log_2FC (P)	Log_2FC (F)	Log_2FC (O)	Function
Down-regulated					
1	**AT1G09350**	–5.3110	–4.7459	–3.2452	**Galactinol synthase 3**
2	AT1G10410	–1.3400	–2.7623	–1.2619	CW14 protein (DUF1336)
3	AT1G16850	–3.3930	–5.0506	–2.3219	Transmembrane protein
4	AT1G20440	–1.2930	–3.2441	–2.1539	Cold-regulated 47
5	AT1G80130	–1.3540	–1.1934	–2.3004	Tetratricopeptide repeat (TPR)-like superfamily protein
6	AT2G21620	–1.0160	–1.8011	–1.3921	Adenine nucleotide α-hydrolases-like superfamily protein
7	AT2G36390	–1.1480	–2.5084	–1.5353	Starch branching enzyme 2
8	**AT2G42530**	–2.2950	–3.7778	–2.0893	**Cold regulated 15b**
9	**AT2G42540**	–1.4440	–3.7451	–4.1712	**Cold-regulated 15a**
10	**AT3G55580**	–3.4330	–3.2975	–1.2723	**Regulator of chromosome condensation (RCC1) family protein, TCF1**
11	AT4G03400	–1.7670	–1.1224	–1.3585	Auxin-responsive GH3 family protein
12	AT4G04330	–1.1050	–2.7443	–1.6666	Chaperonin-like RbcX protein
13	AT4G12470	–5.3270	–8.8845	–3.4112	Azelaic acid induced 1
14	AT4G19120	–1.328	–1.0304	–1.3219	SAM-dependent methyltransferases superfamily protein
15	AT4G30650	–3.3420	–3.5718	–2.1649	Low temperature and salt responsive protein
16	AT4G38580	–2.7600	–1.9848	–1.3884	Farnesylated protein 6
17	**AT5G15650**	–1.1960	–2.4748	–1.3535	**Reversibly glycosylated polypeptide 2**
18	AT5G15960	–3.2340	–4.2797	–2.7486	Stress-induced protein (KIN1)
19	**AT5G15970**	–1.1310	–2.2623	–3.1362	**Stress-induced protein (KIN2)**
20	**AT5G20830**	–1.4370	–4.6919	–1.4860	**Sucrose synthase 1**
21	AT5G25110	–3.6300	–5.4042	–1.7760	CBL-interacting protein kinase 25
22	**AT5G42570**	–1.1900	–2.1643	–1.2584	**B-cell receptor-associated 31-like protein**
23	**AT5G61380**	–1.6990	–3.6902	–1.1488	**CCT motif -containing protein, TOC1**
Up-regulated					
1	AT1G07350	1.6980	1.2064	1.2839	RNA-binding (RRM/RBD/RNP) family protein
2	AT1G48430	1.3980	2.1000	1.0704	Dihydroxyacetone kinase
3	**AT1G51400**	1.0300	5.6705	1.3250	**Photosystem II 5 kD protein**
4	AT1G52190	1.1590	4.2159	1.8424	Major facilitator superfamily protein, nitrate transporter
5	**AT1G62660**	2.6050	2.7248	1.1414	**Glycosyl hydrolases family 32 protein**
6	AT1G73330	2.3480	3.2504	2.0559	Drought-repressed 4
7	AT1G80920	1.4160	2.8915	2.0208	Chaperone DnaJ-domain superfamily protein
8	AT2G05540	2.4190	2.5811	2.6592	Glycine-rich protein family
9	AT2G18050	1.8460	1.1505	1.6722	Histone H1-3
10	AT2G28630	1.4590	2.9248	1.9587	3-ketoacyl-CoA synthase 12
11	**AT2G36830**	1.1040	3.4769	1.6663	**Gamma tonoplast intrinsic protein**
12	AT2G37180	2.7700	3.1986	1.6462	Aquaporin-like superfamily protein, PIPC2
13	AT3G02170	1.4330	3.7024	1.4636	Longifolia2
14	AT3G15950	1.4650	1.5050	1.4558	DNA topoisomerase-like protein
15	AT3G16420	1.4900	5.2012	1.2374	PYK10-binding protein 1
16	AT3G16460	1.3650	5.3848	1.3225	Mannose-binding lectin superfamily protein
17	AT3G61430	1.0220	2.0873	1.8069	Plasma membrane intrinsic protein 1A
18	AT4G23670	2.0900	3.0235	1.6889	Polyketide cyclase/dehydrase, lipid transport superfamily
19	AT4G23680	3.3490	2.9236	1.0398	Polyketide cyclase/dehydrase, lipid transport superfamily
20	AT4G27450	3.1660	4.5428	3.1805	Aluminum induced protein (YGL and LRDR motifs)
21	AT4G35770	1.9890	5.8742	3.1411	Rhodanese/Cell cycle control phosphatase superfamily
22	**AT4G37980**	1.2050	1.1776	2.4132	**Cinnamyl alcohol dehydrogenase 7**
23	AT5G19120	1.3280	1.5411	1.8388	Eukaryotic aspartyl protease family protein
24	AT5G49360	2.6070	4.9231	4.0900	β-xylosidase 1
25	AT5G66040	1.1550	2.1026	1.9321	Sulfurtransferase protein 16

The genes constitute the overlap of the results from three publicly available data sets as shown in Box 2 and Table 1. The log_2FC values are taken from Pagter *et al.* (2017) (P), Firtzlaff *et al.* (2016) (F), and Oono *et al.* (2006) (O). Genes are ordered by AGI code. Genes in bold represent the overlap with ecologically significant temperature-responsive genes identified in *Arabidopsis halleri* (Nagano *et al.*, 2019).

up-regulated under these conditions, indicating that the loss of freezing tolerance and the initiation of growth are transcriptionally interrelated even though there are no phenotypic changes visible after 24 h of deacclimation (Pagter *et al.*, 2017). The core set of up-regulated genes that we have identified here (Table 2) contains genes encoding the aquaporin proteins PIP2C, PIP1A, and gamma tonoplast intrinsic protein, indicating the importance of balancing cell water status during

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deacclimation, when cellular osmolyte concentrations (sugars, amino acids) are drastically reduced. This is also in agreement with the increase in transcript levels of the gene *drought-repressed 4*, which shows reduced expression under drought (Gosti *et al.*, 1995). Unfortunately, to the best of our knowledge, this gene has not been functionally characterized. Similarly, the *β-xylosidase 1* gene has been found to be up-regulated during rehydration after drought stress (Oono *et al.*, 2006). Other core up-regulated genes are related to recovery and repair processes, such as the genes encoding a chaperone DnaJ-domain superfamily protein and the PYK10 binding protein, which is part of a β-glucosidase complex involved in repair, for example after wounding (Yamada *et al.*, 2011).

A recent study using the perennial species *Arabidopsis halleri*, a close relative of *A. thaliana*, determined transcriptome dynamics over 2 years under natural environmental conditions using RNA-seq (Nagano *et al.*, 2019). From these expression profiles, 228 genes were identified as specifically associated with seasonal temperature variation. The overlap between this set of ecologically significant temperature-responsive genes and our core set of 48 deacclimation-related genes comprises 13 genes, nine among the down-regulated and four among the up-regulated genes (highlighted in bold in Table 2). Among the down-regulated genes, we find some of the cold-induced genes described above (*galactinol synthase 3*, *sucrose synthase 1*, *COR15a*, *COR15b*, *KIN2*), but also *TCF1* (*tolerant to chilling and freezing 1*), encoding a CBF-independent chromatin-based regulator of cold-responsive genes (Ji *et al.*, 2015), *RGP2* (*reversibly glycosylated polypeptide 2*), encoding a UDP-arabinose mutase essential for cell wall formation (Rautengarten *et al.*, 2011), and *TOC1*, a gene of the central oscillator of the circadian clock, which is strongly dampened in its expression by low temperature (Bieniawska *et al.*, 2008). With the exception of the gamma tonoplast intrinsic protein referred to above, the up-regulated genes are only poorly characterized. However, the cinnamyl alcohol dehydrogenase is involved in green leaf volatile emission (Tanaka *et al.*, 2018), but it is presently unclear how that may be related to plant freezing tolerance. Nevertheless, these genes are interesting candidates to search for upstream regulators such as transcription factors that may then allow us to identify deacclimation regulons with a functional role in this process.

Reacclimation after deacclimation and cold memory

During sudden cold spells in spring or warm spells in autumn, deacclimation is followed directly by reacclimation. In this sequence, the first cold stress may prime plants for a future stress, leading to increased freezing tolerance due to a cold memory (Hilker *et al.*, 2016; Baier *et al.*, 2019). However, increased tolerance was not found for canola or wheat, which only showed 100% and 39% recovery of acclimated freezing tolerance after reacclimation, respectively (Trischuk *et al.*, 2014). In Arabidopsis, on the other hand, cold memory has recently been demonstrated, as indicated by a higher freezing tolerance after the second compared to the first cold treatment (Zuther *et al.*, 2019). After a 7-d deacclimation phase, no cold-induced changes in lipid content are detectable compared to non-acclimated plants, while some primary metabolites still show increased levels (Zuther *et al.*, 2019). This lack of full reversion of metabolite pools to non-stressed levels could be a sign of metabolic imprinting during cold acclimation (Schwachtje *et al.*, 2019). Similarly, some cold-induced plasma membrane proteins in Arabidopsis remain at elevated levels during deacclimation (Miki *et al.*, 2019). In addition, the chloroplast antioxidant capacity plays an important role in the formation of a cold memory (Baier *et al.*, 2019). RNA-seq analysis reveals specific gene expression patterns associated with reacclimation (Zuther *et al.*, 2019) and further studies will be necessary to establish the functional role of these genes in cold priming and memory.

Conclusions and future directions

As we have outlined above, deacclimation after cold acclimation is a crucial factor in plant winter survival that will increase in importance as global climate change proceeds. However, unlike cold acclimation, deacclimation has attracted comparatively little research interest and therefore its molecular basis is largely—and in the case of woody plants—completely unexplored (see Box 3 for a schematic summary of current knowledge for herbaceous plants). In particular, while a limited number of metabolomic, proteomic, and transcriptomic data sets are available now, no large-scale genetic studies such as QTL or GWA mapping have been performed that could point to interesting novel regulators of this process. Likewise, the screening of mutant populations (chemical or T-DNA insertion mutants) could potentially lead to the identification of important components of deacclimation. Our meta-analysis of a small number of available microarray studies clearly indicates that it should be possible, with a larger number of more comprehensive transcriptomic data sets, to define a core set of deacclimation-related genes that could be prioritized for functional analysis. In addition, similar studies are lacking in woody plants, where just recently the first transcriptional regulators of bud break have been identified in aspen (Maurya *et al.*, 2018; Singh *et al.*, 2018); however, their possible involvement in deacclimation has so far not been explored. Candidate genes for the regulation of deacclimation in herbaceous plants could be interesting starting points to unravel similar gene regulatory networks in woody plants, but also to define specific deacclimation mechanisms in the two groups. In the long term, respective mutants or gene-edited plants could be used to investigate how different levels or speed of deacclimation influence plant fitness under (simulated) global climate change conditions.

Even on the physiological side, many open questions remain. For instance, it will be important to investigate the kinetics of both deacclimation and reacclimation at different temperatures in widely differing plant types, such as annual and perennial herbaceous plants (including grasses), trees, and woody shrubs, as a baseline to define the influence of

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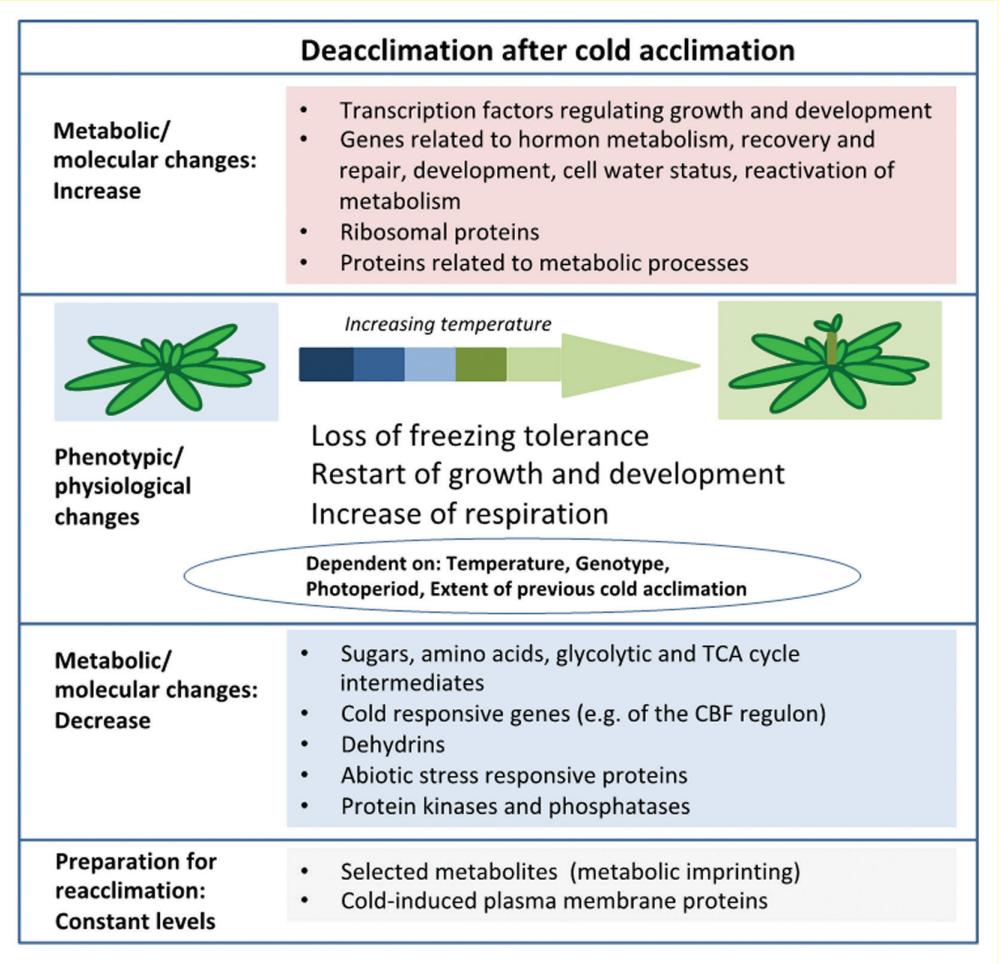
Box 3. Summary of physiological and molecular events identified during deacclimation and reacclimation in herbaceous plants.



developmental stage and dormancy level, and also to investigate external factors related to climate change, such as CO_2 concentration. This would also allow us to make predictions about the effects of further spring warming and erratic spring freezing events on the species composition in different ecosystems. In particular for crop plants, knowledge about the genetic diversity present in cultivars of different species will be crucial to allow breeding of new varieties that are better adapted to the challenges of a rapidly changing climate and thus to ensure sufficient food for an ever-increasing human population.

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