Hormonal balance finely tunes dormancy status in sweet cherry flower buds

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ABSTRACT

In temperate trees, optimal timing and quality of flowering directly depend on adequate winter dormancy progression, regulated by a combination of chilling and warm temperatures. Physiological, genetic and functional genomic studies have shown that hormones play a key role in dormancy establishment, maintenance and release. We combined physiological, transcriptional analyses, quantification of abscisic acid (ABA) and gibberellins (GAs), and modelling to further elucidate how these signaling pathways control dormancy progression in the flower buds of two sweet cherry cultivars.

Our results demonstrated that GA pathways have distinct functions and may differentially regulate dormancy. Following the observations that ABA levels are strongly linked with dormancy depth, we successfully modelled ABA content and dormancy behavior in three cultivars based on the expression of a small set of genes regulating ABA levels. These results underscore the central role of ABA and GA pathways in the control of dormancy progression.

INTRODUCTION

Perennial plants have evolved strategies that enhance survival under the various environmental stresses they face during their growth and reproductive cycles. Among them, dormancy is a quiescent phase that protects meristematic and reproductive tissues from freezing damage. In temperate trees, the transition from active growth to dormancy is triggered by decreasing photoperiod and temperatures (Heide and Prestrud, 2005; Petterle et al., 2013; Rohde et al., 2011; Singh et al., 2016). Subsequently, the bud dormancy process relies on the integration of cold and warm temperatures between endodormancy, when buds are unable to resume growth even under favorable conditions(Rohde and Bhalerao, 2007), and ecodormancy, when bud development is inhibited by unfavorable conditions until optimal growth temperatures and photoperiod are met (Lang et al., 1987). In the current context of climate change, temperate trees are affected by contradictory effects during the dormancy period and shifts in phenological phases are observed: longer growing season and insufficient chilling during winter, both effects potentially having dramatic impact on growth and production (Atkinson et al., 2013; Jochner et al., 2013; Vitasse et al., 2011). In-depth knowledge of mechanisms controlling tree phenology and the response to environment is therefore necessary to anticipate their adaptation to future climates. Dormancy progression and control by temperature and photoperiod in perennial plants have been a focus for decades and physiological, genetic and functional genomic studies have shed some light onto the mechanisms underlying dormancy control in deciduous trees and other perennial plants (Beauvieux et al., 2018; Cooke et al., 2012; Ríos et al., 2014). Bud dormancy is controlled by a complex array of signaling pathways that integrate endogenous and environmental cues towards a rest/growth decision.

Effort to synthesize the available knowledge and data into modelling approaches have led to the development of phenological models based on the endo- and ecodormancy phases regulation by temperature and photoperiod (Chuine et al., 2016; Chuine and Régnière, 2017). However, no new innovative model for dormancy has been proposed since 1990 (Hänninen, 1990) and current process-based models of bud endodormancy rely on very little information about involved mechanisms mainly because so far, robust molecular or physiological markers of dormancy depth and release are lacking. Conceptual models for dormancy progression have been proposed based on interactions between respiratory stresses, ethylene and abscisic acid (ABA), which in turn activate gibberellins (GA)-mediated growth through up-regulation of *FLOWERING LOCUS T* (*FT*) expression and resumption of intercellular transport (Ophir et al., 2009; Rinne et al., 2011). Nevertheless, attempts to integrate these hypotheses into mathematical models are rare.

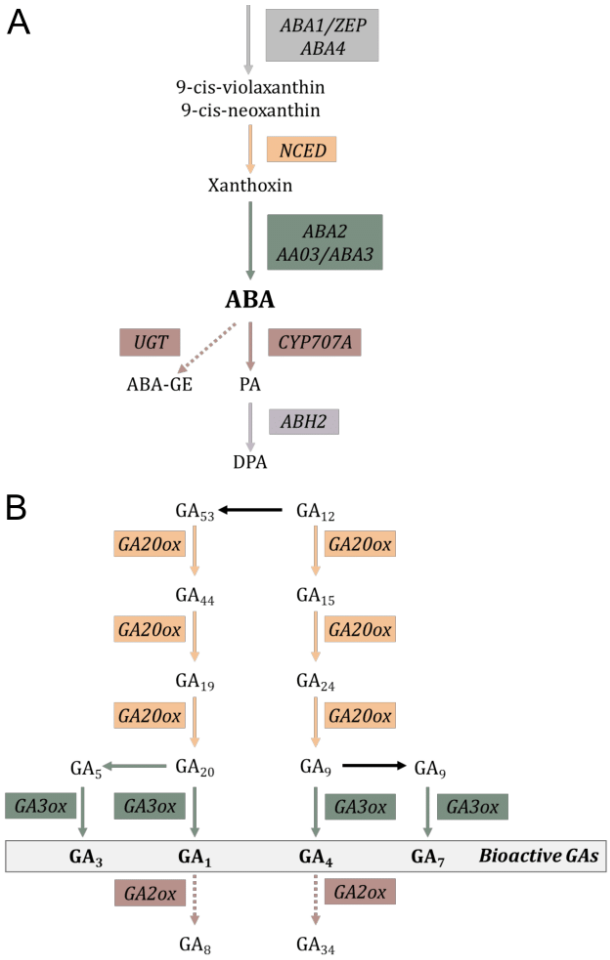

Figure 1
Biosynthesis and catabolism pathway for (A) ABA and (B) GAs. (A) Abscisic acid (ABA) is synthesized through the action of five enzymes: zeaxanthin epoxidase (ZEP/ABA1), ABA-deficient4 (ABA4,) 9-cis epoxycarotenoid dioxygenase (NCED), alcohol dehydrogenase (ABA2) and short-chain dehydrogenase/reductase (AAO3/ABA3). ABA is mainly inactivated by ABA 8′-hydroxylase-catalyzed conversion to 8'hydroxy ABA by cytochrome P450 monooxygenases, encoded by *CYP707A* (Nambara and Marion-Poll, 2005). 8'hydroxy ABA is then spontaneously converted to phaseic acid (PA), which is further catabolized to dihydrophaseic acid DPA by a PA reductase (PAR) encoded by *ABA HYPERSENSITIVE2* (*ABH2*). ABA can be conjugated with glucose to inactive ABA-glucose ester (ABA-GE) by UDP-glycosyltransferases (UGT) (Dietz et al., 2000). (B) Bioactive GAs (GA_1, GA_3, GA_4 and GA_7) are synthetized by GA 20-oxidases (GA20ox) and GA 3-oxidases (GA3ox) and catabolized by GA 2-oxidases (GA2ox) (Yamaguchi, 2008).

Recently, ABA content has been proposed as a determining factor to assess dormancy status in sweet cherry (Chmielewski et al., 2017). Indeed, the major role of hormones in the regulation of bud growth cessation, dormancy and activity resumption has been extensively discussed (e.g. (Beauvieux et al., 2018; Cooke et al., 2012). In particular, seed and bud dormancy show common features in terms of hormonal control (Leida et al., 2012a; Powell, 1987; Wang et al., 2016) and as it has been extensively studied in seed dormancy, gibberellins (GA) and abscisic acid (ABA) balance is often involved in the integration of internal and external cues to control plant growth, (Finkelstein, 2013; Rodríguez-Gacio et al., 2009; Shu et al., 2018): GAs promote growth, whereas ABA promotes dormancy. Multiple physiological and transcriptomic studies have indeed proposed a central role for abscisic acid (ABA) in the repression of bud activity during dormancy. ABA would function as a signal in response to autumn short days and decreasing temperatures to induce dormancy onset (Li et al., 2018; Rohde et al., 2002;

Rohde and Bhalerao, 2007; Ruttink et al., 2007; Tuan et al., 2017; Tylewicz et al., 2018; Wang et al., 2016). Strong correlation was further shown between ABA and dormancy depth with high ABA levels detected during endodormancy, followed by a decrease in endogenous ABA content during the transition from endodormancy to ecodormancy (Chmielewski et al., 2017; Li et al., 2018; Or et al., 2000; Wang et al., 2016; Wen et al., 2016; Zhang et al., 2018; Zheng et al., 2015). Recent transcriptomic analyses of genes involved in the precise balance between biosynthesis and catabolism modulating ABA levels have further defined the involvement of ABA in bud dormancy. Indeed, expression patterns for 9-cis epoxycarotenoid dioxygenases (*NCED*), that catalyze the critical step for ABA biosynthesis, and *CYP707A*, encoding cytochrome P450 monooxygenases that inactivate ABA into 8'hydroxy ABA, as well as ABA signaling genes, support ABA involvement in bud dormancy induction and maintenance (Figure 1A) (Bai et al., 2013; Khalil-Ur-Rehman et al., 2017; Li et al., 2018; Nambara and Marion-Poll, 2005; Wang et al., 2016; Zhang et al., 2018; Zheng et al., 2018a; Zhong et al., 2013; Zhu et al., 2015). Similarly, Until recently, most of the knowledge gathered on the behavior of the GA pathway during dormancy had been obtained in seeds but reports published in the last years have shed some light on GA regulation throughout bud dormancy in perennial plants. Studies have suggested a major role for GAs in maintaining growth before the induction of dormancy (Eriksson et al., 2015; Junttila and Jensen, 1988; Olsen, 2010; Ruttink et al., 2007; Singh et al., 2016) and promoting growth during ecodormancy (Wen et al., 2016; Zhang et al., 2018). Interestingly, GA treatments have a controversial effect on dormancy and bud break as shown in various perennial species since GA application may substitute for chilling (Rinne et al., 2011; Shafer and Monson, 1958; Zhuang et al., 2013), or have delaying effects on shoot growth and bud break (Hoad, 1983; Zheng et al., 2018b), suggesting distinct gibberellin functions during dormancy. Although transcriptomic results for *GA2ox*, *GA3ox* and *GA20ox* vary between studies and therefore suggest complex and distinct functions, general patterns could be identified: expression for GA biosynthesis genes *GA 20-oxidases* (*GA20ox*) and *GA 3-oxidases* (*GA3ox*) decreases during dormancy induction then increases after dormancy release and during ecodormancy while GA deactivation *GA 2-oxidases* (*GA2ox*) genes are up-regulated during endodormancy and inhibited after endodormancy is released (Figure 1B; (Bai et al., 2013; Khalil-Ur-Rehman et al., 2017; Wen et al., 2016; Yamaguchi, 2008; Zhang et al., 2018; Zheng et al., 2018b; Zhong et al., 2013; Zhu et al., 2015).

In this study we explored potential hormonal markers of dormancy using a new modelling approach. We have focused on the involvement of GA and ABA pathways in sweet cherry flower bud dormancy. We examined the effect of exogenous GA and ABA on dormancy status and monitored endogenous contents for GAs and ABA and its metabolites, as well as the expression of genes related to ABA and GA metabolism throughout flower bud dormancy for two cultivars with contrasted dormancy release dates. Following our findings on hormonal control of dormancy, we propose a mathematical model that incorporates the effect of key genes on the dynamics of ABA to estimate dormancy status.

RESULTS

Exogenous GA application accelerates bud dormancy release

To test the effect of exogenous application of hormones on dormancy status, we sprayed dormant branches of the sweet cherry cultivar 'Fertard' with solutions containing GA_3, GA_4, paclobutrazol, ABA and fluridone. Dormancy status was evaluated by the budbreak response to forcing conditions (Figure 2). GA_3 and GA_4 both had significant dormancy alleviating effects, characterized by higher budbreak percentage, which was confirmed for GA_3 in a second experiment in 2018 (Figure 2A). However, no antagonist effect, namely budbreak inhibition, was observed after a treatment with paclobutrazol.

In both seed and bud dormancy, it is hypothesized that GAs and ABA act antagonistically to control growth resumption and inhibition, respectively. We therefore tested the potential inhibiting effect of

ABA on flower bud emergence. We did not observe a significant effect of ABA treatment on dormancy release, but budbreak for ABA-treated branches was slightly higher than the control (Figure 2B). However, inhibiting ABA biosynthesis with fluridone activated bud break, consistent with the established role of ABA in promoting dormancy.

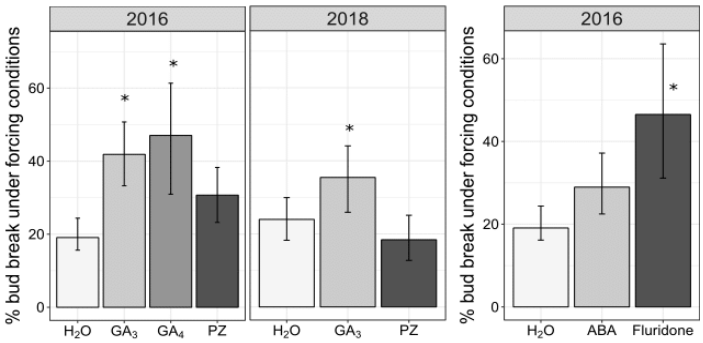

Figure 2
Effect of different GAs, ABA and their inhibitor on the sweet cherry dormancy status. Sweet cherry branches were treated with 5 µM GA_3, 5 µM GA_4, 300 µM, paclobutrazol (GA pathway inhibitor), 400 µM ABA and 5 µM fluridone (ABA pathway inhibitor) and transferred under forcing conditions (25°C, 60-70% humidity, 16 hours light). The percentage of flower bud break was recorded after 20 days. Error bars indicate the data range between the five biological replicates (n=5). Asterisks indicate treatments that differ significantly from untreated branches (Kruskal-Wallis test, $p < 0.05$).

GA content changes during flower bud dormancy progression

In recent studies, distinct functions were identified for gibberellins during bud dormancy (Zheng et al., 2018b; Zhuang et al., 2013). To test whether these results could be confirmed in sweet cherry buds, GA levels were determined over the whole bud development cycle, from flower organogenesis in July to flowering in the spring, in two sweet cherry cultivars, 'Cristobalina' and 'Regina'. Their dormancy status was assessed by forcing tests on branches (Figure 3A). The acute increase in budbreak percentage under forcing conditions indicates that endodormancy is released and flower buds are able to grow under warmer conditions. Here, the two cultivars were much contrasted in their dormancy release dates, 'Cristobalina' exhibiting dormancy release on December 9th, shortly after a marked decrease in temperatures (Figure 3B), ten weeks earlier than February 26th for 'Regina'.

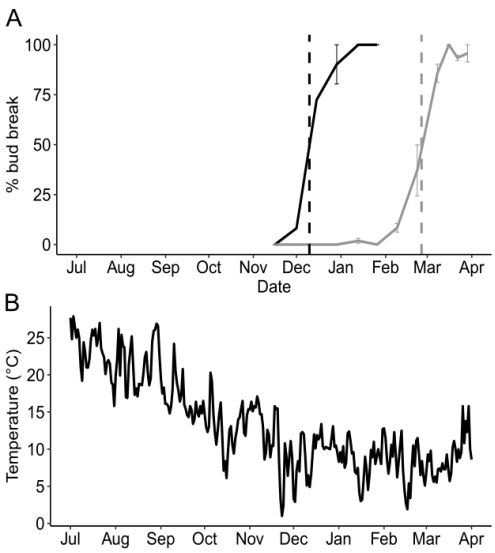

Figure 3
Dormancy status under environmental conditions. (A) Evaluation of bud break percentage under forcing conditions was carried out for two sweet cherry cultivars displaying different flowering dates. Black: 'Cristobalina', grey: 'Regina' for the early and late cultivar, respectively. The dotted line corresponds to the dormancy release dates, estimated at 50% of bud break after ten days under forcing conditions. Error bars indicate the data range between the biological replicates (n=3). (B) Average daily temperature recorded on site during the sampling season 2015/2016.

GA_4, GA_7 and GA_9 have a similar pattern over dormancy progression (Figure 4) rising from July onwards, which corresponds to flower primordia initiation and organogenesis. GA levels are largely flat during endodormancy, decreasing slightly for the late cultivar and more rapidly for the early cultivar during dormancy release. Content for GA_9, which is the direct precursor of GA_4, was significantly higher than for the bioactive GAs (GA_4 and GA_7) with a concentration reaching 163 and 264 pg/mg in October for 'Cristobalina' and 'Regina', respectively. A notable difference in GA_9 content was observed between these two cultivars during the entire time course, in which the late cultivar buds contained more GA_9 than the early cultivar (Figure 4). Among the quantified active GAs, GA_4 was between three and eight times more present than GA_7. GA_4 was the most detected active GA in the early cultivar 'Cristobalina', with a relatively high level reached just after dormancy release in December. By contrast, levels of GA_7 were higher in 'Regina', but with the maximal concentration measured just after dormancy release.

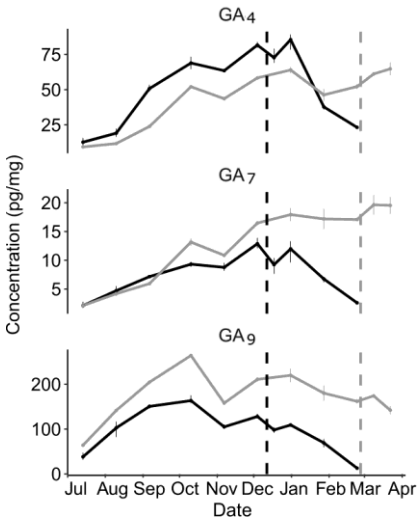

Figure 4
Levels of endogenous bioactive GA_4 and GA_7, and precursor GA_9 in the flower buds of two sweet cherry cultivars during bud development. Black: 'Cristobalina', grey: 'Regina'. Error bars indicate the data range between the biological replicates (n=3 for 'Cristobalina', n=2 for 'Regina'). Dotted lines represent dormancy release.

Expression of GA pathway-related genes have distinct patterns during sweet cherry bud dormancy

To better understand the mechanisms linked to the GA pathway during dormancy progression, we investigated genes involved in GA biosynthesis pathway, degradation, signal transduction and response. We predicted seven *PavGA20ox* and five *PavGA3ox* for GA biosynthesis genes (Supplementary Table 1) but only three genes were differentially regulated throughout flower bud development and dormancy (Figure 5). Interestingly, *PavGA20ox2* displayed two distinct peaks of expression in September and around dormancy release. The marked increase in *PavGA20ox1a* expression could be correlated with the production of GAs after dormancy release. The last step of active GA biosynthesis relies on the activity of GA3ox essentially for the production of GA_1 and GA_4. *PavGA3ox1a* was the only *GA3ox* transcript detected during dormancy (Figure 5) and its expression sharply increases during the early stages of dormancy in October and reaches its highest expression value at maximum dormancy depth in December, for both cultivars. *PavGA3ox1a* is then downregulated after dormancy release, with a marked lag between the two cultivars, potentially linked to their separate dormancy release date (Figure 5). We identified four differentially expressed *PavGA2ox*, potentially involved in GA inactivation (Figure 5). *PavGA2ox1* and *PavGA2ox8b* were highly expressed during endodormancy, concomitantly with *PavGA3ox1a* expression, thus suggesting a balance between synthesis and degradation that closely controls the levels of bioactive GAs. *PavGA2ox8c* and *PavGA2oxb* were expressed before dormancy and during the early stages of dormancy. The expression of *PavGA2ox1* was three times higher for 'Regina' than for 'Cristobalina' while it was the opposite for *PavGA2ox8b*.

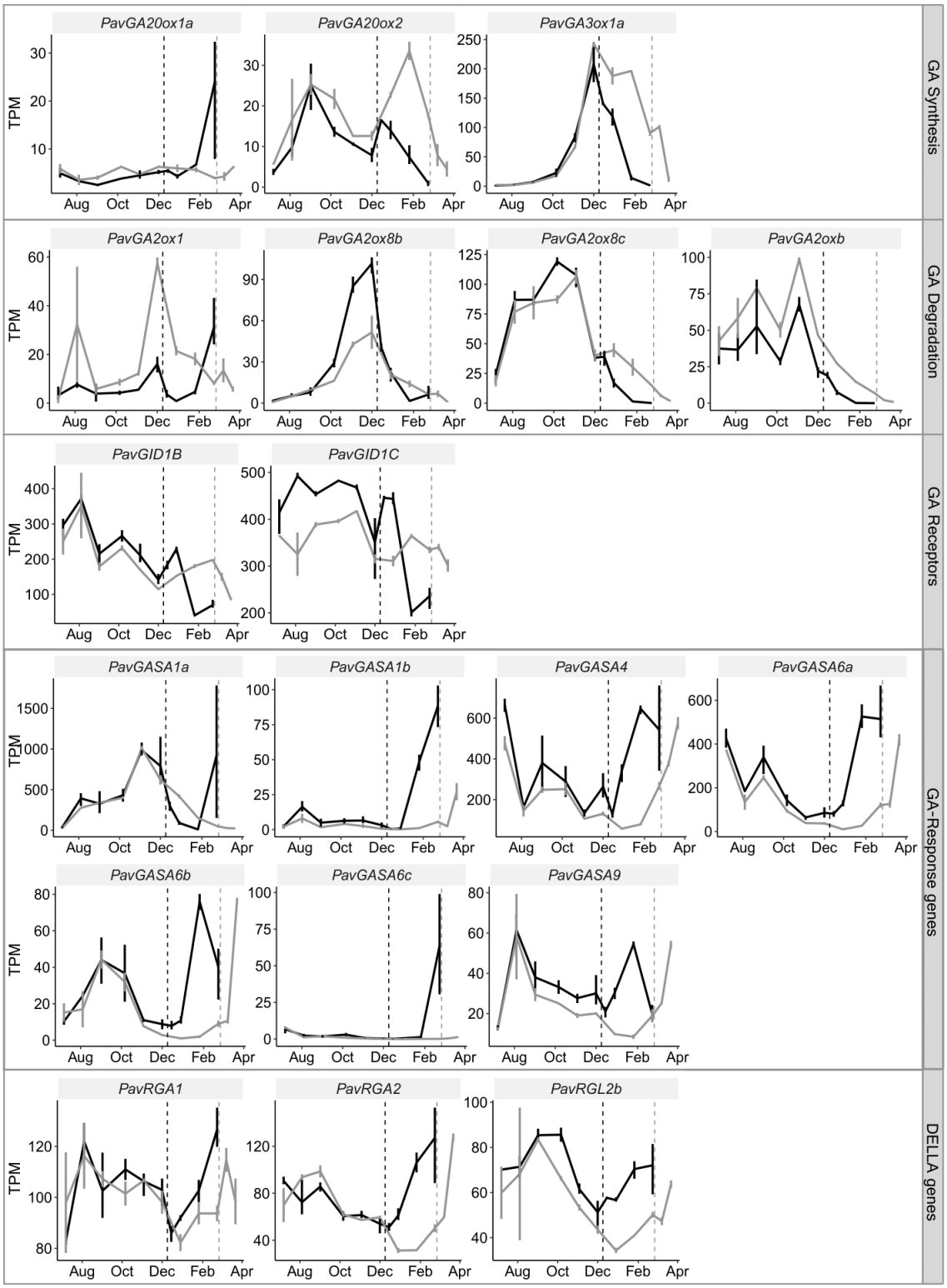

Figure 5

Transcriptional dynamics of genes associated with GA pathway in the flower buds of two sweet cherry cultivars during bud development. Expression of specific genes involved in GA biosynthesis pathway, degradation, signal transduction and response are represented in TPM (Transcripts Per kilobase Million). Black: 'Cristobalina', grey: 'Regina'. Error bars indicate the standard variation between the biological replicates (n=3 for 'Cristobalina', n=2 for 'Regina'). Dotted lines represent dormancy release.

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In terms of GA signaling, our results indicate that the two identified GA receptor-related genes *GA INSENSITIVE DWARF1* (*GID1*), *PavGID1B* and *PavGID1C* are highly expressed during sweet cherry flower bud development (July, August) and early stages of dormancy (September, October; Figure 5). *PavGID1C* expression stays high during dormancy. For 'Cristobalina', expression of both receptor genes sharply decreased after endodormancy is released. Ten GA-response genes, *GA Stimulated Arabidopsis* (*GASA*), potentially regulated by GAs (Aubert et al., 1998), were identified in our transcript dataset and seven were differentially expressed during flower bud development (Figure 5). Expression patterns are diverse but for the majority (*PavGASA1b*, *4*, *6a*, *6b*, *6c*, *9*), a decrease in expression was detected during deep dormancy (November, December), thus suggesting that GA-activated pathways are inhibited during dormancy, despite high contents in GAs (Figure 4). However, one notable exception is *PavGASA1a* that is highly activated specifically during dormancy (Figure 5). The repression of GA by DELLA proteins is well characterized in annuals (Zentella et al., 2007), so to further investigate GA pathway, we identified ten genes coding for predicted DELLAs, namely *PavGAI*, *PavGAI1*, *PavRGA1*, *PavRGA2*, *PavRGL1a, b, c* and *PavRGL2a, b, c* (Supplementary Table 1). Expression patterns for *PavRGA1*, *PavRGA2* and *PavRGL2b* were similar over time and in both cultivars, with a down-regulation during dormancy onset and endodormancy (Figure 5).

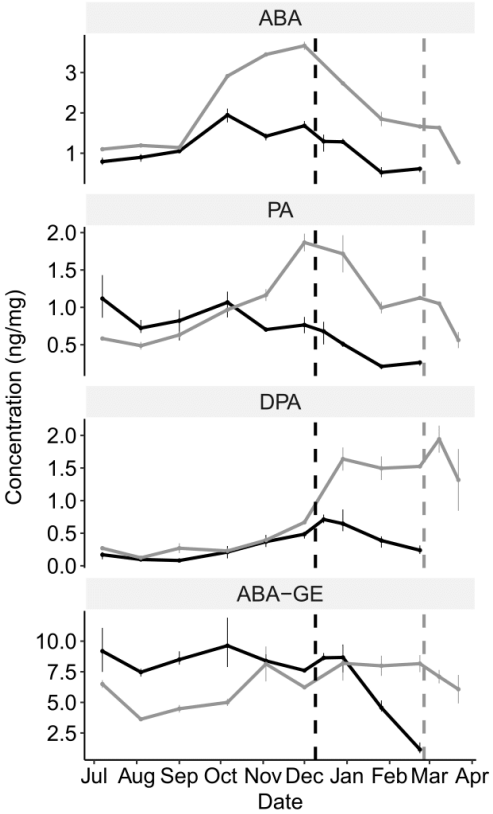

Figure 6
Levels of endogenous bioactive ABA and ABA conjugates in the flower buds of two sweet cherry cultivars during bud development. Black: 'Cristobalina', grey: 'Regina'. Error bars indicate the data range between the biological replicates (n=3 for 'Cristobalina', n=2 for 'Regina'). Dotted lines represent dormancy release. ABA: Abscisic acid; PA: phaseic acid; DPA: dihydrophaseic acid, ABA-GE: ABA-glucose ester.

ABA levels rise at the onset of dormancy

Several studies have highlighted a strong correlation between ABA content and dormancy status. To address this issue in sweet cherry flower buds, we measured ABA levels in both cultivars and results confirmed an increase in ABA content during the early stages of dormancy when photoperiod and temperatures decrease (Figure 6). In 'Regina', ABA levels are related with the dormancy status exhibiting the highest values during the deepest stages of dormancy (December) and a marked decrease under winter chilling conditions. The dormancy release phase, illustrated by an increasing budbreak percentage under forcing conditions (Figure 3), is characterized by decreasing values of ABA content.

ABA levels detected during dormancy for the early cultivar ‘Cristobalina’ are much lower than for the late cultivar, associated with an earlier dormancy release date. We can therefore hypothesize that dormancy depth is highly correlated with ABA contents in sweet cherry buds.

We further investigated the levels of PA and DPA, which are catabolites of ABA, during flower bud dormancy (Figure 6). PA and DPA contents follow a similar pattern as ABA but with a month delay approximately between each, and PA and DPA levels are much lower in ‘Cristobalina’ than in ‘Regina’. Esterification of ABA with glucose was also monitored and the concentrations of ABA-GE were higher in both cultivars compared with ABA and its conjugates. The ABA-GE content was constant in ‘Regina’ over the whole cycle while it decreased in ‘Cristobalina’ after dormancy release (Figure 6). Throughout growth cessation and early stages of dormancy, ABA-GE content was significantly higher in the early cultivar ‘Cristobalina’ compared to the late cultivar ‘Regina’ (Figure 6).

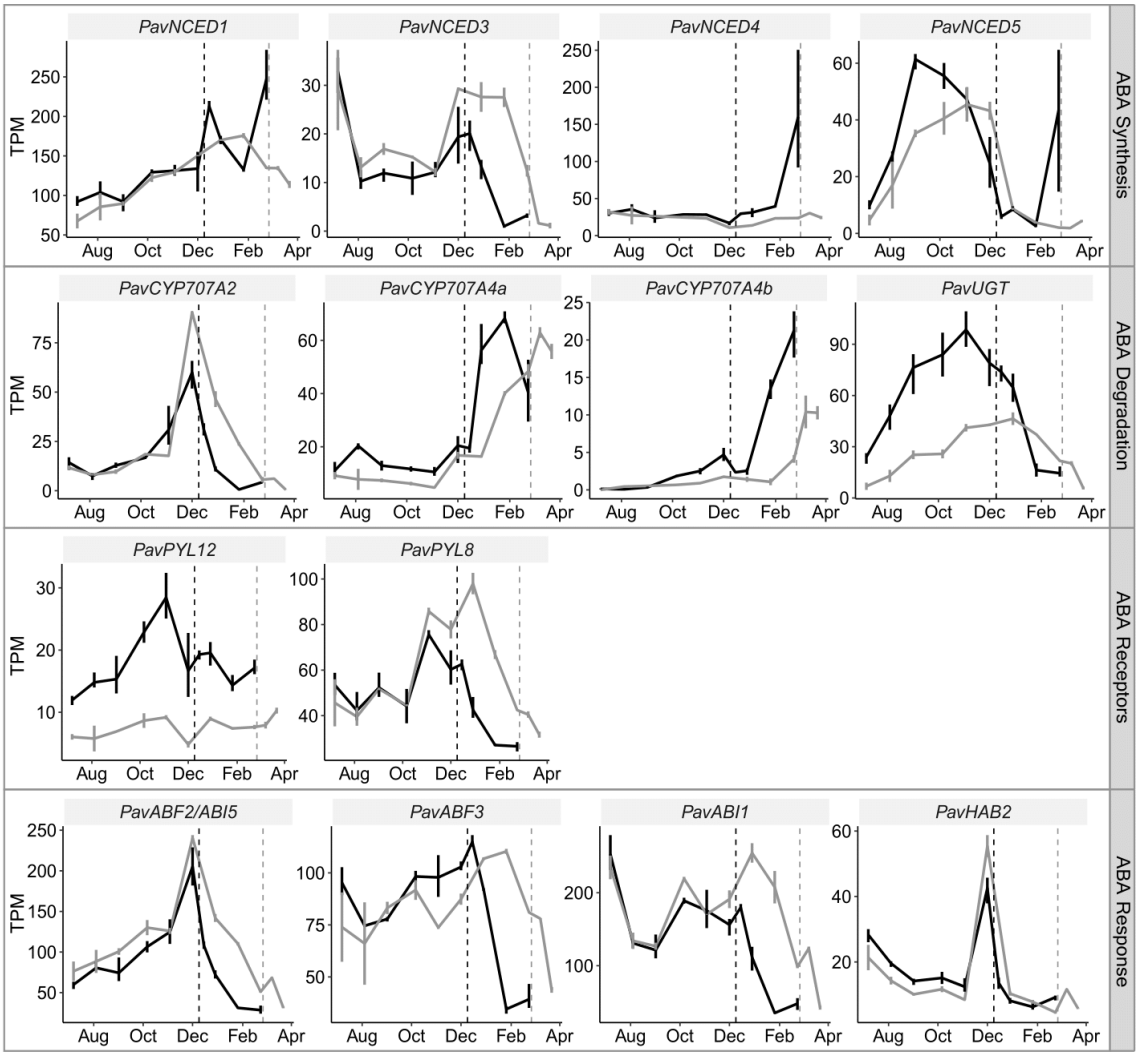

Figure 7
Transcriptional dynamics of genes associated with ABA pathway in the flower buds of two sweet cherry cultivars during bud development. Expression of specific genes involved in ABA biosynthesis pathway, degradation, signal transduction and response are represented in TPM (Transcripts Per kilobase Million). Black: ‘Cristobalina’, grey: ‘Regina’. Error bars indicate the data range between the biological replicates (n=3 for ‘Cristobalina’, n=2 for ‘Regina’). Dotted lines represent dormancy release.

Analysis of differentially expressed genes involved in ABA pathway

Expression for genes involved in the multiple ABA biosynthesis steps, *PavABA1-4*, *PavNCED1* and *PavNCED4*, is not correlated with ABA levels while expression patterns for *NCED3* and *PavNCED5* genes seem strongly linked to ABA contents and dormancy status (Figure 7 and supplements). In particular, *PavNCED5* expression shows a sharp increase during dormancy onset and a marked decay before dormancy release. An increase in *PavNCED3* expression occurs when *PavNCED5* expression decreases. On the other hand, ABA 8'-hydroxylases (*CYP707A* genes) are considered the major regulatory enzymes for ABA catabolism, and indeed, low ABA levels are associated with increased expression for *PavCYP707A1* and *PavCYP707A4* before and after dormancy respectively (Figure 7 and supplements). By contrast, *PavCYP707A2* is characterized by a sharp increase during endodormancy, more specifically in December, but it is not related to a visible decrease in ABA content. In addition, we identified *PavUGT71B6* (Supplementary Table 1), a sweet cherry ortholog of the *Arabidopsis thaliana UDP-glycosyltransferase 71B6*, that preferentially glycosylates ABA into ABA-GE (Priest et al., 2006). *PavUGT71B6* was considerably upregulated in the early cultivar compared to the late cultivar, with a gradual increase between July and deep dormancy followed by a decrease in expression before endodormancy release (Figure 7).

We examined sweet cherry gene predictions for genes involved in ABA signaling. Among the seven ABA receptors *PYR*/*PYL* genes identified for sweet cherry (Supplementary Table 1), six were differentially expressed during flower bud dormancy (Figure 7 and supplements). In particular, *PavPYL8* expression was correlated with ABA levels, increasing after dormancy onset and decaying after dormancy release (Figure 7). A similar pattern was observed for *PavPYL12* in 'Cristobalina' but not for 'Regina' that exhibited very low expression level. Among the differentially expressed ABA response genes (Supplementary Table 1), *PavABF2* and *PavHAB2* have similar patterns with a sharp increase in expression in December while the expression of *PavABF3* and *PavABI1* decrease after dormancy release (Figure 7).

Modelling suggests ABA levels control onset and duration of dormancy

Based on observations that ABA levels are correlated with dormancy depth, ABA content has been proposed as an indicator to assess dormancy status in sweet cherry (Chmielewski et al., 2017). We further investigated the dynamics of ABA to estimate dormancy onset and duration. First, we assumed that ABA synthesis is mainly controlled by PavNCED1, 3, 4 and 5 while ABA is converted to PA by 8'-hydroxylases PavCYP707A4 and esterified into ABA-GE by PavUGT71B6 (Figure 8A). Since data for protein activity are not available, we used transcript levels as a proxy for enzymatic activity. We then used an Ordinary Differential Equation (ODE) approach to model how dormancy may be regulated by ABA levels. The mathematical model, based on identical parameter set for both cultivars, shows a good fit to the data, indicating that the differential in ABA levels between the two cultivars can be solely explained by the differences in gene expression of the relevant enzymes (Figure 8B). To validate the model, we simulated ABA levels for a third cultivar 'Garnet' that was examined along 'Cristobalina' and 'Regina'. Endodormancy was released on January 21st for 'Garnet', an early flowering cultivar. Based on expression data for *PavNCED1*, *3*, *4* and *5*, *PavCYP707A4a*, *PavCYP707A4b* and *PavUGT* genes (Figure 8 – supplement figure 1), the model simulated ABA levels for 'Garnet' during dormancy (Figure 8C). Simulated ABA content for 'Garnet' increases during dormancy onset, reaches high values during endodormancy and decreases before dormancy release, earlier then for the late cultivar 'Regina'. Highest estimated levels for ABA are lower in 'Garnet' than in 'Regina' but higher than 'Cristobalina'.

Observed and simulated levels of endogenous ABA at the date of dormancy release show a good match for both 'Cristobalina' and 'Regina' cultivars (Figures 6 and 8B). ABA levels are low before dormancy

onset but as they increase dormancy is triggered; high ABA levels maintain dormancy but they decrease under chilling temperatures and endodormancy is released as ABA content falls. The Garnet ABA simulated levels lie between those of Cristoballina and Regina levels, thus if ABA content is related to dormancy release, then our modelling predicts that under a chosen fixed threshold of ABA, Garnet dormancy release will occur between the other two release dates. For example, ABA simulated levels of 'Cristobalina', 'Regina' and 'Garnet' would fall below the arbitrary threshold of 1.46 ng/mg on December 7^{h}, February 27st and January 27th, respectively. Choice of any other threshold during this period will result in the same date order. Such ordering is observed in our data for dormancy release (Figures 3A); 'Cristobalina' and 'Regina' release dates are December 9th and February 26th, while 'Garnet' release date is the January 21st; thus opening the possibility that there is a threshold for ABA concentration that determines the dormancy status. Since we can account for the dormancy behavior of a wide-range of cultivars based on the expression of a small number of key genes regulating ABA levels, this underscores the central role of this phytohormone in the control of dormancy progression.

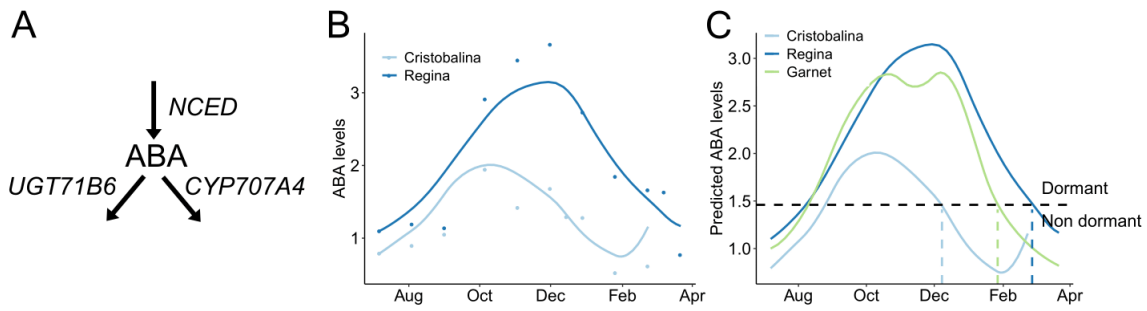

Figure 8
Modelling ABA. (A) Conceptual model used to simulate ABA content. ABA synthesis is controlled by NCED1, NCED3, NCED4 and NCED5 proteins; ABA is deactivated by 8'-hydroxylases CYP707A4 and UGT71B6. The assumption is that enzymatic activity is proportional to gene expression levels. (B) Simulated content of ABA using the model (lines) with means of data (circles) for cultivars 'Cristobalina' and 'Regina'. The model is simulated with parameters: $p^{1,2}_{NCED1} = 0.0029, p^{1,2}_{NCED3} = 0.002241$, $p^{1,2}_{NCED4}$=0.0000218, $p^{1,2}_{NCED5}$= 0.009599, $p^{1,2}_{CYP707A4b}$= 0.00010859, $p^{1,2}_{CYP707A4a}$= 0.00012048 and $p^{1,2}_{UGT}$= 0.0003027. (C) Simulated levels of ABA for cultivars 'Cristobalina' and 'Regina', that were used to calibrate the model, and for 'Garnet'. Arbitrary level of ABA set at 1.46 ng/mg (black dash line) is reached by 'Cristobalina', 'Garnet' and 'Regina' simulations on December 7th, January 27th and February 27th, respectively (colored dash lines).

DISCUSSION

Sweet cherry specific GA signaling during bud dormancy

We have shown that GA_4 has significant dormancy alleviating effects, similar to previous observations in hybrid aspen (*Populus tremula* × *Populus Tremuloides*; (Rinne et al., 2011) and Japanese apricot (*Prunus mume*; (Zhuang et al., 2013). Interestingly, results for GA_3 were similar, with a significant increase in bud break under forcing conditions, similar to dormancy alleviating effects observed in peach (Donoho and Walker, 1957) but opposed to null or inhibitory effects on dormancy release previously reported (Rinne et al., 2011; Zheng et al., 2018b). In addition, using 20 mg of tissue, high levels of GA_4 and GA_7 were detected in sweet cherry flower buds whereas GA_1 and GA_3 were undetectable in the samples. These observations differ from results obtained on grapevine buds with high GA_1 levels during dormancy induction (Zheng et al., 2018b) or on Japanese apricot, characterized with elevated levels of GA_3, especially during ecodormancy (Zhang et al., 2018). Interestingly, this is the first study showing high GA content during endodormancy, as opposed to low GA levels during dormancy maintenance in

grapevine and Japanese apricot buds (Zhang et al., 2018; Zheng et al., 2018b). The results question the role of GAs in growth cessation and resumption. In grapevine buds, application of GA_3 inhibits bud break (Zheng et al., 2018b) so it is possible that at the detected concentrations, GAs may have a role in dormancy induction and maintenance. Nonetheless, contradictory observations on GA regulation during dormancy are consistent with the hypothesis that GAs act in a complex manner, with differential effects depending on concentrations and developmental phases, as discussed in Zheng et al. (Zheng et al., 2018b). Here, we define three phases in GA signaling involving different transcriptional cascades, as revealed by our results on GA content and expression of GA pathway-related genes:

i) During bud development and early stages of dormancy (July to October), an increase in *PavGA20ox2* expression may lead to rising GA_4 and GA_7 content, along with the upregulation of receptors *PavGID1C* and *PavGID1B* similarly to GA response shown in Arabidopsis (Sun, 2011), and the activation of GA signaling, as revealed by the increased expression of GA-response genes *PavGASA4*, *6b*, *6a*, and *9*. Enhanced GA content triggers the expression of *PavGA2ox8c* and *PavGA2oxb* to catalyze GA deactivation as part of the feedback regulation (Yamaguchi, 2008). In Arabidopsis, proteins GASA4 and GASA6 are involved in flower development and cell elongation, respectively, in response to GA signaling (Roxrud et al., 2007; Zhong et al., 2015) therefore we can hypothesize that gibberellin signaling in this phase may control flower bud organogenesis and active growth.

ii) In November, dormancy depth is at its maximum, characterized by high GA_4 and GA_7 contents and a peak in *PavGA3oxa* expression. Gibberellin homeostasis seems to be maintained during deep dormancy through enhanced expression of deactivation genes *PavGA2ox1* and *PavGA2ox8b*, in 'Regina' and 'Cristobalina' respectively. Interestingly, these contrasted patterns between the two cultivars suggest that GAs may be differentially degraded between cultivars resulting in the accumulation of different types of GA. In agreement with acute GA deactivation, only one GA-response gene is activated during this phase, *PavGASA1a*, thus suggesting a very specific response

iii) After dormancy release, namely ecodormancy phase, levels for GA_4 and GA_7 decrease, associated with low, if not null, expression for *PavGA20ox2* and *PavGA3ox2a* synthesis genes, as well as *PavGA2ox8b*, *PavGA2ox8c*, *PavGA2oxb* deactivation genes and *PavGID1B* and *PavGID1C* receptors. However, the seven GA-response genes are activated during the ecodormancy, thus suggesting that GA-stimulated pathways are up-regulated. Indeed, despite increasing GA levels during dormancy, GA-response pathways seem to be mostly activated when *GA2ox* genes are down-regulated after dormancy release so further investigation on the control of GA deactivation during dormancy could unravel a potential regulation by cold accumulation. In fact, another set of genes seems to be activated with enhanced expression for *PavGA20ox1a*, *PavGA2ox1* and genes coding for DELLA proteins, which are GA signaling repressors. So one aspect that should be considered about the GA pathway regulation is the critical homeostasis controlled by the DELLA proteins, that target GA biosynthesis and receptor genes and impacts GA balance through a feedback regulation (Gagne et al., 2002; Ueguchi-Tanaka et al., 2007; Zentella et al., 2007). Indeed, there seems to be a negative correlation between *PavRGA*, *PavRGA2* and *PavRGL2* expression and GAs content, especially during endodormancy. Moreover up-regulation of sweet cherry DELLA-related genes after dormancy release, similarly to results reported in Chinese cherry (Zhu et al., 2015) and Japanese apricot (Zhang et al., 2018), is associated with a decrease in the expression of *PavGID1B*, *PavGID1C* and *PavGA3ox1*, further validating a potential feedback loop down-regulating GA pathways during ecodormancy.

Dormancy depth is correlated with endogenous ABA content

In order to investigate the role of ABA in sweet cherry bud dormancy, we first tested the effect of exogenous application of ABA and fluridone on the sweet cherry bud dormancy status. Buds were not

affected by ABA treatment, which could indicate that endogenous ABA levels in the buds were at the optimal efficiency therefore additional ABA had no effect on dormancy maintenance. Indeed, although delayed bud break was reported after ABA application in birch, sour cherry, grapevine and pear (Li et al., 2018; Mielke and Dennis, 1978; Rinne et al., 1994; Zheng et al., 2015), further assays showed that ABA treatment during dormancy release and ecodormancy have no effect on grapevine buds (Zheng et al., 2015). Besides, we observed that dormancy release was triggered in buds treated with fluridone that inhibits ABA biosynthesis and therefore with lower ABA levels, thus suggesting that high ABA levels act to maintain dormancy. This was further confirmed by the contrasted ABA levels over dormancy progression and between low- and high-chill cultivars. Indeed, elevated ABA levels are correlated with deep dormancy in the late cultivar while ABA levels stay low in the early cultivar. We propose that dormancy is triggered and maintained when ABA levels are above a threshold. Bud dormancy may subsequently be released if ABA levels fall below the threshold. Consequently, such a dormancy release threshold would be reached earlier in the season for early cultivars with less ABA (Wen et al., 2016) or when ABA levels are lower due to various environmental conditions (Chmielewski et al., 2017).

ABA levels are also correlated with the expression patterns for genes involved in ABA biosynthesis and degradation. We show that during early stages of dormancy, an increase in *PavNCED5* expression leads to the synthesis of ABA in both cultivars. When *PavNCED5* expression decreases, *PavNCED3* increases, thus maintaining high ABA levels in sweet cherry flower buds. Consistently with elevated ABA levels, genes encoding ABA receptors (*PavPYL12*, *8*), as well as ABA response genes, are highly expressed during deep dormancy. Afterwards, genes involved in ABA degradation (*CYP707A*) are highly expressed during dormancy release and positively correlated with a decrease of ABA content. Similar results were reported in other *Prunus* species (Tuan et al., 2017; Wang et al., 2016; Zhang et al., 2015) confirming the major role of ABA signaling pathway dormancy. Interestingly, *PavCYP707A2* is up-regulated during deep dormancy for both cultivars, synchronized with the sharp increase in *PavNCED3* expression, thus suggesting a tight regulation of ABA homeostasis in response to low temperatures recorded during this period. Based on their synchronized peak of expression, it is possible that *ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING FACTOR 2* (*PavABF2*) and *HOMOLOGY TO ABI2* (*PavHAB2*) are also involved in this acute ABA signaling response to severe cold rather than dormancy progression. These results thus suggest that ABA functions in two distinct pathways for dormancy regulation and response to cold stress.

A contrasted balance between ABA synthesis and conjugation may explain the differences between early and late cultivars

Overall, although the dynamics of expression for *PavNCED* and *PavCYP707A* genes effectively explain the increasing and decreasing pattern of ABA levels between dormancy induction and release, we further investigated whether they could account for the significant differences observed for ABA levels between the two cultivars. Indeed, *PavNCED3* is more expressed in the late cultivar 'Regina', especially after December. However, the difference in *PavNCED3* expression between the two cultivars is less pronounced at the beginning of endodormancy (October, November) therefore, the higher expression of *PavNCED3* is not sufficient to explain the noticeably contrasted ABA accumulation. In terms of catabolism, *PavCYP7074a* and *PavCYP7074b* are upregulated earlier in 'Cristobalina', thus accounting for the earlier decrease in ABA levels after dormancy release but not the lower levels during endodormancy. Differences in ABA catabolites between cultivars were also observed. PA and DPA levels followed the same pattern as ABA but with one-month delay and the ABA catabolites were higher in the late cultivar 'Regina'. Interestingly, Weng and colleagues (2016) exposed the compensatory effect of PA under low ABA conditions, in which PA is recognized by ABA receptors (PYL5 and 2) allowing a supplementary growth inhibition effect. In sweet cherry, increased *PavCYP707A2* expression induces higher level of PA in dormancy in 'Regina' and might therefore result in even deeper dormancy by the

inhibitory combination of ABA and PA. Since free ABA levels may be controlled through conjugation as well (Chmielewski et al., 2018; El Kayal et al., 2011), we investigated the conversion of ABA to ABA-GE and we found a *PavUGT71B6* gene characterized by strikingly higher expression levels for the early cultivar compared to the late cultivar. UGT71B6 orthologs in Arabidopsis and Adzuki bean act specifically for ABA conjugation into ABA-GE (Priest et al., 2006; Xu et al., 2002), so up-regulation of *PavUGT71B6* expression in 'Cristobalina' during dormancy explains the higher content of ABA-GE. We can therefore hypothesize that low ABA content in the early variety is due to active catabolism of ABA to ABA-GE.

In simplified terms, we propose that the difference of dormancy release dates between these two cultivars is due to the ABA levels present within the buds. In the early stages of dormancy induction, short days and decreasing temperatures activates ABA biosynthesis while active ABA esterification in early cultivars maintains low ABA levels. Endodormancy period is prolonged in the late cultivar by additional ABA synthesis activated by the enhanced expression of *PavNCED3*. In addition, increasing ABA catabolism, indicated by up-regulation of *PavCYP7072A*, allows the accumulation of PA, which can play a growth-limiting role similar to ABA. Consequently, the potential combination of two inhibitory actors (ABA and PA) deepens dormancy and delays dormancy release in late cultivars.

Presently, a remaining question to elucidate is what drives the decrease in ABA levels around dormancy release. Which mechanisms are involved in the down-regulation of *PavNCEDs* and up-regulation of *PavCYP707As* through chill accumulation? Firstly, several reports indicate that ABA might regulate its own accumulation and high levels of ABA attained during endodormancy could up-regulate the expression of catabolic genes such as *PavCYP707A4*, leading to a global decrease in ABA content. Secondly, *DORMANCY-ASSOCIATED MADS-BOX* (*DAM*) genes have been strong candidates for a key role in dormancy promotion and maintenance since they were identified in the non-dormant *evg* mutant of peach (Bielenberg et al., 2008; Rodriguez et al., 1994). *DAM* genes are highly expressed during dormancy and their expression is inhibited by chill accumulation (Hao et al., 2015; Jiménez et al., 2010), but more interestingly, one example of direct interaction between PpDAM1 and *PpNCED3* promoter was reported in pear (Tuan et al., 2017). Further *in vitro* assays suggest a potential regulation between CBF proteins and *DAM* promoters (Niu et al., 2015; Zhao et al., 2018). We therefore hypothesize that chill accumulation induces *CBF* expression and CBF proteins modulate the expression of *DAM* genes that subsequently upregulate *NCED* genes and ABA biosynthesis. Chill accumulation potentially induces chromatin modifications that silences *DAM* genes (de la Fuente et al., 2015; Leida et al., 2012b; Ríos et al., 2014) and inhibits ABA production, consistently with decreasing ABA levels observed as soon as January. Regulation of *DAM* and ABA pathways by cold temperatures should be further investigated to better understand how temperature variations control dormancy progression in sweet cherry flower buds.

Towards new phenology approaches based on molecular mechanisms

Following our observations that ABA levels were highly correlated with dormancy depth and that dynamics of expression for ABA synthesis and catabolism could explain the differences observed between cultivars, we have successfully modeled ABA content and dormancy behavior in three cultivars exhibiting contrasted dormancy release dates. Indeed, ABA had been proposed as an indicator for dormancy release in sweet cherry (Chmielewski et al., 2017) but to our knowledge, this is the first attempt to simulate dormancy onset and duration using molecular data. Only a small number of key genes regulating ABA were sufficient to account for all variations in ABA levels and dormancy progression overtime and between cultivars. Previous analyses have shown that ABA levels are highly

variables between years (Chmielewski et al., 2017) therefore further analyses are needed to explore and validate the current model.

Antagonistic actions of ABA and GA have been extensively studied in seed dormancy (Shu et al., 2018) and the ABA/GA ratio is often proposed as a determinant factor in the control of rest and growth responses, including dormancy release (Zhang et al., 2018). Therefore, integrating GA signaling into the bud dormancy model might be necessary to better account for the regulation of dormancy release. For example, it is possible that high GA levels around dormancy release play a role by overcoming the ABA-dependent growth inhibition. Interaction between GA and ABA pathways might also be critical in the response to environmental conditions during dormancy, including intertwined regulations of hormone biosynthesis (Shu et al., 2013; Yue et al., 2017). This was applied in a very innovative model for seed germination based on the endogenous hormone integration system (Topham et al., 2017). The hormonal balance between GA and ABA is regulated by endogenous and environmental signals towards the developmental switch that triggers termination of dormancy and germination. Accordingly, ON/OFF systems, like dormancy or flower initiation, can be modelled as developmental switches triggered in response to quantitative inputs after a threshold has been reached (Bassel, 2016; Donohue et al., 2015; Wilczek et al., 2009). In our current model, we propose a first step for mechanistic modelling of dormancy onset and release based on expression data and ABA quantification. The next steps, in addition to the integration of GA signaling and its crosstalk with ABA, will be to provide information on temperature-mediated control of the regulatory cascades. Ground-breaking research led on Arabidopsis, allowed by high-throughput sequencing techniques, has hastened the pace for the incorporation of molecular data into phenology models (Kudoh, 2016; Satake et al., 2013), thus opening new roads for perennial studies.

MATERIALS AND METHODS

Plant material

Samples were collected from two different sweet cherry cultivars (*Prunus avium* L.) having contrasted flowering dates. Very low-, low- and high-chilling cultivars (respectively, ‘Cristobalina’, ‘Garnet’ and ‘Regina’) are grown in an orchard located at the Fruit Experimental Unit of INRA in Bourran, South West of France (44° 19′ 56″ N, 0° 24′ 47″ E) under standard agricultural practices. Ten time points spanning the entire period of bud dormancy up to bud break were chosen for phytohormone quantification (for ‘Cristobalina’ and ‘Regina’) and RNA-seq analysis (for the three cultivars). Flower buds were harvested from branches, one branch per replicate from three (‘Cristobalina’ and ‘Garnet’) or two different trees (‘Regina’). Upon harvesting, buds were flash frozen in liquid nitrogen and stored at -80°C prior to performing RNA-seq. Average daily temperatures were recorded by an on-site weather station.

For the exogenous application of hormones, branches were collected from the high-chilling sweet cherry cultivar ‘Fertard’. Trees were grown in an orchard located at the Fruit Experimental Unit of INRA in Toulenne, South West of France (48° 51′ 46″ N, 2° 17′ 15″ E) under standard agricultural practices. Five branches per modality were randomly harvested from ten trees in 2016 and 2018 in mid-January when trees had reached three quarter of their chilling requirements.

Measurements of bud break and estimation of the dormancy release date

Measurements for the dormancy stages were performed on randomly chosen cut branches harvested every two weeks from November 16th 2015 to April 4th 2016 from ‘Cristobalina’, ‘Garnet’ and ‘Regina’ trees. Branches were incubated in water pots placed in a growth chamber (25°C, 16h light/ 8h dark, 60-

70% humidity). The water was replaced every 3-4 days. When the first flower bud reached the BBCH stage 53 (Fadón et al., 2015) phenological observations were carried out on flower buds every two days. The date of dormancy release was estimated when the percentage of bud break reached 50% of buds at the BBCH stage 53. The data are represented in percentage of buds at the BBCH stage 53 for three branches over the course of time.

Treatments with exogenous hormones and antagonists

To investigate the effects of GA and ABA on dormancy, cherry dormant buds were treated with 5 µM GA_3 (Sigma-Aldrich, ref. 48870), 5 µM GA_4 (Sigma-Aldrich, ref. G7276), 400 µM ABA (Sigma-Aldrich, ref. A1049), 300 µM paclobutrazol (Sigma-Aldrich, ref. 46046), an inhibitor of the GA pathway, and 5 µM fluridone (Sigma-Aldrich, ref. 45511), an inhibitor of the ABA pathway.
All chemicals and a water control were freshly prepared to the desired concentrations in 0.5% of surfactant ("Calanque", Action Pin, Castets, France) to ensure the penetration of active molecules through the bud scales. Chemicals were sprayed on buds under a fume-hood and branches were left several minutes to allow them to dry. Branches were then transferred in the growth chamber (25°C, 16h light/ 8h dark, 60-70% humidity) in pots containing water. The water was replaced every 3-4 days. Bud break measurements were performed on flower buds as mentioned above.

Phytohormones extraction

For each sample, 10 mg of frozen pulverised flower buds were weighed in a 2 mL tube. The extraction was carried out by adding 1 mL of cold 70% MeOH / 29% H_2O / 1.0% formic acid, containing isotopically labelled internal standards. Then, the tubes were stirred at room temperature for 30 min and centrifuged (5427R, Eppendorf) at 16,000 rpm for 20 minutes at 4°C. The supernatant of each tubes were transferred into new tubes and evaporated to dryness using a Turbovap LV system (Biotage, Sweden). The dried extracts were dissolved with 1 mL of a 2% formic acid solution. The resuspended extracts were purified using a solid phase extraction (SPE) Evolute express ABN 1ml-30 mg (Biotage, UK). The eluate was evaporated to dryness and resuspended in 200 µL of 0.1% formic acid.

Phytohormones quantification

ABA and conjugates (ABA-GE, PA, DPA) and GAs (GA_1, GA_3, GA_4, GA_7, GA_9) were quantified by UHPLC-MS/MS. ABA, ABA-GE, gibberellins (GA_4, GA_7, GA_9), [$^2H_6$]-ABA, and [$^2H_2$]-GA_4 were purchased from OlchemIn (Olomouc, Czech Republic). DPA, PA, [$^2H_3$]-dihydrophaseic acid (D-DPA), and [$^2H_3$]-phaseic acid (D-PA) were purchased from NRC (Ottawa, Canada). Phytohormones were analyzed by an UHPLC-MS/MS system. The separation and detection were achieved using a Nexera X2 UHPLC system (Shimadzu, Japan) coupled to a QTrap 6500+ mass spectrometer (Sciex, Canada) equipped with an electrospray (ESI) source. Phytohormones separation was carried out by injecting 2 µL into a Kinetex Evo C18 core-shell column (100 x 2.1mm, 2.6µm, Phenomenex, USA) at a flow rate of 0.7 mL/min, and the column oven was maintained at 40°C. The mobile phases were composed of solvent A Milli-Q water (18 MΩ, Millipore, USA) containing 0.1% formic acid (LCMS grade, Fluka analytics, Germany), and solvent B acetonitrile LCMS grade (Fisher Optima, UK) containing 0.1% formic acid. The gradient elution started with 1% B, 0.0-5.0 min 60% B, 5.0-5.5 min 100% B, 5.5-7.0 min 100 % B, 7.0-7.5 min 1% B, and 7.5-9.5 min 1% B. The ionization voltage was set to 5kV for positive mode and -4.5 kV for negative mode producing mainly $[M+H]^+$ and $[M-H]^-$ respectively. The analysis was performed in scheduled multiple reaction monitoring (MRM) mode in positive and negative mode simultaneously with a polarity switching of 5 ms. All quantitative data were processed using MultiQuant software V 3.0.2 (Sciex, Canada). GA_1, GA_3 were not detected in the samples.

RNA extraction and library preparation

Total RNA was extracted from 50-60 mg of frozen and pulverised flower buds using RNeasy Plant Mini kit (Qiagen) with minor modification (1.5% PVP-40 was added in the RLT buffer). RNA quality was evaluated using Tapestation 4200 (Agilent Genomics). Only samples with RNA integrity number equivalent (RINe) superior or equivalent to 8.5 were used for RNA-seq. Library preparation was performed with 1 µg of total RNA using the TruSeq Stranded mRNA Library Prep Kit High Throughput (96 samples, 96 indexes, Illumina cat. no. RS-122-2103). DNA quality from libraries was evaluated using Tapestation 4200 (Agilent Genomics). The libraries were sequenced on a NextSeq500 using paired-end sequencing of 75 bp in length.

RNA-seq data analysis

The raw reads obtained from the sequencing were analysed using several publicly available software and in-house scripts. The quality of reads was assessed using FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc/) and possible adaptor contaminations and low quality trailing sequences were removed using Trimmomatic (Bolger et al., 2014). Trimmed reads were mapped to the peach (*Prunus persica* (L.) Batsch) reference genome v2.0 (Verde et al., 2013) using Tophat (Trapnell et al., 2009) and possible optical duplicates were removed using Picard tools (https://github.com/broadinstitute/picard). For each gene, raw reads and Transcripts Per Million (TPM) were calculated (Wagner et al., 2012). Data were filtered by removing lowly expressed genes and genes that have a little change in expression (coefficient of variation < 0.3). Differentially expressed genes (DEGs) between time-points were assessed using DESeq2 R Bioconductor package (Anders and Huber, 2010) on filtered data. Genes with an adjusted p-value (padj) < 0.05 were assigned as DEGs.

Modelling

In order to explore the differences in the expression of ABA in the two cultivars, 'Cristobalina' and 'Regina', we took a mathematical modelling approach. We constructed a model incorporating information from the genes involved in the ABA signalling pathway.

Since NCEDs and CYP707As and UGT have been implicated in the production and degradation of ABA, respectively, they were considered in the production and decay rates of ABA. ABA level at different times, t, for each cultivar is described by an ordinary differential equation:

$$\begin{aligned}\frac{d\,ABA_i(t)}{dt} = {} & p^i_{NCED1} * NCED1_i(t) + p^i_{NCED3} * NCED3_i(t) + p^i_{NCED4} * NCED4_i(t) + p^i_{NCED5} \\ & * NCED5_i(t) \\ & - \left(p^i_{CYP707A4a} * CYP707A4a_i(t) + p^i_{CYP707A4b} * CYP707A4b_i(t) + p^i_{UGT} \right. \\ & \left. * UGT_i(t)\right) * ABA_i(t)\end{aligned}$$

for i=1,2, where i=1 represents the index of cultivar 'Regina' and i=2 is the index of cultivar 'Cristobalina'.

In both cultivars, for the sake of simplicity, it was assumed that the rates are linearly dependent on the gene levels. For example, the rate of NCED1–dependent ABA production in Regina at a time t is described by $p^1_{NCED1} * NCED1_1(t)$ where p^1_{NCED1} is a non-negative rate constant (model parameter). Genes (NCEDs, CYP707As and UGT) are treated as the time-dependent parameters of the model and their values are taken from the data. More precisely, going back to the earlier example, the level of NCED1 in 'Regina', labelled $NCED1_1(t)$, is a function of time t with values calculated from linearly interpolated mean 'Regina' data values of NCED1. Initial level of ABA at time 0 in each cultivar, i.e. ABA(0), is taken to be the mean level of ABA on the first day of measurement.

In order to show whether the differential in ABA in the two cultivars could be explained solely by the differences in NCEDs, CYP7074As and UGT, we tested whether there exists a set of parameters where the parameter values for both cultivar models are the same (i.e. $p^1_{NCED1} = p^2_{NCED1}, p^1_{NCED3} = p^2_{NCED3}$ and so on) but for which the model simulations can show the ABA differences seen between the two cultivars. Latin Hypercube Sampling was used to select 100,000 parameter sets. Since the gene levels can peak with level (in TPM) of up to 100 times higher than ABA levels (in pg/mg), the production and decay rate constants were bounded above by 0.001 and 0.005, respectively. Once the model solutions were calculated, least squares analysis was performed to calculate the residuals between the models and the mean ABA data of each of the two cultivars. Model with the parameter set that had the lowest sum of the least squares was chosen for simulation and prediction.

Finally, using the mean data measurements for *PavNCEDs*, *PavCYP7074As* and *PavUGT* of the cultivar 'Garnet' and the model parameter set identified above, we used our model to predict the levels of ABA in the 'Garnet' cultivar. Since the initial value of ABA content in 'Garnet' cultivar was not measured, we took it arbitrarily to be 1 at the time 0 (this being a value that also falls within the range of the initial ABA levels of the 'Regina' and 'Cristobalina' cultivars).

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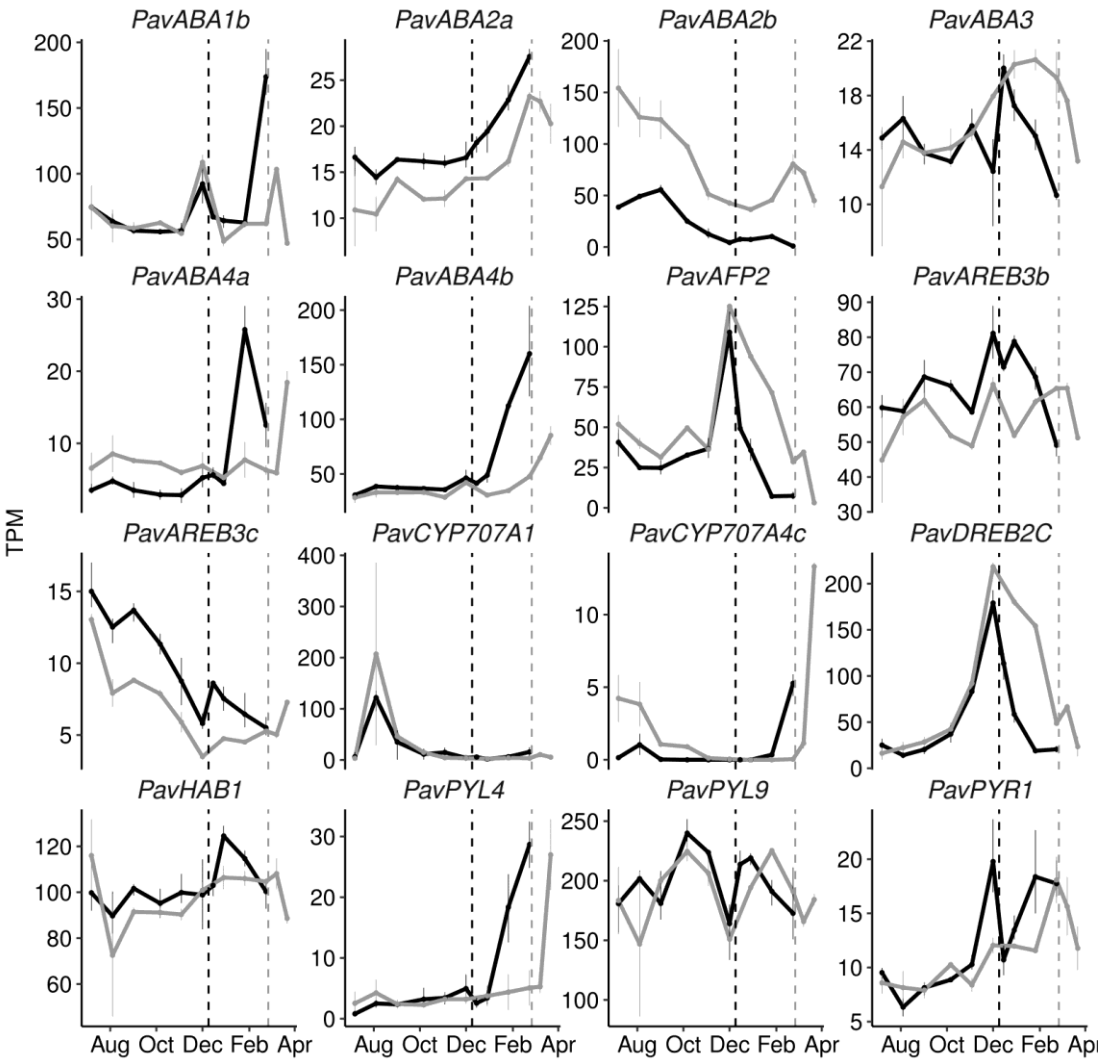

Figure 7 – figure supplement 1 | Transcriptional dynamics of genes associated with ABA pathway in the flower buds of two sweet cherry cultivars during bud development. Expression of specific genes involved in ABA biosynthesis pathway, degradation, signal transduction and response are represented in TPM (Transcripts Per kilobase Million). Black: 'Cristobalina', grey: 'Regina'. Error bars indicate the data range between the biological replicates (n=3 for 'Cristobalina', n=2 for 'Regina'). Dotted lines represent dormancy release. Expression data are available in the Source data 1.

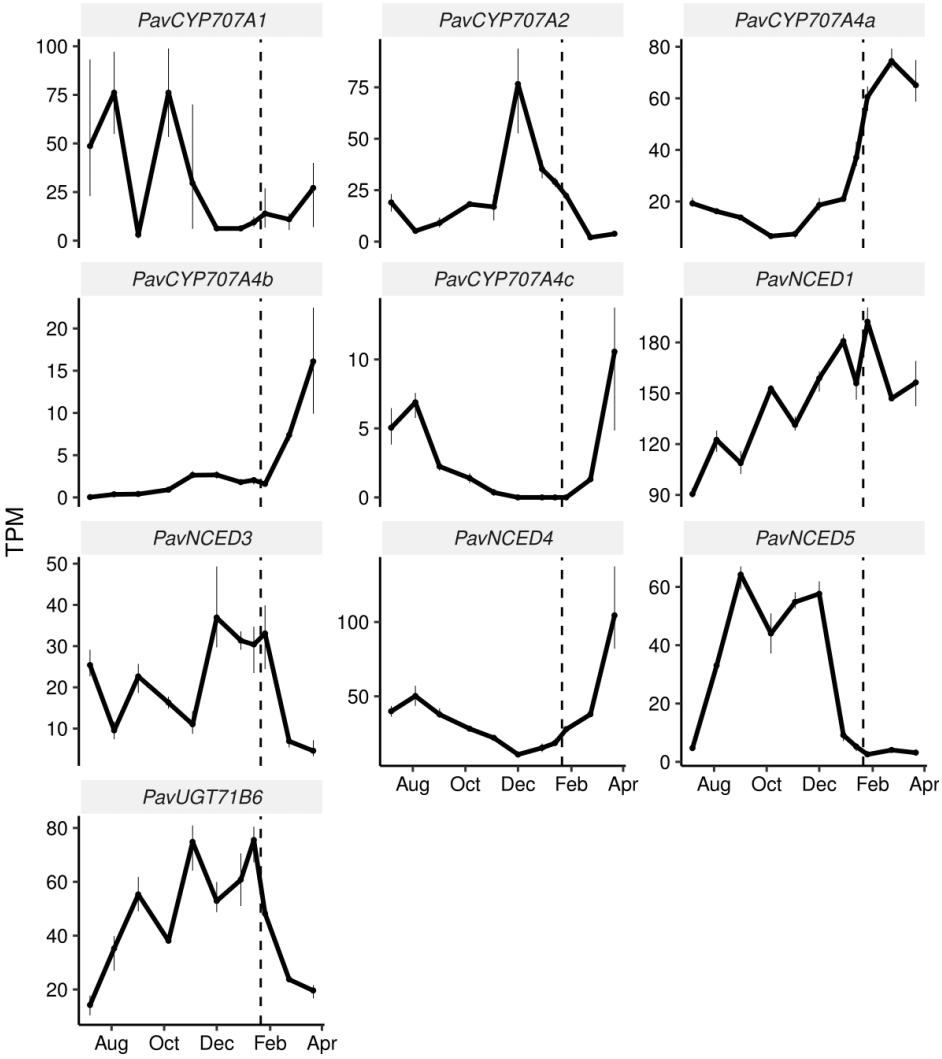

Figure 8 – figure supplement 1 | Transcriptional dynamics of genes involved in ABA synthesis and degradation for the sweet cherry cultivar 'Garnet'. Expression of specific genes involved in ABA biosynthesis pathway, degradation, signal transduction and response are represented in TPM (Transcripts Per kilobase Million). Error bars indicate the data range between the biological replicates (n=3). Dotted lines represent dormancy release. Expression data are available in the Source data 1.

SUPPLEMENTARY MATERIAL

List of genes analysed in the paper are available in SupplTable1.xlsx.