Title: Hormonal balance finely tunes dormancy status in sweet cherry flower buds

Running head: ABA and GA control sweet cherry bud dormancy

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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 bud 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-associated pathways have distinct functions and may differentially regulate dormancy. In addition, ABA levels rise at the onset of dormancy, associated with enhanced expression of ABA biosynthesis *PavNCED* genes, and decreased prior to dormancy release. Following the observations that ABA levels are strongly linked with dormancy depth, we identified *PavUG71B6*, a sweet cherry *UDP-GLYCOSYLTRANSFERASE* gene that up-regulates active catabolism of ABA to ABA-GE in the early cultivar. Subsequently, 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 and open up new perspectives for the development of molecular-based phenological modelling.

KEY WORDS: Abscisic acid, bud dormancy, gibberellic acid, hormones, modelling, *Prunus avium* L.

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 often triggered by decreasing photoperiod and/or temperatures depending on the species (Heide and Prestrud 2005, Rohde et al. 2011, Petterle et al. 2013, 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, 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 (Vitasse et al. 2011, Atkinson et al. 2013, Jochner et al. 2013). 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 (Cooke et al. 2012, Ríos et al. 2014, Beauvieux et al. 2018). 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 dormancy regulation by temperature and photoperiod (Chuine et al. 2016, Chuine and Régnière 2017). However, process-based models of bud dormancy have not changed substantially since 1990 (Hänninen 1990) and the current predictive models rely on very little information about involved mechanisms. 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).

The major role of hormones in the regulation of bud growth cessation, dormancy and activity resumption has been extensively discussed (e.g. (Cooke et al. 2012, Beauvieux et al. 2018, Liu and Sherif 2019). Seed and bud dormancy show common features in terms of hormonal control (Powell 1987, Leida, Conejero, et al. 2012, Wang et al. 2016) and GA and ABA balance is often involved in the integration of internal and external cues to control plant growth (Rodríguez-Gacio et al. 2009, Finkelstein 2013, Shu et al. 2018): GAs promote growth, whereas ABA promotes dormancy. Multiple physiological and transcriptomic studies have indeed proposed a central role for 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 (Rohde et al. 2002, Rohde and Bhalerao 2007, Ruttink et al.

2007, Wang et al. 2016, Tuan et al. 2017, Li et al. 2018, Tylewicz et al. 2018). 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 (Or et al. 2000, Zheng et al. 2015, Wang et al. 2016, Wen et al. 2016, Chmielewski et al. 2017, Li et al. 2018, Zhang et al. 2018, Yamane et al. 2019). Recently, ABA content has been proposed as a determining factor to assess dormancy status in sweet cherry (Chmielewski et al. 2017). 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 (Fig. **1a**) (Nambara and Marion-Poll 2005, Bai et al. 2013, Zhong et al. 2013, Zhu et al. 2015, Wang et al. 2016, Khalil-Ur-Rehman et al. 2017, Li et al. 2018, Zhang et al. 2018, Zheng et al. 2018). 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 (Junttila and Jensen 1988, Ruttink et al. 2007, Olsen 2010, Eriksson et al. 2015, 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 (Shafer and Monson 1958, Rinne et al. 2011, Zhuang et al. 2013), or have delaying effects on shoot growth and bud break (Hoad 1983, Zheng et al. 2018), 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 (Fig. **1b**; Yamaguchi 2008, Bai et al. 2013, Zhong et al. 2013, Zhu et al. 2015, Wen et al. 2016, Khalil-Ur-Rehman et al. 2017, Zhang et al. 2018, Zheng et al. 2018).

In this study we explored potential hormonal markers of dormancy using a combination of physiological and transcriptomic analyses and 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.

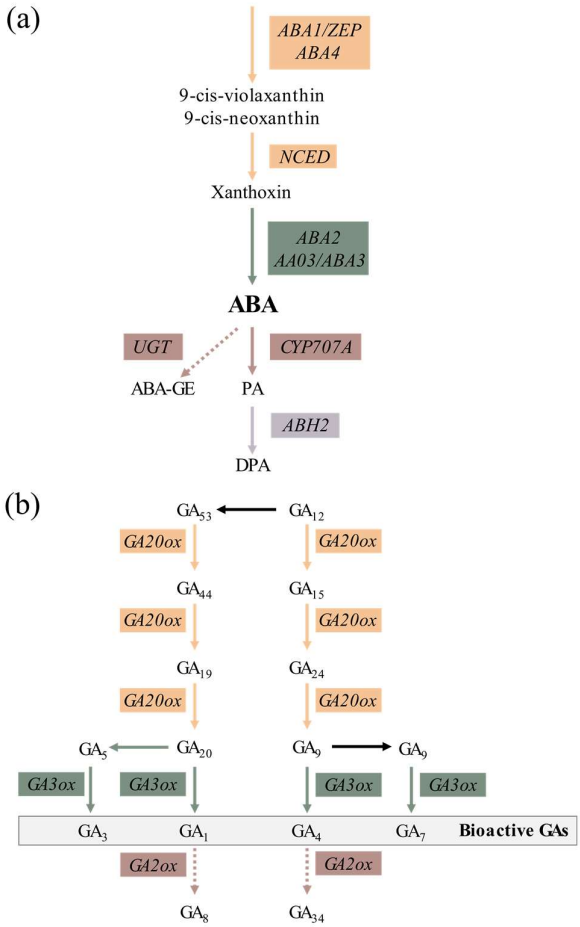

Figure 1 Biosynthesis and catabolism pathway for ABA and GAs. (**a**) ABA is synthesized through the action of five enzymes: zeaxanthin epoxidase (ZEP/ABA1), ABA-deficient4 (ABA4,) 9-cis epoxycarotenoid dioxygenase (NCED), alcohol dehydrogenase (ABA2) and short-chain dehydrogenase/reductase (AAO3/ABA3). 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). ABA: Abscisic acid; GA: Gibberellic acid.

MATERIALS AND METHODS

Plant material

Samples were collected from three different sweet cherry cultivars (*Prunus avium* L.) having very early, early and late flowering dates (respectively, 'Cristobalina', 'Garnet' and 'Regina'). Trees 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. During the sampling season (July 2015 to

March 2016), a mix of randomly chosen flower buds (equivalent to a 2 mL volume) were sampled at ten time points spanning the entire period of bud development (Fig. 2a) for phytohormone quantification (for 'Cristobalina' and 'Regina') and RNA-seq analysis (for the three cultivars). Flower buds were harvested from branches of three ('Cristobalina' and 'Garnet') or two different trees ('Regina'). A total of 29, 31 and 21 samples were analyzed for 'Cristobalina', 'Garnet' and 'Regina' respectively. Details are available in Table S1 (Supplementary file at *Tree Physiology* online). 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.

In addition, for the exogenous application of hormones, branches were collected from the late flowering 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.

Measurements of bud break and estimation of the dormancy release date

Measurements for the dormancy stages were performed on randomly chosen branches cut every two weeks from November 16th 2015 to April 4th 2016 for 'Cristobalina', 'Garnet', 'Regina' and 'Fertard', and between November 21st 2017 and April 4th 2018 for 'Fertard'. 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. After ten days under these forcing conditions, the percentage of bud break, i.e. flower buds at BBCH stage 53 (Fadón et al. 2015), as illustrated in Fig. **S1a**, was recorded. The date of dormancy release was estimated when at least 50% of the flower buds were at the BBCH stage 53 or higher after ten days under forcing conditions.

Treatments with exogenous hormones and antagonists

To investigate the effects of GA and ABA on dormancy, five branches per modality were randomly harvested from ten 'Fertard' dormant trees on January 19th 2016 and January 29th 2018 (Fig. S1; Supplementary file at *Tree Physiology* online). The 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. Bud break measurements were performed on flower buds as mentioned above.

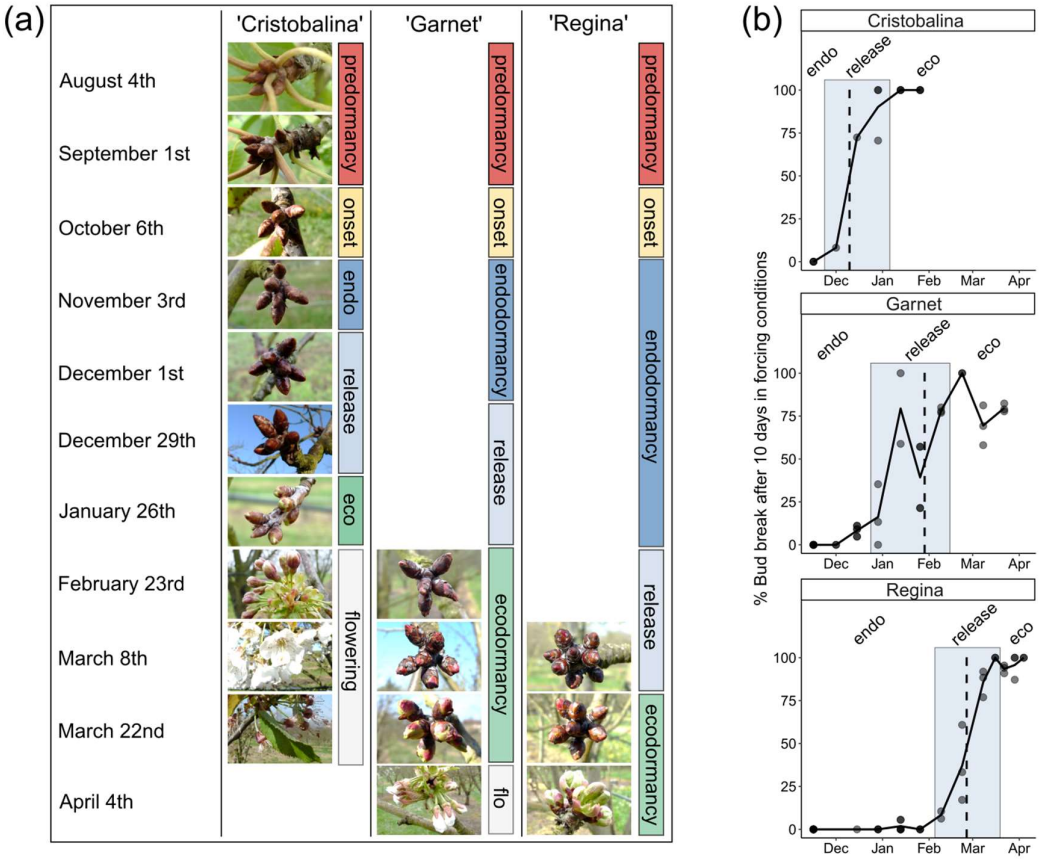

Figure 2 Dormancy status defined for the three sweet cherry cultivars. (a) bud stages at the sampling dates and the corresponding dormancy status as defined in the current study; (b) Evaluation of bud break percentage under forcing conditions was carried out for three sweet cherry cultivars displaying very early, early and late flowering dates: 'Cristobalina', 'Garnet' and 'Regina' respectively. The dotted line corresponds to the dormancy release dates, estimated at 50% of bud break after ten days under forcing conditions. Dots indicate the data points for the biological replicates.

149

150 **Phytohormones extraction**

151 For each sample (see Table S1; Supplementary file at *Tree Physiology* online), 10 mg of frozen
152 pulverised flower buds were weighed in a 2 mL tube. The extraction was carried out as previously
153 described (Ali et al. 2018, Haddad et al. 2018, Lakkis et al. 2019) by adding 1 mL of cold 70% MeOH
154 / 29% H_2O / 1.0% formic acid, containing isotopically labelled internal standards. Then, the tubes were
155 stirred at room temperature for 30 min and centrifuged (5427R, Eppendorf) at 16,000 rpm for 20 minutes
156 at 4°C. The supernatant of each tubes were transferred into new tubes and evaporated to dryness using
157 a Turbovap LV system (Biotage, Sweden). The dried extracts were dissolved with 1 mL of a 2% formic
158 acid solution. The resuspended extracts were purified using a solid phase extraction (SPE) Evolute
159 express ABN 1ml-30 mg (Biotage, UK). The eluate was evaporated to dryness and resuspended in 200
160 µL of 0.1% formic acid before analysis.

161 **Phytohormones quantification**

ABA and conjugates (ABA-GE, PA, DPA) and GAs (GA_1, GA_3, GA_4, GA_7) were quantified by UHPLC-MS/MS as previously described (Lakkis et al. 2019). ABA, ABA-GE, gibberellins (GA_4, GA_7), [$^2H_6$]-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 National Research Council Canada (NRC, Saskatoon, 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 (see Table S1 for the detailed sample list) 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 (Illumina), at the Sainsbury Laboratory Cambridge University (SLCU), using paired-end sequencing of 75 bp in length.

Mapping and differential expression 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. 2017) using Tophat (Trapnell et al. 2009) and possible optical duplicates were removed using Picard tools

197 (https://github.com/broadinstitute/picard). For the number of mapped reads in each sample, please refer
198 to Table S1 (Supplementary file at *Tree Physiology* online). For each gene, Transcripts Per Million
199 (TPM) were calculated (Wagner et al. 2012). TPM for the genes analysed in this study are available in
200 the supplementary data file at *Tree Physiology* online. For the differential expression analysis, genes
201 were first filtered based on their average expression in all samples for 'Cristobalina' and 'Regina'
202 (average TPM > 4) and their variation coefficient C_v ($C_v > 0.3$) calculated as:

203 $$C_v = \frac{\sigma}{\mu}$$

204 where σ and μ are the standard deviation and mean values, respectively, for the TPM counts in all
205 samples for 'Cristobalina' and 'Regina'. Then, differentially expressed genes (DEGs) for each
206 combination of dormancy stages (pre-dormancy, endodormancy, dormancy breaking and ecodormancy)
207 were assessed using DEseq2 R Bioconductor package (Love et al. 2014), in the statistical software R (R
208 Core Team 2018), on filtered data. Genes with an adjusted *p-value* (padj) < 0.05 (Benjamini-Hochberg
209 multiple testing correction method), in at least one of the comparisons, were assigned as DEGs (Table
210 S2).

211 **Candidate gene identification**

212 We selected and investigated genes involved in the hormonal signaling pathways from predicted
213 functions available for the peach genes based on pairwise sequence comparison (blastx algorithm against
214 various protein databases) with *Arabidopsis thaliana* proteins (Verde et al. 2017);
215 https://www.rosaceae.org/analysis/154). Genes were identified in the database by key words and gene
216 names from the literature. Details on the studied genes are available in Table S3 (Supplementary file at
217 *Tree Physiology* online).

218

219 **Modelling**

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

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

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

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 (*NCED*s, *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. Predictions for the best model were evaluated using root mean square error (RMSE).

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).

RESULTS

Exogenous GA application accelerates bud dormancy release

GA and ABA effect on the bud break response to forcing conditions was evaluated on branches carrying dormant flower buds as assessed by forcing tests on the late flowering cultivar 'Fertard' (Fig. S1). Results revealed that GA_3 and GA_4 both had significant dormancy alleviating effects, characterized by higher bud break percentage, which was confirmed for GA_3 in a second experiment in 2018 (Fig. **3**, Fig. **S2**). However, no antagonist effect, namely bud break 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 bud break for ABA-treated branches was slightly higher than the control (Fig. **3**, Fig. **S2**). However, inhibiting ABA biosynthesis with fluridone activated bud break, consistent with the established role of ABA in promoting dormancy.

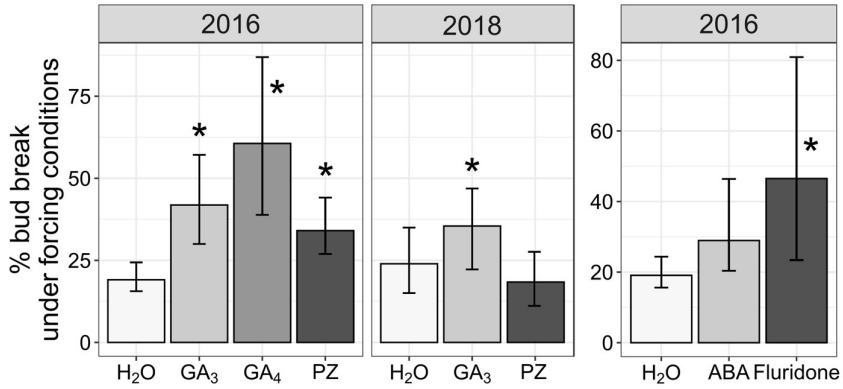

Figure 3 Effect of different GAs, ABA and their inhibitor on the sweet cherry dormancy status. Sweet cherry branches were treated with (a) 5 µM GA_3, 5 µM GA_4, 300 µM, paclobutrazol (GA pathway inhibitor), (b) 400 µM ABA and 5 µM fluridone (ABA pathway inhibitor) and transferred under forcing conditions (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. Asterisks indicate treatments that differ significantly from untreated branches (Kruskal-Wallis test, $p < 0.05$). ABA: Abscisic acid; GA: Gibberellic acid.

Definition of the flower bud dormancy status of three cultivars with different flowering dates

We selected three sweet cherry cultivars based on their different dates of flowering: very early, early and late for 'Cristobalina', 'Garnet' and 'Regina', respectively. The stages of bud dormancy were defined based on anatomical and physiological elements (Fig. **2a**). We associated predormancy with developmental stages of the flower buds along green leaves and active growth (July to September). Dormancy onset is often hypothesized to occur at leaf fall (Chmielewski et al. 2017) and we observed leaf senescence at the beginning of October and complete leaf fall at the end of October. Therefore,

dormancy onset was set in October and the beginning of endodormancy was established in November (Fig. **2a**). These stages were similar for all three cultivars. By definition, endodormancy correspond to the bud inability to fully develop under growth-inducing conditions while dormancy is considered as released when bud break is triggered by warm temperatures and/or long photoperiod (Lang et al. 1987). Consequently, dormancy status was assessed by forcing tests on branches carrying flower buds (Fig. **2b**). Endodormancy was defined for the dates with no bud break under forcing conditions (Fig. 2). Incomplete bud break within the population of flower buds indicated dormancy release stages while ecodormancy was defined by optimal bud break response to growth conditions (90-100% bud break; Fig. 2). Here, the three cultivars were much contrasted in the timing of their dormancy phases, 'Cristobalina' exhibiting dormancy release on December 9th, seven weeks earlier than 'Garnet' (January 29th) and ten weeks earlier than 'Regina' (February 26th) .

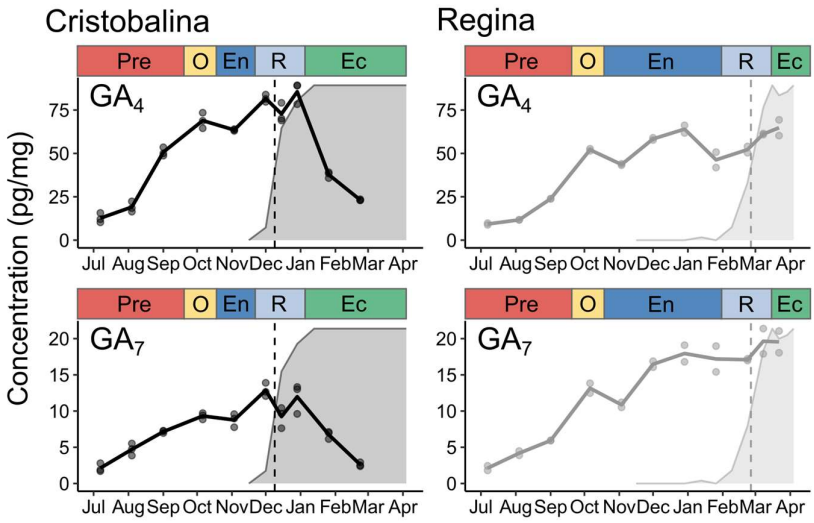

Figure 4 Levels of endogenous bioactive GA_4 and GA_7 in the flower buds of two sweet cherry cultivars during bud development. Black: 'Cristobalina', grey: 'Regina'. Background areas correspond to the dormancy depth evaluated as the percentage of bud break under forcing conditions (see Fig. 2b). Dotted lines represent dormancy release. GA: Gibberellic acid.

GA content changes during flower bud dormancy progression

In recent studies, distinct functions were identified for gibberellins during bud dormancy (Zhuang et al. 2013, Zheng et al. 2018). To test whether these results could be confirmed in sweet cherry buds, GA levels were determined over the whole bud development cycle in the very early and late flowering cultivars, 'Cristobalina' and 'Regina'. In details, we studied the content of bioactive GA_1, GA_3 GA_4 and GA_7 but the levels of GA_1 and GA_3 were undetectable in the samples. GA_4, GA_7 have a similar pattern over dormancy progression (Fig. **4**) rising from July onwards, which corresponds to flower primordia initiation and organogenesis. Bioactive GA levels also increase at the beginning of endodormancy (November – December), reaching their highest concentration during dormancy release. Results show a sharp decrease in the levels of GA_4 and GA_7 overlapping with ecodormancy for 'Cristobalina', which

could not be observed for the late cultivar 'Regina', potentially due to the lack of ecodormancy coverage. Interestingly, the levels of GA_4, and GA_7 significantly differed between the two cultivars, especially during endodormancy and when dormancy release was triggered in the early cultivar (Fig. S**3b**). Notable differences in GA_7 content were observed between these two cultivars during the entire time course, in which the late cultivar buds contained more GA_7 than the early cultivar (Fig. **4**) while levels for GA_4 were significantly higher in the early cultivar during endodormancy (Fig. S**3b**). 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.

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 found seven *PavGA20ox* and five *PavGA3ox* for GA biosynthesis genes in the peach database (**S3**) but only three genes were differentially expressed during flower bud development and dormancy (Fig. **5**, Table **S2**). Interestingly, *PavGA20ox2* expression increased in December for both cultivars, regardless of their dormancy status but decreased prior to 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 and its expression increases as early as dormancy onset (October), followed by a sharp increase during endodormancy, reaching its highest expression value at maximum dormancy depth in December, for both cultivars (Fig. **5**). *PavGA3ox1a* is then downregulated during or after dormancy release, with a marked lag between the two cultivars, potentially linked to their separate dormancy release date (Fig. **5**). We identified four differentially expressed *PavGA2ox* genes, involved in GA inactivation (Fig. **5**, Tables S2, S3). *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*.

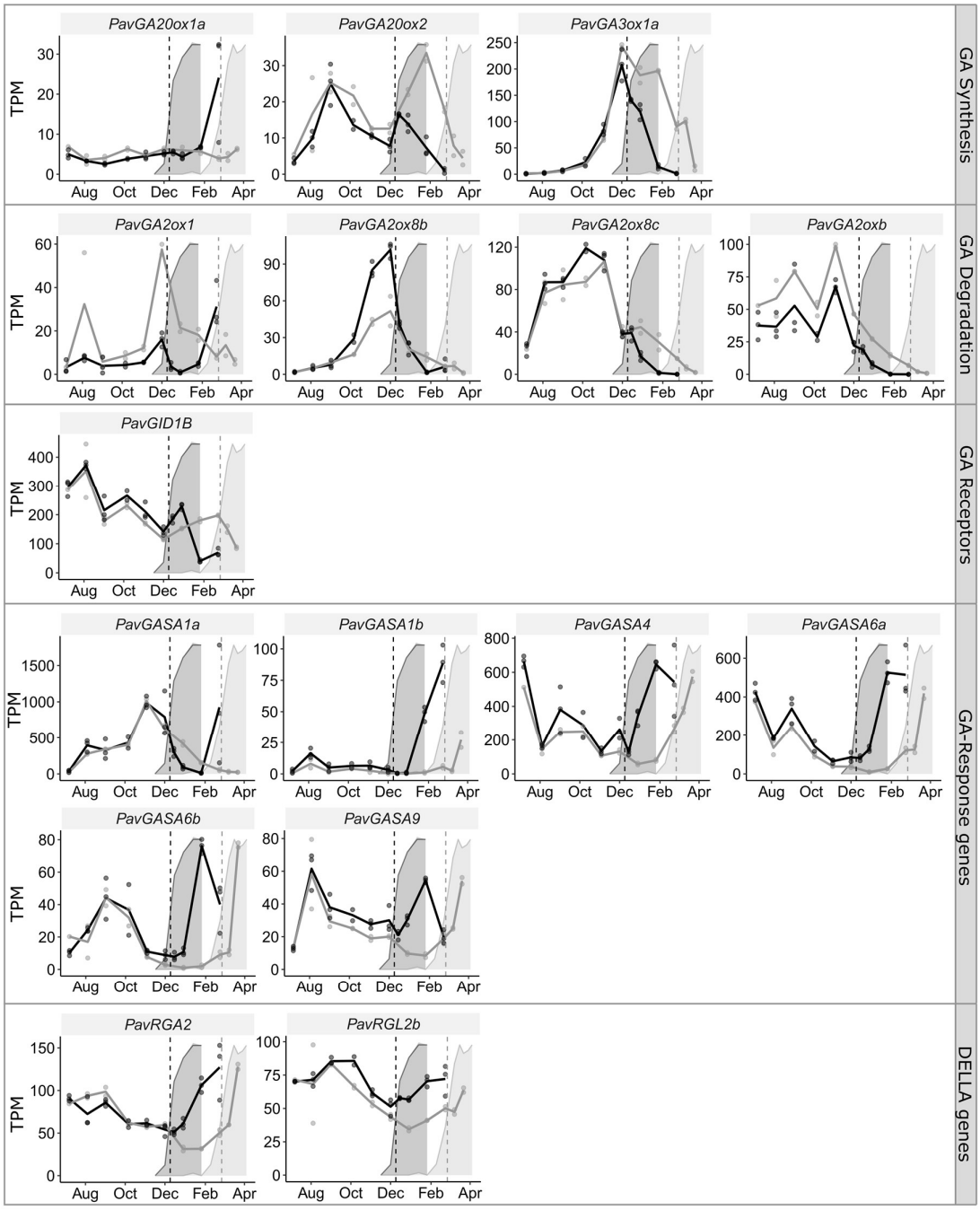

Figure 5 Transcriptional dynamics of genes associated with GA pathway in the flower buds of two sweet cherry cultivars during bud development. Expression of specific genes involved in GA biosynthesis pathway, degradation, signal transduction and response are represented in TPM (Transcripts Per Million reads). Black: 'Cristobalina', grey: 'Regina'. Background areas correspond to the dormancy depth evaluated as the percentage of bud break under forcing conditions (see Fig. 2b). Dotted lines represent dormancy release.GA: Gibberellic acid; GA20ox: GA 20-oxidases, GA3ox: GA 3-oxidases; GA2ox: GA 2-oxidases; GID: GA INSENSITIVE DWARF; GASA: GA Stimulated Arabidopsis; RGA: REPRESSOR OF GA; RGL: RGA-like.

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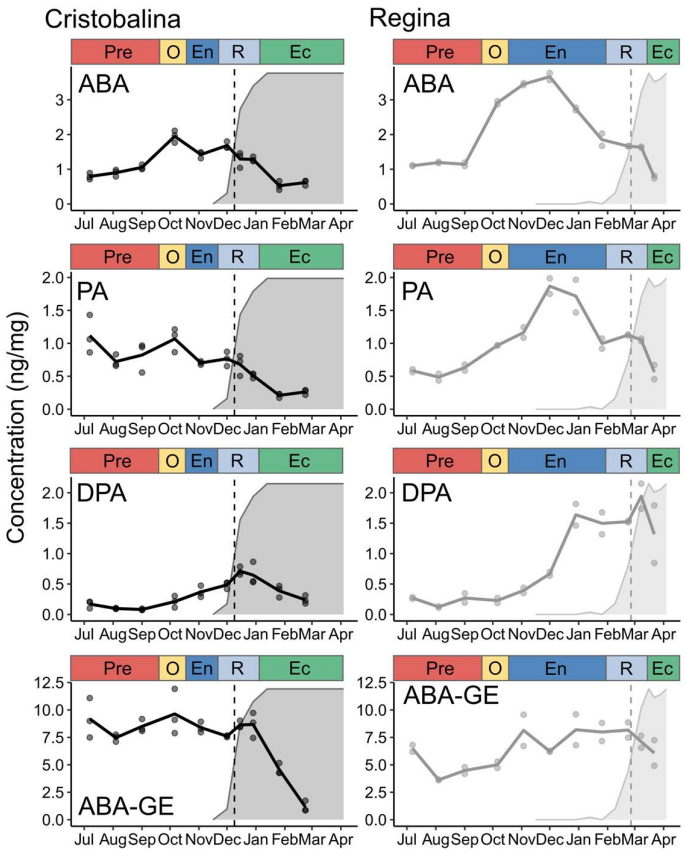

Figure 6 Levels of endogenous bioactive ABA and ABA conjugates in the flower buds of two sweet cherry cultivars during bud development. Black: 'Cristobalina', grey: 'Regina'. Background areas correspond to the dormancy depth evaluated as the percentage of bud break under forcing conditions (see Fig. 2b). Dotted lines represent dormancy release. ABA: Abscisic acid; PA: phaseic acid; DPA: dihydrophaseic acid, ABA-GE: ABA-glucose ester.

In terms of GA signaling, our results show that the identified GA receptor-related *GA INSENSITIVE DWARF1B* (*PavGID1B*) is highly expressed during the predormancy (July, August) and early stages of dormancy (September, October; Fig. **5**). For 'Cristobalina', expression of the receptor gene sharply decreased after endodormancy was released. Ten GA-response genes, *GA Stimulated Arabidopsis* (*GASA*), potentially regulated by GAs (Aubert et al. 1998), were identified in the transcript dataset (Table **S3**) and we analyzed the seven genes differentially expressed during flower bud cycle (Fig. **5**, Table **S2**). 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 (Fig. **4**). More strikingly, all *PavGASA* genes, except for *PavGASA1b*, were sharply upregulated during dormancy release. However, one notable exception is *PavGASA1a* that is highly activated specifically during dormancy (Fig. **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* (Table **S3**).

Among them, *PavRGA2* and *PavRGL2b* genes were differentially expressed during bud development, characterized by a down-regulation during dormancy onset and endodormancy, followed by a marked increase during dormancy release and ecodormancy (Table S2, Fig. **5**).

ABA levels rise at the onset of dormancy

Several studies have highlighted a strong correlation between ABA content and dormancy status and to address this issue in sweet cherry flower buds, we measured ABA levels, as well as PA and DPA, which are catabolites of ABA, in both cultivars. Results show an increase in ABA and PA content during the early stages of dormancy , reaching their highest levels in October and December for 'Cristobalina' and 'Regina', respectively, which is approximately two months prior to dormancy release for both cultivars. This ABA peak is followed by a decrease in ABA levels, accompanied by increased levels of DPA, preceding dormancy release (Fig. **6**). ABA, PA and DPA levels detected during dormancy for the early cultivar 'Cristobalina' are significantly lower than for the late cultivar (Fig. S**3a**). We can therefore hypothesize that dormancy depth is highly correlated with ABA contents in sweet cherry buds.

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 constantly high in 'Regina' over the whole cycle, with a slight increase during endodormancy induction, while it markedly decreased in 'Cristobalina' during ecodormancy (Fig. **6**, Fig. S**3a**). However, this observation could be due to a low coverage of ecodormancy in the 'Regina' samples. Throughout pre-dormancy stages, ABA-GE content was significantly higher in the early cultivar 'Cristobalina' compared to the late cultivar 'Regina' (Fig. S**3a**).

Analysis of 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 *PavNCED3* and *PavNCED5* genes seem strongly linked to ABA contents and dormancy status (Fig. **7**, Fig. **S4**). In particular, *PavNCED5* expression shows a sharp increase during dormancy onset and a marked decay before dormancy release. On the other hand, low ABA levels are associated with increased expression for ABA catabolism genes *PavCYP707A1* and *PavCYP707A4* before and after dormancy respectively (Fig. **7**, Fig. **S4**). By contrast, *PavCYP707A2* is characterized by a sharp increase in December, followed by a marked decrease during dormancy release in 'Cristobalina' but before induction of dormancy release in 'Regina'. In addition, we identified *PavUGT71B6* (Supporting Information Table **S2**), 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 (Fig. **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 (Table **S3**), six were differentially expressed during flower bud dormancy (Fig. **7**, Fig. **S4,** Table **S2**). In particular, *PavPYL8* expression was correlated with ABA levels, increasing after dormancy onset and decaying after dormancy release (Fig. **7**). A similar pattern was observed for *PavPYL12* in 'Cristobalina'. Among the differentially expressed ABA response genes (Supporting Information Tables **S2**, **S3**), *PavABF2* and *PavHAB2* had similar patterns with a sharp increase in expression in December while the expression of *PavABF3* and *PavABI1* decreased after dormancy release (Fig. **7**).

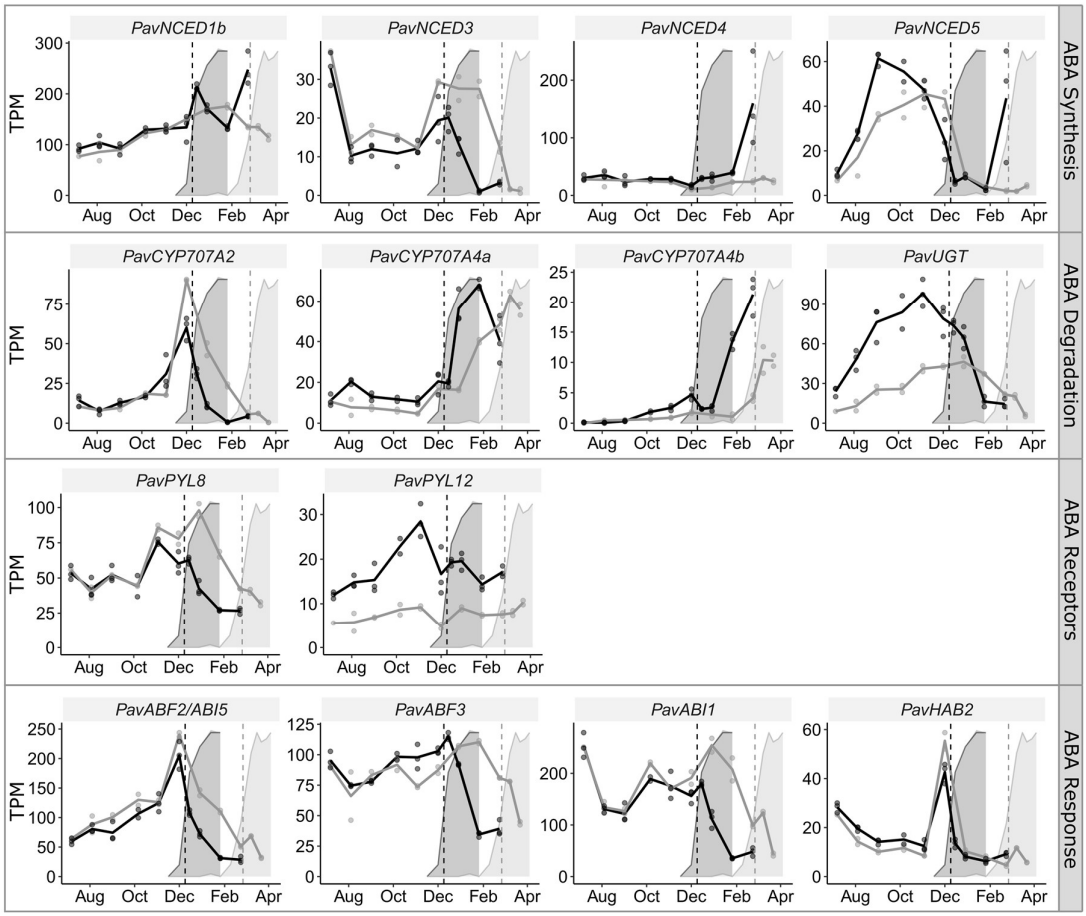

Figure 7 Transcriptional dynamics of genes associated with ABA pathway in the flower buds of two sweet cherry cultivars during bud development. Expression of specific genes involved in ABA biosynthesis pathway, degradation, signal transduction and response are represented in TPM (Transcripts Per Million reads). Black: 'Cristobalina', grey: 'Regina'. Background areas correspond to the dormancy depth evaluated as the percentage of bud break under forcing conditions (see Fig. 2b). Dotted lines represent dormancy release. ABA: Abscisic acid; NCED: 9-cis epoxycarotenoid dioxygenase; PYL: PYR-like; ABF: ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING PROTEIN; ABI; ABA INSENSITIVE; HAB: homology to ABI2; UGT: UDP-GLYCOSYLTRANSFERASE.

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 biosynthesis and catabolism 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* (Fig. **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, with a global RMSE equal to 0.3468 for all data, and 0.3142 and 0.3627 for 'Cristobalina' and 'Regina' respectively 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 (Fig. **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 (Fig. **8**, Fig. **S5**), the model simulated ABA levels for 'Garnet' during dormancy (Fig. **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 (Fig. **6**, **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 (Fig. **2a**); 'Cristobalina' and 'Regina' release dates are December 9th and February 26th, while 'Garnet' release date is the January 29st; 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.

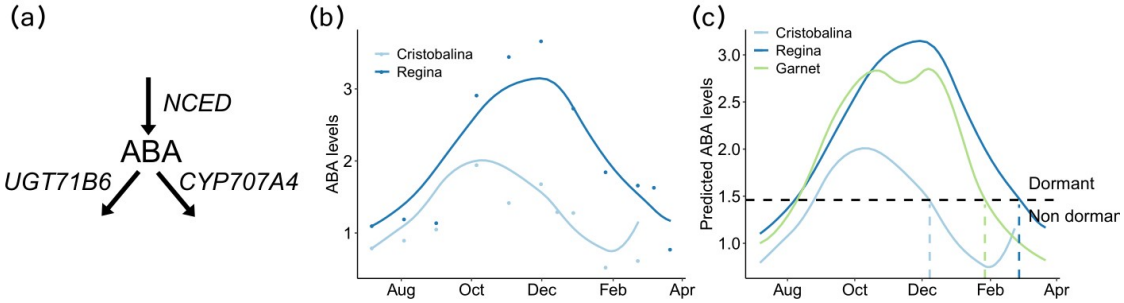

Figure 8 Modelling ABA. (**a**) Conceptual model used to simulate ABA content. ABA synthesis is controlled by NCED1, NCED3, NCED4 and NCED5 proteins; ABA is deactivated by 8'-hydroxylases CYP707A4 and UGT71B6. The assumption is that enzymatic activity is proportional to gene expression levels. (**b**) Simulated content of ABA using the model (lines) with means of data (circles) for cultivars 'Cristobalina' and 'Regina'. The model is simulated with parameters: $p_{NCED}^{1,2} = 0.0029, p_{NCED3}^{1,2} = 0.002241$, $p_{NCED}^{1,2}$ =0.0000218, $p_{NCED5}^{1,2}$= 0.009599, $p_{CYP707A4b}^{1,2}$= 0.00010859, $p_{CYP707A4a}^{1,2}$= 0.00012048 and $p_{UGT}^{1,2}$= 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). ABA: Abscisic acid; NCED: 9-cis epoxycarotenoid dioxygenase; UGT: UDP-GLYCOSYLTRANSFERASE.

DISCUSSION

Sweet cherry specific GA signaling during bud dormancy

We have shown that GA_3 and GA_4 have significant dormancy alleviating effects in sweet cherry cultivars, similar to previous observations in hybrid aspen (*Populus tremula* × *Populus Tremuloides*; Rinne et al. 2011), Japanese apricot (*Prunus mume*; Zhuang et al. 2013) and peach (Donoho and Walker 1957), but opposed to null or inhibitory effects on dormancy release previously reported in hybrid aspen and grapevine (Rinne et al. 2011, Zheng, et al. 2018). In addition, high levels of GA_4 and GA_7 were detected in sweet cherry flower buds, similarly to results obtained in pear (Ito et al. 2019), whereas GA_1 and GA_3 were undetectable in the samples, contradictory to the high GA_1 and GA_3 levels recorded in grapevine and Japanese apricot buds during dormancy (Zhang et al. 2018, Zheng et al. 2018). These observations 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. 2018). In addition, GA effects might be species or even cultivar-specific, with complex interactions with environmental factors, such as temperature or photoperiod. Further investigation on the regulation of the GA-related pathway during dormancy in sweet cherry, under contrasted environmental conditions, will bring key elements to the current hypotheses. Furthermore, given the complexity of GA quantification, it is possible that key GA dynamics might have been missed in the present study and therefore, expression of GA-pathway-related genes might be more reliable. Here, based on our results, we propose our hypotheses on GA signaling involving different transcriptional cascades during sweet cherry flower bud dormancy:

i) During predormancy stages (July to September), GA_4 and GA_7 content progressively increases, associated with an increase in *PavGA20ox2* expression, and the activation of GA signaling, as revealed by the increased expression of GA-response genes *PavGASA1a*, *4*, *6b*, and *9*. Enhanced GA content potentially triggers the expression of *PavGA2ox8c* 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, mostly driven by *PavGA20ox2* in this phase may control flower bud organogenesis and development.

ii) After endodormancy is induced, there is a marked increase in GA_4 and GA_7 contents. Gibberellin homeostasis seems to be actively controlled during endodormancy through enhanced expression of biosynthesis gene *PavGA3oxa* and deactivation genes *PavGA2ox1*, *PavGA2ox8b* and *PavGA2ox8c*. Interestingly, previous studies have shown that cellular transport is blocked during dormancy (Rinne et al. 2011, Tylewicz et al. 2018) and we can therefore hypothesize that although GAs may be present in the bud, their growth-promoting effect is physically inhibited. In agreement with acute GA deactivation, only one GA-response gene is activated during this phase, *PavGASA1a*, thus suggesting a very specific response. Expression for the five GA-related genes up-regulated during endodormancy sharply decrease during dormancy release, thus supporting the hypothesis that this is an endodormancy-specific regulation.

iii) As observed in the early cultivar 'Cristobalina', levels for GA_4 and GA_7 decrease during ecodormancy, associated with low, if not null, expression for *PavGA20ox2* and *PavGA3ox2a* synthesis genes, as well as *PavGA2ox8b*, *PavGA2ox8c*, *PavGA2oxb* deactivation genes and *PavGID1B* receptor. Interestingly, the GA-response *PavGASA* genes and the DELLA genes *PavRGA2* and *PavRGL2* are markedly activated during ecodormancy, thus suggesting that GA-stimulated pathways are up-regulated. Indeed, despite decreasing GA_4 and GA_7 levels during ecodormancy, 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 addition, bioactive GAs, including GA_1 and GA_3, that were not detected in the current study might be key actors in the growth resumption stage of ecodormancy in sweet cherry. Acute GA response occurring during ecodormancy, including activation of *GASA* genes, was previously reported in grapevine (Zheng et al. 2018), oak (Ueno et al. 2013), pear (Yang et al. 2019) and Japanese apricot (Zhang et al. 2018). Despite the fact that DELLA genes' activity is mainly regulated by protein stability, the up-regulation of *PavRGA2* and *PavRGL2* genes is consistent with the GA response during ecodormancy, associated with a decrease in *PavGIDB* expression. These results suggest that the GA homeostasis, critical during active growth, is controlled in sweet cherry by the DELLA proteins, that target GA biosynthesis and receptor genes and impacts GA balance through a feedback regulation, as

previously shown in Arabidopsis and rice (Gagne et al. 2002, Ueguchi-Tanaka et al. 2007, Zentella et al. 2007).

Dormancy depth is correlated with endogenous ABA content

Exogenous application of ABA on the sweet cherry dormant bud showed that buds at this specific stage were not affected by ABA treatment, in contrast to the observation that ABA treatment had a significant effect on grapevine bud break (Zheng et al. 2015). However, as shown by the high expression of genes involved in ABA degradation, including *PavCYP707A2*, high catabolism ability during endodormancy might have limited the effect of exogenous ABA. It might also suggest that ABA response is saturated in the context of very high ABA levels during endodormancy, therefore limiting the effect of additional ABA. Nevertheless, we observed that dormancy release was triggered in buds treated with fluridone that inhibits ABA biosynthesis, thus suggesting that high ABA levels may act to maintain dormancy. This was further confirmed by the observed elevated ABA levels correlated with endodormancy in both cultivars, as well as the differences in ABA levels between the early and late flowering cultivars. Our results are consistent with the hypothesis that dormancy is triggered and maintained when ABA levels are above a threshold, taking into account the potential heterogeneity within a population of flower buds. 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). Recent reports have indeed shown how low or high levels of ABA closely drive the depth of dormancy by controlling the blockage of cellular transports (Tylewicz et al. 2018, Singh et al. 2019). We show that during the predormancy and dormancy onset stages, ABA levels are correlated with an increase in the expression of *PavNCED5*, then *PavNCED3* genes in both cultivars. Consistently with elevated ABA levels, genes encoding ABA receptors (*PavPYL12*, *8*), as well as ABA response genes, are highly expressed during deep dormancy. One interesting result is that *PavNCED5* expression peak coincides with the highest ABA levels in October in 'Cristobalina' and in December in 'Regina', approximately two months before dormancy is released. This suggests that the deepest dormancy state might occur earlier in 'Cristobalina' than in 'Regina' and therefore question whether endodormancy is indeed induced simultaneously in both cultivars. Further physiological observations during the early stages of dormancy induction, including flower primordia developmental context (Fadón et al. 2018), could help better understanding the observed differences. 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 (Zhang et al. 2015, Wang et al. 2016, Tuan et al. 2017) confirming the major role of ABA signaling pathways on the regulation of dormancy induction, maintenance and release.

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 (Rodriguez et al. 1994, Bielenberg et al. 2008). *DAM* genes are highly expressed during dormancy and their expression is inhibited by chill accumulation (Jiménez et al. 2010, Hao et al. 2015), but more interestingly, recent studies have highlighted the direct effect of *DAM* on the activation of ABA biosynthesis (Tuan et al. 2017, Yamane et al. 2019). 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 (Leida et al. 2012, Ríos et al. 2014, de la Fuente et al. 2015) and inhibits ABA production, consistently with decreasing ABA levels observed as soon as January. Interestingly, a recent study conducted in hybrid aspen shows that chilling and long photoperiods induces a decrease in ABA levels, which subsequently drives the decrease in *SHORT VEGETATIVE PHASE-LIKE* (*SVL*) expression (Singh et al. 2018, 2019). *SVL* shows similarity to *SHORT VEGETATIVE PHASE* (*SVP*) and *DAM* genes and this mechanism suggests that ABA might also act on the expression of *DAM* genes. 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.

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. Contrasted expression patterns for *PavNCED3*, *PavCYP7074a* and *PavCYP7074b* were not sufficient to explain the noticeably contrasted ABA accumulation during endodormancy between the two cultivars. Differences in ABA catabolites between cultivars were also observed. While PA content followed the same pattern as ABA, DPA was particularly high during dormancy release, inversely correlated with the decreasing ABA levels. Interestingly, Weng and colleagues (Weng et al. 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 may explain the higher levels of PA during dormancy in 'Regina' and might therefore result in even deeper dormancy by the inhibitory combination of ABA and PA.

552 Since free ABA levels may be controlled through conjugation as well (El Kayal et al. 2011, Chmielewski
553 et al. 2018, Liu and Sherif 2019), we investigated the conversion of ABA to ABA-GE and we found a
554 *PavUGT71B6* gene characterized by strikingly higher expression levels for the early cultivar compared
555 to the late cultivar. UGT71B6 orthologs in Arabidopsis and Adzuki bean act specifically for ABA
556 conjugation into ABA-GE (Xu et al. 2002, Priest et al. 2006), so up-regulation of *PavUGT71B6*
557 expression in 'Cristobalina' during dormancy explains the higher content of ABA-GE. We can therefore
558 hypothesize that the low ABA content in the early cultivar may be due to active catabolism of ABA to
559 ABA-GE.

560 **Towards new phenology approaches based on molecular mechanisms**

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

571 Antagonistic actions of ABA and GA have been extensively studied in seed dormancy (Shu et al. 2018)
572 and the ABA/GA ratio is often proposed as a determinant factor in the control of rest and growth
573 responses, including dormancy release (Zhang et al. 2018). Therefore, integrating GA signaling into the
574 bud dormancy model might be necessary to better account for the regulation of dormancy release. For
575 example, it is possible that high GA levels around dormancy release play a role by overcoming the ABA-
576 dependent growth inhibition. Interaction between GA and ABA pathways might also be critical in the
577 response to environmental conditions during dormancy, including intertwined regulations of hormone
578 biosynthesis (Shu et al. 2013, Yue et al. 2017). This was applied in a very innovative model for seed
579 germination based on the endogenous hormone integration system (Topham et al. 2017). The hormonal
580 balance between GA and ABA is regulated by endogenous and environmental signals towards the
581 developmental switch that triggers termination of dormancy and germination. Accordingly, ON/OFF
582 systems, like dormancy or flower initiation, can be modelled as developmental switches triggered in
583 response to quantitative inputs after a threshold has been reached (Wilczek et al. 2009, Donohue et al.
584 2015, Bassel 2016). In our current model, we propose a first step for mechanistic modelling of dormancy
585 onset and release based on expression data and ABA quantification. The next steps, in addition to the
586 integration of GA signaling and its crosstalk with ABA, will be to provide information on temperature-

mediated control of the regulatory cascades. Recent research led on Arabidopsis, allowed by high-throughput sequencing techniques, has hastened the pace for the incorporation of molecular data into phenology models (Satake et al. 2013, Kudoh 2016), thus opening new roads for perennial studies.

DATA AVAILABILITY

RNA-seq data: Gene Expression Omnibus GSE130426

Number of mapped reads for all samples and expression data are available in the **supplementary datafile**

SUPPLEMENTARY DATA

Fig. S1 Chilling and dormancy status during the treatments with exogenous hormones and antagonist on sweet cherry cultivar 'Fertard'

Fig. S2 Effect of GAs, ABA and their inhibitor on the bud break percentage under forcing conditions

Fig. S3 Concentration ratio of ABA, ABA conjugates and GAs levels between 'Regina' and 'Cristobalina'

Fig. S4 Transcriptional dynamics of genes associated with ABA pathway in the flower buds of two sweet cherry cultivars during bud development

Fig. S5 Transcriptional dynamics of genes involved in ABA synthesis and degradation for the sweet cherry cultivar 'Garnet'

Table S1 Description of the flower bud samples used for hormone quantification and RNA-seq and total number of mapped reads for RNA-seq data

Table S2 RNA-seq filtering and Differential expression analysis

Table S3 Details of the genes related to ABA and GA pathways analyzed in the project

Supplementary Data File Expression data in transcripts per million reads (TPM) for the genes analyzed in the project

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AUTHORS' CONTRIBUTION

BW, SC, ED and PAW designed the original research. NV produced and analyzed the transcriptional data under the supervision of SC. RB conducted the analysis on exogenous application. AS and NV performed the phytohormones extraction and quantification. MA, FJ and JCY supervised the phytohormones analyses. NV and BW wrote the manuscript with the assistance of all the authors.

REFERENCES

Ali N, Schwarzenberg A, Yvin JC, Hosseini SA (2018) Regulatory role of silicon in mediating differential stress tolerance responses in two contrasting tomato genotypes under osmotic stress. Front Plant Sci 9:1–16.

Atkinson CJ, Brennan RM, Jones HG (2013) Declining chilling and its impact on temperate perennial crops. Environ Exp Bot 91:48–62.

Aubert D, Chevillard M, Dorne AM, Arlaud G, Herzog M (1998) Expression patterns of *GASA* genes in Arabidopsis thaliana: The *GASA4* gene is up-regulated by gibberellins in meristematic regions. Plant Mol Biol 36:871–883.

Bai S, Saito T, Sakamoto D, Ito A, Fujii H, Moriguchi T (2013) Transcriptome Analysis of Japanese Pear (*Pyrus pyrifolia* Nakai) Flower Buds Transitioning Through Endodormancy. Plant Cell Physiol 54:1132–1151.

Bassel GW (2016) To grow or not to grow. Trends Plant Sci 21:498–505.

Beauvieux R, Wenden B, Dirlewanger E (2018) Bud Dormancy in Perennial Fruit Tree Species : A Pivotal Role for Oxidative Cues. Front Plant Sci 9:1–13.

Bielenberg DG, Wang Y, Li Z, Zhebentyayeva T, Fan S, Reighard GL, Scorza R, Abbott AG (2008) Sequencing and annotation of the evergrowing locus in peach [*Prunus persica* (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genet Genomes 4:495–507.

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120.

Chmielewski FM, Baldermann S, Götz K, Homann T, Gödeke K, Schumacher F, Huschek G, Rawel H (2018) Abscisic Acid Related Metabolites in Sweet Cherry Buds (*Prunus avium* L.). J Hortic 05.

Chmielewski FM, Götz K, Homann T, Huschek G, Rawel H (2017) Identification of Endodormancy Release for Cherries (*Prunus Avium* L.) by Abscisic Acid and Sugars. J Hortic 04.

Chuine I, Bonhomme M, Legave J-M, García de Cortázar-Atauri I, Charrier G, Lacointe A, Améglio T (2016) Can phenological models predict tree phenology accurately in the future? The unrevealed hurdle of endodormancy break. Glob Chang Biol 22:3444–3460.

Chuine I, Régnière J (2017) Process-Based Models of Phenology for Plants and Animals. Annu Rev Ecol Evol Syst 48:159–82.

Cooke JEK, Eriksson ME, Junttila O (2012) The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Env 35:1707–1728.

Donoho CWJ, Walker DR (1957) Effect of Gibberellic Acid on Breaking of Rest Period in Elberta Peach. Science (80-) 126:1178–1179.

Donohue K, Burghardt LT, Runcie D, Bradford KJ, Schmitt J (2015) Applying developmental threshold models to evolutionary ecology. Trends Ecol Evol 30:66–77.

Eriksson ME, Hoffman D, Kaduk M, Mauriat M, Moritz T (2015) Transgenic hybrid aspen trees with increased gibberellin (GA) concentrations suggest that GA acts in parallel with FLOWERING LOCUS T2 to control shoot elongation. New Phytol 205:1288–1295.

Fadón E, Herrero M, Rodrigo J (2015) Flower development in sweet cherry framed in the BBCH scale. Sci Hortic (Amsterdam) 192:141–147.

Fadón E, Rodrigo J, Herrero M (2018) Is there a specific stage to rest? Morphological changes in flower primordia in relation to endodormancy in sweet cherry (*Prunus avium* L.). Trees 32:1583–1594.

Finkelstein R (2013) Abscisic Acid Synthesis and Response. The Arabidopsis Book 11:e0166.

Gagne JM, Downes BP, Shiu S-H, Durski AM, Vierstra RD (2002) The F-box subunit of the SCF E3 complex is encoded by a diverse superfamily of genes in Arabidopsis. Proc Natl Acad Sci 99:11519–11524.

Haddad C, Arkoun M, Jamois F, Schwarzenberg A, Yvin JC, Etienne P, Laîné P (2018) Silicon promotes growth of *Brassica napus* L. And delays leaf senescence induced by nitrogen

679 starvation. Front Plant Sci 9:1–13.

680 Hänninen H (1990) Modelling bud dormancy release in trees from cool and temperate regions. Acta
681 For Fenn 213:1–47.

682 Hao X, Chao WS, Yang Y, Horvath DP (2015) Coordinated expression of *FLOWERING LOCUS T*
683 and *DORMANCY ASSOCIATED MADS-BOX*-like genes in leafy spurge. PLoS One 10:1–18.

684 Heide OM, Prestrud AK (2005) Low temperature, but not photoperiod, controls growth cessation and
685 dormancy induction and release in apple and pear. Tree Physiol 25:109–114.

686 Hoad G V (1983) Hormonal regulation of fruit-bud formation in fruit trees. Acta Hortic 149:13–24.

687 Ito A, Tuan PA, Saito T, Bai S, Kita M, Moriguchi T (2019) Changes in Phytohormone Content and
688 Associated Gene Expression Throughout the Stages of Pear (*Pyrus pyrifolia* Nakai) Dormancy.
689 Tree Physiol tpz101

690 Jiménez S, Reighard GL, Bielenberg DG (2010) Gene expression of *DAM5* and *DAM6* is suppressed
691 by chilling temperatures and inversely correlated with bud break rate. Plant Mol Biol 73:157–67.

692 Jochner S, Caffarra A, Menzel A (2013) Can spatial data substitute temporal data in phenological
693 modelling? A survey using birch flowering. Tree Physiol 33:1256–1268.

694 Junttila O, Jensen E (1988) Gibberellins and photoperiodic control of shoot elongation in *Salix*.
695 Physiol Plant 74:371–376.

696 El Kayal W, Allen CCG, Ju CJT, Adams E, King-Jones S, Zaharia LI, Abrams SR, Cooke JEK (2011)
697 Molecular events of apical bud formation in white spruce, *Picea glauca*. Plant Cell Environ
698 34:480–500.

699 Khalil-Ur-Rehman M, Sun L, Li CX, Faheem M, Wang W, Tao JM (2017) Comparative RNA-seq
700 based transcriptomic analysis of bud dormancy in grape. BMC Plant Biol 17:1–11.

701 Kudoh H (2016) Molecular phenology in plants: In natura systems biology for the comprehensive
702 understanding of seasonal responses under natural environments. New Phytol 210:399–412.

703 de la Fuente L, Conesa A, Lloret A, Badenes ML, Ríos G (2015) Genome-wide changes in histone H3
704 lysine 27 trimethylation associated with bud dormancy release in peach. Tree Genet Genomes
705 11:45.

706 Lakkis S, Trotel-Aziz P, Rabenoelina F, Schwarzenberg A, Nguema-Ona E, Clément C, Aziz A
707 (2019) Strengthening Grapevine Resistance by Pseudomonas fluorescens PTA-CT2 Relies on
708 Distinct Defense Pathways in Susceptible and Partially Resistant Genotypes to Downy Mildew
709 and Gray Mold Diseases. Front Plant Sci 10:1–18.

710 Lang G, Early J, Martin G, Darnell R (1987) Endo-, para-, and ecodormancy: physiological
711 terminology and classification for dormancy research. Hort Sci 22:371–377.

712 Leida C, Conejero A, Arbona V, Gómez-Cadenas A, Llácer G, Badenes ML, Ríos G (2012) Chilling-
713 dependent release of seed and bud dormancy in peach associates to common changes in gene
714 expression. PLoS One 7:e35777.

715 Leida C, Conesa A, Llácer G, Badenes ML, Ríos G (2012) Histone modifications and expression of
716 *DAM6* gene in peach are modulated during bud dormancy release in a cultivar-dependent
717 manner. New Phytol 193:67–80.

718 Li J, Xu Y, Niu Q, He L, Teng Y, Bai S (2018) Abscisic acid (ABA) promotes the induction and
719 maintenance of pear (*Pyrus pyrifolia* white pear group) flower bud endodormancy. Int J Mol Sci
720 19

721 Liu J, Sherif SM (2019) Hormonal Orchestration of Bud Dormancy Cycle in Deciduous Woody
722 Perennials. Front Plant Sci 10:1–21.

723 Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-
724 seq data with DESeq2. Genome Biol 15:1–21.

725 Nambara E, Marion-Poll A (2005) Abscisic Acid Biosynthesis and Catabolism. Annu Rev Plant Biol
726 56:165–185.

727 Niu Q, Li J, Cai D, Qian M, Jia H, Bai S, Hussain S, Liu G, Teng Y, Zheng X (2015) Dormancy-
728 associated MADS-box genes and microRNAs jointly control dormancy transition in pear (*Pyrus*
729 *pyrifolia* white pear group) flower bud. J Exp Bot 67:erv454.

730 Olsen JE (2010) Light and temperature sensing and signaling in induction of bud dormancy in woody
731 plants. Plant Mol Biol 73:37–47.

732 Ophir R, Pang X, Halaly T, Venkateswari J, Lavee S, Galbraith D, Or E (2009) Gene-expression
733 profiling of grape bud response to two alternative dormancy-release stimuli expose possible links
734 between impaired mitochondrial activity, hypoxia, ethylene-ABA interplay and cell enlargement.
735 Plant Mol Biol 71:403–423.

736 Or E, Belausov E, Popilevsky I, Ben Tal Y (2000) Changes in endogenous ABA level in relation to
737 the dormancy cycle in grapevines grown in a hot climate. J Hortic Sci Biotechnol 75:190–194.

738 Petterle A, Karlberg A, Bhalerao RP (2013) Daylength mediated control of seasonal growth patterns in
739 perennial trees. Curr Opin Plant Biol 16:301–306.

740 Powell LE (1987) The hormonal control of bud and seed dormancy in woody plants. In: Davies P (ed)
741 Plant Hormones and Their Role in Plant Growth and Development. Martinus Nijhoff Publishers,

742 Dordrecht, pp 539–552.

743 Priest DM, Ambrose SJ, Vaistij FE, Elias L, Higgins GS, Ross ARS, Abrams SR, Bowles DJ (2006)
744 Use of the glucosyltransferase UGT71B6 to disturb abscisic acid homeostasis in Arabidopsis
745 thaliana. Plant J 46:492–502.

746 Rinne PLH, Welling A, Vahala J, Ripel L, Ruonala R, Kangasjarvi J, van der Schoot C (2011)
747 Chilling of dormant buds hyperinduces *FLOWERING LOCUS T* and recruits GA-inducible 1,3-
748 beta-glucanases to reopen signal conduits and release dormancy in *Populus*. Plant Cell 23:130–
749 146.

750 Ríos G, Leida C, Conejero A, Badenes ML (2014) Epigenetic regulation of bud dormancy events in
751 perennial plants. Front Plant Sci 5:247.

752 Rodríguez-Gacio MDC, Matilla-Vázquez M a, Matilla AJ (2009) Seed dormancy and ABA signaling:
753 the breakthrough goes on. Plant Signal Behav 4:1035–1049.

754 Rodriguez A, Sherman W, Scorza R, Wisniewski M, Okie WR (1994) 'Evergreen' peach, its
755 inheritance and dormant behavior. J Am Soc hort Sci 119:789–792.

756 Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12:217–223.

757 Rohde A, Prinsen E, Rycke R De, Engler G, Montagu M Van, Boerjan W (2002) PtABI3 Impinges on
758 the Growth and Differentiation of Embryonic Leaves during Bud Set in Poplar. Plant Cell
759 14:1885–1901.

760 Rohde A, Storme V, Jorge V, Gaudet M, Vitacolonna N, Fabbrini F, Ruttink T, Zaina G, Marron N,
761 Dillen S, Steenackers M, Sabatti M, Morgante M, Boerjan W, Bastien C (2011) Bud set in
762 poplar--genetic dissection of a complex trait in natural and hybrid populations. New Phytol
763 189:106–121.

764 Roxrud I, Lid SE, Fletcher JC, Schmidt EDL, Opsahl-Sorteberg HG (2007) *GASA4*, one of the 14-
765 member Arabidopsis GASA family of small polypeptides, regulates flowering and seed
766 development. Plant Cell Physiol 48:471–483.

767 Ruttink T, Arend M, Morreel K, Storme V, Rombauts S, Fromm J, Bhalerao RP, Boerjan W, Rohde A
768 (2007) A molecular timetable for apical bud formation and dormancy induction in poplar. Plant
769 Cell 19:2370–2390.

770 Satake A, Kawagoe T, Saburi Y, Chiba Y, Sakurai G, Kudoh H (2013) Forecasting flowering
771 phenology under climate warming by modelling the regulatory dynamics of flowering-time
772 genes. Nat Commun 4:2303.

773 Shafer N, Monson WG (1958) The Role og Gibberellic Acid in Overcoming Bud Dormancy in

774 Perennial Weeds. I. Leafy Spurge (*Euphorbia esulta* L.) and Ironweed (*Vernonia Baldwini*
775 Torr.). Weeds 6:172–178.

776 Shu K, Zhang H, Wang S, Chen M, Wu Y, Tang S, Liu C, Feng Y, Cao X, Xie Q (2013) ABI4
777 Regulates Primary Seed Dormancy by Regulating the Biogenesis of Abscisic Acid and
778 Gibberellins in Arabidopsis. PLoS Genet 9

779 Shu K, Zhou W, Yang W (2018) APETALA 2-domain-containing transcription factors: focusing on
780 abscisic acid and gibberellins antagonism. New Phytol 217:977–983.

781 Singh RK, Maurya JP, Azeez A, Miskolczi P, Tylewicz S, Stojkovič K, Delhomme N, Busov V,
782 Bhalerao RP (2018) A genetic network mediating the control of bud break in hybrid aspen. Nat
783 Commun 9

784 Singh RK, Miskolczi P, Maurya JP, Bhalerao RP (2019) A Tree Ortholog of *SHORT VEGETATIVE*
785 *PHASE* Floral Repressor Mediates Photoperiodic Control of Bud Dormancy. Curr Biol 29:128-
786 133.e2.

787 Singh RK, Svystun T, AlDahmash B, Jönsson AM, Bhalerao RP (2016) Photoperiod- and
788 temperature-mediated control of growth cessation and dormancy in trees: A molecular
789 perspective. New Phytol 213:511-524.

790 Topham AT, Taylor RE, Yan D, Nambara E, Johnston IG, Bassel GW (2017) Temperature variability
791 is integrated by a spatially embedded decision-making center to break dormancy in Arabidopsis
792 seeds. Proc Natl Acad Sci 114:6629–6634.

793 Trapnell C, Pachter L, Salzberg SL (2009) TopHat: Discovering splice junctions with RNA-Seq.
794 Bioinformatics 25:1105–1111.

795 Tuan PA, Bai S, Saito T, Ito A, Moriguchi T (2017) Dormancy-Associated MADS-Box (DAM) and
796 the Abscisic Acid Pathway Regulate Pear Endodormancy Through a Feedback Mechanism. Plant
797 Cell Physiol 58:1378–1390.

798 Tylewicz S, Petterle A, Marttila S, Miskolczi P, Azeez A, Singh RK, Immanen J, Mähler N, Hvidsten
799 TR, Eklund DM, Bowman JL, Helariutta Y, Bhalerao RP (2018) Photoperiodic control of
800 seasonal growth is mediated by ABA acting on cell-cell communication. Science (80-) 360:212–
801 215.

802 Ueguchi-Tanaka M, Nakajima M, Katoh E, Ohmiya H, Asano K, Saji S, Hongyu X, Ashikari M,
803 Kitano H, Yamaguchi I, Matsuoka M (2007) Molecular Interactions of a Soluble Gibberellin
804 Receptor, GID1, with a Rice DELLA Protein, SLR1, and Gibberellin. Plant Cell Online
805 19:2140–2155.

806 Ueno S, Klopp C, Leplé JC, Derory J, Noirot C, Léger V, Prince E, Kremer A, Plomion C, Le Provost
807 G (2013) Transcriptional profiling of bud dormancy induction and release in oak by next-
808 generation sequencing. BMC Genomics 14:236.

809 Verde I, Jenkins J, Dondini L, Micali S, Pagliarani G, Vendramin E, Paris R, Aramini V, Gaza L,
810 Rossini L, Bassi D, Troggio M, Shu S, Grimwood J, Tartarini S, Dettori MT, Schmutz J (2017)
811 The Peach v2.0 release: high-resolution linkage mapping and deep resequencing improve
812 chromosome-scale assembly and contiguity. BMC Genomics 18:225.

813 Vitasse Y, François C, Delpierre N, Dufrêne E, Kremer A, Chuine I, Delzon S (2011) Assessing the
814 effects of climate change on the phenology of European temperate trees. Agric For Meteorol
815 151:969–980.

816 Wagner GP, Kin K, Lynch VJ (2012) Measurement of mRNA abundance using RNA-seq data: RPKM
817 measure is inconsistent among samples. Theory Biosci 131:281–285.

818 Wang D, Gao Z, Du P, Xiao W, Tan Q, Chen X, Li L, Gao D (2016) Expression of ABA Metabolism-
819 Related Genes Suggests Similarities and Differences Between Seed Dormancy and Bud
820 Dormancy of Peach (*Prunus persica*). Front Plant Sci 6:1–17.

821 Wen LH, Zhong WJ, Huo XM, Zhuang WB, Ni ZJ, Gao ZH (2016) Expression analysis of ABA- and
822 GA-related genes during four stages of bud dormancy in Japanese apricot (*Prunus mume* Sieb. et
823 Zucc). J Hortic Sci Biotechnol 91:362–369.

824 Weng JK, Ye M, Li B, Noel JP (2016) Co-evolution of Hormone Metabolism and Signaling Networks
825 Expands Plant Adaptive Plasticity. Cell 166:881–893.

826 Wilczek AM, Roe JL, Knapp MC, Cooper MD, Lopez-Gallego C, Martin LJ, Muir CD, Sim S, Walker
827 A, Anderson J, Egan JF, Moyers BT, Petipas R, Giakountis A, Charbit E, Coupland G, Welch
828 SM, Schmitt J, Franklin Egan J, Moyers BT, Petipas R, Giakountis A, Charbit E, Coupland G,
829 Welch SM, Schmitt J (2009) Effects of genetic perturbation on seasonal life history plasticity.
830 Science (80-) 323:930–935.

831 Xu ZJ, Nakajima M, Suzuki Y, Yamaguchi I (2002) Cloning and Characterization of the Abscisic
832 Acid-Specific Glucosyltransferase Gene from Adzuki Bean Seedlings. Plant Physiol 129:1285–
833 1295.

834 Yamaguchi S (2008) Gibberellin Metabolism and its Regulation. Annu Rev Plant Biol 59:225–251.

835 Yamane H, Wada M, Honda C, Matsuura T, Ikeda Y, Hirayama T, Osako Y, Gao-Takai M, Kojima
836 M, Sakakibara H, Tao R (2019) Overexpression of *Prunus DAM6* inhibits growth, represses bud
837 break competency of dormant buds and delays bud outgrowth in apple plants. PLoS One 14:1–
838 24.

839 Yang Q, Niu Q, Tang Y, Ma Y, Yan X, Li J, Tian J, Bai S, Teng Y (2019) PpyGAST1 is potentially
840 involved in bud dormancy release by integrating the GA biosynthesis and ABA signaling in
841 'Suli' pear (*Pyrus pyrifolia* White Pear Group). Environ Exp Bot 162:302–312.

842 Yue C, Cao H, Hao X, Zeng J, Qian W, Guo Y, Ye N, Yang Y, Wang X (2017) Differential
843 expression of gibberellin- and abscisic acid-related genes implies their roles in the bud activity-
844 dormancy transition of tea plants. Plant Cell Rep 37:425–441.

845 Zentella R, Zhang Z-L, Park M, Thomas SG, Endo A, Murase K, Fleet CM, Jikumaru Y, Nambara E,
846 Kamiya Y, Sun T (2007) Global Analysis of DELLA Direct Targets in Early Gibberellin
847 Signaling in *Arabidopsis*. Plant Cell 19:3037–3057.

848 Zhang X, An L, Nguyen TH, Liang H, Wang R, Liu X, Li T, Qi Y, Yu F (2015) The cloning and
849 functional characterization of peach *CONSTANS* and *FLOWERING LOCUS T* homologous genes
850 *PpCO* and *PpFT*. PLoS One 10:1–16.

851 Zhang Z, Zhuo X, Zhao K, Zheng T, Han Y, Yuan C, Zhang Q (2018) Transcriptome Profiles Reveal
852 the Crucial Roles of Hormone and Sugar in the Bud Dormancy of *Prunus mume*. Sci Rep 8:1–15.

853 Zhao K, Zhou Y, Ahmad S, Yong X, Xie X, Han Y, Li Y, Sun L, Zhang Q (2018) PmCBFs
854 synthetically affect PmDAM6 by alternative promoter binding and protein complexes towards
855 the dormancy of bud for *Prunus mume.* Sci Rep 8:4527.

856 Zheng C, Acheampong AK, Shi Z, Halaly T, Kamiya Y, Ophir R, Galbraith DW, Or E (2018) Distinct
857 gibberellin functions during and after grapevine bud dormancy release. J Exp Bot 69:1635–1648.

858 Zheng C, Acheampong AK, Shi Z, Mugzech A, Halaly-Basha T, Sun Y, Colova V, Mosquna A, Ophir
859 R, Galbraith DW, Or E (2018) Abscisic Acid Catabolism Enhances Dormancy Release of
860 Grapevine Buds. Plant Cell Environ 41:2490–2503.

861 Zheng C, Halaly T, Acheampong AK, Takebayashi Y, Jikumaru Y, Kamiya Y, Or E (2015) Abscisic
862 acid (ABA) regulates grape bud dormancy, and dormancy release stimuli may act through
863 modification of ABA metabolism. J Exp Bot 66:1527–1542.

864 Zhong W, Gao Z, Zhuang W, Shi T, Zhang Z, Ni Z (2013) Genome-wide expression profiles of
865 seasonal bud dormancy at four critical stages in Japanese apricot. Plant Mol Biol 83:247–64.

866 Zhong C, Xu H, Ye S, Wang S, Li L, Zhang S, Wang X (2015) AtGASA6 Serves as an Integrator of
867 Gibberellin-, Abscisic Acid- and Glucose-Signaling during Seed Germination in Arabidopsis.
868 Plant Physiol 169:pp.00858.2015.

869 Zhu Y, Li Y, Xin D, Chen W, Shao X, Wang Y, Guo W (2015) RNA-Seq-based transcriptome
870 analysis of dormant flower buds of Chinese cherry (*Prunus pseudocerasus*). Gene 555:362–376.

871 Zhuang W, Gao Z, Wang L, Zhong W, Ni Z, Zhang Z (2013) Comparative proteomic and
872 transcriptomic approaches to address the active role of GA4 in Japanese apricot flower bud
873 dormancy release. J Exp Bot 64:4953–4966.

874

875