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.

72 2007, Wang et al. 2016, Tuan et al. 2017, Li et al. 2018, Tylewicz et al. 2018). Strong correlation was
73 further shown between ABA and dormancy depth with high ABA levels detected during endodormancy,
74 followed by a decrease in endogenous ABA content during the transition from endodormancy to
75 ecodormancy (Or et al. 2000, Zheng et al. 2015, Wang et al. 2016, Wen et al. 2016, Chmielewski et al.
76 2017, Li et al. 2018, Zhang et al. 2018, Yamane et al. 2019). Recently, ABA content has been proposed
77 as a determining factor to assess dormancy status in sweet cherry (Chmielewski et al. 2017). Recent
78 transcriptomic analyses of genes involved in the precise balance between biosynthesis and catabolism
79 modulating ABA levels have further defined the involvement of ABA in bud dormancy. Indeed,
80 expression patterns for 9-cis epoxycarotenoid dioxygenases (*NCED*), that catalyze the critical step for
81 ABA biosynthesis, and *CYP707A*, encoding cytochrome P450 monooxygenases that inactivate ABA
82 into 8'hydroxy ABA, as well as ABA signaling genes, support ABA involvement in bud dormancy
83 induction and maintenance (Fig. **1a**) (Nambara and Marion-Poll 2005, Bai et al. 2013, Zhong et al. 2013,
84 Zhu et al. 2015, Wang et al. 2016, Khalil-Ur-Rehman et al. 2017, Li et al. 2018, Zhang et al. 2018,
85 Zheng et al. 2018). Similarly, until recently, most of the knowledge gathered on the behavior of the GA
86 pathway during dormancy had been obtained in seeds but reports published in the last years have shed
87 some light on GA regulation throughout bud dormancy in perennial plants. Studies have suggested a
88 major role for GAs in maintaining growth before the induction of dormancy (Junttila and Jensen 1988,
89 Ruttink et al. 2007, Olsen 2010, Eriksson et al. 2015, Singh et al. 2016) and promoting growth during
90 ecodormancy (Wen et al. 2016, Zhang et al. 2018). Interestingly, GA treatments have a controversial
91 effect on dormancy and bud break as shown in various perennial species since GA application may
92 substitute for chilling (Shafer and Monson 1958, Rinne et al. 2011, Zhuang et al. 2013), or have delaying
93 effects on shoot growth and bud break (Hoad 1983, Zheng et al. 2018), suggesting distinct gibberellin
94 functions during dormancy. Although transcriptomic results for *GA2ox*, *GA3ox* and *GA20ox* vary
95 between studies and therefore suggest complex and distinct functions, general patterns could be
96 identified: expression for GA biosynthesis genes *GA 20-oxidases* (*GA20ox*) and *GA 3-oxidases* (*GA3ox*)
97 decreases during dormancy induction then increases after dormancy release and during ecodormancy
98 while GA deactivation *GA 2-oxidases* (*GA2ox*) genes are up-regulated during endodormancy and
99 inhibited after endodormancy is released (Fig. **1b**; Yamaguchi 2008, Bai et al. 2013, Zhong et al. 2013,
100 Zhu et al. 2015, Wen et al. 2016, Khalil-Ur-Rehman et al. 2017, Zhang et al. 2018, Zheng et al. 2018).

101 In this study we explored potential hormonal markers of dormancy using a combination of physiological
102 and transcriptomic analyses and a new modelling approach. We have focused on the involvement of GA
103 and ABA pathways in sweet cherry flower bud dormancy. We examined the effect of exogenous GA
104 and ABA on dormancy status and monitored endogenous contents for GAs and ABA and its metabolites,
105 as well as the expression of genes related to ABA and GA metabolism throughout flower bud dormancy
106 for two cultivars with contrasted dormancy release dates. Following our findings on hormonal control

107 of dormancy, we propose a mathematical model that incorporates the effect of key genes on the
108 dynamics of ABA to estimate dormancy status.

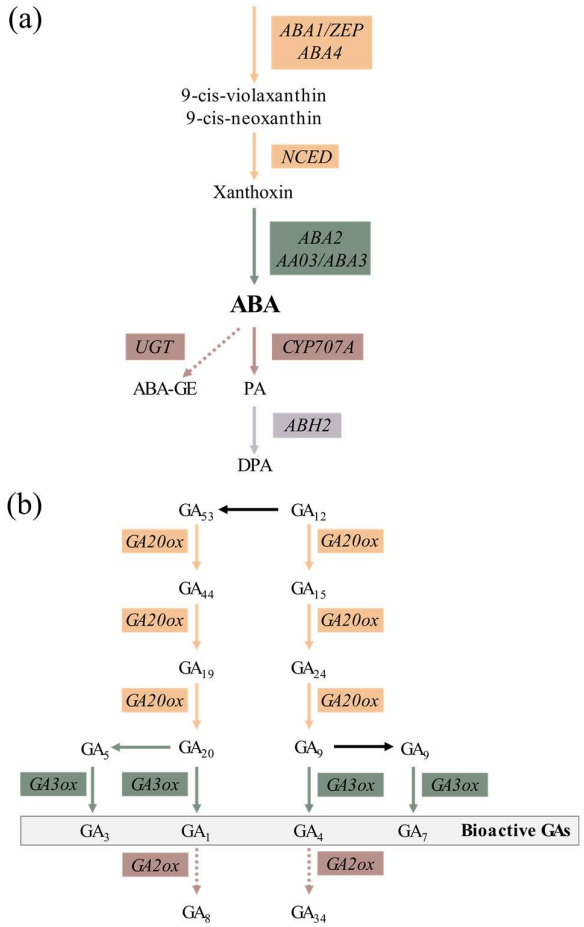

Figure 1 Biosynthesis and catabolism pathway for ABA and GAs. (**a**) ABA is synthesized through the action of five enzymes: zeaxanthin epoxidase (ZEP/ABA1), ABA-deficient4 (ABA4,) 9-cis epoxycarotenoid dioxygenase (NCED), alcohol dehydrogenase (ABA2) and short-chain dehydrogenase/reductase (AAO3/ABA3).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

109 **MATERIALS AND METHODS**

110 **Plant material**

111 Samples were collected from three different sweet cherry cultivars (*Prunus avium* L.) having very early,
112 early and late flowering dates (respectively, 'Cristobalina', 'Garnet' and 'Regina'). Trees are grown in
113 an orchard located at the Fruit Experimental Unit of INRA in Bourran, South West of France (44° 19′
114 56″ N, 0° 24′ 47″ E) under standard agricultural practices. During the sampling season (July 2015 to

115 March 2016), a mix of randomly chosen flower buds (equivalent to a 2 mL volume) were sampled at
116 ten time points spanning the entire period of bud development (Fig. 2a) for phytohormone quantification
117 (for 'Cristobalina' and 'Regina') and RNA-seq analysis (for the three cultivars). Flower buds were
118 harvested from branches of three ('Cristobalina' and 'Garnet') or two different trees ('Regina'). A total
119 of 29, 31 and 21 samples were analyzed for 'Cristobalina', 'Garnet' and 'Regina' respectively. Details
120 are available in Table S1 (Supplementary file at *Tree Physiology* online). Upon harvesting, buds were
121 flash frozen in liquid nitrogen and stored at -80°C prior to performing RNA-seq. Average daily
122 temperatures were recorded by an on-site weather station.

123 In addition, for the exogenous application of hormones, branches were collected from the late flowering
124 sweet cherry cultivar 'Fertard'. Trees were grown in an orchard located at the Fruit Experimental Unit
125 of INRA in Toulenne, South West of France (48° 51′ 46″ N, 2° 17′ 15″ E) under standard agricultural
126 practices.

127 **Measurements of bud break and estimation of the dormancy release date**

128 Measurements for the dormancy stages were performed on randomly chosen branches cut every two
129 weeks from November 16th 2015 to April 4th 2016 for 'Cristobalina', 'Garnet', 'Regina' and 'Fertard',
130 and between November 21st 2017 and April 4th 2018 for 'Fertard'. Branches were incubated in water
131 pots placed in a growth chamber (25°C, 16h light/ 8h dark, 60-70% humidity). The water was replaced
132 every 3-4 days. After ten days under these forcing conditions, the percentage of bud break, i.e. flower
133 buds at BBCH stage 53 (Fadón et al. 2015), as illustrated in Fig. **S1a**, was recorded. The date of
134 dormancy release was estimated when at least 50% of the flower buds were at the BBCH stage 53 or
135 higher after ten days under forcing conditions.

136 **Treatments with exogenous hormones and antagonists**

137 To investigate the effects of GA and ABA on dormancy, five branches per modality were randomly
138 harvested from ten 'Fertard' dormant trees on January 19th 2016 and January 29th 2018 (Fig. S1;
139 Supplementary file at *Tree Physiology* online). The cherry dormant buds were treated with 5 µM GA_3
140 (Sigma-Aldrich, ref. 48870), 5 µM GA_4 (Sigma-Aldrich, ref. G7276), 400 µM ABA (Sigma-Aldrich,
141 ref. A1049), 300 µM paclobutrazol (Sigma-Aldrich, ref. 46046), an inhibitor of the GA pathway, and 5
142 µM fluridone (Sigma-Aldrich, ref. 45511), an inhibitor of the ABA pathway.

143 All chemicals and a water control were freshly prepared to the desired concentrations in 0.5% of
144 surfactant ("Calanque", Action Pin, Castets, France) to ensure the penetration of active molecules
145 through the bud scales. Chemicals were sprayed on buds under a fume-hood and branches were left
146 several minutes to allow them to dry. Branches were then transferred in the growth chamber (25°C, 16h
147 light/ 8h dark, 60-70% humidity) in pots containing water. Bud break measurements were performed on
148 flower buds as mentioned above.

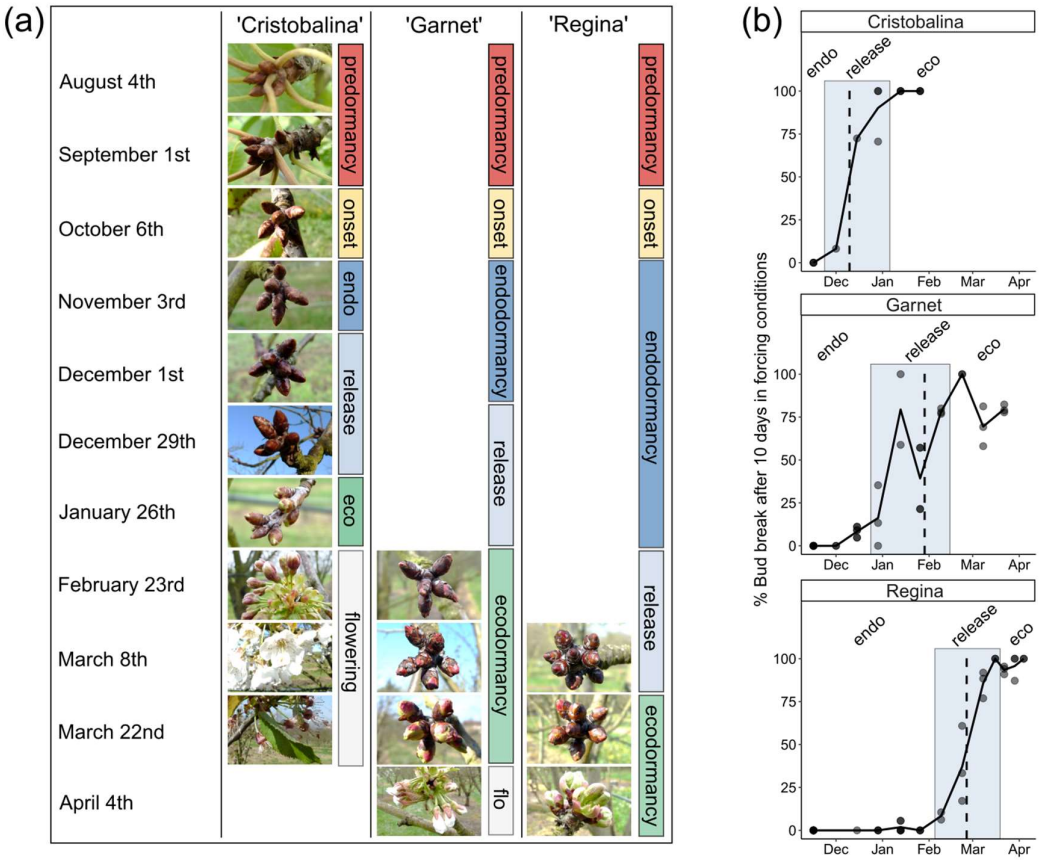

Figure 2 Dormancy status defined for the three sweet cherry cultivars. (a) bud stages at the sampling dates and the corresponding dormancy status as defined in the current study; (b) Evaluation of bud break percentage under forcing conditions was carried out for three sweet cherry cultivars displaying 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**

162 ABA and conjugates (ABA-GE, PA, DPA) and GAs (GA_1, GA_3, GA_4, GA_7) were quantified by UHPLC-
163 MS/MS as previously described (Lakkis et al. 2019). ABA, ABA-GE, gibberellins (GA_4, GA_7), [$^2H_6$]-
164 ABA, and [$^2H_2$]-GA_4 were purchased from OlchemIn (Olomouc, Czech Republic). DPA, PA, [$^2H_3$]-
165 dihydrophaseic acid (D-DPA), and [$^2H_3$]-phaseic acid (D-PA) were purchased from National Research
166 Council Canada (NRC, Saskatoon, Canada). Phytohormones were analyzed by an UHPLC-MS/MS
167 system. The separation and detection were achieved using a Nexera X2 UHPLC system (Shimadzu,
168 Japan) coupled to a QTrap 6500+ mass spectrometer (Sciex, Canada) equipped with an electrospray
169 (ESI) source. Phytohormones separation was carried out by injecting 2 µL into a Kinetex Evo C18 core-
170 shell column (100 x 2.1mm, 2.6µm, Phenomenex, USA) at a flow rate of 0.7 mL/min, and the column
171 oven was maintained at 40°C. The mobile phases were composed of solvent A Milli-Q water (18 MΩ,
172 Millipore, USA) containing 0.1% formic acid (LCMS grade, Fluka analytics, Germany), and solvent B
173 acetonitrile LCMS grade (Fisher Optima, UK) containing 0.1% formic acid. The gradient elution started
174 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-
175 9.5 min 1% B. The ionization voltage was set to 5kV for positive mode and -4.5 kV for negative mode
176 producing mainly $[M+H]^+$ and $[M-H]^-$ respectively. The analysis was performed in scheduled multiple
177 reaction monitoring (MRM) mode in positive and negative mode simultaneously with a polarity
178 switching of 5 ms. All quantitative data were processed using MultiQuant software V 3.0.2 (Sciex,
179 Canada). GA_1, GA_3 were not detected in the samples.

180 **RNA extraction and library preparation**

181 Total RNA was extracted from 50-60 mg of frozen and pulverised flower buds (see Table S1 for the
182 detailed sample list) using RNeasy Plant Mini kit (Qiagen) with minor modification (1.5% PVP-40 was
183 added in the RLT buffer). RNA quality was evaluated using Tapestation 4200 (Agilent Genomics). Only
184 samples with RNA integrity number equivalent (RINe) superior or equivalent to 8.5 were used for RNA-
185 seq. Library preparation was performed with 1 µg of total RNA using the TruSeq Stranded mRNA
186 Library Prep Kit High Throughput (96 samples, 96 indexes, Illumina cat. no. RS-122-2103). DNA
187 quality from libraries was evaluated using Tapestation 4200 (Agilent Genomics). The libraries were
188 sequenced on a NextSeq500 (Illumina), at the Sainsbury Laboratory Cambridge University (SLCU),
189 using paired-end sequencing of 75 bp in length.

190 **Mapping and differential expression analysis**

191 The raw reads obtained from the sequencing were analysed using several publicly available software
192 and in-house scripts. The quality of reads was assessed using FastQC
193 (www.bioinformatics.babraham.ac.uk/projects/fastqc/) and possible adaptor contaminations and low
194 quality trailing sequences were removed using Trimmomatic (Bolger et al. 2014). Trimmed reads were
195 mapped to the peach (*Prunus persica* (L.) Batsch) reference genome v2.0 (Verde et al. 2017) using
196 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

258 **Exogenous GA application accelerates bud dormancy release**

259 GA and ABA effect on the bud break response to forcing conditions was evaluated on branches carrying
260 dormant flower buds as assessed by forcing tests on the late flowering cultivar 'Fertard' (Fig. S1).
261 Results revealed that GA_3 and GA_4 both had significant dormancy alleviating effects, characterized by
262 higher bud break percentage, which was confirmed for GA_3 in a second experiment in 2018 (Fig. **3**, Fig.
263 **S2**). However, no antagonist effect, namely bud break inhibition, was observed after a treatment with
264 paclobutrazol.

265 In both seed and bud dormancy, it is hypothesized that GAs and ABA act antagonistically to control
266 growth resumption and inhibition, respectively. We therefore tested the potential inhibiting effect of
267 ABA on flower bud emergence. We did not observe a significant effect of ABA treatment on dormancy
268 release, but bud break for ABA-treated branches was slightly higher than the control (Fig. **3**, Fig. **S2**).
269 However, inhibiting ABA biosynthesis with fluridone activated bud break, consistent with the
270 established role of ABA in promoting dormancy.

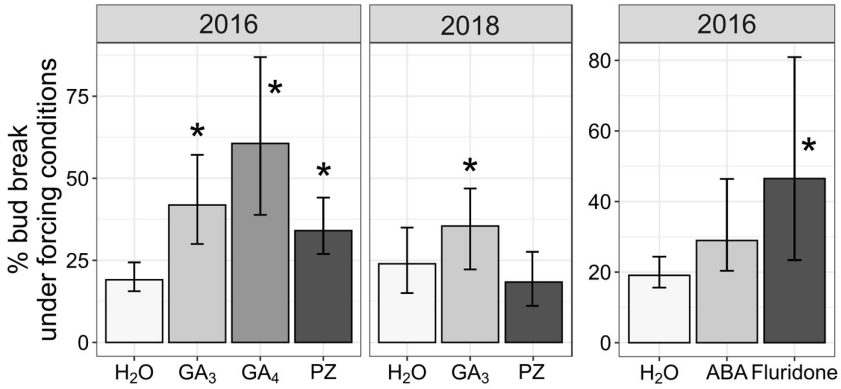

Figure 3 Effect of different GAs, ABA and their inhibitor on the sweet cherry dormancy status. Sweet cherry branches were treated with (a) 5 µM GA_3, 5 µM GA_4, 300 µM, paclobutrazol (GA pathway inhibitor), (b) 400 µM ABA and 5 µM fluridone (ABA pathway inhibitor) and transferred under forcing conditions (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.

271

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

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

279 dormancy onset was set in October and the beginning of endodormancy was established in November
280 (Fig. **2a**). These stages were similar for all three cultivars. By definition, endodormancy correspond to
281 the bud inability to fully develop under growth-inducing conditions while dormancy is considered as
282 released when bud break is triggered by warm temperatures and/or long photoperiod (Lang et al. 1987).
283 Consequently, dormancy status was assessed by forcing tests on branches carrying flower buds (Fig.
284 **2b**). Endodormancy was defined for the dates with no bud break under forcing conditions (Fig. 2).
285 Incomplete bud break within the population of flower buds indicated dormancy release stages while
286 ecodormancy was defined by optimal bud break response to growth conditions (90-100% bud break;
287 Fig. 2). Here, the three cultivars were much contrasted in the timing of their dormancy phases,
288 'Cristobalina' exhibiting dormancy release on December 9th, seven weeks earlier than 'Garnet' (January
289 29th) and ten weeks earlier than 'Regina' (February 26th) .

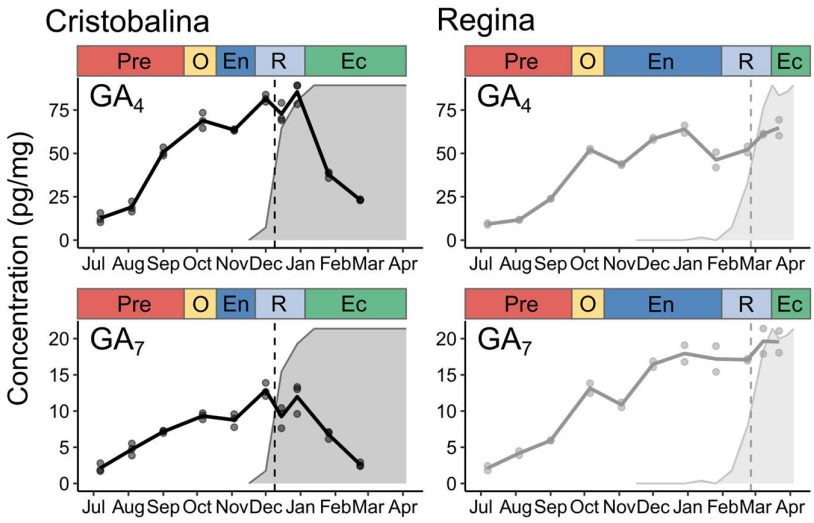

Figure 4 Levels of endogenous bioactive GA_4 and GA_7 in the flower buds of two sweet cherry cultivars during bud development. Black: 'Cristobalina', grey: 'Regina'. Background areas correspond to the dormancy depth evaluated as the percentage of bud break under forcing conditions (see Fig. 2b). Dotted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

290 **GA content changes during flower bud dormancy progression**

291 In recent studies, distinct functions were identified for gibberellins during bud dormancy (Zhuang et al.
292 2013, Zheng et al. 2018). To test whether these results could be confirmed in sweet cherry buds, GA
293 levels were determined over the whole bud development cycle in the very early and late flowering
294 cultivars, 'Cristobalina' and 'Regina'. In details, we studied the content of bioactive GA_1, GA_3 GA_4 and
295 GA_7 but the levels of GA_1 and GA_3 were undetectable in the samples. GA_4, GA_7 have a similar pattern
296 over dormancy progression (Fig. **4**) rising from July onwards, which corresponds to flower primordia
297 initiation and organogenesis. Bioactive GA levels also increase at the beginning of endodormancy
298 (November – December), reaching their highest concentration during dormancy release. Results show
299 a sharp decrease in the levels of GA_4 and GA_7 overlapping with ecodormancy for 'Cristobalina', which

300 could not be observed for the late cultivar 'Regina', potentially due to the lack of ecodormancy coverage.
301 Interestingly, the levels of GA_4, and GA_7 significantly differed between the two cultivars, especially
302 during endodormancy and when dormancy release was triggered in the early cultivar (Fig. S**3b**). Notable
303 differences in GA_7 content were observed between these two cultivars during the entire time course, in
304 which the late cultivar buds contained more GA_7 than the early cultivar (Fig. **4**) while levels for GA_4
305 were significantly higher in the early cultivar during endodormancy (Fig. S**3b**). Among the quantified
306 active GAs, GA_4 was between three and eight times more present than GA_7. GA_4 was the most detected
307 active GA in the early cultivar 'Cristobalina', with a relatively high level reached just after dormancy
308 release in December. By contrast, levels of GA_7 were higher in 'Regina', but with the maximal
309 concentration measured just after dormancy release.

310

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

313 To better understand the mechanisms linked to the GA pathway during dormancy progression, we
314 investigated genes involved in GA biosynthesis pathway, degradation, signal transduction and response.
315 We found seven *PavGA20ox* and five *PavGA3ox* for GA biosynthesis genes in the peach database (**S3**)
316 but only three genes were differentially expressed during flower bud development and dormancy (Fig.
317 **5**, Table **S2**). Interestingly, *PavGA20ox2* expression increased in December for both cultivars, regardless
318 of their dormancy status but decreased prior to dormancy release. The marked increase in *PavGA20ox1a*
319 expression could be correlated with the production of GAs after dormancy release. The last step of active
320 GA biosynthesis relies on the activity of GA3ox essentially for the production of GA_1 and GA_4.
321 *PavGA3ox1a* was the only *GA3ox* transcript detected during dormancy and its expression increases as
322 early as dormancy onset (October), followed by a sharp increase during endodormancy, reaching its
323 highest expression value at maximum dormancy depth in December, for both cultivars (Fig. **5**).
324 *PavGA3ox1a* is then downregulated during or after dormancy release, with a marked lag between the
325 two cultivars, potentially linked to their separate dormancy release date (Fig. **5**). We identified four
326 differentially expressed *PavGA2ox* genes, involved in GA inactivation (Fig. **5**, Tables S2, S3).
327 *PavGA2ox1* and *PavGA2ox8b* were highly expressed during endodormancy, concomitantly with
328 *PavGA3ox1a* expression, thus suggesting a balance between synthesis and degradation that closely
329 controls the levels of bioactive GAs. *PavGA2ox8c* and *PavGA2oxb* were expressed before dormancy
330 and during the early stages of dormancy. The expression of *PavGA2ox1* was three times higher for
331 'Regina' than for 'Cristobalina' while it was the opposite for *PavGA2ox8b*.

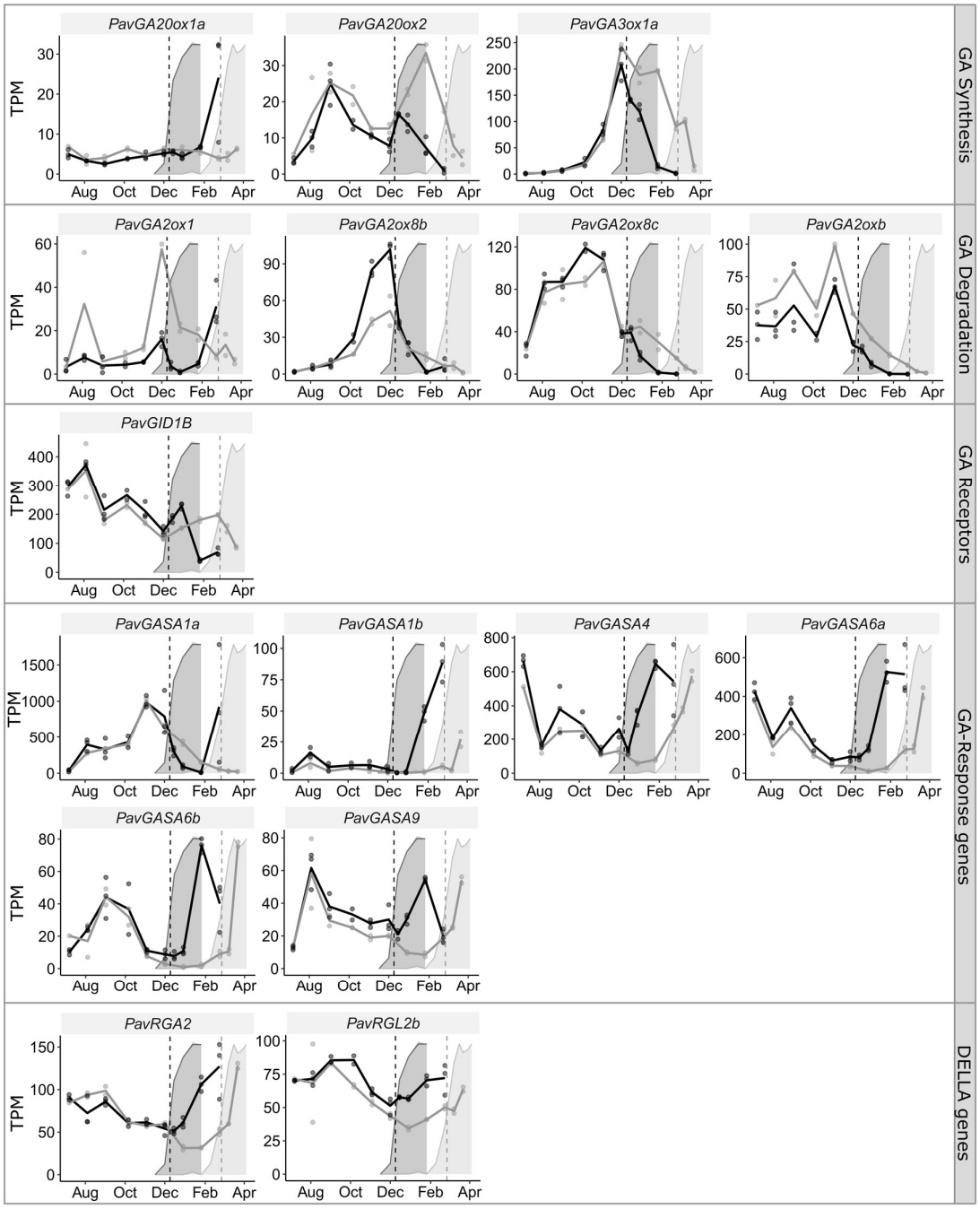

Figure 5 Transcriptional dynamics of genes associated with GA pathway in the flower buds of two sweet cherry cultivars during bud development. Expression of specific genes involved in GA biosynthesis pathway, degradation, signal transduction and response are represented in TPM (Transcripts Per Million 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.

332

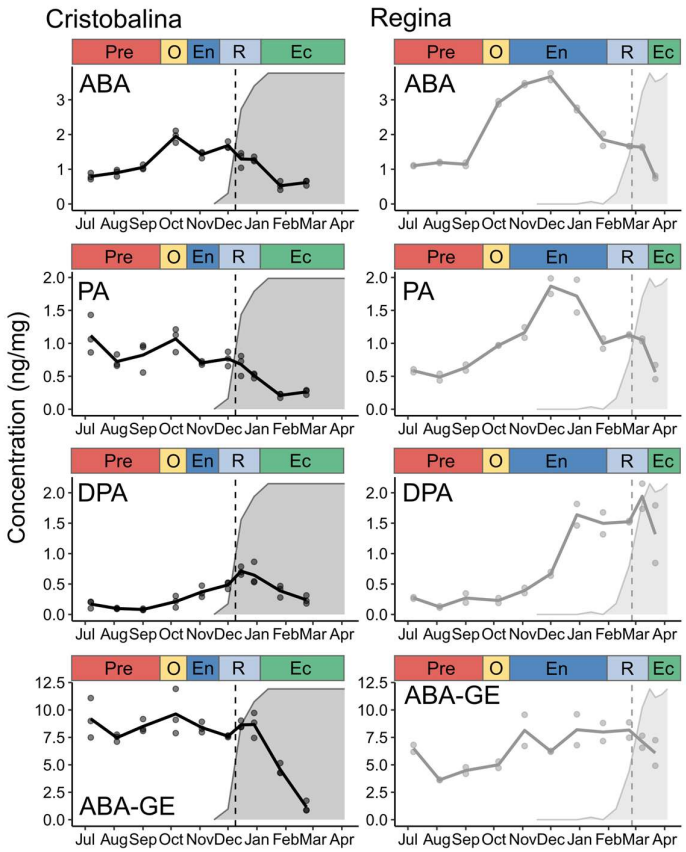

Figure 6 Levels of endogenous bioactive ABA and ABA conjugates in the flower buds of two sweet cherry cultivars during bud development. Black: 'Cristobalina', grey: 'Regina'. Background areas correspond to the dormancy depth evaluated as the percentage of bud break under forcing conditions (see Fig.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

333 In terms of GA signaling, our results show that the identified GA receptor-related *GA INSENSITIVE*
334 *DWARF1B* (*PavGID1B*) is highly expressed during the predormancy (July, August) and early stages of
335 dormancy (September, October; Fig. **5**). For 'Cristobalina', expression of the receptor gene sharply
336 decreased after endodormancy was released. Ten GA-response genes, *GA Stimulated Arabidopsis*
337 (*GASA*), potentially regulated by GAs (Aubert et al. 1998), were identified in the transcript dataset
338 (Table **S3**) and we analyzed the seven genes differentially expressed during flower bud cycle (Fig. **5**,
339 Table **S2**). Expression patterns are diverse but for the majority (*PavGASA1b*, *4*, *6a*, *6b*, *6c*, *9*), a decrease
340 in expression was detected during deep dormancy (November, December), thus suggesting that GA-
341 activated pathways are inhibited during dormancy, despite high contents in GAs (Fig. **4**). More
342 strikingly, all *PavGASA* genes, except for *PavGASA1b*, were sharply upregulated during dormancy
343 release. However, one notable exception is *PavGASA1a* that is highly activated specifically during
344 dormancy (Fig. **5**). The repression of GA by DELLA proteins is well characterized in annuals (Zentella
345 et al. 2007), so to further investigate GA pathway, we identified ten genes coding for predicted DELLAs,
346 namely *PavGAI*, *PavGAI1*, *PavRGA1*, *PavRGA2*, *PavRGL1a, b, c* and *PavRGL2a, b, c* (Table **S3**).

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

350

351 **ABA levels rise at the onset of dormancy**

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

361 Esterification of ABA with glucose was also monitored and the concentrations of ABA-GE were higher
362 in both cultivars compared with ABA and its conjugates. The ABA-GE content was constantly high in
363 'Regina' over the whole cycle, with a slight increase during endodormancy induction, while it markedly
364 decreased in 'Cristobalina' during ecodormancy (Fig. **6**, Fig. S**3a**). However, this observation could be
365 due to a low coverage of ecodormancy in the 'Regina' samples. Throughout pre-dormancy stages, ABA-
366 GE content was significantly higher in the early cultivar 'Cristobalina' compared to the late cultivar
367 'Regina' (Fig. S**3a**).

368

369 **Analysis of genes involved in ABA pathway**

370 Expression for genes involved in the multiple ABA biosynthesis steps, *PavABA1-4*, *PavNCED1* and
371 *PavNCED4*, is not correlated with ABA levels while expression patterns for *PavNCED3* and
372 *PavNCED5* genes seem strongly linked to ABA contents and dormancy status (Fig. **7**, Fig. **S4**). In
373 particular, *PavNCED5* expression shows a sharp increase during dormancy onset and a marked decay
374 before dormancy release. On the other hand, low ABA levels are associated with increased expression
375 for ABA catabolism genes *PavCYP707A1* and *PavCYP707A4* before and after dormancy respectively
376 (Fig. **7**, Fig. **S4**). By contrast, *PavCYP707A2* is characterized by a sharp increase in December, followed
377 by a marked decrease during dormancy release in 'Cristobalina' but before induction of dormancy
378 release in 'Regina'. In addition, we identified *PavUGT71B6* (Supporting Information Table **S2**), a sweet
379 cherry ortholog of the *Arabidopsis thaliana UDP-glycosyltransferase 71B6*, that preferentially
380 glycosylates ABA into ABA-GE (Priest et al., 2006). *PavUGT71B6* was considerably upregulated in

381 the early cultivar compared to the late cultivar, with a gradual increase between July and deep dormancy
382 followed by a decrease in expression before endodormancy release (Fig. **7**).

383 We examined sweet cherry gene predictions for genes involved in ABA signaling. Among the seven
384 ABA receptors *PYR*/*PYL* genes identified for sweet cherry (Table **S3**), six were differentially expressed
385 during flower bud dormancy (Fig. **7**, Fig. **S4,** Table **S2**). In particular, *PavPYL8* expression was
386 correlated with ABA levels, increasing after dormancy onset and decaying after dormancy release (Fig.
387 **7**). A similar pattern was observed for *PavPYL12* in 'Cristobalina'. Among the differentially expressed
388 ABA response genes (Supporting Information Tables **S2**, **S3**), *PavABF2* and *PavHAB2* had similar
389 patterns with a sharp increase in expression in December while the expression of *PavABF3* and *PavABI1*
390 decreased after dormancy release (Fig. **7**).

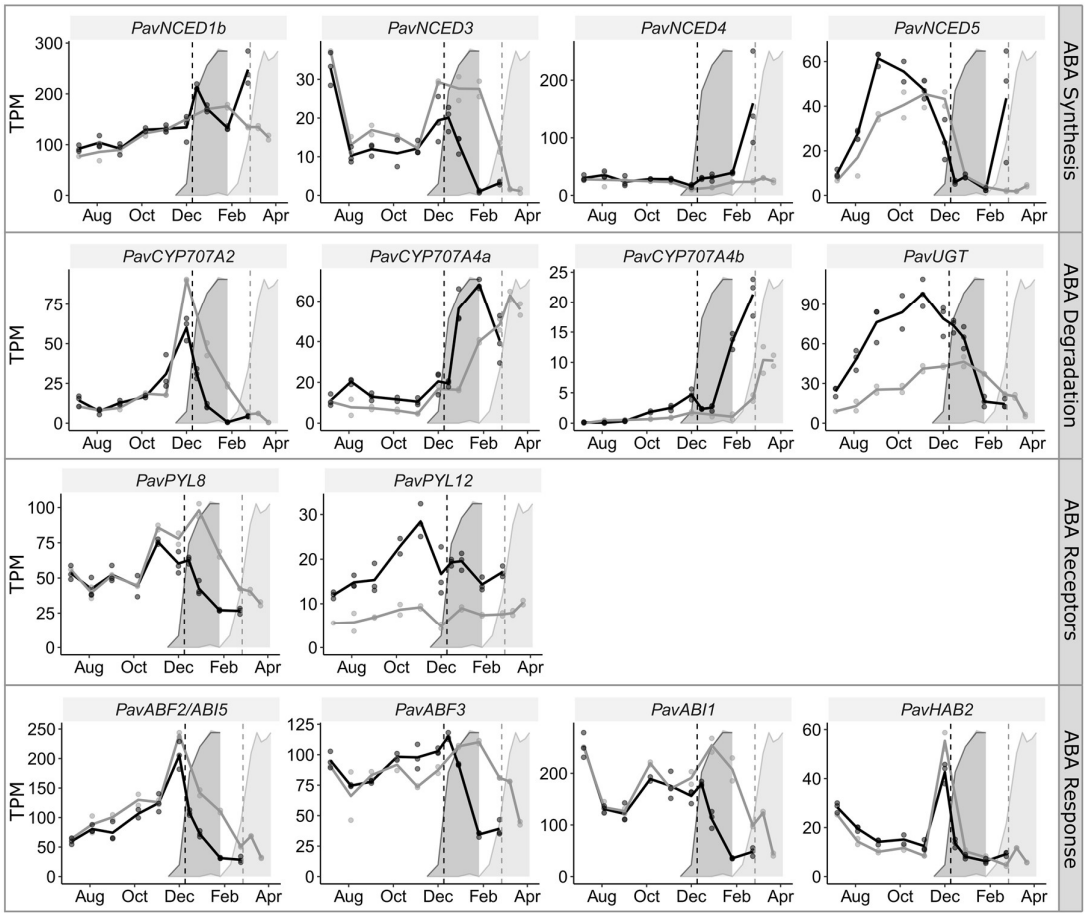

Figure 7 Transcriptional dynamics of genes associated with ABA pathway in the flower buds of two sweet cherry cultivars during bud development. Expression of specific genes involved in ABA biosynthesis pathway, degradation, signal transduction and response are represented in TPM (Transcripts Per Million 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.

391 **Modelling suggests ABA levels control onset and duration of dormancy**

392 Based on observations that ABA levels are correlated with dormancy depth, ABA content has been
393 proposed as an indicator to assess dormancy status in sweet cherry (Chmielewski et al. 2017). We further
394 investigated the dynamics of ABA biosynthesis and catabolism to estimate dormancy onset and duration.
395 First, we assumed that ABA synthesis is mainly controlled by *PavNCED1*, *3*, *4* and *5* while ABA is
396 converted to PA by 8'-hydroxylases *PavCYP707A4* and esterified into ABA-GE by *PavUGT71B6* (Fig.
397 **8a**). Since data for protein activity are not available, we used transcript levels as a proxy for enzymatic
398 activity. We then used an Ordinary Differential Equation (ODE) approach to model how dormancy may
399 be regulated by ABA levels. The mathematical model, based on identical parameter set for both
400 cultivars, shows a good fit to the data, with a global RMSE equal to 0.3468 for all data, and 0.3142 and
401 0.3627 for 'Cristobalina' and 'Regina' respectively indicating that the differential in ABA levels
402 between the two cultivars can be solely explained by the differences in gene expression of the relevant
403 enzymes (Fig. **8b**). To validate the model, we simulated ABA levels for a third cultivar 'Garnet' that
404 was examined along 'Cristobalina' and 'Regina'. Endodormancy was released on January 21st for
405 'Garnet', an early flowering cultivar. Based on expression data for *PavNCED1*, *3*, *4* and *5*,
406 *PavCYP707A4a*, *PavCYP707A4b* and *PavUGT* genes (Fig. **8**, Fig. **S5**), the model simulated ABA levels
407 for 'Garnet' during dormancy (Fig. **8c**). Simulated ABA content for 'Garnet' increases during dormancy
408 onset, reaches high values during endodormancy and decreases before dormancy release, earlier then
409 for the late cultivar 'Regina'. Highest estimated levels for ABA are lower in 'Garnet' than in 'Regina'
410 but higher than 'Cristobalina'.

411 Observed and simulated levels of endogenous ABA at the date of dormancy release show a good match
412 for both 'Cristobalina' and 'Regina' cultivars (Fig. **6**, **8b**). ABA levels are low before dormancy onset
413 but as they increase dormancy is triggered; high ABA levels maintain dormancy but they decrease under
414 chilling temperatures and endodormancy is released as ABA content falls. The Garnet ABA simulated
415 levels lie between those of Cristoballina and Regina levels, thus if ABA content is related to dormancy
416 release, then our modelling predicts that under a chosen fixed threshold of ABA, Garnet dormancy
417 release will occur between the other two release dates. For example, ABA simulated levels of
418 'Cristobalina', 'Regina' and 'Garnet' would fall below the arbitrary threshold of 1.46 ng/mg on
419 December 7^{h}, February 27st and January 27th, respectively. Choice of any other threshold during this
420 period will result in the same date order. Such ordering is observed in our data for dormancy release
421 (Fig. **2a**); 'Cristobalina' and 'Regina' release dates are December 9th and February 26th, while 'Garnet'
422 release date is the January 29st; thus opening the possibility that there is a threshold for ABA
423 concentration that determines the dormancy status. Since we can account for the dormancy behavior of
424 a wide-range of cultivars based on the expression of a small number of key genes regulating ABA levels,
425 this underscores the central role of this phytohormone in the control of dormancy progression.

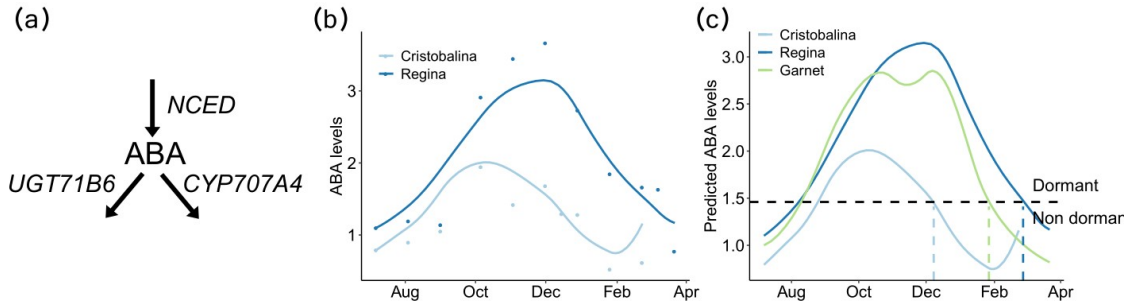

Figure 8 Modelling ABA. (**a**) Conceptual model used to simulate ABA content. ABA synthesis is controlled by NCED1, NCED3, NCED4 and NCED5 proteins; ABA is deactivated by 8'-hydroxylases CYP707A4 and UGT71B6. The assumption is that enzymatic activity is proportional to gene expression levels. (**b**) 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:

447 i) During predormancy stages (July to September), GA_4 and GA_7 content progressively increases,
448 associated with an increase in *PavGA20ox2* expression, and the activation of GA signaling, as revealed
449 by the increased expression of GA-response genes *PavGASA1a*, *4*, *6b*, and *9*. Enhanced GA content
450 potentially triggers the expression of *PavGA2ox8c* to catalyze GA deactivation as part of the feedback
451 regulation (Yamaguchi 2008). In Arabidopsis, proteins GASA4 and GASA6 are involved in flower
452 development and cell elongation, respectively, in response to GA signaling (Roxrud et al. 2007, Zhong
453 et al. 2015) therefore we can hypothesize that gibberellin signaling, mostly driven by *PavGA20ox2* in
454 this phase may control flower bud organogenesis and development.

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

465 iii) As observed in the early cultivar 'Cristobalina', levels for GA_4 and GA_7 decrease during
466 ecodormancy, associated with low, if not null, expression for *PavGA20ox2* and *PavGA3ox2a* synthesis
467 genes, as well as *PavGA2ox8b*, *PavGA2ox8c*, *PavGA2oxb* deactivation genes and *PavGID1B* receptor.
468 Interestingly, the GA-response *PavGASA* genes and the DELLA genes *PavRGA2* and *PavRGL2* are
469 markedly activated during ecodormancy, thus suggesting that GA-stimulated pathways are up-regulated.
470 Indeed, despite decreasing GA_4 and GA_7 levels during ecodormancy, GA-response pathways seem to
471 be mostly activated when *GA2ox* genes are down-regulated after dormancy release so further
472 investigation on the control of GA deactivation during dormancy could unravel a potential regulation
473 by cold accumulation. In addition, bioactive GAs, including GA_1 and GA_3, that were not detected in the
474 current study might be key actors in the growth resumption stage of ecodormancy in sweet cherry. Acute
475 GA response occurring during ecodormancy, including activation of *GASA* genes, was previously
476 reported in grapevine (Zheng et al. 2018), oak (Ueno et al. 2013), pear (Yang et al. 2019) and Japanese
477 apricot (Zhang et al. 2018). Despite the fact that DELLA genes' activity is mainly regulated by protein
478 stability, the up-regulation of *PavRGA2* and *PavRGL2* genes is consistent with the GA response during
479 ecodormancy, associated with a decrease in *PavGIDB* expression. These results suggest that the GA
480 homeostasis, critical during active growth, is controlled in sweet cherry by the DELLA proteins, that
481 target GA biosynthesis and receptor genes and impacts GA balance through a feedback regulation, as

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

484 **Dormancy depth is correlated with endogenous ABA content**

485 Exogenous application of ABA on the sweet cherry dormant bud showed that buds at this specific stage
486 were not affected by ABA treatment, in contrast to the observation that ABA treatment had a significant
487 effect on grapevine bud break (Zheng et al. 2015). However, as shown by the high expression of genes
488 involved in ABA degradation, including *PavCYP707A2*, high catabolism ability during endodormancy
489 might have limited the effect of exogenous ABA. It might also suggest that ABA response is saturated
490 in the context of very high ABA levels during endodormancy, therefore limiting the effect of additional
491 ABA. Nevertheless, we observed that dormancy release was triggered in buds treated with fluridone that
492 inhibits ABA biosynthesis, thus suggesting that high ABA levels may act to maintain dormancy. This
493 was further confirmed by the observed elevated ABA levels correlated with endodormancy in both
494 cultivars, as well as the differences in ABA levels between the early and late flowering cultivars. Our
495 results are consistent with the hypothesis that dormancy is triggered and maintained when ABA levels
496 are above a threshold, taking into account the potential heterogeneity within a population of flower buds.
497 Bud dormancy may subsequently be released if ABA levels fall below the threshold. Consequently, such
498 a dormancy release threshold would be reached earlier in the season for early cultivars with less ABA
499 (Wen et al. 2016) or when ABA levels are lower due to various environmental conditions (Chmielewski
500 et al. 2017). Recent reports have indeed shown how low or high levels of ABA closely drive the depth
501 of dormancy by controlling the blockage of cellular transports (Tylewicz et al. 2018, Singh et al. 2019).
502 We show that during the predormancy and dormancy onset stages, ABA levels are correlated with an
503 increase in the expression of *PavNCED5*, then *PavNCED3* genes in both cultivars. Consistently with
504 elevated ABA levels, genes encoding ABA receptors (*PavPYL12*, *8*), as well as ABA response genes,
505 are highly expressed during deep dormancy. One interesting result is that *PavNCED5* expression peak
506 coincides with the highest ABA levels in October in 'Cristobalina' and in December in 'Regina',
507 approximately two months before dormancy is released. This suggests that the deepest dormancy state
508 might occur earlier in 'Cristobalina' than in 'Regina' and therefore question whether endodormancy is
509 indeed induced simultaneously in both cultivars. Further physiological observations during the early
510 stages of dormancy induction, including flower primordia developmental context (Fadón et al. 2018),
511 could help better understanding the observed differences. Afterwards, genes involved in ABA
512 degradation (*CYP707A*) are highly expressed during dormancy release and positively correlated with a
513 decrease of ABA content. Similar results were reported in other *Prunus* species (Zhang et al. 2015,
514 Wang et al. 2016, Tuan et al. 2017) confirming the major role of ABA signaling pathways on the
515 regulation of dormancy induction, maintenance and release.

516 Presently, a remaining question to elucidate is what drives the decrease in ABA levels around dormancy
517 release. Which mechanisms are involved in the down-regulation of *PavNCEDs* and up-regulation of
518 *PavCYP707As* through chill accumulation? Firstly, several reports indicate that ABA might regulate its
519 own accumulation and high levels of ABA attained during endodormancy could up-regulate the
520 expression of catabolic genes such as *PavCYP707A4*, leading to a global decrease in ABA content.
521 Secondly, *DORMANCY-ASSOCIATED MADS-BOX* (*DAM*) genes have been strong candidates for a
522 key role in dormancy promotion and maintenance (Rodriguez et al. 1994, Bielenberg et al. 2008). *DAM*
523 genes are highly expressed during dormancy and their expression is inhibited by chill accumulation
524 (Jiménez et al. 2010, Hao et al. 2015), but more interestingly, recent studies have highlighted the direct
525 effect of *DAM* on the activation of ABA biosynthesis (Tuan et al. 2017, Yamane et al. 2019). Further *in*
526 *vitro* assays suggest a potential regulation between CBF proteins and *DAM* promoters (Niu et al. 2015,
527 Zhao et al. 2018). We therefore hypothesize that chill accumulation induces *CBF* expression and CBF
528 proteins modulate the expression of *DAM* genes that subsequently upregulate *NCED* genes and ABA
529 biosynthesis. Chill accumulation potentially induces chromatin modifications that silences *DAM* genes
530 (Leida et al. 2012, Ríos et al. 2014, de la Fuente et al. 2015) and inhibits ABA production, consistently
531 with decreasing ABA levels observed as soon as January. Interestingly, a recent study conducted in
532 hybrid aspen shows that chilling and long photoperiods induces a decrease in ABA levels, which
533 subsequently drives the decrease in *SHORT VEGETATIVE PHASE-LIKE* (*SVL*) expression (Singh et al.
534 2018, 2019). *SVL* shows similarity to *SHORT VEGETATIVE PHASE* (*SVP*) and *DAM* genes and this
535 mechanism suggests that ABA might also act on the expression of *DAM* genes. Regulation of *DAM* and
536 ABA pathways by cold temperatures should be further investigated to better understand how
537 temperature variations control dormancy progression in sweet cherry flower buds.

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

540 Overall, although the dynamics of expression for *PavNCED* and *PavCYP707A* genes effectively explain
541 the increasing and decreasing pattern of ABA levels between dormancy induction and release, we further
542 investigated whether they could account for the significant differences observed for ABA levels between
543 the two cultivars. Contrasted expression patterns for *PavNCED3*, *PavCYP7074a* and *PavCYP7074b*
544 were not sufficient to explain the noticeably contrasted ABA accumulation during endodormancy
545 between the two cultivars. Differences in ABA catabolites between cultivars were also observed. While
546 PA content followed the same pattern as ABA, DPA was particularly high during dormancy release,
547 inversely correlated with the decreasing ABA levels. Interestingly, Weng and colleagues (Weng et al.
548 2016) exposed the compensatory effect of PA under low ABA conditions, in which PA is recognized
549 by ABA receptors (PYL5 and 2) allowing a supplementary growth inhibition effect. In sweet cherry,
550 increased *PavCYP707A2* expression may explain the higher levels of PA during dormancy in 'Regina'
551 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-

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

590 **DATA AVAILABILITY**

591 RNA-seq data: Gene Expression Omnibus GSE130426

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

594

595 **SUPPLEMENTARY DATA**

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

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

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

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

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

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

607 **Table S2** RNA-seq filtering and Differential expression analysis

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

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

611

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623 **AUTHORS' CONTRIBUTION**

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

628

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