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Physiological and biochemical characterization of bud dormancy: Evolution of carbohydrate and antioxidant metabolisms and hormonal profile in a low chill peach variety

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ABSTRACT

In this work, we studied the interplay among the levels of starch, sugars, abscisic acid (ABA) and bioactive gibberellins (GAs), and the antioxidant metabolism during bud dormancy release in a low chill peach variety. The experiments were performed in two consecutive years and two geographical areas with different annual media temperature.

In the cold area, peach plants accumulated more starch and soluble sugars than in the temperate area, sorbitol and sucrose being the most abundant sugars.

The enzymatic activities of the ascorbate-glutathione (ASC-GSH) cycle and the ROS-scavengers enzymes superoxide dismutase (SOD), catalase and peroxidase (POX) were measured. The increased activity of the ASC-GSH cycle enzymes and POX suggested a mild oxidative stress at endodormancy and at dormancy release periods. In this sense, the presence of H_2O_2-sensitive antioxidant enzymes, including Fe-SOD, Cu,Zn-SOD, POX and APX in the floral buds could trigger the oxidative signaling leading to dormancy release. Interestingly, the induction of a new POX isoenzyme occurred at dormancy release, suggesting its use as a marker for this event in low chill peach varieties.

GAs levels decreased at the end of the dormancy period, whereas ABA levels showed a strong decline throughout the dormancy period, especially in buds from the cold area. As a consequence, a sharp decrease in the ABA/GAs ratio was observed, a phenomenon closely related to dormancy release. This response was more evident in the cold area. Results suggested that a decrease in ABA levels, as well as in the ABA/GAs ratio, may be necessary for the bud dormancy release in peach, at least in low chill varieties. Overall, we observed an interaction among sugars, antioxidant enzymes and plant hormones in the dormancy period. Glucose levels correlated with chill accumulation, peaking at ecodormancy and dormancy release. This response was parallel with increases in some antioxidant enzymes, the induction of a new POX isoenzyme and the decline in ABA levels and the ratio ABA/GAs.

1. Introduction

Bud dormancy in woody deciduous plants is a physiological stage that enables plants to survive long periods under adverse conditions and is characterized by growth cessation, arrest of cell division and reduced metabolic and respiratory activities (Faust et al., 1997, Rhode and Bhalerao, 2007). Dormancy is a prerequisite for proper flowering and fruit setting (Campoy et al., 2011; Fadon and Rodrigo, 2018). There is a relationship between dormancy release and action of low temperatures (referred as chilling requirements) (Coville, 1920). However, when chilling requirements are fulfilled and dormancy release takes place, a certain period under mild temperature is required for growth

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resumption (referred as heat requirements) (Campoy et al., 2011; Fadon and Rodrigo, 2018). Lang (1987) distinguished three types of dormancy: (1) Paradormancy, where the growth inhibition arises from another part of the plant; (2) endodormancy (or true dormancy), where the growth inhibition resides in the dormant structure itself; and (3) ecodormancy, where the growth is limited by environmental factors. The knowledge on the physiological, biochemical and molecular basis of flower bud dormancy in template zones is of crucial importance in order to ensure the fruit production, mainly in a climate change context (Prudencio et al., 2019). In the recent years, different works has studied this process in woody plants (Rohde and Bhalerao, 2007; Campoy et al., 2011; Gholizadeh et al., 2017; Fadon and Rodrigo, 2018). However, the regulation of bud dormancy in stone fruit trees remains relatively unknown (Fadon and Rodrigo, 2018).

Carbohydrates play an important role during bud growth and development and in the process of bud dormancy release (Takemura et al., 2015; Gholizadeh et al., 2017), by acting as the primary source of carbon and energy (Anderson et al., 2010). However, while the function of sugars as energy source is well known, its role mediating bud dormancy evolution is still poorly understood (Zhang et al., 2018). During endodormancy and dormancy release, starch is hydrolyzed into soluble sugars (Ito et al., 2012; Gholizadeh et al., 2017; Kaufman and Blanke, 2017, Fadón et al., 2018). In that regards, it has been reported that the application of chemicals used for breaking bud dormancy induced α-amylase expression (Rubio et al., 2014).

Moreover, signals or cycles of oxidation and reduction are crucial for dormancy release, activation of the cell cycle and expression of genes related to growth and differentiation. Redox signaling and phytohormones networks were described to synergistically control growth, development and differentiation (Considine and Foyer, 2014). Changes in starch and sugars as well as in the antioxidant metabolism have been studied in walnut buds and the authors proposed that redox interaction rather than sugar metabolism governs bud dormancy release (Gholizadeh et al., 2017). Pérez et al. (2008) reported a H_2O_2-mediated effect of hydrogen cyanamide as a dormancy-breaking compound. In that regards, it has been reported that the regulation of redox and oxygen metabolism is critical to organ development (Meitha et al., 2015). More recently Beauvieux et al. (2018) highlighted a pivotal role of reactive oxygen species (ROS) and antioxidant metabolism in dormancy release.

In a recent work, Prudencio et al. (2019) proposed the *PdP40* gene, encoding for a Class III peroxidase (POX), as well as the POX activity as two candidate markers to monitoring the transition from endodormancy to ecodormancy in almond flower buds from several cultivars with different chilling requirements. Moreover, by proteomics approaches, Takemura et al., (2011) showed that the majority of proteins expressed in the pre-breaking period of endodormancy in pear floral buds were involved in redox processes. In addition, the protein expression of floral and vegetative buds from peach has been also analysed (Prassinos et al., 2011). Floral peach buds up-regulated proteins were involved in the mobilization of energy and nitrogen reserves as well as in plant morphological development. Other enzymes, related to H_2O_2 homeostasis, were also differentially accumulated, detecting catalase (CAT) up-regulation only in floral buds whereas POX was up-regulated in vegetative buds (Prassinos et al., 2011). The authors suggested that H_2O_2 might activate the bud-break process, acting as a signaling molecule triggering the mentioned process. Different evidences supported this hypothesis, *e.g.* link between CAT inhibition, H_2O_2 accumulation and grapevine bud-break (Pérez et al., 2008; Sudawan et al., 2016). Additionally, the transcription of genes encoding for H_2O_2-scavenging enzymes, and/or the corresponding enzymatic activities, were induced after dormancy release, in order to prevent the accumulation of lethal levels of H_2O_2. In this sense, the expression of the entire battery of antioxidant defenses has been detected in peach floral buds (Prassinos et al., 2011).

The relative abundance of phytohormones such as abscisic acid (ABA) and gibberellins (GAs), together with oxidative signaling processes and sugar metabolism determines the regulation of dormancy and bud burst (Considine and Foyer, 2014). The role of plant hormones, especially ABA, in the dormancy cycle has been widely discussed by different authors (Cooke et al., 2012; Zheng et al., 2015, 2018a,b). ABA catabolism enhances dormancy release of grapevine buds mediated by the up-regulation of *CYP707A4* gene, which encodes for ABA 8′-hydroxylase (Zheng et al., 2018a, b). In addition, there is extensive information on the involvement of GAs in the induction of growth cessation, for which a decrease in the levels of active GAs is a prerequisite. A short photoperiod causes down-regulation of GA_{20} oxidase, a key enzyme for GA biosynthesis, but is unknown whether GA biosynthesis is activated during chilling or after dormancy release (Cooke et al., 2012). An inhibition of flower induction was observed in peach, apricot, almond, sweet cherry and apple after treatment with GA_3 (Hoad, 1983). However, the effect of GAs on bud break remains controversial, considering that both promoting and inhibiting effects have been reported (Ionescu et al., 2017; Zheng et al., 2018a). Nevertheless, it seems that ratio the ABA / GAs and not the absolute hormone contents could be more relevant in the control of dormancy release, as described in dormant seeds (Finch-Savage and Leubner-Metzger, 2006),sweet cherry floral buds (Duan et al., 2004) and *Prunus mume* (Zhang et al., 2018). In sweet cherry, the endogenous ABA/GA_3 ratio increased in flower buds during natural dormancy induction and decreased during dormancy release (Duan et al., 2004).

In this work, the levels of the main antioxidant enzyme activities, abscisic acid (ABA), bioactive gibberellins (GAs) and soluble sugars were monitored in buds of a low chill peach variety grown in two geographical areas with different annual media temperature, in order to elucidate the interplay among these variables on bud dormancy maintenance and release.

2. Material and methods

2.1. Plant material

Floral buds of peach tree (*Prunus persica* L.) variety 'Okinawa' [GEM065 (OKINAWA CAIN, DPRU 2695 PI 673729, ref from Davis repository, USDA)], characterized by its low chill requirements [100–150 chill units (Okie, 1998), equivalent to 10–13 chill portions], were used. Plants were cultivated in the experimental collections of BAGERIM-IMIDA in two geographical areas: one located in a temperate zone (El Jimenado, Torre Pacheco, Murcia 37°46′13.6″N 1°01′16.9″W) and the other located in an area with colder temperatures, especially in winter (El Chaparral, Bullas, Murcia 38°06′36.0″N 1°41′19.8″W). In the temperate zone, the average daily temperature from October to December ranged from 18.8 to 10.6 °C in 2017 and from 17.5 to 12.1 °C in 2018. In the cold zone, the corresponding average daily temperature ranged from 16.8 to 5.8 °C in 2017 and from 15.1 to 6.5 °C in 2018.

Chill portions (CP) were used for quantification of chilling accumulation according to the Dynamic Model (Fishman et al., 1987a, b). This model was developed for an accurate good estimation of chilling accumulation in warm winter areas (Campoy et al., 2011).

Flower buds were collected from October to December in two consecutive years (2017 and 2018, year 1 and year 2, respectively) in dates comprising paradormancy (PD) (up to 5th October in year 1, and 10th October, year 2), endodormancy (ED) (31th October, and 16th and 27th November in year 1; 30th October and 14th November in year 2), ecodormancy (EC) (4th and 14th December in year 1; 4th December in year 2) and dormancy release (DR) (28th December in year 1; 18th December in year 2). At least 200 buds per tree were taken and used for further analysis in each sampling period.

For all analysis, four biological samples were used each year. For the determination of starch, sugars and hormones, and for histological study, each biological sample consisted of 10–12 flower buds for a total fresh weight of ca. 0.1 g, after removing most of the bracts. For the antioxidant enzymes analyses, each biological sample consisted of

20–25 flower buds for a total fresh weight of ca. 0.25 g, after removing most of the bracts. All the flower buds batches, previously devoid of their scales, were weighted, and either immediately used for the histological study or frozen in liquid nitrogen and stored at -80 °C for further analysis.

2.2. Histological study

Flower buds, previously devoid of their bracts, were fixed in 2.5 % glutaraldehyde-4 % paraformaldehyde diluted in 0.1 M sodium phosphate, pH 7.2, for 24 h. Subsequently, they were embedded in methacrylate JB-4. Serial sections of 3 μm were mounted on slides impregnated with an adhesive of gelatin, glycerin and 3 % formaldehyde. Samples were stained with toluidine blue (0.03 %) and observed with a light microscope.

2.3. Starch and sugar determinations

Flower buds were crushed in liquid nitrogen and extracted with MilliQ water (1/10, w/v). Extracts were incubated at 90 °C for 20 min, and then centrifuged at 4000 *g* for 5 min at room temperature. The resulting supernatant was mixed with absolute ethanol (1/3, v/v; PanReac AppliChem, Spain) and centrifuged at 10,000 *g* for 10 min.

For the starch determination, the resulting pellet was re-suspended in 1 ml Milli-Q H_2O and mixed with 50 μL lugol solution (Sigma-Aldrich, Gillingham, UK). The optical density of the mix was recorded at 595 nm using an Epoch Gene-5 plate reader (BioTek Instruments, Vermont, USA). The starch concentration was calculated using a standard curve of pure rice starch (Sigma-Aldrich) (0–200 μg/mL).

For the sugar determination, the resulting supernatant from the ethanol extraction was passed through a Minisart RC15 cellulose filter (45 μm, Sartorius AG, Göttingen, Germany), and subjected to HPLC (series 1100, Hewlett-Packard, Palo Alto, CA, USA). The mobile phase consisted of 0.1 % phosphoric acid eluted isocratically with a flow rate of 0.5 mL/min. The sugars were eluted through a Supelco column (Supelcogel C-610H, 30 cm x7.8 mm, Supelco Park, Bellefonte, PA, USA) and detected by a refractive index detector. A standard curve of pure sugars (glucose, fructose, sucrose and sorbitol) (Sigma-Aldrich) prepared in the range of 0–1.00 % (w/v) was used for the quantification of the different sugars.

2.4. Enzyme analysis

Flower buds were crushed with liquid nitrogen, and extracted with 50 mM Tris-acetate buffer, pH 6.0 (1/8 w/v), containing 2 mM Cys, 0.1 mM EDTA, 1 % PVP (w/v), 2 % PVPP (w/v) and 0.2 % Triton X-100 (w/v). For the APX activity, 20 mM sodium ascorbate was added to the extraction buffer. The extracts were centrifuged at 10,000 *g* for 15 min at 4 °C. The supernatant fraction was filtered on Sephadex G-25 NAP columns (ThermoFisher Scientific, Hampton, NH, USA) equilibrated with the same buffer used for homogenization, and used for the enzymatic determinations. For the APX activity, 2 mM sodium ascorbate was added to the equilibration buffer. The activities of the ASC-GSH cycle enzymes, POX, CAT, and SOD were assayed as described previously (Barba-Espin et al., 2012; Acosta-Motos et al., 2015). Protein contents were determined according to Bradford (1976).

2.5. Electrophoretic analysis

To separate POX and SOD isozymes, non-denaturing PAGE was performed on 10 % acrylamide gels, using a Mini-protean III dual slab cell (Bio-Rad, Hercules, CA, USA). In all cases, 10 μg of protein per line was used in native gels. Staining of peroxidase isoenzymes with 4-methoxy-1-naphtol was performed as described by Ros-Barceló et al. (2006). SOD isozymes were localised by the photochemical method of Weissiger and Fridovich (1993). SOD isoenzyme identification was performed by selective inhibition with KCN or H_2O_2 (Hernández et al., 1999).

2.6. Plant hormone determination

Flower buds were crushed in liquid nitrogen, and suspended in 80 % methanol-1% acetic acid containing deuterium labeled GAs and ABA as internal standards, and mixed by shaking for one hour at 4 °C. The resulting extract was kept at 20 °C overnight and then centrifuged, and the supernatant was dried in a vacuum evaporator. The dry residue was dissolved in 1% acetic acid and passed through an Oasis HLB (reverse phase) column (Waters Corp., Milford, MA, USA) as described in Seo et al. (2011). For GAs and ABA quantification, the dried eluate was dissolved in 5 % acetonitrile-1% acetic acid, and the hormones were separated using an auto-sampler and a reverse phase UHPLC chromatographer (2.6 μm Accucore RP-MS column, 50 mm length×2.1 mm i.d.; ThermoFisher Scientific) with a 5–50 % acetonitrile gradient containing 0.05 % acetic acid, at a flow rate of 400 μL/min over 14 min. The hormones were analysed with a Q-Exactive mass spectrometer (Orbitrap detector; ThermoFisher Scientific) by targeted Selected Ion Monitoring (SIM). The concentrations of hormones in the extracts were determined using embedded calibration curves and the Xcalibur 2.2 SP1 build 48 and TraceFinder programs. The deuterium-labeled hormones were the internal standards for quantification of each of the different plant hormones.

2.7. Statistical analysis

The data were analysed by one-way ANOVA followed by Tukey's Multiple Range Test ($P \leq 0.05$), using the SPSS 20.0 software (SPSS Inc., 2002) software. A principal component analysis (PCA), followed by a partial least squares discriminant analysis were conducted to assign the principal components displaying eigenvalues greater than or equal to 1.0, using the StatGraphics Centurion XV software (StatPoint Technologies,Warrenton, VA, USA).

3. Results

3.1. Chill requirements

GEM065 [OKINAWA CAIN, DPRU 2695 PI 673729, ref. from Davis repository, USDA] is a peach variety with very low chill requirements [100–150 chill units, (Okie, 1998), equivalent to 10–13 chill portions). In the temperate area (El Jimenado), by the end of December 2017 and 2018, the accumulation of chill portions was scarce but enough to overcome dormancy. In both areas, the chill accumulation began before in 2017 than in 2018, and, as expected, the chill accumulation was greater in the cold zone than in the temperate area. Dormancy release occurred at the end of December in 2017 and at the middle of December in 2018, once the plants had satisfied their chill requirements. The data of the accumulated chill portions can be consulted in all Figures.

3.2. Histological study

The pollen development was monitored in 2018 in both areas (Fig. 1). At the end of October, there was no evidence of pollen development in the anthers (Fig. 1A and D). At the beginning of December, the developmental stage of anthers was similar in both locations and the pollen mother cell was observed (Fig. 1B and E). At the middle of December, when the dormancy release took place, anthers either contained pollen cells at tetrad stage or showed vacuolated microspores after meiosis in the temperate area (Fig. 1C). However, only in the cold location, pollen grains were observed (Fig. 1F).

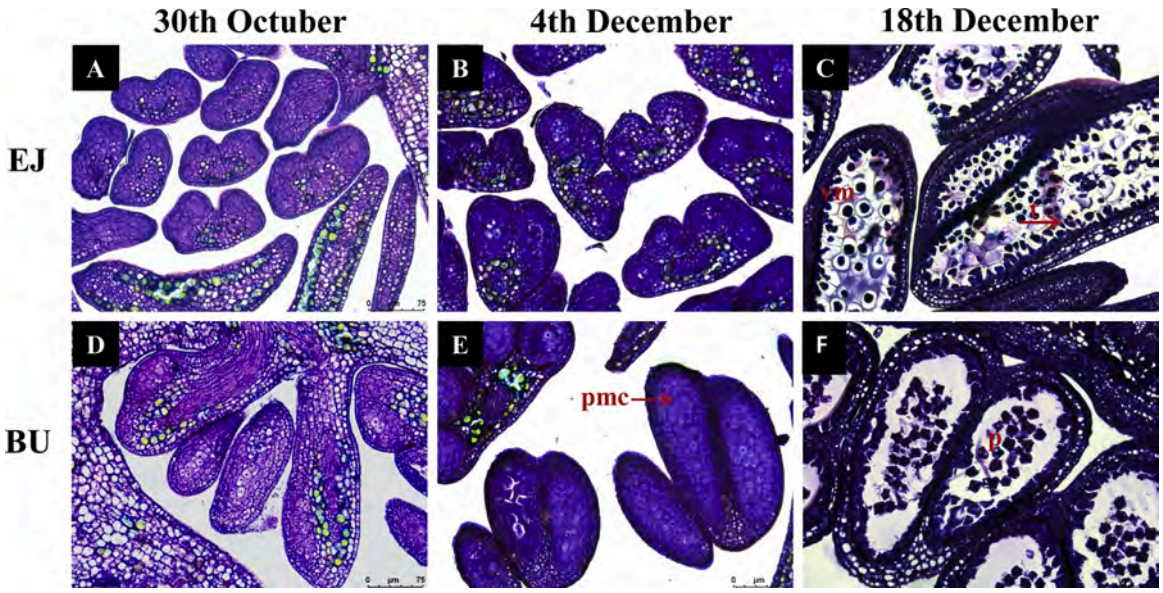

Fig. 1. Histological analysis of flower bud development from peach cultivar '065'. Samples colleted at El Jimenado (A, B and C) and Bullas (C, E and F) on October (A and D), February (B and E) and March 2018 (C and F). vm = vacuolated microspora; pmc = pollen mother cell; p = pollen grain; t = tetrad.

3.3. Carbohydrate metabolism

The growth of peach trees in two zones with a differential climatology was reflected on the starch content of their flower buds. The differences were also evident between the two years of study (Fig. 2). In 2017, the starch content was higher in the cold zone than in the temperate one, at least during endodormancy. However, in ecodormancy and at dormancy release, starch concentration was similar in both areas (Fig. 2A). In the temperate zone, starch content was similar between stages, showing a slight increase immediately before dormancy release (14th December) and at dormancy release. In the cold area, the starch content increased during endodormancy, decreased during ecodormancy and finally rose coinciding with the dormancy release (Fig. 2A). In 2018, the differences in starch content in relation to the previous year, as well as starch temporal evolution, were evident. In addition, the initial starch levels were much higher in 2018 than in 2017 for both geographical locations (Fig. 2B). In the temperate zone, the starch levels peaked at the end October, and then decreased, maintaining the levels until the dormancy release. A similar response was observed in the cold zone, although a non-significant slight upward trend in the starch levels was observed at the dormancy breaking period (Fig. 2B).

The evolution of soluble sugars was also different between the two years of study. Regarding sucrose, during the first year and in both geographical locations, a progressive increase was observed previous to the dormancy release, followed by a decline concomitant with the bud break process (Supplemental Fig. 1A). However, in the second year, no significant changes occurred (Supplemental Fig. 1B).

Fructose levels were, in general, higher in the cold zone than in the temperate area. In year 1, in the temperate area, a 1.6-fold decrease occurred at the end of the endodormancy period, whereas at dormancy release, the fructose levels reached the initial values. No significant changes occurred in the cold area (Supplemental Fig. 2A). In the year 2, in contrast, an opposite behavior was observed: fructose remained unchanged in the temperate zone, while a 2.4-fold increase occurred in the cold area at endodormancy (30th October), followed by a not significant decrease in ecodormancy and at dormancy release (Supplemental Fig. 2B).

In general, sorbitol contents were lower in the temperate zone than in the cold area. In the temperate area, during the first year, sorbitol levels slightly increased coinciding with dormancy release, although the changes were not significant (Supplemental Fig. 3 A). In the cold area, flower buds showed the lowest sorbitol content at paradormancy. The values peaked at the beginning of endodormancy (31th October), then a decrease occurred, but again values increased until dormancy released, showing a 2.3-fold increase in relation to the initial values (Supplemental Fig. 3A). However, in the year 2, sorbitol contents decreased in the temperate zone during the dormancy process, reaching a 40 % decline at dormancy release, in relation to the initial values. In the cold zone, a 40 % increase in sorbitol concentration was observed in endodormancy (30th October), and then a progressive decrease occurred until dormancy release, leading to a 25 % drop in relation to the initial values (Supplemental Fig. 3B).

In year 1, the glucose levels remained unchanged until dormancy release in the temperate zone, when glucose levels significantly increased (30 %) with respect to the initial values. A similar situation occurred in the cold zone, where glucose levels remaining constant during paradormancy and endodormancy, but showed an important increase at ecodormancy and dormancy release of 68 % and 50 %, respectively (Fig. 3A). In the second year, a remarkable increase of glucose levels was observed in both areas, reaching the highest values just before dormancy release (ecodormancy), when glucose concentration showed a 40 % rise (Fig. 3B).

3.4. Antioxidant metabolism

The activity of some antioxidant enzymes was monitored during the same periods (2017–2018). Starting from 31th October 2017, the APX activity showed a 5-fold increase at endodormancy in the temperate zone, followed by a progressive decline until the dormancy release (Fig. 4A). In year 2, APX activity also increased in bud samples at endodormancy, although the rise was up to 2.3-fold in the temperate zone. After this increase, APX activity remained constant until dormancy release, but showing activity values much higher than the initial ones at endodormancy (Fig. 4B). In the cold zone, during the first year, APX activity decreases strongly at the end of endodormancy, and remained low until dormancy release (Fig. 4A). In 2018, APX activity showed much lower starting values compared to 2017. In the cold zone, APX activity showed a 12-fold increase in endodormancy (30th October), and then a significant decline occurred at the end of endodormancy, maintaining the activity values until dormancy release (Fig. 4B).

In year 1, and in both zones, MDHAR activity values were elevated at

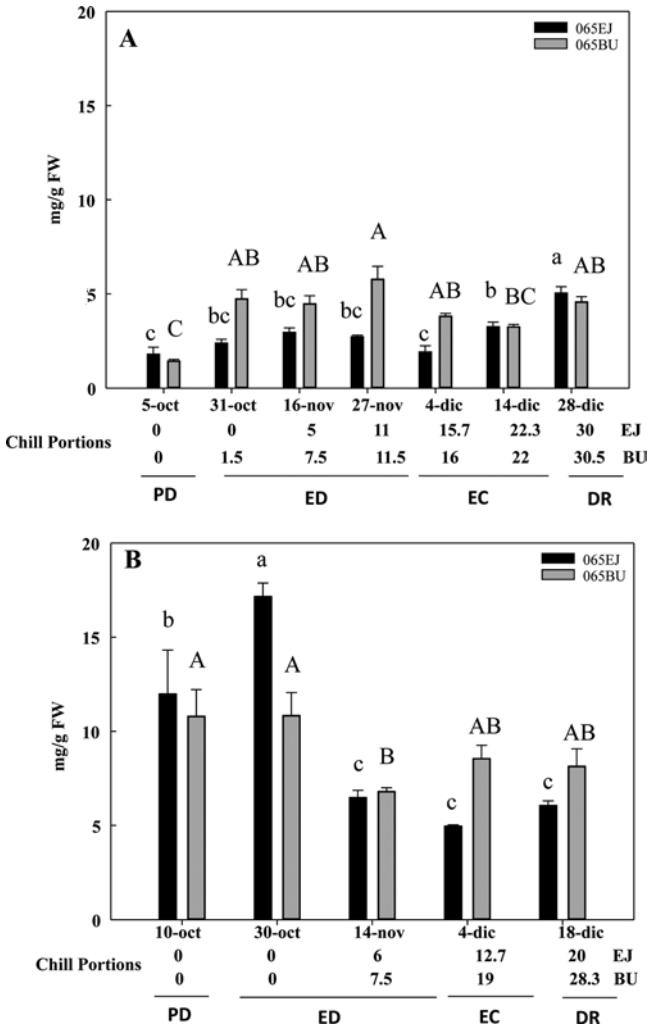

Fig. 2. Evolution of starch contents in flower bud from peach 'GEM 065' cultivated in two different geographical areas during the dormancy cycle on two different years and sampling dates. A: year 1 (2017); B: year 2 (2018). Different letters (lowercase and uppercase for the samples for the EJ and BU samples, respectively) indicate significant differences according to the Tukey's multiple test ($P \leq 0.05$). EJ, temperate area; BU, cold area. The different phases of dormancy, paradormancy (PD), endodormancy (ED), ecodormancy (EC) and dormancy release (DR) are indicated at the bottom of Fig. 2A and B. For more information, please consult 'Plant Material' section.

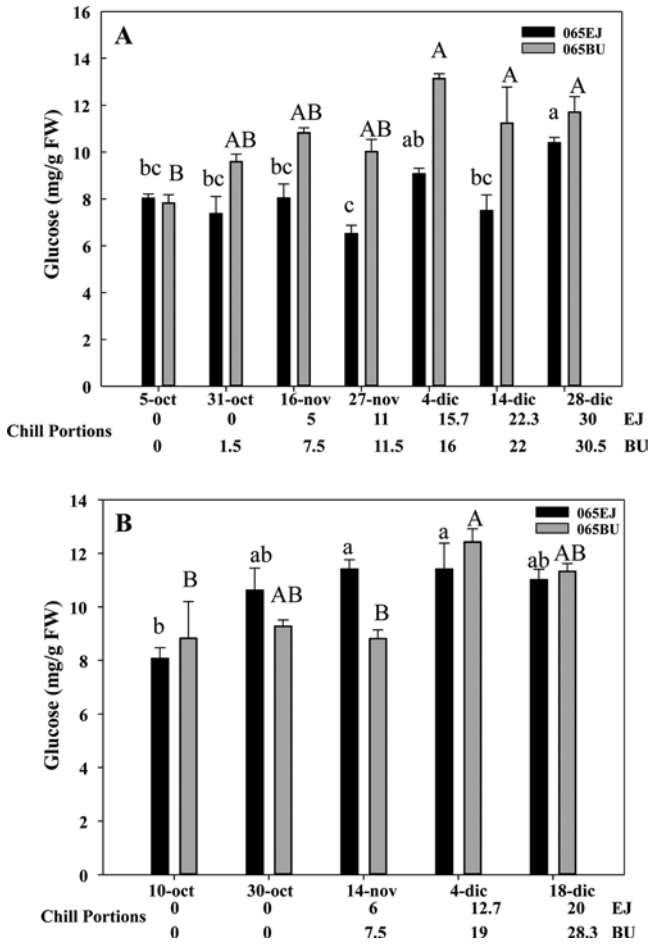

Fig. 3. Evolution of glucose contents in flower bud of peach 'GEM 065' cultivated in two different geographical areas during the dormancy cycle on two different years and sampling dates. A: year 1; B: year 2. Different letters (lowercase and uppercase for the samples for the EJ and BU samples, respectively) indicate significant differences according to Tukey's multiple test ($P \leq 0.05$). EJ, temperate area; BU, cold area. For more information, please consult Fig. 2.

the beginning of endodormancy, and then declined at ecodormancy about 8-fold and 13-fold for the temperate and cold zones, respectively (Fig. 5A). Finally, an increase in MDHAR was observed, coinciding with the end of ecodormancy and the floral bud break (Fig. 5A). In the second year, the highest values of MDHAR activity were also observed in endodormancy. Then, MDHAR activity decreased in ecodormancy. Finally, MDHAR activity increased simultaneously to the dormancy release (Fig. 5B). In the cold zone, the behavior of MDHAR activity was similar to that observed in the temperate zone in the first and the second year of experiment (Fig. 5A, B), showing its highest values in endodormancy and a decline in ecodormancy following by an increase at dormancy release (Fig. 5B).

In 2017, DHAR activity increased a 50 % in endodormancy in the temperate area with respect to the initial values, then the activity was maintained and finally declined at the beginning of ecodormancy (4th December), Finally, a new increase was observed at bud break stage (Fig. 6A). In the year 2, DHAR activity showed a 4-fold decrease at the end of endodormancy (14th November 2018), followed by a progressive increase at the dormancy release (Fig. 6B). In the cold area, DHAR activity followed a similar pattern to that observed in the temperate zone in both years of the experiment, that is, an initial increase in DHAR activity, followed by a significant decline and an increase at dormancy release, although statistically not significant (Fig. 6A, B).

GR activity, involved in the DHAR-mediated ascorbate recycling, behaved similarly to DHAR activity. In the temperate zone, during 2017, GR levels were constant during endodormancy, and then a significant drop was observed at ecodormancy. Finally, at dormancy release, GR activity recovered its initial values (Fig. 7A). In year 2, GR activity increased significantly during endodormancy in the temperate area, followed by a decrease at dormancy release, although no significant differences were observed in relation the initial values (Fig. 7B). In the cold area, GR activity maintained a similar pattern than in the temperate area with some differences. In this case, the increase in GR activity during endodormancy was more pronounced in the cold area than in the temperate zone, for both experimental years. Then, the activity decreased (year 1) or remained constant (year 2) at the end of endodormancy. In both cases, GR activity dropped at ecodormancy to rise again coinciding with the bud break (Fig. 7A, B).

The evolution of SOD activity was different in both years. During 2017, SOD activity reached its maximum activity at dormancy release in the temperate zone, whereas in the second year, SOD activity was higher at the endodormancy stage, and then a progressive decline occurred (Supplemental Fig. 4A, B). In the cold area, SOD activity peaked at the

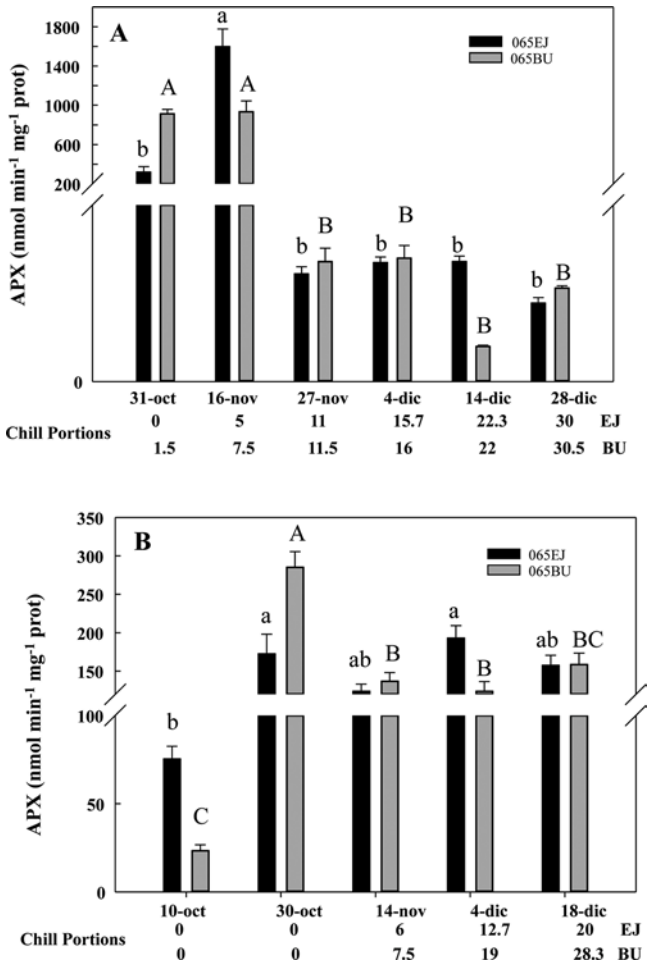

Fig. 4. Evolution of APX activity in flower bud from peach 'GEM 065' cultivated in two different geographical areas during the dormancy cycle on two different years and sampling dates. A: year 1; B: year 2. Different letters (lowercase and uppercase for the samples for the EJ and BU samples, respectively) indicate significant differences according to the Tukey's multiple test ($P \leq 0.05$). EJ, temperate area; BU, cold area. For more information, please consult Fig. 2.

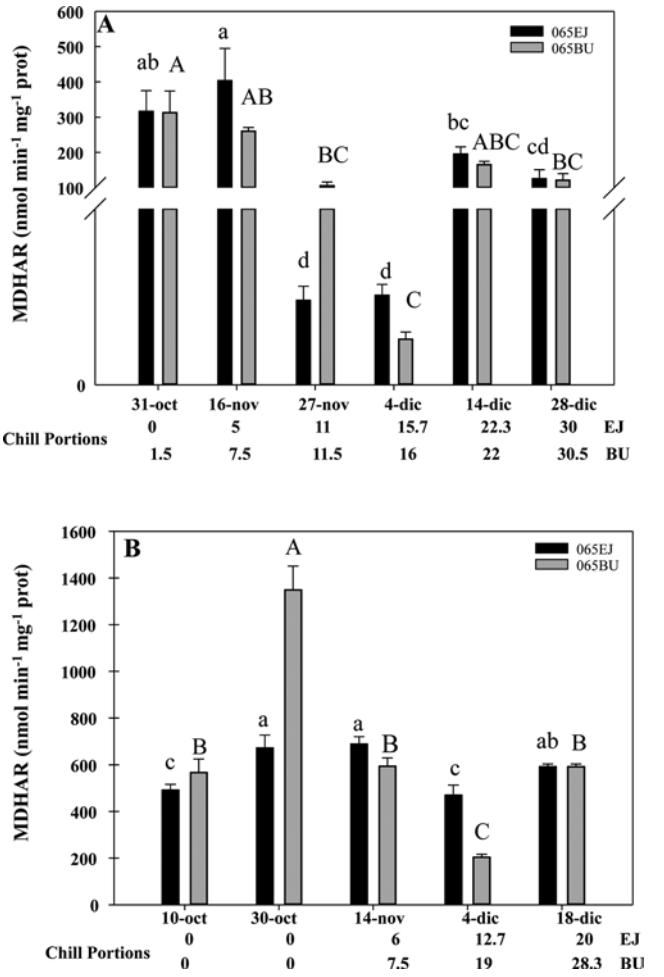

Fig. 5. Evolution of MDHAR activity in flower bud from peach 'GEM 065' cultivated in two different geographical areas during the dormancy cycle on two different years and sampling dates. A: year 1; B: year 2. Different letters (lowercase and uppercase for the samples for the EJ and BU samples, respectively) indicate significant differences according to the Tukey's multiple test ($P \leq 0.05$). EJ, temperate area; BU, cold area. For more information, please consult Fig. 2.

beginning of endodormancy and dormancy release phases, and maintained a lower and constant activity during the rest of the period (Supplemental Fig. 4A). In 2018, SOD activity behaved similarly in the temperate area with respect to 2017, but showing higher activity values than in the first year (Supplemental Fig. 4A, B). Native PAGE revealed the presence of three different SOD isoenzymes in floral buds. According to the results obtained with the selective inhibitors (KCN and H_2O_2), a Fe-containing SOD and two Cu,Zn-containing SODs, named I and II in order of increasing mobility, were present (Fig. 8).

In the temperate zone, POX activity rose at endodormancy and before dormancy release in both years of study, descending just at breaking of bud dormancy (Fig. 9A, B). In the cold area, POX activity resembled to that observed in the temperate zone. In that regards, in year 1, POX activity showed a near 2-fold increase at endodormancy, followed by a progressive decrease until the beginning of ecodormancy, and then the activity remained constant until dormancy release (Fig. 9A). During the year 2, POX activity again increased near 2-fold at endodormancy, then remained constant, followed by an increase of about 3.2-fold just before dormancy release. Finally, POX activity strongly dropped, with a 7-fold decrease in relation to the previous activity data at dormancy release (Fig. 9B). By native PAGE and POX staining, three main bands with POX activity were detected in floral buds during the dormancy period for both locations. However, and coinciding with dormancy release, a new POX isoenzyme was detected, showing lower intensity and higher mobility. In addition, this new isoenzyme was also observed prior to dormancy release (Fig. 10).

CAT activity was difficult to monitor in flower bud samples, and its behavior was totally different in the two years of study (Supplemental Fig. 5). In the temperate zone, during year 1, CAT activity peaked at endodormancy (27th November) followed by a sharp decrease at ecodormance and dormancy release. In the year 2, activity peaked in ecodormancy and decreased at dormancy release. In the cold zone, the highest CAT activity value occurred at the beginning of endodormancy, decreasing until the beginning of ecodormancy. Thereafter, CAT activity progressively increased until dormancy release, but keeping lower values than the initial ones (Supplemental Fig. 5). In the second year, CAT activity was maintained constant in endodormancy and ecodormancy, but a decrease at dormancy release occurred (Supplemental Fig. 5).

3.5. Plant hormones

In the temperate zone and in the first year of study, the most important changes in relation to bioactive GAs levels was a decrease in GA_1, ranging between 3 to 8-fold, from endormancy to dormancy release (Fig. 11A). However, no significant changes were recorded for GA_4. It is

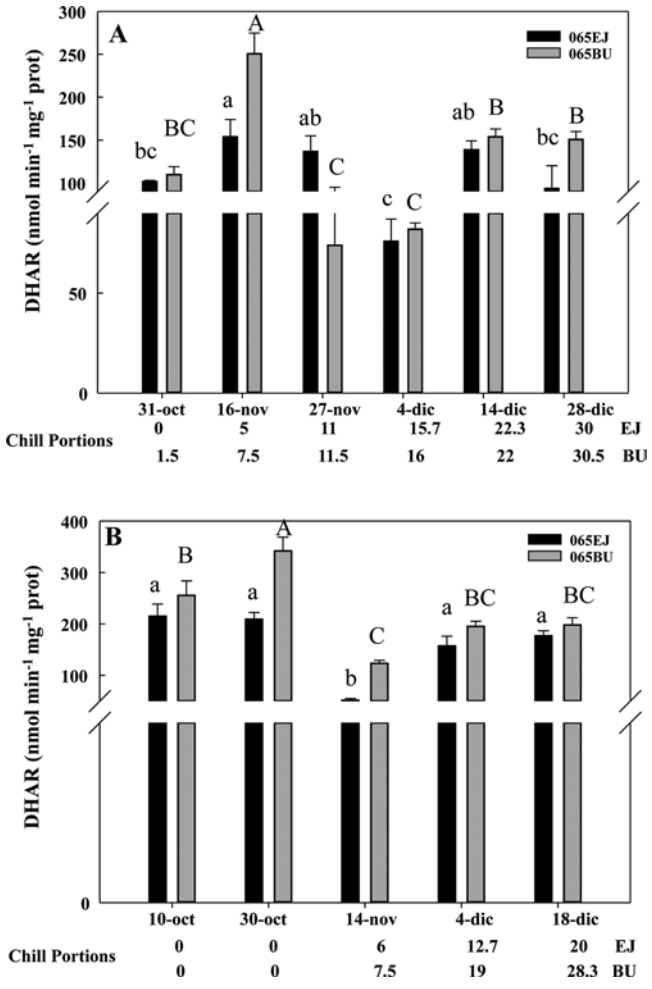

Fig. 6. Evolution of DHAR activity in flower bud from peach 'GEM 065' cultivated in two different geographical areas during the dormancy cycle on two different years and sampling dates. A: year 1; B: year 2. Different letters (lowercase and uppercase for the samples for the EJ and BU samples, respectively) indicate significant differences according to the Tukey's multiple test ($P \leq 0.05$). EJ, temperate area; BU, cold area. For more information, please consult Fig. 2.

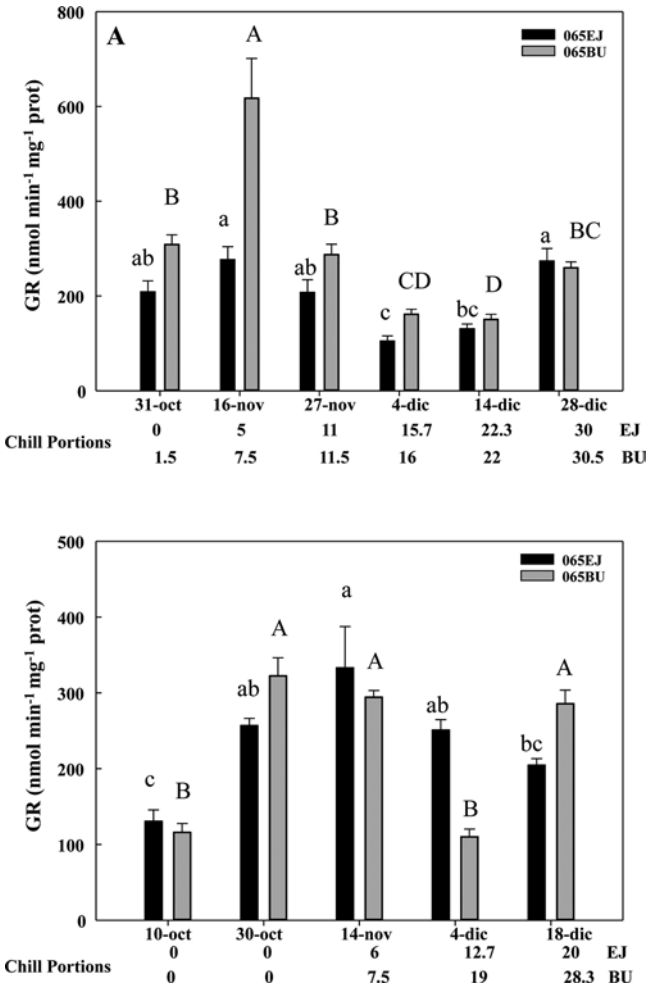

Fig. 7. Evolution of GR activity in flower bud of peach 'GEM 065' cultivated in two different geographical areas during the dormancy cycle on two different years and sampling dates. A: year 1; B: year 2. Different letters (lowercase and uppercase for the samples for the EJ and BU samples, respectively) indicate significant differences according to Tukey's multiple test ($P \leq 0.05$). EJ, temperate area; BU, cold area. For more information, please consult Fig. 2.

interesting to remark that the GA_7 was the most abundant bioactive GA in peach buds in all cases. In 2017, GA_7 levels were high until the end of ecodormancy and dormancy release, when levels dropped near a 50 % (Fig. 11A). During the second year of study, some differences were observed in relation to GA_1 and GA_4 (Fig. 11B). The initial GA_1 contents, unlike the first year, were very low, increasing 5-fold at the end of endodormancy and decreasing again as flower buds approached to dormancy release. GA_4, whose levels were higher than in the first year, behaved similarly to GA_1 levels (Fig. 11B). The most interesting results corresponded to the changes observed in GA_7 levels. This bioactive GA behaved similar than in the first year, and its levels remained constant during endodormancy and decreased at ecodormancy and, especially, during dormancy release (Fig. 11B).

In the cold area, and during the year 1, the GA_1 levels were similar to those observed in the temperate area, showing a peak at the beginning of endodormancy, followed by a strong decrease at dormancy period and constant values thereafter until dormancy release (Fig. 12A). GA_4 did not show pronounced changes, but its levels were statistically higher at the end of endodormancy than at dormancy release (Fig. 12A). GA_7 showed a peak in endodormancy, at 16th November, and then values progressively decreased, reaching the initial values at dormancy release. During the second year of the study, GA_1 contents were unchanged

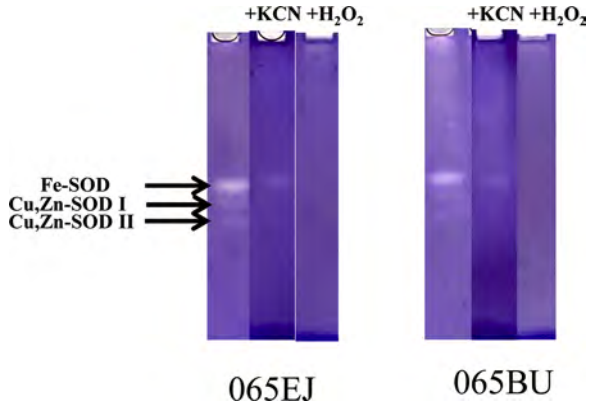

Fig. 8. Pattern of SOD isoenzymes in flower buds of peach 'GEM 065', cultivated in two different geographical areas, by native PAGE. Gels were stained in the presence and in the absence of the selective inhibitors KCN or H_2O_2. Ten micrograms of proteins were loaded in each lane.

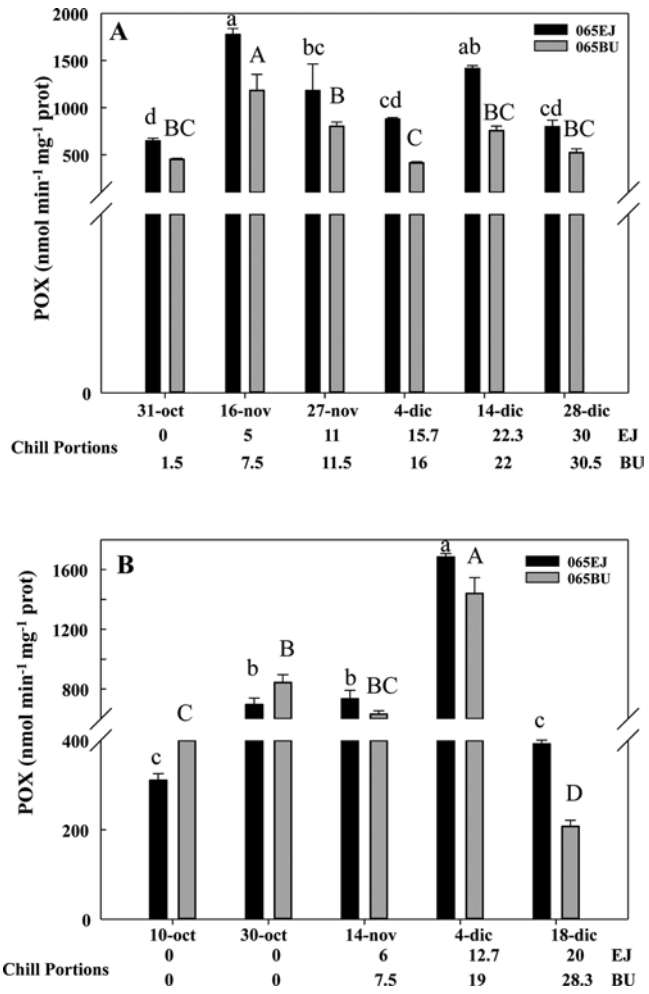

Fig. 9. Evolution of total POX activity in flower bud from peach 'GEM 065' cultivated in two different geographical areas during the dormancy cycle on two different years and sampling dates. A: year 1; B: year 2. Different letters (lowercase and uppercase for the samples for the EJ and BU samples, respectively) indicate significant differences according to the Tukey's multiple test ($P \leq 0.05$). EJ, temperate area; BU, cold area. For more information, please consult Fig. 2.

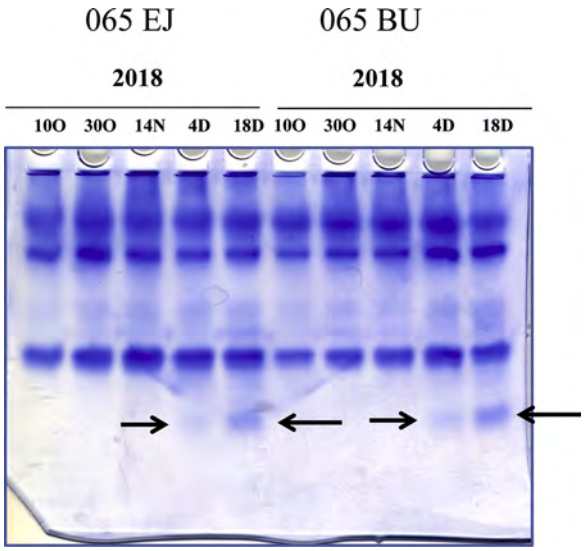

Fig. 10. Pattern of POX isoenzymes in flower buds of peach 'GEM 065', cultivated in two different geographical areas, by native PAGE. Ten micrograms of proteins were loaded in each lane. For more information, please consult Fig. 2.

(Fig. 12B). GA_4 peaked at the beginning of endodormancy, and then values showed a 4-fold decrease that was maintained until dormancy release. Finally, GA_7 levels remained unchanged during the dormancy period and declined at dormancy release (Fig. 12B).

In 2017, at the beginning of endodormancy, ABA concentration was about 3-fold higher in the cold area than in the temperate zone (Fig. 13A). As expected, in both cases, ABA contents progressively declined until dormancy release. During the year 2, the initial ABA content was also higher in the cold area than in the temperate zone (Fig. 13B), and again its levels dropped until dormancy release. As a consequence of these changes, the ABA/total GAs ratio also decreased during the dormancy period in both locations, the lowest values being observed at dormancy release (Fig. 14A, B).

Complementarily, PCA was utilized as a mathematical tool to determine associations among the different variables studied. In the model, SOD, POX and CAT activities, and the minor GAs (GA1 and GA4) were excluded. For the temperate area and in the year 1, the first component (PC1), which explains 43 % of the variability of the experiment (Supplemental Table S1A and Supplemental Fig. 6A), indicated that the greater relevance corresponded to ABA and the ratio ABA/GAs, APX and GR activities and starch, sucrose contents. The second component (PC2), explaining 19.3 % of the variability of the experiment, gives the greater relevance to the sugars glucose, fructose and sorbitol and MDHAR and DHAR activities (Supplemental Table S1 and Supplemental Fig. 6A). During the year 2, the PC1, which explains 45.0 % of the variability of the experiment (Supplemental Table S2 and Supplemental Fig. 6B), indicated that the greater relevance corresponded again to ABA and ABA/GAs ratio, but included also GA7 as well as sorbitol and DHAR activity (Supplemental Table S2 and Supplemental Fig. 6B). The PC2 that explained 15.8 % of the variability gives the greater importance to MDHAR activity, again to the sugars glucose, fructose and sorbitol as well as GA7 contents (Table S2 and Supplemental Fig. 6B). For the cold area, in 2017, the PC1, which explains 37.6 % of the variability of the experiment, indicated that the greater relevance corresponded to ABA and the ratio ABA/GAs, but also the sugars sorbitol, glucose and sucrose and the starch contents (Supplemental Table S3 and Supplemental Fig. 6C). The PC2 that explained 16.8 % of the variability gives the greater importance to GR and APX activities but also to fructose contents (Supplemental Table S3 and Supplemental Fig. 6C). Finally, in 2018, the PC1, which explains 39.1 % of the variability of the experiment (Supplemental Table S4 and Supplemental Fig. 6D), indicated that the greater relevance corresponded again to ABA and the ratio ABA/GAs as well as to APX activity and sorbitol levels. The PC2, explaining 17.5 % of the variability of the experiment, indicated the higher importance corresponded to fructose and glucose contents and the DHAR activity (Supplemental Table S4 and Supplemental Fig. 6D).

4. Discussion

The term bud dormancy applies to the mechanism that woody plants have developed to survive the adverse conditions of winter in temperate and cold climates. In addition, the dormancy onset is of pivotal importance prior to the arrival of the adverse conditions (Campoy et al., 2011). For stone fruit species, dormancy release may be a crucial issue in coming years due to decreased chill accumulation in a context of climate change. This situation may produce a negative effect in flowering quality and uniformity, therefore causing an ominous consequence on fruit production (Campoy et al., 2011; Beauvieux et al., 2018). As an alternative, the use of commercial varieties with low chill requirements, such as the one used in the present study, could partially alleviate the aforementioned fruit production problems.

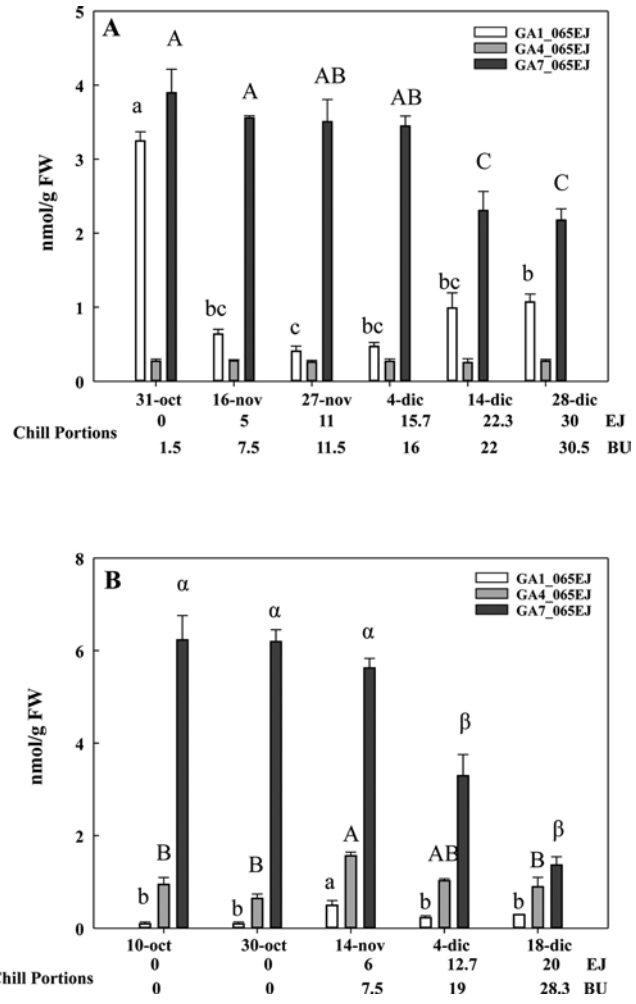

Fig. 11. Evolution of gibberellins (GA_1, GA_4 and GA_7) in flower bud of peach 'GEM 065' cultivated in a temperate zone (EJ). A: year 1; B: year 2. Different letters indicate significant differences according to the Tukey's multiple test ($P \leq 0.05$). (2017: lowercase and uppercase for GA1 and GA7, respectively. 2018, lowercase, uppercase and Greek letters for GA1, GA4 and GA7, respectively. Absence of letters mean not-significant differences. For more information, please consult Fig. 2.

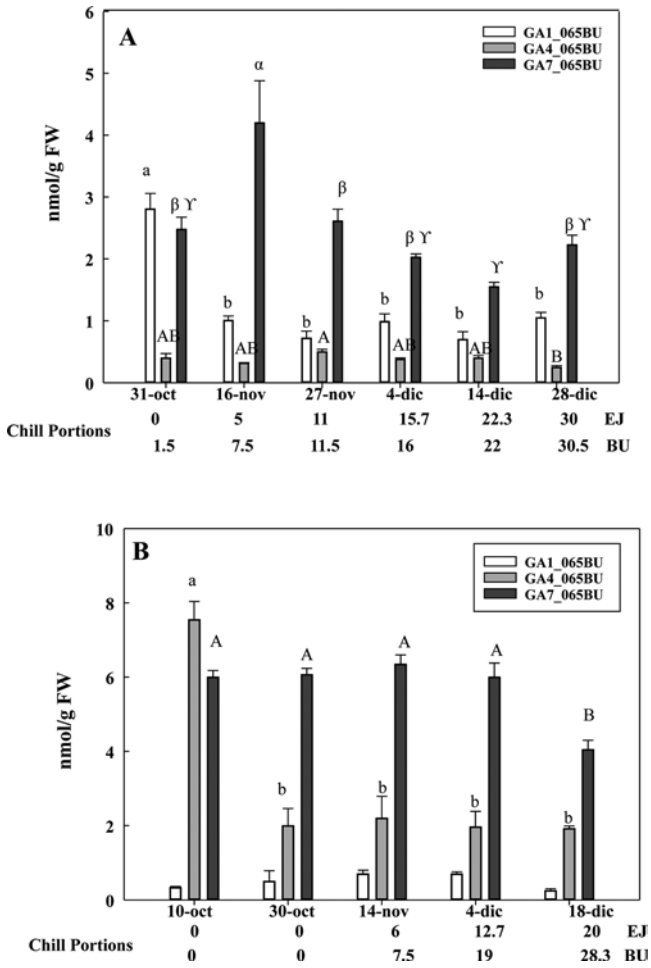

Fig. 12. Evolution of gibberellins (GA1, GA4 and GA7) in flower bud of peach 'GEM 065' cultivated in a cold zone (BU). A: year 1; B: year 2. Different letters indicate significant differences according to the Tukey's multiple test ($P \leq 0.05$). (2017: lowercase, uppercase and Greek letters for GA1, GA4 and GA7, respectively. 2018, lowercase and uppercase for GA4 and GA7, respectively. Absence of letters means not-significant differences. For more information, please consult Fig. 2.

4.1. Histological study

Different authors reported that anthers enter microsporogenesis after dormancy in apricot (Julian et al., 2011) and peach (Ríos et al., 2013). Accordingly, microspores were observed in the anthers of the low chilling peach 'GEM065' at dormancy release (end of December). In the coldest location, the development of the anthers was faster than in the warm location, and pollen grains appeared at the middle of December.

4.2. Carbohydrate metabolism

During the arrest period, flower buds need to accumulate reserve carbohydrates (starch and sugars) that allow them to start their growth once buds reach a minimum of chilling requirements. In that regards, carbohydrate metabolism seems to have a role in the control of bud growth and development during dormancy and dormancy release (Kaufmann and Blanke, 2017). In general, starch accumulates during endodormancy in species with high chill requirements, and then gradually decreases from dormant bud to dormancy release. This occurred in sweet cherry and in walnut buds (Gholizadeh et al., 2017; Kaufmann and Blanke, 2017, Fadón et al., 2018), and this response correlated with an increase in soluble sugars (Gholizadeh et al., 2017, Kaufmann and Blanke, 2017).

In the present work, carried out with a low chill peach variety, the starch evolution behaved different in the two years of study. In the year 1, starch contents remained constant in the flower buds during endodormancy and ecodormancy, showing an increase at dormancy release in the temperate area. In the cold area, starch content reached its maximum levels at the end of endodormancy, maintaining its levels until dormancy release. In the year 2, we observed higher initial starch levels than in the first year at both locations. In 2018, flower buds reached the dormancy release earlier and with lower accumulation of chill portions than in 2017. Probably, this different behavior could be influenced by the starch contents accumulated in the flower buds, that was much higher in the second year of the experiment. The evolution of the starch levels that we observed in year 2 is similar to that described in a variety of walnut with low chill requirements (Gholizadeh et al., 2017). These authors also observed the highest starch levels at the beginning of dormancy, followed by a progressive decrease until dormancy release. In Japanese pear, the starch concentration in stems reached its maximum at the beginning of dormancy process, and them a progressive decline occurred until endodormancy release (Ito et al., 2012).

In relation to soluble sugars content, sorbitol was the more abundant soluble sugar in peach buds, mainly in the cold area. In pear, apple and sweet cherry buds, sorbitol was the major metabolizing sugar (Ito et al.,

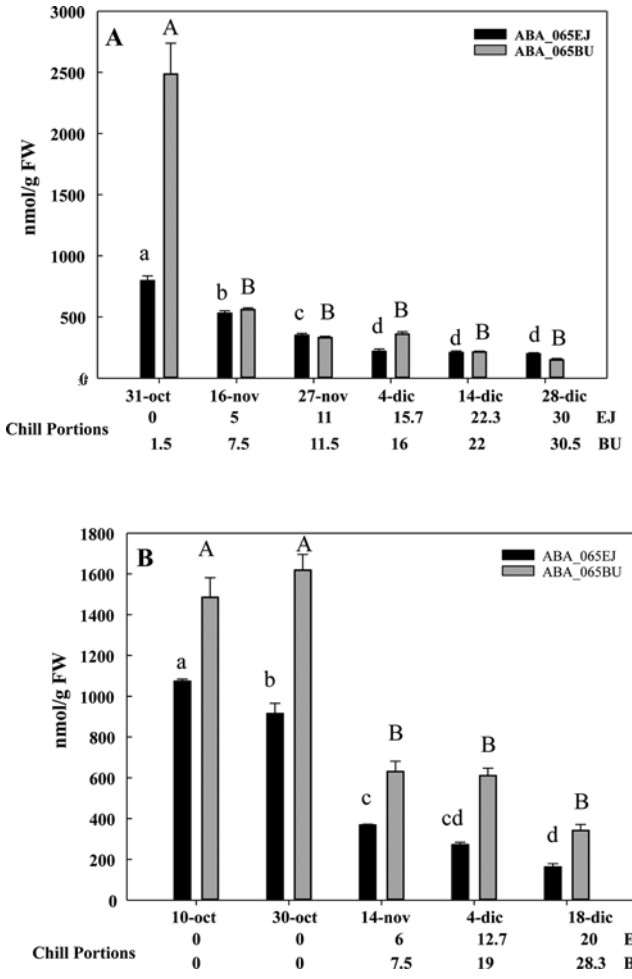

Fig. 13. Evolution of ABA in flower bud of peach 'GEM 065' cultivated in two different geographical areas during the dormancy cycle on two different years and sampling dates. A: year 1; B: year 2. Different letters (lowercase and uppercase for the samples for the EJ and BU samples, respectively) indicate significant differences according to Tukey's multiple test ($P \leq 0.05$). EJ, temperate area; BU, cold area. For more information, please consult Fig. 2.

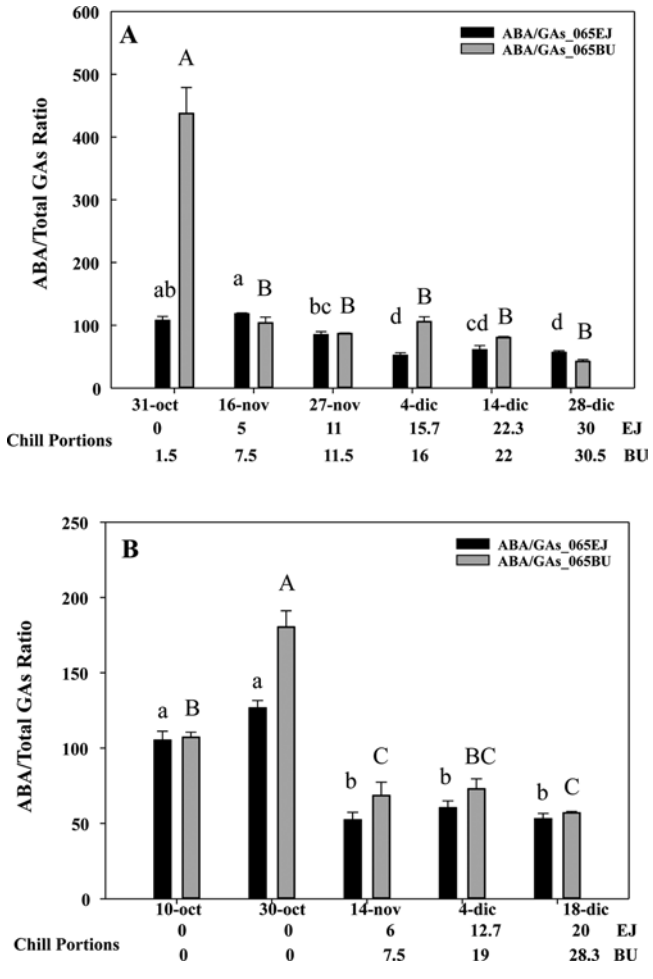

Fig. 14. Evolution in the ABA/total GAs in flower bud o peach 'GEM 065' cultivated in two different geographical areas during the dormancy cycle on two different years and sampling dates. A: year 1; B: year 2. Different letters (lowercase and uppercase for the samples for the EJ and BU samples, respectively) indicate significant differences according to Tukey's multiple test ($P \leq 0.05$). EJ, temperate area; BU, cold area. For more information, please consult Fig. 2.

2012, Rady and Seif El-Yazal, 2013, Kaufmann and Blanke, 2017), and it has been also reported that the enzyme sorbitol dehydrogenase was up-regulated during endodormancy release (Ito et al., 2012; Rady and Seif El-Yazal, 2013). As occurred for the starch contents, the initial sorbitol concentration in bud flower was also higher in 2018 than in 2017, especially during the endodormancy period. From a biochemical point of view, sorbitol has advantages over sucrose as a translocable and storage sugar, including its important role in osmotic adjustment in cell cytoplasm under stress conditions (Kanayama, 2009), being able to stabilize membranes. This suggests that sorbitol may participate in plant protection during winter by enhancing tolerance to chilling stress (Ito et al., 2013). In addition, the levels of glucose, fructose and sorbitol were statistically higher in the cold zone than in the temperate zone (F values 5.49; 71.52 and 11.61, respectively). This response could be attributed to an acquisition of cold hardening or to meet the demand of carbon under cold temperatures, as has been described in other plant species (Gholizadeh et al., 2017, Kaufmann and Blanke, 2017). It has been reported that glucose and fructose levels correlated with the chill accumulation in *Prunus* ssp (González-Rossia et al., 2008). In stem cutting from apricot, peach, nectarine and plum, glucose contents significantly increased in the bark tissue by the effect of artificial chilling. A similar response has been observed in peach flower buds. In that regards, in the present work and in the two years of study, glucose levels increased from endodormancy to dormancy release in both locations.

4.3. Antioxidant metabolism

The analysis of the antioxidants metabolism during the transition endodormancy-dormancy release in flower buds is scarce, especially in flower buds from fruit stone trees, as peach. Different authors reported that sub-lethal oxidative stress can be involved in the bud dormancy release, and increased H_2O_2 levels may activate the bud burst acting as a signaling molecule triggering this process. (Pérez and Burgos, 2004; Kuroda et al., 2005; Prassinos et al., 2011; Sudawan et al., 2016; Beauvieux et al., 2018). Different evidences support this fact. For example, the inhibition and/or decrease of H_2O_2-scavenging enzymes, such as CAT, increased H_2O_2 levels and correlated with bud-break induction in different plant species (Pérez and Burgos, 2004; Takemura et al., 2015; Sudawan et al., 2016). In that regards, we also observed decreased CAT levels at dormancy release in both locations.

Additionally, the up-regulation or accumulation of ROS-scavenging enzymes after or at dormancy release stage suggests the need to eliminate ROS to avoid ROS toxicity (Prassinos et al., 2011; Takemura et al., 2015; Sudawan et al., 2016). Takemura et al. (2015) observed a non-significant increase in H_2O_2 concentration in pear floral buds in the late-breaking period (at 96 % of bud break) parallel to a significant

decline in CAT activity. Interestingly, this small increase in H_2O_2 levels led to an important increase in APX activity, an enzyme involved in the fine control of H_2O_2 contents. These results suggested that transition from to dormancy release might involve the removal of ROS (especially H_2O_2) by using reduced ascorbate coupled to APX activity (Takemura et al., 2015). However, Hussain et al. (2015) observed an inverse correlation between H_2O_2 contents and APX and POX activities in pear floral buds. These authors reported an increase in H_2O_2 during dormancy period but a decrease at bud break stage, accompanied by an increase in APX activity and, to a lesser extent, in POX activity (Hussain et al., 2015). The maintenance and / or increase of some antioxidant enzymes was also observed in the present work. In general, we observed the highest antioxidant activities at endodormancy period. That was the case for the ASC-GSH cycle enzymes and POX. In addition, MDHAR, DHAR and GR also showed increases at pre-breaking and/or dormancy release phases. These results suggested that at both endodormancy and dormancy release periods a controlled oxidative stress may occur. In addition, we observed the up-regulation of a minor POX isoenzyme at dormancy release in both locations, suggesting a possible role in the dormancy release process and its use as a marker for monitoring dormancy release at least in low chill peach varieties. These result agree with a recent work showing increased expression of a gene encoding for a Class III peroxidase (named *PdP40*) before endodormancy release in flower buds from different almond species displaying different chilling requirements (Prudencio et al., 2019). In parallel, endodormancy release was preceded by an increase in total POX activity (Prudencio et al., 2019). Taken into consideration these results, the authors proposed the *PdP40* gene, and therefore POX activity, as markers for monitoring bud dormancy in almond as well as in other *Prunus* species. In dormancy-released peach buds, Leida et al. (2010) found the up-regulation of different peroxidase-like genes, suggesting also a role for H_2O_2 in the process of bud-breaking regulation.

In lateral buds from low chill walnut trees, Gholizadeh et al. (2017) analysed the evolution of NADPH oxidase, SOD, soluble and cell wall-bound POX and APX activities as well as H_2O_2 contents and lipid peroxidation. They observed that SOD and APX progressively increased, reaching its highest values at dormancy release. NADPH oxidase also showed a gradual increase, but a significant decline was produced just at dormancy release. In contrast, both soluble and cell wall-bound POX activity progressively dropped. As a consequence of the changes in the antioxidant defenses, H_2O_2 contents and lipid peroxidation in the lateral buds decreased at dormancy release. According to the authors, the lack of oxidative stress at dormancy release could be mediated by the decrease in NADPH oxidase [superoxide ($O_2^{\cdot-}$)-generating enzyme] as well as the important increase in APX activity (H_2O_2-scavenging enzyme) in this period. The increase in APX has been interpreted as a transition from endodormancy to ecodormancy (Takemura et al., 2015), and even to dormancy release (Gholizadeh et al., 2017). These authors proposed that redox interactions could trigger bud dormancy release, suggesting that this period requires an oxidative signaling networking, the cultivars with lower chilling requirements being able to develop and react to this oxidative signalling earlier that cultivars with higher chilling needs (Gholizadeh et al., 2017). This finding, regarding APX and POX activities, contrasts with our results. In the present work, we observed that APX and POX activities declined from endodormancy to dormancy release. We suggest that this response could produce a H_2O_2-accumulation triggering the oxidative signaling required for growth, development and differentiation during dormancy release in peach buds.

In our work, we detected that floral peach buds contained much more MDHAR than DHAR activity, both of them involved in the ascorbate recycling. That finding suggested that peach buds mainly uses NADH to recycle reduced ascorbate, which is much more efficient, from an energy point of view for the plant, than the use of GSH (Acosta-Motos et al., 2019).

Surprisingly, flower buds from peach contained a Fe-SOD isoenzyme but no Mn-SOD isoenzyme. Fe-SOD has been located in chloroplasts (Hernández et al., 1999), whereas Mn-SOD has a mitochondrial and peroxisomal location (Hernández et al., 1999). However, in carnation petals Fe-SOD was also found in mitochondria and peroxisomes (Droillard and Paulin, 1990). Fe-SOD and Cu,Zn-SOD isoenzymes are inhibited by H_2O_2 whereas Mn-SOD isoenzymes are H_2O_2-resistant (Hernández et al., 1999). It has been reported that the regulation of redox and oxygen metabolism is critical to organ development (Considine and Foyer, 2014; Meitha et al., 2015), and a sub-lethal oxidative stress could be involved in the bud dormancy release. Probably, the presence of H_2O_2-sensitive antioxidant enzymes, including Fe-SOD and Cu,Zn-SOD, besides APX and POX, could help to create a situation of controlled oxidative stress (i.e., increased $O_2^{\cdot-}$ accumulation) that could be involved in dormancy release. This hypothesis is supported by the mentioned putative role for oxidative signaling in dormancy release. Therefore, it is likely that peach blossoms may also contain Fe-SOD, as reported in carnation (Droillard and Paulin, 1990). Fe-SODs are considered as enzymes of prokaryotic origin, although they have also been identified in nine plant families: *Brassicaceae, Ginkgoaceae, Nymphaeaceae, Solanaceae, Fabaceae, Cariophylaceae, Rubiaceae, Chenopodiaceae* and *Rutaceae* (Bridges and Salin, 1981; Kwiatowski et al., 1985, Almansa et al., 1994; Kurepa et al., 1997; Hernández et al., 1999). Now, we can add the *Rosaceae* family to the list of higher plants containing Fe-SOD isoenzymes.

4.4. Plant hormones

The central role of ABA and GAs in seed dormancy regulation is well known (Chahtane et al., 2017), but their role in bud dormancy is more uncertain. Different studies support a function for ABA in the induction of bud endodormancy (Zheng et al., 2015, 2018b, Ruttink et al., 2007). By using a transgenic approach, Zheng et al. (2018b) demonstrated that endogenous ABA inhibited dormancy release. The overexpression of *Vv8H-CYP707A4* gene, involved in ABA catabolism, significantly enhanced bud break in grapevine (Zheng et al., 2018b). In different plant species, including apple, pear and grape, endogenous ABA was higher during endodormancy induction, but during dormancy release its levels declined (Seeley and Powell, 1981; Li et al., 2018; Ito et al., 2019, Zheng et al., 2015). This finding agrees with our results, in which elevated ABA levels were found at endodormancy, but they were significant reduced at dormancy release. In addition, in the cold zone, ABA accumulation was much more important than in the temperate zone, suggesting an effect of the geographical location on ABA accumulation at endodormancy stage.

The effect of GAs in breaking dormancy is still uncertain, and both stimulating and inhibitory effects have been reported (Ionescu et al., 2017). In a recent work, Zheng et al. (2018a) noticed the decline in the level of *VvGA3ox*, *VvGA20ox* and *VvGASA2* transcripts, all of them involved in GA metabolism, during the induction of natural dormancy in grapevine. Additionally, and during dormancy release, the expression of those genes was induced, and in parallel, a decline in *VvGA2ox* gene expression, involved in GA deactivation, took place. These results suggested a role for bioactive GAs in dormancy release of grapevine floral buds (Zhang et al., 2018a). In contrast to the aforementioned results, the direct application of GA had inhibitory effects in bud break in grapevine and persimmon (Zhang et al., 2018; Kang et al., 1998). Nevertheless, it seemed that GA effects on dormancy release depended on bud dormancy status (Zhang et al., 2018a). In other plant species, such as peach and apricot, GA application increased bud break (Donoho and Walker, 1957; Zhuang et al., 2013). In Japanese pear buds, GA_4 levels were higher at the induction of endodormancy, but decreased at the end of endodorrmancy, remained low at ecodormancy and gradually rose as the flowering period approached (Ito et al., 2019).

According to our results, we suggest that GAs were not the key plant hormones in dormancy release in low chill peach. In both locations, GA_7 was the major bioactive GA, whose levels were always higher at

endodormancy stage than at dormancy release phase. Similarly, GA_4 and GA_1, being the minor GAs, did not increase at dormancy release stage in any year of the study.

The clear decline in ABA contents in peach buds correlated with a decrease in the ratio ABA/total GAs, but mainly due to the drop of ABA. Our results are in agreement with those observed in sweet cherry and in *Prunus mume* (Duan et al., 2004; Zhang et al., 2018). These authors reported a raise in ABA/GA_3 ratio in flower buds during natural dormancy induction, but a decline at dormancy release. Thus, we suggest that the decrease in ABA levels, as well as in the ABA/GAs ratio, may be necessary for the bud dormancy release in peach, at least in low chill varieties.

The results obtained by PCA confirmed the importance of ABA and the ABA/GAs ratio for the dormancy process, but also of the sugars glucose and sorbitol and the ASC-GSH cycle enzymes, for both locations and experimental years.

Our results agree with a recent molecular approach carried out in peach and apricot floral buds (Yu et al., 2020). The study of the transcriptomic profiles from dormant buds, revealed that both ROS-responsive genes and genes involved in the metabolism and signaling of plant hormones are involved in the endodormancy to ecodormancy transition (Yu et al., 2020).

5. Conclusions

Both starch and sorbitol contents in flower buds were higher in the second year of the experiment (2018) than in the first year (2017) in both location. This could be related to the fact that peach buds broke the dormancy in 2018 with lower chill portions accumulation than in 2017. Sorbitol was one of the more abundant soluble sugars in peach floral buds. In addition, the levels of glucose, fructose and sorbitol were statistically higher in the cold zone than in the temperate zone. This response could be attributed to an acquisition of cold hardening or to meet the demand of carbon under cold temperatures, as it has been described in other plant species (Gholizadeh et al., 2017, Kaufmann and Blanke, 2017). In relation to the latter, glucose levels correlated with the chill accumulation in both locations and studied seasons. In the present work, in general, we observed increased antioxidants activities in endodormancy. In addition, MDHAR, DHAR and GR also showed increases in pre-breaking and/or dormancy release phases. These results suggested that at both endodormancy and dormancy release, a controlled oxidative stress may occur. The up-regulation of a minor POX isoenzyme at dormancy release in both locations was observed, suggesting a possible role in the dormancy release process and the possibility of being used as marker for monitoring dormancy release at least in low chill peach varieties.Peach floral buds contains the H_2O_2-sensitive Fe-SOD and Cu,Zn-SOD isoenzymes but not the H_2O_2-resistant Mn-SOD. In this sense, the presence of H_2O_2-sensitive antioxidant enzymes could lead to the establishment of a sub-lethal oxidative stress involved in dormancy release. Finally, and according to the results observed in plant hormones, we can suggest that the decrease in ABA levels and in ABA/GAs ratio may be necessary for the bud dormancy release in peach, at least in low chill varieties.

CRediT authorship contribution statement

José A. Hernandez: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Pedro Díaz-Vivancos:** Conceptualization, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Gema Martínez-Sánchez:** Investigation, Methodology, Resources. **Nuria Alburquerque:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Domingo Martínez:** Investigation, Methodology, Resources. **Gregorio Barba-Espín:** Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **José Ramón Acosta-Motos:** Data curation, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Esther Carrera:** Investigation, Methodology, Resources. **Jesús García-Bruntón:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.scienta.2021.109957.

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