Vitis **58** (Special Issue), 95–101 (2019) DOI: 10.5073/vitis.2019.58.special-issue.95-101

Forcing vine regrowth to delay ripening and its association to changes in the hormonal balance

A. Pou[1)], P. Balda[2)], A. Albacete[3)] and F. Martínez de Toda[1)]

[1)] Departamento de Viticultura, Instituto de Ciencias de la Vid y del Vino (Gobierno de la Rioja, CSIC, Universidad de La Rioja), Logroño, La Rioja, Spain
[2)] Departamento de Fisiología Vegetal, Universidad de les Illes Balears (UIB), Palma, Illes Balears, Spain
[3)] Departamento de Nutrición Vegetal, Centro de Edafología y Biología Aplicada del Segura, Murcia, Spain

Summary

The quality and typicality of wines, strongly depends on the management techniques used for grapevine cultivation. Actually, the increment in the average world temperature due to climate change induces not only bigger irrigation necessities, but also earlier grape-ripening processes, which take place in warmer days and shorter nights. Thus, with the aim of delaying grape ripeness of at least two months, a technique has been proposed based on forcing vine regrowth. This technique consists on forcing vine regrowth from the formed latent buds after cutting the green shoots between the second and the third node; lateral shoots, leaves, and primary clusters are also removed. In this study, a forcing treatment was carried out at three different phenological stages (G, I and J). Depending on the phenological stage of vines during the forcing treatment, we wanted to determine the berry ripening delay and to explore how this mechanical pruning interacts with the hormonal balance to modulate bud growth just before shoot decapitation (Control) and later, within the following 7 and 14 days after cutting the green shoots. Forcing treatments carried out at stages G, I and J succeeded to delay ripening 18, 27 and 45 days respectively, as compared to unforced plants. Vine yield was significantly reduced in all treatments as compared to control plants, resulting in a high level of acidity in berries which might be associated with the loss of flowers, a reduction in the fruit set percentage or a combination of both. Endogenous cytokinin (CK) content in control latent buds decreased during the vine vegetative cycle. Contrarily, abscisic acid (ABA) and Jasmonic acid (JA) increased, while minor changes were found in the concentration of gibberellins (GAs), salicylic acid (SA) and the ethylene precursor 1-aminocyclopropane-1-carboxilic acid (ACC). Moreover, a clear modification of the hormonal balance was found in latent buds 7 and 14 days after forcing regrowth. CK content significantly increased while ABA rapidly decreased after pruning in all treatments. Thus, vine regrowth from the formed latent buds might have been upregulated by CK and promoted by the absence of ABA.

K e y w o r d s : climate warming; grapevine; delayed maturation; hormonal balance.

Introduction

Global climate change is projected to produce warmer temperatures, as well as longer and more frequent droughts in many regions of the world (IPCC 2014). In viticulture, climate change has the potential to greatly influence the suitability of a region for grapevine cultivation and the production of wine, since it may advance the harvest period. Indeed, changes in grapevine phenology during the past decades have been reported for several grape-growing areas (Duchêne and Schneider 2005, Petrie and Sadras 2008, Ramos *et al.* 2008). Such effects can trigger early maturation of grapes, which will have to deal with a warmer ripening period that entails higher acid degradation during the night and higher accumulation of sugars in the fruit during the day. This results in musts with lower anthocyanin content and lower acidity, which produce unbalanced wines with a lack of colour, less freshness and, therefore, of lower quality (Jackson and Lombard 1993, Mateus *et al.* 2002, Hannah *et al.* 2013).

Various management techniques have been proposed for delaying grape ripening, such as late winter pruning (Zheng *et al.* 2017a), shoot trimming (Martínez de Toda *et al.* 2014, Zheng *et al.* 2017b) and minimal pruning (Zheng *et al.* 2017c). Each of these techniques allows delaying the ripening of the grape between 15 and 20 d. However, considering the increase in temperatures that has occurred in these recent years and the foreseeable increase that is projected to come in the following years, the effects of the already mentioned techniques, may not be enough to counteract the global warming in certain areas. In this sense, and aiming to delay grape maturation for at least two months, several forcing techniques have been proposed conducted by hedging, growing shoots to several nodes and removing summer laterals and leaves (Gu *et al.* 2012, Martínez de Toda *et al.* 2019). Under conventional conditions, the formation of clusters for next year's crop begins concurrently with the formation of leaf primordia within the compound

Correspondence to: Dr. A. Pou, Departamento de Viticultura, Instituto de Ciencias de la Vid y del Vino (Gobierno de la Rioja, CSIC, Universidad de La Rioja), Finca La Grajera, Carretera de Burgos, km 6, 26071 Logroño, La Rioja, Spain. E-mail: alicia.pou@icvv.es

bud. The differentiation of flowers on the cluster primordium begins after the dormant compound buds are activated in the spring. However, during the first phase of bud predormancy, while the primary shoots of the grapevine are green and actively growing, compound buds can be forced to break up during the current season, as they are not fully dormant and do not require chilling. In order to force bud break and the subsequent shoot regrowth, the source of inhibition played by shoot tips, lateral shoots, and/or leaves (Lavée and May 1997) needs to be physically removed by means of release of the apical dominance (Cline 1994). Thus, following shoot decapitation, initial bud outgrowth will take place within a few hours or longer depending on the specie and upon the degree of inhibition and the stage of the cell cycle at the time of inhibition (Tamas 1987). In the days following decapitation, subsequent elongation and development of the lateral bud into a new lateral shoot occur. Concretely, in grapevines, Gu *et al.* (2012) and others (Zheng *et al.* 2017a, Petrie *et al.* 2017, Martínez de Toda *et al.* 2019), described shifted phenological development of up to 2 months when performing forcing treatments, which caused delayed fruit ripening in the cooler portion of the growing season, thus, with more suitable temperatures for ripening. As expected, forced vines gave smaller berries and their juice showed a lower pH, higher acidity, and higher contents of anthocyanins, tannins, and total phenolics, compared to non-forced vines.

These foregoing developmental steps are differently affected by plant hormones. Plant hormones most commonly associated with growth promotion of lateral buds are gibberellic acid (GA_3) and cytokinins (CKs), while those associated with inhibition are abscisic acid (ABA) and auxins, mainly indoleacetic acid (IAA). The IAA-CK balance may regulate the degree of expression of apical dominance in intact plants, and may also be involved in bud growth responses to shoot decapitation (Sachs and Thimann, 1967, Srinivasan and Mullins 1981, Mader *et al.* 2003). Indeed, some studies have demonstrated a release of the apical dominance by direct application of CK to the lateral bud (Pillay and Railton 1983, Turunbull *et al.* 1997) or a repression by auxin treatment of the decapitated stump just above the lateral bud (Thimann and Skoog 1934). However, it appears that this model in its simple form fits under certain defined physiological circumstances, but not in others, so, it should be also taking into account the complexity of processes contributing to axillary bud release (Cline 1991) and the contribution of other hormones related with inhibition of bud growth (Gocal *et al.* 1991). In this context, few studies have considered the hormonal balance of the buds with simultaneous measurements of other correlative factors or potential alterations involved in lateral bud growth. Moreover, information on endogenous hormones is especially difficult to obtain in grapevine. Indeed, most of the conclusions on the role of hormones in bud growth have been based on inferences from the effects of exogenous hormones and growth regulators. Therefore, with this in mind, the present study aims at investigating changes in the hormonal balance of grapevine during bud break after three shoot decapitation processes in different stages of vine growth. Accordingly, the dominant shoot was decapitated at stages G, I and J (according to the Baillod and Baggiolini 1993 system) and CKs, gibberellins (GAs), ABA, IAA, jasmonic acid (JA) and salicylic acid (SA) were carefully monitored on a time-scale from 0 h to 14 d following the decapitation.

Material and Methods

Plant material: The experiment was conducted in the experimental field of the University of the Balearic Islands (Mallorca, Spain) on grapevine plants of 'Tempranillo' during the summer of 2017. Plants were 7 years old grafted on Richter-110 and planted with a spacing of 2.5 m (between rows) and 1 m (within rows). Vines were trained in a bilateral double cordon having between 7 and 8 shoots per plant. Drip irrigation was applied to all the treatments with an average amount of 5 $L \cdot vine \cdot day^{-1}$ from the beginning of June, when about 70 % of the control shoots ceased growing, until the end of August.

Forcing treatments: Forcing consisted of trimming green shoots to two nodes and removing, when developed, the summer lateral shoots. Forcing treatments were applied on 25 May (stage G; treatment G), 15 June (stage I; treatment I) and 25 June (stage J; treatment J) according to Baillod and Baggiolini system (1993) and compared to vines grown under conventional practices (Control). Each treatment was set out in randomized blocks with six replicates, each of a single vine, for vine yield and berry composition evaluation and another six replicates for hormonal analysis.

Hormonal response to decapitation: For this experiment, six plants were trimmed to two nodes at each previously described stage. Thus, a total of 18 plants was used for hormonal analyses. Within each forcing date, between 2 and 4 buds from node 1 were excised just before shoot decapitation (0 h; control) and snap-frozen in liquid nitrogen. Later on, 7 and 12 d after shoot decapitation (treatments 7 and 12 d, respectively), other 2 to 4 buds per plant were excised following the same procedure as before. Through this, six replicates per sampling date were obtained from different plants and each sample comprised between 2 and 4 buds. Control buds permitted to evaluate the normal changes occurring in hormonal content along time. Plant material was then ground to a powder using a Mixer Mill MM200 tissue homogenizer (Retsch, Haan, Germany) and weighed into a 2 mL vial.

Active cytokinins (trans-zeatin, TZ, zeatin riboside, ZR and isopentenyl adenine, iP), gibberellins (GA1, GA3 and GA4), indole-3-acetic acid (IAA), abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA) and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) were analysed according to Albacete *et al.* (2008) with some modifications. Briefly, 100 mg of homogenized plant material was dropped in 1 mL of cold (-20 °C) extraction mixture of methanol/water (80/20, v/v). Solids were separated by centrifugation (20,000 g, 15 min) and re-extracted for 30 min at 4 °C in an additional 1 mL of the same extraction solution. Pooled supernatants were passed through Sep-Pak

Plus †C18 cartridge (SepPak Plus, Waters, USA) to remove interfering lipids and part of plant pigments and evaporated at 40 °C under vacuum either to near dryness or until organic solvent was removed. The residue was dissolved in 0.5 mL methanol/water (20/80, v/v) solution using an ultrasonic bath. The dissolved samples were filtered through 13 mm diameter Millex filters with 0.22 µm pore size nylon membrane (Millipore, Bedford, MA, USA).

Ten µL of filtrated extract were injected in a U-HPLC-MS system consisting of an Accela Series U-HPLC (ThermoFisher Scientific, Waltham, MA, USA) coupled to an Exactive mass spectrometer (ThermoFisher Scientific, Waltham, MA, USA) using a heated electrospray ionization (HESI) interface. Mass spectra were obtained using the Xcalibur software version 2.2 (ThermoFisher Scientific, Waltham, MA, USA). For quantification of the plant hormones, calibration curves were constructed for each analysed component (1, 10, 50, and 100 $\mu g \cdot L^{-1}$) and corrected for 10 $\mu g \cdot L^{-1}$ deuterated internal standards. Recovery percentages ranged between 92 and 95 %.

Vine yield and berry composition: Grapes from all the treatments were harvested and analysed at the same total soluble solids (TSS) level (22-23 °Brix, which is a common range for commercial grapes in the region). At this time, fruit composition was evaluated using a sample of 200 berries per plant. The berries were crushed and the juice analysed for pH, soluble solids (°Brix) and titratable acidity (TA) according to the OIV (1990) procedures. At harvest, the number of clusters and their total weight per vine were recorded on six previously selected vines per treatment. The time of harvest was recorded for each replicate.

Statistical analysis: Statistical differences between means were assessed by one-way analysis of variance (ANOVA). When differences were significant, a multiple comparison of means post hoc Duncan ($P < 0.05$) was performed with SPSS 22.0 (IBM Corp., Armonk, NY, USA).

Results

Effects of forcing treatments timing on bud hormonal responses: The changes occurring in control buds (obtained before shoot decapitation; time 0 h) from preflowering (end of April) onwards were accompanied by an increase in the content of ABA and JA and a decrease of active CKs (Figure). Less changes were found between control buds (*i.e.* sampled along time) in the amounts of GAs, SA and the ethylene precursor ACC (Figure, 0 h). Auxin concentration was not detected in buds in any of the treatments (data not shown).

Moreover, hormone concentration in buds at 7 and 14 d after shoot decapitation was quantified within each treatment. Depending on the forcing date, the resulted hormone concentrations vary over time (Figure).

CKs content significantly increased in all the established treatments after 7 d of forcing vine regrowth. However, after 14 d of forcing, CKs concentration significantly decreased for treatments G and I, while continued to increase in treatment J (Figure). By contrast, ABA concentration was significantly reduced after 7 d of forcing as compared to control and remained low after 14 d for all the treatments (Figure).

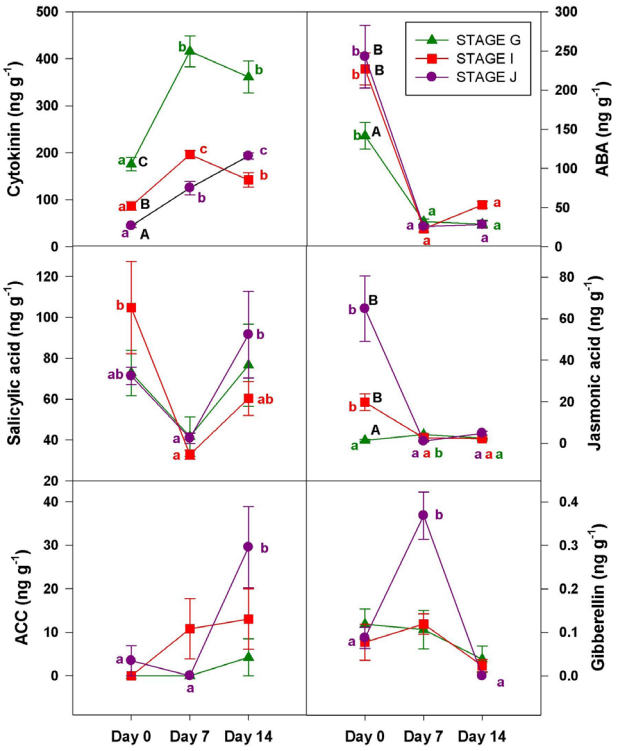

Figure: Cytokinin, abscisic acid (ABA), Salicilic acid, Jasmonic acid, ACC (1-aminocyclopropane-1-carboxylic acid) and Gibberellin concentrations in buds of forced plants at 0 h, 7 d and 14 d after forcing treatment. Different shoot trimming treatments were done at Stage G (in green), Stage I (in red) and Stage J (in purple). Data are mean of six plants ± SE. Means that do not share the same lower case letter are significantly different when comparing within the same shoot trimming treatment throughout the experiment ($P < 0.05$). The different capital letters in day 0 relates to the differences between unforced buds within the different phenological stages.

In the same way as ABA, after 7 d of forcing, bud hormonal concentration of JA was significantly reduced in treatments I and J but increased in treatment G. This reduction seems to be less important as the vegetative cycle advanced until reaching a turning point in stage G. Later, 14 d after forcing, JA concentration remained low regardless of treatment.

GA concentrations in the buds changed very little in treatments G and I but increased in treatment J during the first 7 d after forcing. In contrast, ACC concentration increased with time in treatment J and remained stable for treatments G and I.

Finally, SA was reduced after 7 d of forcing and returned to its initial values after 14 d of decapitation. Although treatment G showed the same tendency, SA concentrations did not vary significantly.

Effects of forcing treatments on delaying harvest and on yield and berry composition: Forcing treatments shifted harvest dates compared to control vines, with the effect increasing as the forcing treatment was delayed (Tab. 1). Indeed, forcing

Table 1

Effects of forcing treatments timing on phenology and yield components

Forcing Treatments	Harvest date	No. of clusters/vine	Total yield ($g \cdot vine^{-1}$)	Cluster weight (g)
Forced on 25 April. Stage G	09 Sept.	10.0 ± 2.8^{b}	799.6 ± 192.3^{c}	89.5 ± 15.5^{b}
Forced on 15 May. Stage I	18 Sept.	16.0 ± 2.3^{ab}	1520.2 ± 278.4^{bc}	90.7 ± 7.5^{b}
Forced on 25 May. Stage J	06 Oct.	27.3 ± 4.6^{a}	2200.0 ± 466.5^{b}	79.3 ± 5.8^{b}
Unforced/ control	22 Aug	12.5 ± 1.3^{b}	3915.7 ± 617.0^{a}	308.8 ± 35.8^{a}

Within each column, different letters indicate significant differences according to Duncan's multiple range test at the 95 % confidence level.

treatments carried out at stages G (25 May), I (15 June) and J (25 June) (Baillod and Baggiolini 1993) and appointed from now on as treatments G, I and J, respectively, succeeded to delay ripening 18, 27 and 45 days respectively, as compared to unforced plants (Tab. 1).

All the three forced treatments showed statistically lower values for yield components as compared with the unforced plants, with the lowest yield at 0.7 kg (for treatment G) and the highest at 2.2 kg (for treatment J) (Tab. 1). Consequently, although the number of clusters per vine highly increased for treatments I and J, the cluster weight was significantly reduced for all forced treatments as compared to control vines.

As a result, at harvest, pH values were lower and TA values were higher in treatments G, I and J compared to control vines with the effect increasing as the forcing treatment was delayed (Tab. 2).

Discussion

Effects of forcing treatments on phenology, growth and grape yield and chemical composition: With the aim of delaying the maturation of the grape by at least 1.5 months, a technique consisting of trimming shoots to two nodes has been studied. This technique forced the breaking of compound buds and induced the regrowth of fruitful shoots. Previously, Martínez de Toda *et al.* (2019) used the same technique and they effectively delayed all the phenological stages of 'Tempranillo' and 'Maturana Tinta' grapevines to a great extent causing the ripening to occur at temperatures considerably lower than the unforced vines. Moreover, in a similar experiment conducted in 'Cabernet Sauvignon', Gu *et al.* (2012) also got a shifted fruit ripening from the hot (July and August) to the cool (October through Early-November) portion of the growing season.

Table 2

Technological maturity parameters of the must of grapes for regular pruned vines (unforced/control) and forcing treatments: TSS (total soluble solids, °Brix), TA (Titratable acidity)

Forcing treatments	TSS (°Brix)	pH	TA ($g \cdot L^{-1}$)
Unforced/control	22.1 ± 0.1^{ab}	3.7 ± 0.0^{a}	4.0 ± 0.3^{b}
Forced on 25 April. Stage G	22.6 ± 0.2^{a}	3.5 ± 0.0^{b}	4.2 ± 0.1^{b}
Forced on 15 May. Stage I	22.6 ± 0.2^{a}	3.4 ± 0.0^{c}	5.6 ± 0.1^{a}
Forced on 25 May. Stage J	21.8 ± 0.2^{ab}	3.3 ± 0.0^{c}	5.9 ± 0.1^{a}

Within each column, different letters indicate significant differences according to Duncan's multiple range test at the 95 % confidence level.

Both studies emphasized the importance of selecting the proper timing of forcing, since fruit could not ripen and sprouting of the shoots may not occur if forcing is too late, depending on the temperature and heat distribution during the vine vegetative cycle. However, varietal differences may also have to be taken into account when selecting timing of forcing. Indeed, Martínez de Toda *et al.* (2019) showed that in Maturana Tinta variety forced in stage K, there was a lack of sprouting, while it was not the same in 'Tempranillo'. These different responses between varieties under forcing conditions could be attributed to the important role of roots and their phytohormone balance during the bud break process and/or that the installation of dormancy occurred earlier in 'Maturana Tinta' than in 'Tempranillo'. In such a case, the timing of forcing is of crucial importance since it is described that dormant buds gradually lose the ability to break in 2-3 weeks along with the slowing down of shoot growth (Reynier 2002).

In our study, forced shoots developed normally regardless of treatment and we succeeded to delay ripening from 18 up to 45 d depending on the timing of forcing. However, the number and weight of the clusters as well as the total yield were generally lower than in the unforced vines. Thus, to keep more than two nodes per shoot during trimming is likely to be an effective way of reaching a yield comparable to control vines (Martínez de Toda *et al.* 2019), however further studies should be carried out to determine the optimal number of nodes left to obtain a desired level of yield. Regarding the chemical composition of the grape, the longer period of fruit ripening under cooler weather resulted in a lower pH and a higher TA, even for the same level of TSS, the possible reason being that low temperatures reduced the respiratory malate degradation as compared to the control (Keller 2015). These results agree with those of Gu *et al.* (2012) and Martínez de Toda *et al.* (2019) which

also described higher levels of TA and lower pH for vines pruned at later stages in comparison with unforced vines. Moreover, Petrie *et al.* (2017), in a similar experiment, also obtained higher levels of TA in the latter pruning treatments, which suggests that the later pruning treatments may have maintained a higher tartaric acid concentration (Gatti *et al.* 2016). Further investigation is required to determine the cause of these differences; which may be due to altered acid synthesis during development, differences in dilution due to variation in yield [the later pruning treatments had significantly smaller yield (Tab. 1)] or shifts in fruit development relative to the temperature cycle (Petrie *et al.* 2017).

Effects of forcing treatments timing on endogenous hormonal regulation: It becomes evident that there is a basic molecular mechanism controlling the development of latent buds in grapevine and that this basic developmental program can be switched with another in response to environmental cues (photoperiod, temperature) or internal cues (e.g. plant hormones). In this study, the hypothesis that hormones are involved in regulating the development of the buds and that shoot decapitation may exert their enhancing effects on bud break by causing a rapid bud hormonal regulation was tested. Moreover, careful attention was paid to the timing of events, whereby endogenous hormone measurements were taken in the critical time window preceding, and leading up to, lateral bud release.

In literature, a major focus involves auxin (mainly IAA) and CKs as key players for the fate of the bud, *i.e.* whether buds remain dormant or start growing. Indeed, pioneering work with exogenous hormones (Sachs and Thimann 1967) led to an apical dominance model in which auxin, originating from the shoot apex, represses bud break and root-supplied CKs promote axillary bud break. However, it appears that the model in its simple form fits only under certain defined physiological circumstances. Other cases may comprise more complex processes contributing to bud break, as can be the critical timing leading up to bud release, or the analysis of multiple groups of hormones. Thus, to better elucidate such switches, a multihormonal approach was combined with a reliable way of inducing and tracking bud break. In this sense, the forcing experiment enabled us to disrupt the xylem flow following shoot decapitation which lead to a hormonal disequilibrium in the sampled buds during the bud break process in *Vitis vinifera*.

Increased translocation of xylem CKs into buds after decapitation was previously demonstrated in chickpea (Mader *et al.* 2003). These authors showed that when a pulse of labelled *trans*-ZR was introduced into the xylem stream, active CKs in the xylem were diverted to cause bud break directly after the loss of the dominant apex. This assumption could be translated in our study since the CK concentrations in the latent bud decreased with time over the vine vegetative cycle. Furthermore, when forcing was applied in stages G, I and J, grapevine CK concentrations significantly increased after 7 d of forcing, resulting in a newly developed shoot. Therefore, with these results, we may suggest a potential involvement of CKs in the cascade that leads to grapevine bud break. However, it seems plausible that a high number of factors controls bud break and that it may be triggered by the concomitant increase in the levels of initiating factors and a decline in the levels of inhibiting factors. In lupine, detailed studies on branching patterns revealed that lateral branch development was controlled by interactive effects of endogenous CKs, IAA and ABA rather than by any single hormone (Emery *et al.* 1998). Indeed, ABA appears to be also involved in bud release since increased degradation capacity of ABA, and levels of its degradation products were previously recorded in grapevine buds during dormancy release (Zheng *et al.* 2015). Moreover, other studies have shown that, in general, deficiency in ABA and its synthesis, as well as interference in ABA signalling, lead to dormancy loss, while suppression of ABA inactivation leads to increased depth of dormancy (Nambara and Marion-Poll 2005, Nambara *et al.* 2010, Fennel *et al.* 2015, Vergara *et al.* 2017). Our data supports this assumption since rapid decrease of ABA content after shoot decapitation seems crucial for the resumption of bud break of the newly formed basal compound buds, thus suggesting that ABA inhibits dormancy release in grape buds. Moreover, as the season progresses, an increase was recorded in the degree of inhibition exerted by endogenous ABA on dormancy release of control buds (data obtained at time 0h over the vine vegetative cycle) supporting the resulted inhibitory effect of ABA on bud break of dormant grape buds.

Moreover, a recent study by Zhong *et al.* (2013), revealed the comprehensive mechanism of seasonal bud dormancy in Japanese apricot (*Prunus mume*), by applying next-generation sequencing to study differentially expressed genes at the transcriptional level. As a result, the authors of this study demonstrated that hormone response genes, such as IAA, ABA, ethylene and JA, were possibly involved in seasonal bud dormancy in Japanese apricot. These results may contribute to further understanding of the mechanism of bud dormancy in grapevine, although the role of hormones in dormancy varies according to the species. In our study, JA seems also to exert an inhibitory effect of bud break, as its response was similar to that of ABA. However, after seven days of forcing, JA increased in treatment G, which may represent an artifact or it may be due to its role in regulating plant resistance to abiotic and biotic stresses (Browse 2009).

The current study also examined the role of GAs, SA and ACC in mediating bud break in grapevine buds since there is much less information in literature.

GAs are known for their antagonistic relationships with ABA in regulating various developmental processes (Weiss and Ori 2007), such as seed dormancy and germination. The transcript profiles of GA metabolism genes recorded in the buds throughout the natural dormancy cycle generally agree with the assumption that leads to dormancy release (Ophir *et al.* 2009). However, both negative and positive effects of GAs were reported on outgrowth of paradormant buds during the natural bud endodormancy cycle (Lionakis and Schwabe 1984, Zhuang *et al.* 2013). In our study, GA concentrations were similar in the moment that decapitation treatments took place (0 h for stages G, I and J) in the different treatments. The subsequent induction of bud break in the latent buds was accompanied by an increase in GA levels only after 7 d of shoot decapitation in treatment J, but not in the others. This may simply stem from the absence of a temperature appropriate for synthesis in treatments G

and I or could reflect the assumption that the effects of GA treatment are a complex function that specifically depends on bud dormancy status (Zheng *et al.* 2018).

Finally, both SA and ACC have also shown remarkable changes after forcing. On the one hand, SA showed a marked decrease after 7 d of shoot decapitation in treatments I and J which values returned to the control levels 14 d after forcing. Therefore, in this case, SA may have a role by acting as an essential plant growth regulator (Díaz-Riquelme *et al.* 2012, Agtuca *et al.* 2014). On the other hand, ACC is a non-protein amino acid acting as the direct precursor of ethylene, a plant hormone regulating a wide variety of vegetative and developmental processes, ranging from seed germination to organ senescence (Van de Poel and Van der Straeten 2014). In this sense, a significant increase after 14 d of forcing in treatment J could contribute to favoring plant growth. However, there is not much information on the direct relationship of those hormones with bud break.

Conclusion

Forcing shifted fruit ripening up to 1.5 months, from the warm August to the cooler October. Forced vines produced smaller berries with lower pH and higher acidity as compared to non-forced vines. Thus, forcing may be a promising way to restore the balance between alcohol content and titratable acidity, decoupled by the warming climate and thus, to get better quality wines.

Moreover, by releasing the apical dominance after shoot decapitation, we promoted a clear and rapid hormonal disequilibrium, which would be a key to identify the so-called switches that initiate bud growth. We conclude that CKs enhances bud break, whereas ABA prevents bud break.

Nonetheless, this is a technique that still has many questions to be studied: on the one hand, the floral induction on side buds, as well as the hormonal equilibria that affect such processes and, on the other hand, the quantification of the irrigation water need, due to possible differences in the foliar surface of the forced treatments. Moreover, further studies should be carried out to evaluate the long-term effects of this technique on grapevine, focusing on the ratio between the leaf area and the grape yield and more specifically in the plant reserves accumulation.

Acknowledgements

Financial support was provided by a post-doctoral grant from the University of the Balearic Islands and the Spanish Ministry of Education and a postdoctoral contract from the Spanish Ministry of Economy and Competitiveness (Juan de la Cierva program).

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Received September 12, 2019