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Chapter 7

Phytohormonal Control over the Grapevine Berry Development

Francisca Parada, Carmen Espinoza and Patricio Arce-Johnson

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Abstract

Grapevine (*Vitis vinifera*) is one of the most important commercial plants since its berries are used for wine production or consumed as fresh fruit or dry fruit. Many studies have focused on berry development and have pointed out the hormonal regulation on the three phases, from early development to maturity. Grapevine fruit has been classified as non-climacteric based on the low levels of ethylene present around *véraison*, although recent evidence has suggested a role for this hormone during grape berry ripening. The control of different physiological processes depends on a complex integration between environmental cues and endogenous factors, which is mediated by a phytohormone crosstalk. In this chapter, we will focus on phytohormones, their signaling pathways, and their association to berry development in *V. vinifera*; in particular, we will refer to auxins, abscisic acid, brassinosteroids, ethylene, gibberellins, and cytokinins.

Keywords: grapevine, berry, development, hormones, auxins, abscisic acid, brassinosteroids, ethylene, gibberellins, cytokinins

1. Introduction

The genus *Vitis* comprises 60–70 species and the majority of cultivated varieties pertain to *Vitis vinifera* L. The most studied process in this species is berry development, since it has a direct economic impact for wine, fresh fruit, and raisin production [1]. Berry development consists of two sigmoidal growth periods with a lag phase in between. The first stage (phase I) is characterized by a rapid cellular division and cell enlargement, establishing the final number of cells in the berries. At the end of this period, the seed embryos and berries have been defined. This stage is accompanied by the accumulation of metabolites, such as malic, tartaric,

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and hydroxycinnamic acids; minerals; tannins; flavonols; and volatile compounds, all essentials for normal berry development. During the lag stage (phase II), berry growth markedly diminishes, the concentration of organic acids reaches their highest level, and berries start to lose the chlorophyll accumulated during phase I. Finally, ripening stage (phase III) begins with the berry softening or *véraison*, and a gradual increase in sugar content occurs. Moreover, aroma and flavor compounds are accumulated during this stage, and anthocyanin accumulation takes place in red varieties. Also, berries increase their size by cell expansion without cellular division [2–4].

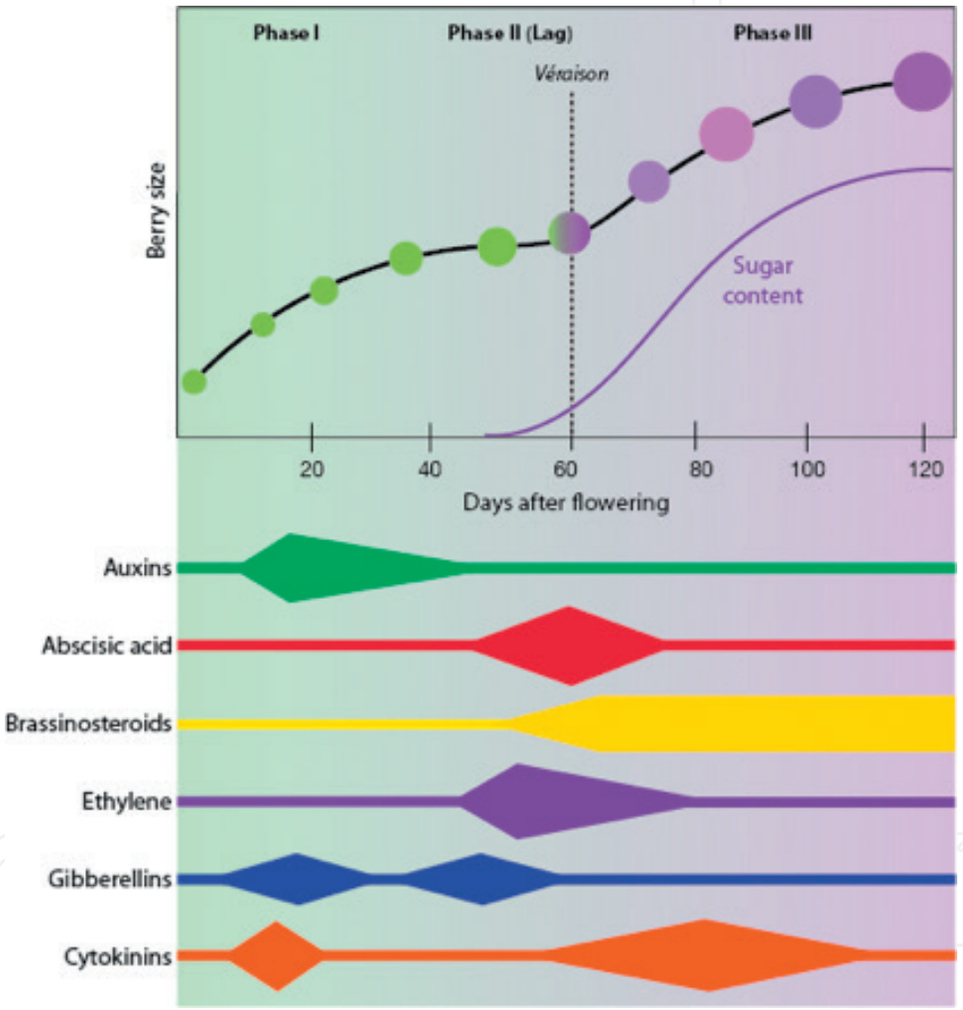

Figure 1. Schematic representation of hormonal content in grape berry development stages. Changes in hormone levels are shown for auxins, abscisic acid, brassinosteroids, ethylene, gibberellins, and cytokinins at phases I, II, and III of grape berry development. Several studies have shown a main role for auxins, cytokinins, and gibberellins in early phases of development associated to cell division process and fruit set. At *véraison*, there is a marked increase in abscisic acid levels, essential for ripening-associated physiological changes. High levels of brassinosteroids at *véraison* and phase III are consistent with a ripening-promoting role for this hormone. Moreover, a peak in ethylene has been detected prior to *véraison* that suggests a role in the initiation of this stage. Recently, a possible role for cytokinin signaling in phase III has been reported, due to its high levels at maturation. Changes in sugar content are depicted in purple curve, with an increase around *véraison* and its maximum in full mature berries. *Véraison* is denoted by a dashed line. For more details, see the text.

All the developmental phases of grapevine berry and the metabolic changes associated with them are tightly controlled by complex interactions between environmental factors, such as temperature [5–7], light [8–10], UV-B radiation [11–14], and water availability [15, 16], and endogenous factor, such as phytohormones [17–21]. Classically, fruits have been classified into climacteric and non-climacteric, where the first ones correspond to tomatoes, bananas, avocados, apples, and others that show a marked peak of ethylene associated with an increase in respiration rate during the onset of ripening. In opposition, non-climacteric fruits such as strawberry, citrus, and grapevines do not exhibit this correlation between ethylene and respiration [22–24]. However, recent evidence has suggested an unexpected role for ethylene in non-climacteric fruits [25]. In this chapter, we will discuss the current understanding of hormonal influence over berry development in grapevine, from early processes to ripening stage. In particular, the role of auxins, abscisic acid, brassinosteroids, ethylene, gibberellins, and cytokinins and its complex interaction network will be analyzed according to classic evidences and recent advances based on transcriptomic and proteomic approaches. The hormone levels at different stages of berry development are summarized in **Figure 1**.

2. Hormonal control over berry development

2.1. Auxins

Auxins are involved in several plant physiological processes, such as cell elongation and differentiation, responses to pathogen and abiotic stresses, and gravitropic and light responses, among others [26]. Indole-3-acetic acid (IAA), the main auxin in higher plants, has an essential role in initial stages of berry development. The IAA highest concentration has been observed in flowers and young berries, and it gradually decreases to a minimum low level at *véraison* [27]. Considering the high rate of cell division described during the first stage of berry development, these results are consistent with the role of auxin on this cellular process [4, 28]. The fruit set is the changeover that takes place in the ovary of a mature flower that includes the decision to abort or continue with the development of a functional fruit [29]. In grapevines, this process seems to be controlled by a hormonal balance between auxin and gibberellin (GA) [17]. Auxin induces the generation of seedless berries by parthenocarpy, a process where fruits are formed in the absence of fertilization. It has been described that genes encoding for negative regulators of fruit set initiation, AUX-IAA protein *VvIAA9* and auxin response factor (ARF) *VvARF7*, decrease their expression level during parthenocarpic berry process in cv. Tamnara treated with GA at pre-bloom stage [30]. Exogenous application of 4-chlorophenoxyacetic acid (4-CPA), a compound chemically similar to auxins, in ovaries of Fenghou grapevine, induced fruit set, but it depends on subsequent biosynthesis of gibberellin GA3 [31]. The GA signaling could be integrated to auxins by a DELLA protein mediation, since GA application increases expression of a DELLA gene [30] and the inhibition of gibberellin biosynthesis and auxin responses affected the normal physiological processes associated with fruit set [17]. In grapevine, fruit abscission occurs within 3 weeks after flowering [32], and differences in berry abscission are dependent on polar auxin transport. Experimental

evidence showed a negative correlation between abscission rate and polar auxin transport, suggesting that IAA maintains the "first berries" in the cluster, i.e., those berries derived from flowers that opened the same day that flowering starts [33]. IAA delays fruitlet abscission, preventing the formation of the abscission zone by reducing its ethylene sensitivity [34]. Abscission requires a continuous polar IAA transport to the pedicel, which is controlled by IAA itself through changes in the expression and localization of PIN-FORMED (PIN) auxin efflux proteins [35]. A lower polar auxin transport rate in excised fruitlets and a decrease in the expression of *VvPINs* were correlated with an increase in abscission [35]. Taken together, these results show a key role for auxins during abscission process in grapevine.

The most studied process in berry development is the ripening. It is characterized by global transcriptomic and hormonal changes [3, 18]. Physiological changes associated with this stage have been attributed to a proper balance between ABA and auxin levels [18, 36]. IAA content is high from anthesis to *véraison* and then declines at maturation [19]. Nevertheless, there is some discrepancy about IAA variations during grapevine berry development, since no significant changes in IAA content throughout phases I and II of berry development have also been reported [37]. The maintenance of IAA low levels has been explained by a hormone inactivation mechanism, where an increase of aspartic acid-IAA conjugated form (IAA-Asp) occurs during ripening. Since this form is inactive, no biologically active IAA form will be present at this stage [38]. This is consistent with the increase in *Gretchen Hagen 1* (*GH3-1*) gene expression at *véraison*, which encodes for an IAA-amido synthetase that conjugates IAA, preferentially to aspartic acid and tryptophan [38]. Also, grape berries treated with the synthetic auxin 1-naphthaleneacetic acid (NAA) show an upregulation of GH3 gene expression [36]. In agreement to this, transcriptomic analysis using Affymetrix platform using clusters of cv. Pinot Noir collected at different development stages showed a repression in the expression of two auxin response factors (ARFs) and an auxin receptor during ripening [39]. Treatments with NAA in grape berries downregulated different metabolic pathways, such as carbohydrates, lipids, cell wall metabolism, secondary metabolites, and amino acids, and upregulated light reaction pathways [36]. This suggests that auxins might delay ripening by a negative regulation of several essential metabolic processes for normal berry maturation. Exogenous IAA application delayed the ripening process, and treatment of pre-*véraison* berries with NAA diminished the accumulation of total soluble solids (TSS) and anthocyanins levels [40–42]. Dipping of cv. Shiraz berries in a synthetic auxin-like compound benzothiazole-2-oxyacetic acid (BTOA) solution decreased the expression of chalcone synthase (*CHS*) gene of flavonoid biosynthesis pathway prior to *véraison* [43]. Moreover, BTOA caused a 2-week retardment in the onset of ripening, measured as a delay of normal increase in berry weight, color, deformability, hexose concentration, and abscisic acid content [43]. All this evidence indicates a repressor role for auxin in the typical ripening-associated physiological processes. Spraying berries with NAA delays the increase in berry size post-*véraison*, but these fruits were larger than control fruit at harvest [40]. In addition, NAA seems to delay ripening by regulation at transcriptional level, since hormone application over cv. Merlot bunches changed the expression of about 1500 genes in berries [36]. Moreover, application of NAA downregulated expression of genes encoding for 9-cis-epoxycarotenoid dioxygenase 3 (NCED3) and abscisic acid insensitive 3 (ABI3) involved in ABA biosynthesis and perception, respectively [36]. Also, NAA strongly induces

the expression of genes involved in processes of conjugation, transport, and signal transduction of auxin. Particularly, NAA treatment upregulates genes coding for an Indole-3-acetic acid amido synthetase (GH3-like), protein transport inhibitor response 1 (TIR1-like), protein PIN-FORMED for polar transport (PIN3), two AUX/IAA proteins (IAA4-like and IAA31-like) known by repressed transcription of auxin-responsive genes, and an auxin response factor 8 (ARF8) [36]. On the other hand, an antagonistic relationship between auxin and ethylene has been observed. Genes encoding key enzymes of ethylene biosynthesis were upregulated in NAA-treated berries during *véraison*, as observed for 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS6) and ACC oxidase (ACO2) [36]. Also, genes encoding ethylene receptors, such as ethylene insensitive 4 (EIN4-like) and ethylene response sensor 1 (ERS1-like), showed an earlier increase in untreated berries compared with NAA-treated [36]. Related to the regulation of ethylene response, three genes encoding for ethylene response factors (ERF3-like, ERF-AP2-like, and ERF5-1) increase their expression in response to NAA [36]. This evidence illustrates the complex network of transcriptional responses regulating ripening process in grapevine berries.

2.2. Abscisic acid

Abscisic acid (ABA) has shown to regulate several developmental processes in plants, such as seed germination, dormancy, floral induction, and responses to environmental stresses [44]. ABA levels have been directly associated with changes in expression of the marker gene 9-cis-epoxycarotenoid dioxygenases (NCEDs), coding for the rate-limiting enzyme in ABA biosynthesis [15, 21, 45]. Moreover, ABA can be inactivated in several ways that include conjugation with glucose or by hydroxylation into phaseic acid (PA) and dihydrophaseic acid (DPA) [44]. Thus, the active ABA content depends on the balance between biosynthesis and inactivation. Experimental evidence strongly suggests that ABA is the main signal for ripening induction in grapevine [46]. ABA levels in grape berries gradually and strongly increase just before *véraison*, accumulating in berry skin during maturity [41, 43, 45]. In agreement with this, *ABI3* expression, a transcription factor involved in ABA signaling, rises during lag phase of berry development [16, 44, 47]. Also, a correlation between the increase in anthocyanin amount and ABA content has been observed during ripening in red varieties [18, 21, 48]. An increase in ABA concentration accompanied with an upregulation of anthocyanin biosynthetic genes and anthocyanin accumulation in the skin during grape berry ripening has been reported in cv. Cabernet Sauvignon ([49] and references therein). Consistently, spraying synthetic ABA in cv. Cabernet Sauvignon immature berries at fruit set promotes anthocyanin accumulation in fruits [50]. Moreover, ABA enhances anthocyanins levels in the berry skin of cv. Cabernet Sauvignon together with an increase in *VvMYBA1* mRNA accumulation, a positive regulator of anthocyanin biosynthesis [42]. Application of ABA at *véraison* stage in cv. Crimson Seedless accelerates coloring process in berries, allowing an earlier harvesting and superior appearance compared with ethephon-treated or control berries [51]. Exogenous treatment with ABA in cv. Cabernet Sauvignon berries increases the expression of genes related to flavonoid biosynthesis, such as those encoding for stilbene synthases (STS) [52]. All this results are consistent with previous reports showing the accumulation of stilbenes, anthocyanins, and flavonols in the skin of cv. Cabernet Sauvignon berries, in response to ABA application [53]. A 2-DE proteomic approach revealed that ABA treatment increases the

amount of different proteins before véraison. These proteins included vacuolar invertase GIN1, involved in hexose accumulation in the berry, and alcohol dehydrogenase 2 (ADH2); three proteins involved in flavonoid biosynthesis, chalcone isomerase, dihydroflavonol-4-reductase, and anthocyanidin reductase; and xyloglucan endotransglycosylase (XET) protein involved in cell wall modification [48]. ABA could regulate the synthesis of cell wall-modifying enzymes such as polygalacturonase [54]. The peak of activity of this enzyme coincides with the peak of ABA, and it is detected during color change period [54]. All the evidence is consistent with a role of ABA promoting berry ripening, regulating both structural and secondary metabolisms associated with berry development.

Interestingly, there is an increase in anthocyanin biosynthesis during ripening when cv. Merlot grapevines are grown under drought conditions in field experiments [15]. *NCED1* and *NCED2* genes, key enzymes from ABA biosynthesis pathway, were upregulated at the onset of *véraison* in water-deficient plants [15]. Drought stress increased ABA, proline, sugars, and anthocyanin concentration in cultivars such as Cabernet Sauvignon [45]. It has been proposed that the increase in ABA levels precedes the hexose accumulation [45]. Water deficit increased expression of genes coding for enzymes from ABA metabolism, including β-carotene hydroxylase (BHASE), involved in ABA precursors biosynthesis and NCED and (+)-abscisic acid 8′-hydroxylase (ABAHASE), involved in ABA oxidative catabolism [45]. The ABSCISIC ACID RESPONSE ELEMENT-BINDING FACTOR2 (AREB/ABFs) are a family of transcription factors involved in ABA-dependent gene activation and have been associated with ABA and abiotic stress signaling in different plant species [55]. In a recent work, the characterization of VvABF2, an AREB/ABF-like transcription factor from grape, was performed in order to understand its role during berry ripening in cv. Cabernet Sauvignon. The *VvABF2* expression increased during *véraison* and remains high during ripening until harvesting stage [55]. Interestingly, the overexpression of *VvABF2* in grape cells increased the content of the stilbenes resveratrol and piceid [55]. Berry softening is a process associated with ripening that initiates at stage II of berry development, and it depends on cell wall-modifying enzymes, including expansins, pectin methylesterase, pectate lyase, and xyloglucan endotransglycosylase/hydrolase ([56] and references therein). An early increase in ABA content during softening occurs and seems to be associated with a decrease in catabolism more than an increase in biosynthesis [56]. On the other hand, sugar unloading is a key process during berry development in which source organs, as leaves, deliver sugars to sink organs, as fruits [57]. The distribution of photoassimilates determines if the plant will go to vegetative or reproductive growth or it will accumulate starch as carbon reserve [58]. Foliar spraying of ABA in grapevines cv. Malbec demonstrated that this hormone increases glucose and fructose levels in berries and roots, probably functioning as a stress signal and enhancing sugar transport [58]. These changes in ABA-mediated sugar allocation could be an essential process favoring berry development. Just before *véraison* in Kyoho grapes, sucrose is transported into apoplast through specific carriers from apoplastic path [57]. The unloading process is possible by acid invertase enzymes. There are two types of acid invertases: the cell wall-bound invertase (CWI) that favors sucrose transport from source to sink cells and the soluble acid invertase (SAI) that has a role in storage and metabolism of sucrose in the vacuole [59]. An ABA-induced activation mechanism for these invertase regulations where ABA could regulate acid invertases at transcriptional, translational, and posttranslational levels, activating both the cell wall-bound and the soluble enzymes, has been proposed [59].

2.3. Brassinosteroids

Brassinosteroids (BRs) are a family of polyhydroxilated sterol derivatives that regulate several physiological processes in plants, such as cell elongation, biotic and abiotic stress responses, flowering, photomorphogenesis in darkness, and stomata development, among others [60, 61]. This phytohormone is the most recent being implicated in ripening of non-climacteric fruits [37, 62]. Transcriptomic analysis in cv. Merlot has suggested that BRs act as an early and key signal for ripening processes, perhaps by modulation of ethylene levels [36]. It has been shown that levels of castasterone, the bioactive BR, and its precursor 6-deoxo-castasterone increase at *véraison* and remain high during ripening in cv. Cabernet Sauvignon berries [37]. Moreover, the expression of *VvBR6OX* that encodes for brassinosteroid 6-oxidase that transform 6-deoxo-castasterone into bioactive castasterone increases until *véraison* suggesting that the high levels of castasterone and its precursor 6-deoxo-castasterone, detected at ripening, could be in part due to the increase in BR6OX enzyme [37]. Transcriptomic analysis of cv. Cabernet Sauvignon and Trincadeira has shown that the gene coding for VvBRI1, the putative brassinosteroid receptor insensitive BRI1, peaks at lag phase and then decreases, while *VvBR6OX1* peaks just prior to *véraison* and decreases thereafter [16, 63]. Moreover, putative gene coding for steroid 5 alpha reductase (*VvDET2*) was downregulated pre- and at *véraison* [63]. All the evidence suggests that the biosynthesis of brassinosteroid increases prior to *véraison* and is regulated by a feedback inhibition dependent on BR production. It has been reported that exogenous application of brassinosteroids promotes coloration of the berry skin in cv. Cabernet Sauvignon, suggesting an earlier ripening [37]. In agreement with this, application of brassinazole, an inhibitor of BR biosynthesis, delayed ripening [37]. Also, the application of exogenous brassinosteroid increases the total anthocyanin content in two cultivars, Yan73 and Cabernet Sauvignon [64, 65], and the full coloration of grapes occurred 7 days earlier in BR-treated samples [64]. BR has a major effect over genes of anthocyanin synthesis pathway, including flavonoid-3′-hydroxylase (*F3′H*), flavanone-3β-hydroxylase (*F3H*), flavonoid- 3′,5′-hydroxylase (*F3′5′H*), dihydroflavonol-4-reductase (*DFR*), anthocyanidin synthase (*ANS*), and UDP-Glc-flavonoid-3-O-glucosyl transferase (*UFGT*), and their expression peaks come ahead in about 14 days [64]. In addition to the previously described ABA role in sugar unloading in grape berries, the involvement of BR in this process has been recently reported [66]. Exogenous treatment of Cabernet Sauvignon berries with BR (24-epibrassinolide) increases the soluble sugar content; increases the activities of enzymes related to sugar unloading, neutral and acidic invertases, and sucrose synthase during ripening; and upregulates the expression of sucrose transporter genes [66]. In conclusion, BR has a role in promoting berry ripening, but more research is required to elucidate the role of BRs in berry development and its interaction network.

2.4. Ethylene

Ethylene is a gaseous hormone that can regulate several processes in plants, including carbon assimilation, flower and leaf senescence, germination, responses to abiotic and biotic stresses, organ abscission, and fruit ripening in climacteric species like apples, bananas, and tomatoes [67]. Classically, grapevine has been classified as a non-climacteric fruit due to the low levels of ethylene observed around *véraison*, suggesting that this hormone is not responsible for triggering ripening [46]. However, recent evidence showed that ethylene is present in high concentrations

at anthesis and declines thereafter but clearly displayed a transient increase just prior *véraison*, suggesting now a role for this hormone in ripening initiation [25]. During early berry development, an increase in ethylene levels has been correlated with the onset of fruitlet abscission in cv. Chardonnay, which is consistent with a gain in ACC oxidase activity and high levels of ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) [68]. The positive effect of ethylene over berry abscission was previously reported in cv. Pinot Noir [69]. Transcriptomic analysis has shown that the gene coding for ACC synthase, from ethylene biosynthetic pathway, was repressed post-*véraison* [39]. Moreover, the ACC oxidase gene, involved in the last step of ethylene biosynthesis, showed an increased expression at lag phase with a peak around *véraison* [16, 39]. Treatments with low doses of ethylene stimulated grape berry expansion in Cabernet Sauvignon, and there is an increased expression of auxin-responsive factor 8 (ARF8) near to *véraison* in ethylene-treated berries that could suggest an interaction between ethylene and auxin signaling to control berry expansion process [70]. A possible function for ethylene signaling post-*véraison* has been suggested since some ethylene response factors (ERFs) were induced in ripening stage. However, other members of AP2/EREBP transcription factors were downregulated, indicating a complex regulation mechanism of ethylene signaling during ripening [39]. Ethylene has also been associated with anthocyanin production, since there is an increased expression of genes such as *CHS*, *F3H*, and *UFGT*, when cv. Cabernet Sauvignon berries were treated with 2-chloro-ethylphosphonic acid (2-CEPA), an ethylene-releasing compound [71]. Recently, a role of ethylene in cv. Cabernet Sauvignon fruit senescence has been postulated, since changes in expression of biosynthetic and signaling genes have been found at late ripening [49]. Taken together, this evidence suggests that ethylene has a more important role for berry development in non-climacteric species than the one though a few years ago.

2.5. Gibberellins

Gibberellins (GAs) are hormones that participate in leaf expansion, pollen maturation, seed germination, and induction of flowering, among others [72]. GAs increase early in the first phase of berry development but decrease to very low levels before *véraison* [18, 19, 37, 73], while a second peak of GAs has been reported at lag phase [74]. A role of this hormone in berry enlargement in cv. Centennial Seedless during stage I has been suggested, since GA3-treated berries showed an increased expression of xyloglucan endotransglycosylase (*XET*) genes, which participate in cell wall expansion [75]. A cross talk between GA3-ABA and GA3-ethylene during berry enlargement period has been reported [75]. An RNA-sequencing analysis of cv. Centennial seedless berries after GA3 application showed a decrease in negative regulators of ABA content and signaling, suggesting that GA3 promotes ABA signaling, while there is an upregulation of ethylene response factors (ERFs) at 1 and 3 days post GA3 treatment with a downregulation observed 7 days post treatment [75]. Moreover, GAs have been involved in mesocarp cell expansion, and treatment with paclobutrazol (PAC), an inhibitor of GA biosynthesis, reduces fruit set [31]. An analysis of different GA3-oxidases revealed that *VvGA3ox1*, involved in GA biosynthesis, increases its expression at fruit set [76]. It has been reported that GA3 increases glucose and fructose content in berries of cv. Malbec at *véraison*, therefore affecting sugar distribution [58]. Also, experimental results proposed that fruitlet abscission could be coregulated by levels of auxin and GA, since both reduce the polar transport of auxin [35]. On the other hand,

there is not much information implicating GAs in ripening process. However, GA3 soaking of cv. Kyoho clusters strongly accelerates berry coloration compared with non-treated ones [77]. Thus, it is possible that GAs could participate in anthocyanin biosynthesis regulation, probably in a complex interaction with other hormones. A transcriptomic analysis of cv. Cabernet Sauvignon berries showed that GA β-hydroxylase gene, involved in GA biosynthesis, has its highest expression at phase I and then decreases in phases II and III [16]. Also, there is a gradual increase in gibberellin receptor *GIDL1* expression, which is more marked for *GIDL2,* as phases II and III progress [16]. These results are consistent with an increase in *GILD2* expression at *véraison* in cv. Trincadeira berries [63]. Nevertheless, more studies are needed to determine the precise function of this hormone during berry development, especially during ripening stage.

2.6. Cytokinins

Cytokinins are involved in seed germination, cell proliferation and differentiation, light responses, delayed of senescence, and others [78]. During early grapevine development, cytokinin 6-benzyladenine (6-BA) treatment induces seedless berry formation through parthenocarpy [31]. The levels of zeatin and zeatin riboside, the more active forms of cytokinins, were high in early phase I and then decrease in berry flesh to undetectable levels post-*véraison* [27]. This suggests that cytokinins do not participate in ripening stage. This is consistent with reports that exogenous application of cytokinins in cv. Delaware did not affect fructose content in berries, a key characteristic of ripening [79]. Furthermore, the expression of genes encoding for dehydrogenases, putatively involved in cytokinin degradation, decreased around *véraison* in cv. Trincadeira berries [63]. However, recent evidence indicates that cytokinins could have a role in ripening. For instance, isopentenyladenine (iP), a biological active cytokinin, increases in cv. Shiraz berries during ripening, while, during late ripening, there is a higher amount of t-ZOG, a glycosylated form of inactive cytokinin [80]. Recently, an analysis of cytokinin levels was performed in grape cultivars Cabernet Sauvignon, Riesling, and Pinot Noir, as well as in strawberry and tomato. The results indicated a markedly increase in iP concentration in red firm tomato, red ripe strawberry, and post-*véraison* grapes, based on which the hypothesis of a common role for cytokinin signaling during ripening in climacteric and non-climacteric fruits was postulated [81]. Throughout berry development, genes related to biosynthesis, catabolism, perception, and signaling of cytokinins are expressed [81]. Also, the repression of the gene encoding for cytokinin-repressed 9 protein (CR9) has been reported in ripening, supporting the activation of cytokinin signaling during this stage [39, 63]. The gene expression of nuclear regulators of cytokinin signaling (ARR) had different patterns around *véraison* in cv. Trincadeira grapes, complicating the interpretation [63]. So, it is still needed to clarify cytokinins' exact participation in phase III of berry development.

3. Conclusions

The role of the different hormones during grapevine ripening seems to be complex. Currently, some technical difficulties limit a better understanding of physiological processes occurring during grapevine berry development and the hormonal control. There is no availability

of a mutant collection of *V. vinifera* that could support the study of hormonal signaling or biosynthetic pathways to clearly understand hormone control of berry formation and maturity allowing to establish direct cause-effect relationships. Transgenesis in grapevine is not an easygoing procedure, and the generation of overexpressing or silencing lines in genes of interest is a difficult task. At the moment, correlations between hormone levels and gene expression at different phases of berry development are the best approach to unravel hormonal regulation of berry development. So far, a successful approach has been exogenous treatments with hormones, which have allowed to suggest conclusions and discern the complex interaction network. In addition, global analyses including genomics, transcriptomics, and proteomics have improved our knowledge about this process. It is expected that the progress of new technologies could help to overcome technical limitations and give more insight into control of berry development mediated by plant hormones.

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Author details

Francisca Parada, Carmen Espinoza and Patricio Arce-Johnson*

*Address all correspondence to: parce@bio.puc.cl

Department of Molecular Genetics and Microbiology, Faculty of Biological Sciences, Pontifical Catholic University of Chile, Santiago, Chile

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