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# Relationship between seed content and berry ripening of wine grape (*Vitis vinifera* L.)

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ABSTRACT

Cabernet Sauvignon and Cabernet Gernischt (*Vitis vinifera* L.) berries were sampled at seven stages from pre-veraison to harvest. According to seed weight to berry weight (SB) of berries, the sampled berries were divided into two groups as Low-SB and High-SB grapes. The results showed 0074hat most of colored grapes were Low-SB grapes while under-ripe green berries belonged to High-SB group at mid-veraison. What's more, compared with that of High-SB, there were more accumulation of sugar and anthocyanin and faster decreased total phenols in Low-SB berries. These were indicated that grapes with Low-SB ripened quicker. Abscisic acid (ABA) and auxin (IAA) were two main hormones in regulating berry ripening. Higher ABA and lower IAA concentration were observed in Low-SB grapes during maturation. To further illustrate the relationship of seed content with berry ripening, Cabernet Gernischt clusters were sprayed with 50 mg/L IAA. The results of this study suggested that IAA treatment delayed sugar and anthocyanin accumulation and retarded berry ripening, but increased the synchronicity of maturity by depressing the difference in sugar and anthocyanin contents between Low-SB and High-SB grapes. The delayed grapes had lower ABA and higher IAA content by reducing the expression of *VvBG1*, *VvNCED*, *VvYUC1* and enhancing the expression of *VvGH3-1*. When the same amount of the control and IAA treatment grapes were mixed together, there were more grapes of IAA treatment belonged to the High-SB category and more berries of the control were Low-SB, further suggesting that seed content negatively correlated with berry ripening.

## 1. Introduction

Wine quality highly depends on the consistency of the quality (berry maturation, berry size)of the wine grape berries and the first priority for premium wine is to have a uniform maturity and a suitable berry size (Gladstones, 1992; Hardie and Martin, 1990; Matthews and Anderson, 1989), so the study about the ripening of wine grape has important significance. There are many studies about the relationship between seed and fruit development and ripening. For the grape varieties, the normal number of seeds per berry should be four, but in fact much less than that (Hardie and Aggenbach, 1996; Gray and Coombe, 2009; Ristic and Iland, 2005), which mainly caused by species genetic characteristic and flowering environment (Ebadi et al., 2010a, b; Fernández et al., 2015). Normal seeded berry is described by a double sigmoid curve and divided into three phases (Coombe, 2003). Seed development can also be divided into three phase (Cadot et al., 2006), and the seed weight of which increases at first phase but almost not change from veraison to harvest. It has been suggested that seed content and developmental inconsistencies caused by differences in flowering may be related to uneven ripening of grapes (Coombe, 1992; Friend et al., 2009). While Vondras et al. (2016) found that grapes with the same seed content but different flowering can also enter the maturity synchronously. So compared with flowering, seed content has a greater impact on the maturity of grapes.

In the early stages of fruit development, the number and weight of seeds are associated with the development of the fruit and the seed growth rate is positively correlated with the development of the pericarp tissue (Scienza et al., 1978; Cawthon, 1982; Friend et al., 2009; Ristic and Iland, 2005). In these studies, the lower seed weight in Shiraz (Ristic and Iland, 2005) and the lower seed number in Concord (Cawthon, 1982) lead grapes to mature slowly, and the more seeds in Cabernet Sauvignon (Scienza et al., 1978), the quicker the maturing. While Gouthu and Deluc (2015) found that the green immature berries not only had higher total seed weight but also significantly higher

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single seed weight compared with the red berries in Pinot Noir. These results are contradictory maybe to the fact that the definition of the seed content is different (without taking berry weight into account) and only the seed weight and seed number are considered. So we considered the weight of the berry, and divided the grapes into high-SB group and low-SB group according to the seed weight to berry weight to discover the effect of seed content on the ripening of grapes.

Though existence of seed is highly related to berry development and ripening, parthenocarpic fruit can still mature, indicating that a developmental regulation may be involved (Mcatee et al., 2013). Seeds are generally considered to be the metabolic centers of plant hormones (Nitsch, 1970) and berry ripening is regulated by hormones. ABA and IAA, two main regulators of the onset of ripening, have an abrupt increase and decrease at veraison respectively (Kondo and Kawai, 1988; Wheeler et al., 2009; Zhang et al., 2003; Deytieux et al., 2007). Produced in integument, ABA is an important regulator of seed maturation and embryo formation (Eiji et al., 2010) as well as one of the major hormones that promote ripening of grapes (Koyama et al., 2010; Peppi and Retamales, 2010) and can interact with sugar to enhance the expression of genes related to anthocyanin synthesis (Loreti et al., 2008). IAA is the main active ingredient of auxin and the first hormone to promote plants to grow which is regulated by two forms, free state and binding state, and only the free auxin plays a role. Binding auxin is a storage form, which can be hydrolyzed to free auxin, and the main binding form is IAA-amino acids (Epstein et al., 1986). Auxin is synthetized in the ovule and sent to the pericarp by fertilization to promote cell division and differentiation and also induce GA synthesis, one hormone that degrades DELLA protein which can depress fruit development (Chandler et al., 2002; Harberd, 2003; Achard and Genschik, 2009). When seeds fully mature, the transport of auxin will be inhibited and the fruit gets to mature (Mcatee et al., 2013). So this study not only explored the effect of seed content on berry ripening but discovered how ABA, IAA and the related genes regulated berry ripening.

## 2. Materials and methods

### 2.1. Plant materials and sampling

This experiment was carried out in Xianyang, Shaanxi Province, China (34°650′ N, 108°750′ E). Cabernet Sauvignon and Cabernet Gernischt (*Vitis vinifera* L.), planted in 2009, were selected as samples. Calculation the rate of grape colored, which was the ratio of the number of colored grape and the total number of fruits, randomly collected thirty clusters during fruit development. Six clusters selected randomly from eight vines at 56, 68 (veraison), 72 (mid-veraison, when 50% berries were colored), 79, 86, 100, 114 (harvest) days after anthesis (DAA), each period comprised three independent biological replicates. The berries used for hormone and gene expression detections were stored at −80 ℃ and the others for physiological parameters tests were stored at −20 ℃. There were about 50% and 40% berries having one seed and two seeds separately in Cabernet Sauvignon and 70% berries having one seed in Cabernet Gernischt. To eliminate the effect of seed number on ripening, one-seeded berries and two-seeded berries in Cabernet Sauvignon and one-seeded berries in Cabernet Gernischt were researched separately. At mid-veraison, berries were classified as hard green, hard soft, pink, red grapes on the color and firmness, which had been reported in some studies (Lund et al., 2008; Zenoni et al., 2010; Gouthu and Deluc, 2015). Each berry was weighted and after which seeds were separated and weighted. According to the range of SB values, 40% berries with higher and lower SB were classified as High-SB and Low-SB respectively.

### 2.2. IAA spray experiment of Cabernet Gernischt

IAA stock solution (0.29 mM) was prepared by dissolving 50 mg IAA in 1 mL of 98% ethanol and the control group (CK) only contained 1 mL of 98% ethanol. IAA treatment and the control stock solutions were mixed with 1 mL of Tween 80 and diluted to 1 L with deionized water. The treatment was carried out on 56 DAT and clusters were selected randomly from vines on 68, 76, 85, 100, 117 DAA respectively. The grapes were classified following the method mentioned above.

### 2.3. The determination of berry size, total soluble solids, reducing sugar and titrable acid

A vernier caliper was used to determine the diameter and a digital refractometer for the TSS of each berry. The reducing sugar was measured by Fehling reagent titration and the content of tritrable acidity (expressed as tartaric acid) was determined by Sodium hydroxide titration (0.5 M), both of them were according to OIV method (2012).

### 2.4. Polyphenol extraction and determination

#### 2.4.1. Polyphenol extraction

The polyphenol was extracted using the method proposed by Di Stefano and Cravero (1991) with slight modification according to the experimental conditions. Skin, seeds were separated, dried and weighted and after which 30 mL buffer (12% v/v ethanol, 600 mg/L sodium metbisulfite, 5 g/L tartaric acid, adjust pH to 3.20 with 1 M NaOH) were added respectively, shaken at 25 ℃ for 3 days. The supernatant was decanted and the volume was recorded as 30 mL. The extracted solution was stored at −20 ℃ without light for the following detection.

#### 2.4.2. Polyphenol determination

The total phenols were determined by the Folin-Ciocalteu method (Jayaprakasha et al., 2001), expressed as gallic acid (mg/g); Tannin content test was performed according to the descriptions (Jayaprakasha et al., 2001; Lee et al., 2005) and the results were counted by catechin (mg/g); The determination of anthocyanin in the grape skin was carried out by the pH differential method (Lee et al., 2005).

### 2.5. Hormone analysis in pericarp

Five deseeded grapes each from Low-SB and High-SB with high difference in seed weight to berry weight were as a biological replicate. The extraction and purification of ABA, IAA followed the methods described by Bollmark et al. (1988) and Yang et al. (2001). The determination of ABA and IAA contents were conducted using Enzyme-linked Immunosorbent Assays (ELISA) kit produced in the Phytohormones Research Institute of China Agricultural University.

### 2.6. RNA extraction, reverse transcription and purification

The samples used to determine hormones were also chosen for RNA extraction according to the method of RNA extraction kit (TaKaRa, Dalian, China). The integrity of the RNA sample was analyzed using electrophoresis with 1% agarose gel and the concentration was identified by Ultra low volume spectrometer-BioDrop Touch. 400 pg of total RNA was purified and reverse transcribed using PrimeScript®RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China).

### 2.7. Real-time RT-PCR

Real-time PCR was performed with SYBR Green I chimeric fluorescence method to determine the expression level of the genes (TaKaRa, Dalian, China). The three-step reaction system was used, including 12.5 μL 2 × Premix, 1.0 μL template, 0.5 μL forward primer and reverse primer respectively and 10.5 μL RNA-free water. Genes expression were calculated using the equation $2^{-\triangle\triangle CT}$, shown in the study of Livak and Schmittgen (2001).