

Changes in Endogenous Hormone Levels during the Ripening of Grape Cultivars Having Different Berry Set Mechanisms

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Abstract: Concentrations of indole-3-acetic acid (IAA), gibberellic acid (GA₃) and abscisic acid (ABA) were measured by gas chromatography (GC) in the berries of 3 grape cultivars having different berry set mechanisms to elucidate the possible relationship between endogenous hormones and berry set mechanisms. The results obtained showed that the levels of IAA, GA₃ and ABA in seeded Italia, empty seeded Çavuş and stenospermocarpic seedless Perlette changed during ripening. While Italia exhibited a hormonal pattern similar to that of Çavuş, Perlette showed a different IAA and ABA pattern compared to the other cultivars. Generally, IAA and GA₃ were high in the early stages of berry growth. The concentrations of IAA and GA₃ in the berries declined to very low levels at the time of ripening. On the other hand, changes in ABA levels were closely associated with ripening. The highest contents of hormones were found in Italia and the lowest in Perlette. Çavuş was placed between them with intermediate concentrations. From the results, it was concluded that there may be a close relationship between hormone contents in berries and the degree of seed development.

Key Words: grape, berry set, indole-3-acetic acid, gibberellic acid, abscisic acid

Olgunlaşma Süresince Farklı Tane Tutum Şekillerine Sahip Üzüm Çeşitlerinin İçsel Hormon Seviyelerindeki Değişimler

Özet: Bu araştırmada, içsel hormonlar ile tane tutum şekilleri arasındaki olası ilişkiyi aydınlatmak amacıyla, farklı tane tutum şekillerine sahip üç üzüm çeşidine ait tanelerde, olgunlaşma süresince indol-3-asetik asit (IAA), gibberellik asit (GA₃) ve absizik asit (ABA) miktarları gaz kromatografisi kullanılarak ölçülmüştür. Çekirdekli Italia, stenospermocarpik çekirdeksiz Perlette ve boş çekirdekli Çavuş üzüm çeşidinde IAA, GA₃ ve ABA seviyelerinin olgunlaşma süresince değiştiği tespit edilmiştir. Italia, Çavuş üzüm çeşidi ile benzer bir hormonal süreç gösterirken, Perlette diğer çeşitlerle kıyaslandığında IAA ve ABA bakımından farklı bir hormonal seyir göstermiştir. Genel olarak, IAA ve GA₃ tane büyümesinin erken devrelerinde yüksek bulunmuştur. Olgunlaşma döneminde ise tanelerdeki IAA ve GA₃ konsantrasyonu çok düşük seviyelere kadar inmiştir. Diğer taraftan, ABA seviyesindeki değişikliklerin olgunlaşma ile yakından ilişkili olduğu belirlenmiştir. Her üç hormon da en fazla Italia; en az da Perlette çeşidine ait tanelerde bulunurken; Çavuş üzüm çeşidi bu iki çeşit arasında yer almıştır. Araştırmada elde edilen bu sonuçlara göre, tanelerde bulunan hormonların seviyesi ile çekirdeğin gelişme derecesi arasında yakın bir ilişkinin bulunabileceği sonucuna varılmıştır.

Anahtar Sözcükler: Üzüm, tane tutumu, indol-3-asetik asit, gibberellik asit, absizik asit

Introduction

There are 4 different berry set mechanisms in grapes. In most cultivars, there is a normal berry set and these grapes have normal and viable seeds. Another mechanism is parthenocarpy and cultivars showing this type of set have completely seedless berries. In stenospermocarpic, another type of set, seed development is terminated by early embryo abortion and the berries are considered seedless. The last mechanism is also empty seededness. In

this berry set type, seed development proceeds until hard seed coats of about normal size are formed, and then the embryo aborts, leading to seed emptyness (Winkler et al., 1997; Ağaoğlu, 1999).

Çavuş, one of the most popular white table grape cultivars in Turkey, shows empty seededness. According to Olmo (1934), 99.5% of its seeds are empty. In the development of Çavuş seeds, pollination and fertilization proceed normally. Zygotes, before abortion, grow

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sufficiently to result in the formation of a seed coat of almost normal size and texture. However, these seeds have aborted embryo development, and shrivelled and degenerated endosperms (Winkler et al., 1997).

Differences in the types of berry set affect the growth of berries and their size. It is well known that there is an important relationship between seed development and berry growth (Winkler and Williams, 1936; Scienza et al., 1978). This relationship has been attributed to hormones, such as auxins, gibberellins and cytokinins, formed in the seeds (Coombe, 1960; Alleweldt and Hifny, 1972; Inaba et al., 1976). After endogenous plant hormones were understood to play a significant role in plant growth and development, a number of studies on the presence of hormones in seeded and seedless grape cultivars were reported (Coombe, 1960; Farmahan and Pandey, 1976; Inaba et al., 1976; Lilov and Andonova, 1976; Scienza et al., 1978; Kondo and Kawai, 1998; Perez et al., 2000), while no report has been published on hormone levels and their changes in empty seeded berries.

Plant hormones may play an important role in the growth and development of grape berries (Pandey and Singh, 1989; Tromp, 1989). In this study, the changes in the levels of endogenous indole acetic acid (IAA), gibberellic acid (GA_3) and abscisic acid (ABA) during berry development in empty seeded Çavuş, seeded Italia and stenospermocarpic seedless Perlette were determined. We quantified the IAA, GA_3 and ABA contents of these grape cultivars with the aim of investigating the possible relationship between endogenous hormones and berry set mechanisms in grapes.

Materials and Methods

Materials

This study was carried out on Çavuş (empty seeded), Italia (seeded) and Perlette (stenospermocarpic seedless) grape cultivars. Ten vines of each cultivar were selected in the vineyard of the Horticultural Research Center in Isparta, Turkey. The vines were 14 years old and Guyot trained. Hormone analyses were conducted during the season on berry samples. Berries were picked from these vines every 15 days, starting 25 days after anthesis (DAA) for Italia, 16 DAA for Çavuş and 20 DAA for Perlette until ripeness. The sampling dates were 4 and 18 July; 1, 15 and 30 August; and 14 and 29 September. After

collection, the samples were immediately used for hormone extraction.

Methods

Hormone analyses: Analyses of IAA, GA_3 and ABA were determined according to Topçuoğlu and Ünyayar (1995). This method is a combination of the methods used by Ames et al. (1979), Weiler et al. (1981), Zieslin and Geller (1983), Staden and Nicholson (1989) and Prakash and Prathapasenan (1990). Two-gram berry samples were homogenized in a mixture of methanol:chloroform (14:6 v/v) and maintained for 1 week at $-20\text{ }^{\circ}\text{C}$. The extracts were filtered through a Whatman no. 5 filter paper and the residue was re-homogenized with the same solution mixture, and the extracts were combined. The aqueous residue was adjusted to pH 8.5 with 1 N NaOH and transferred through a separating funnel to separate chloroform from the methanol. The chloroform phase was discarded. The methanol phase was reduced to the aqueous phase in a rotary evaporator at $40\text{ }^{\circ}\text{C}$. The aqueous phase was then adjusted to pH 2.5 with 1 N HCl and partitioned 3 times against ethyl acetate to take acidic free hormones, and dried under vacuum at $40\text{ }^{\circ}\text{C}$. The residue was dissolved in 1 ml of absolute methanol and transferred into an Eppendorf tube. The methanol was reduced to 100 μl under vacuum, and then line-loaded onto a 20 x 20 cm, 0.25 mm thick silica gel 60 F_{254} thin layer chromatography (TLC) plate (Merck, Darmstadt, Germany). Standard IAA, GA_3 and ABA were also spot-loaded in scored strips at both edges of the plates. The plate was allowed to develop for 15 cm in the vertical direction using isopropyl alcohol:ammonia:water (84:4:4 v/v/v) as the solvent system. After development, the positions of IAA, GA_3 and ABA were detected under UV light (254 nm wavelength) and marked. A band of silica corresponding to the relative fluidity (R_f) values of standards was scraped off, dissolved in 0.5 ml of methanol, and then dried under vacuum. The purified samples were methylated with diazomethane (Schlenk and Gellerman, 1960) dissolved in ethyl ether and methanol (9:1 v/v). The derivatives were dried under vacuum and re-dissolved in 100 μl of EtOAc for GC analysis.

IAA, GA_3 and ABA levels were determined with a Fisons 8560 HRGC Mega 2 series equipped with a flame

ionization detector (FID) and using a SPB-1 (30 m x 0.32 mm, ID) capillary column. Injection and detector temperatures were 200 °C and 300 °C, respectively. Samples (1 µl) were injected into the column at 80 °C, and the temperature was programmed to 5 °C min⁻¹ until the column was at 280 °C. Helium flow rate was 1 ml min⁻¹, and inlet pressure was 22 psi. IAA, GA₃ and ABA were quantified using peak areas. The ratio of detector response to putative IAA, GA₃ and ABA peaks in berry samples was compared to the response ratio of the detector for authentic IAA, GA₃ and ABA standards (Sigma).

Statistical Analysis: All experiments were replicated 3 times, and means and standard errors were calculated.

Results and Discussion

Changes in IAA during berry development

The IAA contents of Çavuş, Italia and Perlette are presented in Figure 1. In Çavuş berries, IAA content increased until the beginning of stage II (lag phase), and the highest value was detected at 44 DAA (66.34 ng g⁻¹ fresh weight (FW)). After a sharp reduction in IAA content until 59 DAA, a second increase but of lower magnitude was obtained in the berries at 74 DAA. Subsequently, the amounts of IAA dropped again in the berries and reached their minimum levels at the time of ripening. The levels of IAA in Italia berries rose during stage I (first berry growth phase) and reached their peak value at the end of this stage (92.50 ng g⁻¹ FW). After IAA content decreased until 68 DAA, a slight increase occurred before the initiation of ripening (83 DAA). IAA declined again until ripening and the lowest value was detected at that time. Sacher (1973) and Zhang et al. (2003) also found decreases in auxin content in seeded grape cultivars during ripening. Çavuş showed a pattern of IAA changes similar to that of Italia. The level of IAA in berries was higher at stage I compared to at the other stages of berry growth. Perlette had a different curve compared to the other cultivars. In Perlette, IAA content was at its highest level at 48 DAA. Then IAA declined continuously until ripening and reached its minimum level. These results agree to some degree with those reported by Alleweldt et al. (1975).

In seeded and seedless grape berries endogenous auxin levels are relatively high during the early stages of berry development. This may be related to the rapid

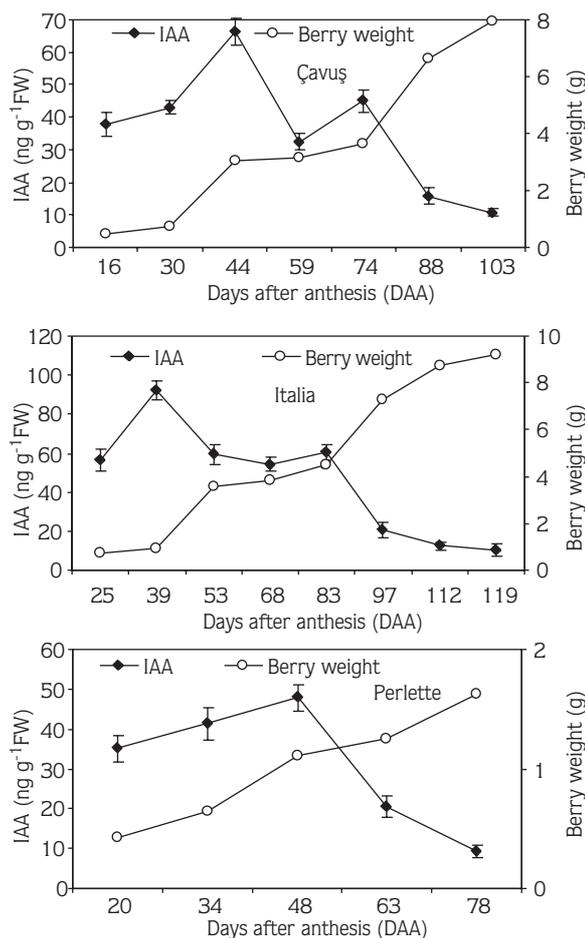


Figure 1. Changes in berry weights and IAA contents in berries of Çavuş, Italia and Perlette during berry development. Each data point represents the average of 3 experiments and bars represent the SE.

growth phase of the berry for enhancing cell division or enlargement, possibly due to the regulation of assimilate partitioning within the grapevine (Brenner et al., 1989). On the other hand, to our knowledge, there is no information on the levels of IAA in empty seeded berries.

Comparing IAA contents in the berries of cultivars, it is seen that there are some differences among the cultivars. Italia showed the highest IAA levels, while Perlette had the lowest IAA content. Çavuş, with intermediate concentrations of IAA, is placed between the 2 other cultivars. Similarly, Coombe (1960), Nitsch et al. (1960) and Farmahan and Pandey (1976) found higher auxin content in seeded berries than in seedless berries. The low IAA level in seedless fruits may be due to a lack of seeds, a site for IAA synthesis (Karanov et al., 1992).

Changes in GA₃ during berry development

The changes in GA₃ contents of the berries are presented in Figure 2. The highest GA₃ level determined in the berries of Çavuş was at 16 DAA (4.47 ng g⁻¹ FW). It declined greatly during the first growth phase of berry development. This decrease continued until ripening. It was determined that only a temporary increase in GA₃ was found at 59 DAA and the minimum GA₃ content was reached during ripening (0.14 ng g⁻¹ FW). As was found for Çavuş, in Italia the maximum GA₃ was detected in the berries collected at the first sampling date (25 DAA). Then the level of GA₃ decreased sharply at stages I and II.

The GA₃ content increased again to 2.94 ng g⁻¹ FW at 68 DAA, before it started to decline. The minimum level of GA₃ was also detected at the time of ripening. In Perlette, GA₃ content was higher at 20 DAA compared to the other phases of berry development (2.25 ng g⁻¹ FW). GA₃ content abruptly declined from this time to 34 DAA. A slight rise was shown at about veraison, and then it started to fall again. The minimum GA₃ content was detected during ripening (0.26 ng g⁻¹ FW).

In this study, the trend of changes in the GA₃ content of grape cultivars having different berry set mechanisms remained similar throughout the various phases. GA₃ in all cultivars was at its peak value in berries taken at the first sampling dates (from 16 to 25 DAA). Similar results were reported by Coombe (1960) and Perez et al. (2000). However, Scienza et al. (1978) determined the maximum GA₃ level at 45 DAA. In GA₃ content, a second peak was also observed at the beginning of stage III in our study, as reported by many authors working on seeded and seedless grape cultivars (Iwahori et al., 1968; Farmaham and Pandey, 1976; Perez et al., 2000). The drastic changes in the concentration of gibberellins occurring during ripening could be indicative of their importance in controlling crucial aspects of berry growth and development (Perez et al., 2000).

With respect to GA₃ concentrations, major differences were found between cultivars. The highest GA₃ level was determined in Italia, and the lowest in Perlette. As in IAA, Çavuş was placed between these 2 cultivars. Weaver and Pool (1965) reported that more gibberellin was present in the seeded than in the seedless berries, indicating that seed may be a source of gibberellins. This concept is supported by a previous report stating that developing seeds contain high levels of gibberellins (Ingram et al., 1985). Iwahori et al. (1968), Farmaham and Pandey (1976), and Perez et al. (2000) also found higher GA₃ levels in seeded cultivars than in seedless ones and they claimed that gibberellins produced by seeds can contribute to berry development and growth.

Changes in ABA during berry development

ABA concentrations in the berries of the grape cultivars are presented in Figure 3. The levels of ABA in Çavuş were low until 44 DAA. Subsequently, a gradual increase was noted, and then a sharp one through 74 DAA. ABA content reached its peak at that time (174.14 ng g⁻¹ FW). Then ABA concentration went on declining for the duration of the ripening. In the berries of Italia, ABA

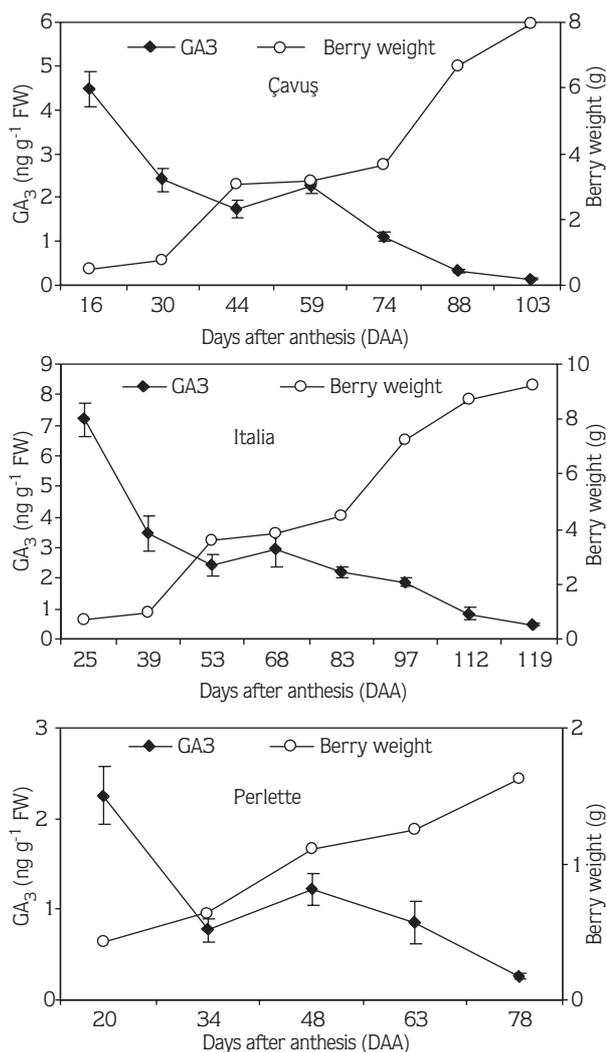


Figure 2. Changes in berry weights and GA₃ contents in berries of Çavuş, Italia and Perlette during berry development. Each data point represents the average of 3 experiments and bars represent the SE.

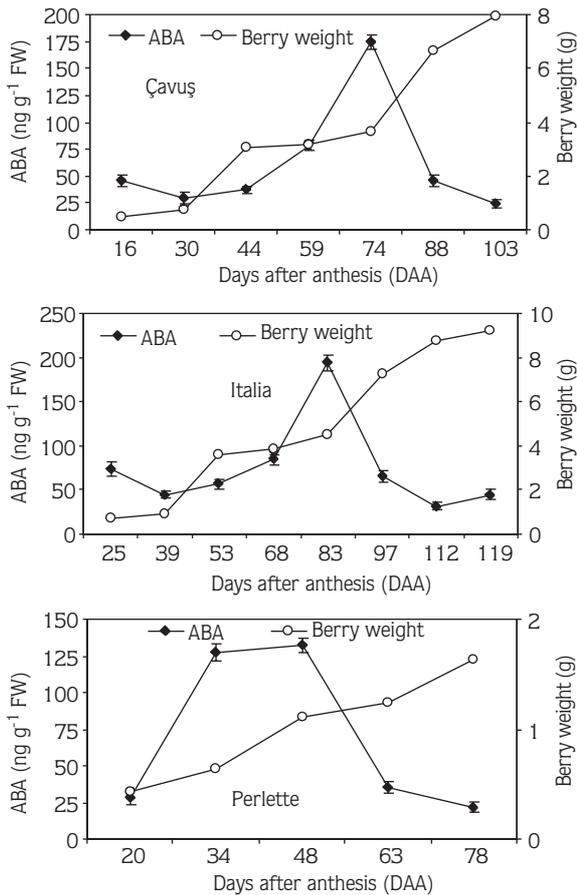


Figure 3. Changes in berry weights and ABA contents in berries of Çavuş, Italia and Perlette during berry development. Each data point represents the average of 3 experiments and bars represent the SE.

content was also low at the beginning of veraison, showing a pattern similar to that of Çavuş. ABA reached a peak at 83 DAA (194.16 ng g^{-1} FW) and it started to decline again and the lowest ABA content was reached during ripening. In Perlette, berries contained a small amount of ABA at 20 DAA, and it increased sharply and reached its peak value at 48 DAA (132.33 ng g^{-1} FW). Subsequently, a considerable fall was noted, which particularly marked the ripening of the grapes. The minimum ABA content was reached during ripening in Perlette.

In all cultivars ABA reached maximum levels at the beginning of ripening (stage III) and then started to decline again during ripening, as deduced from the experiments by Coombe and Hale (1973), Alleweldt et al. (1975) and Scienza et al. (1978). The onset and rate of ripening in the grape berry are a function of the

accumulation of ABA and there is a positive correlation between ABA content and ripening (Coombe and Hale, 1973). Zhang et al. (2003) also reported that changes in ABA levels were closely associated with ripening. ABA may help to trigger a mechanism responsible for the entry of metabolites from the leaves to the berries (Farmahan and Pandey, 1976).

Another result obtained in this study is that there was a relationship between seed development and ABA contents in berries. The highest levels of ABA were found in the berries of Italia, followed by Çavuş and Perlette in that order. Kondo and Kawai (1998) also found higher ABA content in skins of seeded Pione grapes than in those of seedless Pione grapes.

Conclusion

When investigating changes in hormone levels in the berries of grape cultivars during berry development, similar patterns were found between Italia and Çavuş. Only small differences, however, were observed between them at the beginning of the stages of berry development. On the other hand, except GA_3 , the hormones in Perlette showed patterns different from those of the other cultivars. These differences could be due to a different extension of the berry growth phases and time to ripening. Ripening occurred earlier in Perlette (78 DAA) than in Italia (119 DAA) and Çavuş (103 DAA). The data presented in this paper show that there are quantitative changes in endogenous hormones according to different berry set mechanisms.

In grapes, the role of seeds in berry development and growth is not fully understood (Perez et al., 2000). The highest concentrations of hormones were found in the berries of Italia, having fully developed seeds, while the lowest concentrations were detected in those of Perlette, having seedless berries. Moderate concentrations of hormone were detected in Çavuş, having empty seeded berries. Endogenous hormones such as auxins and gibberellins biosynthesized by the seeds are transported to the pericarp and regulate their growth (Van Huizen et al., 1996). Based on these results, it can be concluded that there is a positive relationship between the levels of hormones and development degrees of seeds. This effect may originate from being sources of hormones or their encouragement roles in the transportation of hormones from leaves to berries.

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