Berry development of grapevines: Relations between the growth of berries and their DNA content indicate cell multiplication and enlargement

by

H. Ojeda1, 2, A. Deloire1, A. Carbonneau1, Agnes Ageorges2 and C. Romieu2

1) Agro-M-INRA, UFR de Viticulture, Montpellier, France
2) IPV-INRA, Laboratoire de Biochimie Météabolique et Technologie, Montpellier, France

Summary: DNA of berries (cv. Shiraz) was extracted and quantified to determine, indirectly, the rate of cell division and enlargement in the grape pericarp. The increase of total DNA in the pericarp begins at anthesis in the ovary of grapevine flowers (day 0, 100% of flowers at full bloom). This increase in DNA continues during the herbaceous growth period until 35 d after anthesis (day 35, 19 d before the onset of veraison). Total DNA per berry pericarp does not increase linearly during this growth period since 75% of the DNA has already accumulated before day 20. We determined a cell enlargement index (CEI), to estimate the mean cellular volume. The pericarp cell size increases 16-fold during the whole growth of berries. Volume increase is nearly linear from berry set to the beginning of veraison and thereafter until maturity. The importance of determination of cell division and enlargement of berry pericarp based on the DNA content and its possible application in studies on the influence of environmental factors on berry growth is discussed.

Key words: Vitis vinifera, DNA, berry growth, fruit set, veraison, cell division, cell enlargement.

Introduction

The dynamics of berry growth and composition can be considered as a key determinant of harvest quality (Champagnol 1998). In fact, specific grape substances such as phenolic and aroma compounds, which give wine its originality, are mainly localized in the skin, while the «unspecific» substances, e.g. sugars, acids and cations, are abundant in the pulp (Cordonnier 1976).

The size of the mature berry influences the ratio of skin surface to berry volume and, herewith, the dilution of specific substances of the skin in the must (Singleton 1972).

Many studies have shown that different factors such as temperature (Hale and Buttrose 1974; Kliewer 1977), light (Dokoozlian and Kliewer 1996), plant water status (Hardie and Considine 1976; Matthews et al. 1987; McCarthy 1997), leaf area (Ollat and Gaubillere 1998) and foliage exposure (Carbonneau et al. 1978) can affect berry size, depending on the developmental period and/or the intensity of the considered factor.

The size and number of cells in the pericarp (exocarp + mesocarp + endocarp, according to Hardie et al. 1996) are the basic criteria to explain the final berry size; however, only some papers deal with cell division and cell enlargement during grape berry development. Difficulties and limits in the microscopic observation techniques (numbering of cells from pericarp sections, haemocytometer) and some particularities of the grape berry tissue have contributed to this lack of understanding (Coome 1960; Harris et al. 1968; Considine and Knox 1981; Staudt et al. 1986; Jona and Bott 1988). Referring to literature, there is a relationship between the amount of total DNA in a tissue and its cell number (Gardner and Nieman 1964; Kirkham et al. 1972; Hsiao 1973).

In this article, we present a method for extracting and measuring DNA in the berry pericarp (cv. Shiraz) in order to determine the periods of cell multiplication and enlargement during berry development.

Material and Methods

Plant material: Clusters were harvested on 6-year-old Vitis vinifera L. cv. Shiraz vines, grafted on 110 Richter rootstocks, trained on a vertical trellis and pruned as bilateral cordon (experimental vineyard of Domaine du Chapitre, ENSA.M/INRA, Montpellier, France).

Sampling and preparation of berries: Grapevines with similar vigour were selected. All rank I clusters were harvested, to reach 30 clusters per sample. Eighteen samples were harvested weekly from May 25 to September 1, 1997.

Classification and characterization of berry population: All berries of a cluster were cut at the pedicel base, counted, weighed and classified according to their diameter by sieving. Each class was characterized by its frequency and the mean weight of berries. The mean diameter and weight of the berry populations were determined. The berry mean volume of the major class was measured by water displacement.

One week after the beginning of veraison (assessed by the softness of 10% of berries), berries were classified ac-
According to their density by floating in solutions of sucrose at different concentrations. The average population density was calculated for each sample.

**Sampling for DNA analyses:** About 50 berries from the major class were weighed, then cut transversely to remove the seeds. The seeds were weighed and the pericarp mean weight was calculated. Pericarps were frozen in liquid nitrogen and stored at -80 °C.

**Extraction, purification and estimation of DNA:** Extraction and purification was based on the method described by Thuis et al. (1997) for grapevine wood, with some modifications. From each sample triplicate extractions were made.

**Extraction:** Pericarps were ground for 2 min under liquid nitrogen, with a steel roll-on mechanical grinder (Dangoumeau, France). 2 g of the frozen powder was mixed with 20 ml of extraction buffer [1 M Tris pH 8.0, 1.4 M NaCl, 2% (w/v) CTAB (cetyltrimethyl ethyl ammonium bromide), 20 mM EDTA] and 200 µl of 2-β-mercapto-ethanol (1% v/v) in a 50 ml tube. The tubes were incubated for 60 min at 65 °C with a manual agitation every 10 min. The solution was extracted with one volume of chloroform/isoamyl alcohol (24:1 v/v), mixed slowly for 15 min. at room temperature, then centrifuged for 20 min at 37 °C in the presence of 150 µl of 0.1M NaCl, 0.01M EDTA, containing 33 ng µl⁻¹ of DAPI. Fluorescence was measured at 30 °C, in an Amino Bowman Series 2 spectrofluorimeter (excitation 360 nm, emission 455 nm, slit width 4 nm, PM voltage 700). DNA concentrations were determined by reference to a salmon sperm standard.

**Results and Discussion**

**Berry growth:** The growth of Shiraz berries followed a typical double sigmoid curve (Fig. 1). The first growth phase (phase I) stopped 42 d after anthesis (day 42), when the daily mean temperature summation (basis 10 °C) reached 412 °C. At this stage, the weight of the berries had reached 52% of its maximum. Seeds reached their maximal weights before day 35; and thereafter the weight remained constant until the end of berry growth. Phase II (lag phase) was relatively long (14 d). The second growth period (phase III or ripening phase) started when the temperature sum reached 600 °C (day 56), 2 d after the beginning of veraison (day 54, evaluated from softening of 10% of berries), and continued until maturity.

![Fig. 1: Changes in fresh weight, diameter, total seed weight and mean sugar content of Shiraz berries from anthesis to maturity, related to the average daily air temperature sum (basis 10 °C) and the number of days after anthesis. The arrow indicates the onset of veraison (softening of 10% of the berries). The dotted vertical lines delimit phases I, II and III.](image-url)
Fig. 2: Changes in the absolute growth rates of berries (AGR-berry) and seeds (AGR-seeds) of Shiraz from anthesis to maturity, related to the average daily air temperature sum (basis 10 °C) and the number of days after anthesis. For details see Fig. 1.

During phase I, the absolute growth rates of the berries (AGR-berry) and seeds (AGR-seeds) reached their maximum simultaneously, toward day 28 (Fig. 2). The slowdown of berry growth observed toward day 9, corresponded to a period of significant reduction in air temperature. During this period, the average daily air temperature decreased from 25 to 15 °C between day 2 and day 7 (Fig. 3). This growth depression was not observed on seeds.

During the second growth period, AGR-berry reached a maximum 10 d after the onset of ripening. A second peak was also observed toward day 80, 2 d after a strong rain (Fig. 3).

Changes in total DNA and cellular division: Total DNA of the pericarp increased from anthesis until the average daily temperature sum (basis 10 °C) reached about 340 °C, approximately 35 d after anthesis, before the end of phase I (Fig. 4). From this stage, the total amount of pericarp DNA was constant for two months (5.5 µg total DNA per pericarp), until maturity was reached. The increase in DNA related to the berry volume gives a more regular curve, indicating that DNA reaches a maximal value before the lag phase (Fig. 5).

The changes of the relative DNA content in Fig. 4 indicate that the highest mitotic activity occurred before day 5. This confirms results obtained by COOMB (1960) (cv. Muscat of Alexandria) and by JONA and BOSTA (1988) (Barbera and Freisa) with histological methods based on cell numbering of pericarp sections. The important decrease in DNA observed thereafter, could be associated with a reduction of air temperature by 10 °C (Fig. 3), as has already been noticed by JONA and BOSTA (1988). If this is true, the role of temperature on cell division of berry pericarp would be an important phenomenon which would need more detailed examination.

The return to normal temperatures was not followed by a recovery in the relative rate of DNA increase. The steady increase of DNA from day 11 to day 35, suggests that the mitotic activity is limited to some peripheral cell layers, while the other pericarp cells have already begun to enlarge (see further). These results corroborate the microscopic observations of CONSIDINE and KNOX (1981) and FOUGERE-RIFOT et al. (1997), who have shown that cell division continues in the peripheral tissues localized between the external epidermis and the vascular bundles.

The similarities between our results obtained by DNA estimation, and the results obtained by direct counting seem to exclude the hypothesis of DNA endoreduplication (post-mitotic nuclear DNA synthesis) in berry pericarp cells, in contrast to tomatoes (BEGEROVOET et al. 1996) or maize (KNOWLES and PHILIPS 1988).

The amount of total pericarp DNA increased by a factor of 33 between day 0 and day 35, which is distinctly more important than the 1.5 division cycles shown by HARRIS et al.
and bis the pericarp mean density (g·ml−1) at anthesis, indicating an important cell multiplication rate during this period. Under our experimental conditions, seed growth and the increase of total DNA in the pericarp stopped simultaneously at day 35 (Figs. 1, 4).

It is well known that seed number and berry growth are positively correlated which is an argument in favor of a hormonal control of berry development by seeds (Coombé 1960; Nitsch et al. 1960; Coombé 1973). Our results suggest that seed growth could have a positive effect on cell mitosis, rather than on cell enlargement.

Evolution of the DNA quantity related to the pericarp weight: Fig. 6 shows the DNA evolution on a fresh weight basis. The relative DNA quantity increased during the first weeks after anthesis, indicating an important cell multiplication rate during this period. From day 5, the DNA quantity per gram of pericarp decreased until maturity as a consequence of cell enlargement in relation to vacuolar hypertrophy (Storey 1987).

We propose a cell enlargement index (CEI), that allows to appreciate the mean cell enlargement, according to the following formula:

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CEI = \frac{a}{b^2} \cdot \frac{1}{g^{-1}}
\]

\(a\) is the DNA quantity per pericarp fresh weight (µg·g−1), and \(b\) is the pericarp mean density (g·ml−1) (in this test we have taken into account the mean density of the whole berry).

The CEI (ml·µg−1) represents the pericarp volume per DNA weight unit.

The CEI evolution (Fig. 7) shows that the mean size of the pericarp cells did not increase during the first 15 d. The CEI variation indicates that the mean volume of the pericarp cells increased by a factor of 16 between anthesis and maturity: 8.6 times during phase I, and 7.4 times during phase III. These results are similar to those presented by Coombé (1960) for Muscat of Alexandria, which is also a seeded cultivar. With regard to seedless vines, Harris et al. (1968) have shown that the pericarp cell size of Sultana already increased 20 times during the first 16 d of berry growth.

The CEI mean daily rate, clearly shows two periods of intense cell enlargement (Fig. 7). The first started one week after anthesis (70°C·d) and stopped suddenly at the end of phase I, at 420°C·d. The second period started at veraison, at about 550°C·d, and continued during phase III with a peak towards day 80 (935°C·d). Possibly this peak was the consequence of a modification of the grapevine water status, as it occurred after a strong rain (22 mm at day 77; Fig. 3). The influence of the plant water status on pericarp cell enlargement at different development stages is studied at the moment by our team.

Cell enlargement exhibited comparable rates in phases I and III, although the vacuolar expansion modes are expected to be very different between these periods. There is also a change in the water transport system, i.e. the periph-
eral xylem is no longer functional after veraison and during ripening phloem becomes the main water transport system to the berry (Düring et al. 1987; Creasy et al. 1993; Creasy and Lombard 1993; Ollat and Gaudillère 1996). At this developmental stage, berries loose water by transpiration only (Greenspan et al. 1996).

During the first 5 d, mean daily rates of CEI were negative, but increased thereafter. At the end of phase III, with decreasing cell volume, the CEI variation rate is also negative. The decrease of berry volume by loss of water at the end of the ripening stage is often observed with Shiraz and Carignan (Smart et al. 1974; Freeman and Kliewer 1983; Davis and Robinson 1996; McCarthy 1997, 1999). This phenomenon of over-ripening is accompanied by an increase of sugar concentration (McCarty and Combe 1999). However, the DNA content remains constant, showing that over-ripening, often considered as a senescence process (Sacher 1973), is not associated with cell self-autolysis.

The evolution of cell multiplication (Fig. 4) and enlargement (Fig. 7) allows to distinguish between three periods during phase I. The first, from anthesis to day 5, is characterized by intense mitosis and insignificant cell enlargement. Berry growth seems to be due exclusively to cell multiplication and not to cell enlargement. The second period, from day 5 to day 35, is characterized by a reduction of mitosis and by the induction of a strong cell enlargement. Cell division stops definitely one week before the onset of phase II. The third period corresponds to the last week of phase I, during which berry growth depends only on cell enlargement. During phase III, the berry growth is only due to cell enlargement as well.

**Conclusion**

Analysis of the DNA content of grape berries (cv. Shiraz), presented in this work, has allowed to indirectly quantify the number and size of the pericarp cells. This has enabled us to analyze precisely the beginning and the end of mitosis during phase I, and to quantify and compare the periods of cell enlargement during berry development.

Our attempts to get more precise data by flow cytometry (data not shown) were not successful. However, determination of the DNA content can overcome major disadvantages of methods previously used on grapes. The cell separation technique by steeping and numbering with a haemocytometer is difficult due to the extreme fragility and the compartmentation breakdown of pulp cells, during the berry ripening phase (Harris et al. 1968; Lang and Düring 1991). The histological procedure of direct counting the number of cells across a transverse section of the pericarp (Combe 1960; Considine and Knox 1981; Jona and Botta 1988) permits to estimate the volume and the number of cells, but the problem is also the fragility of pericarp cells, and these methods are not adapted to analyze large numbers of berries. Extraction and estimation of DNA permits to work on frozen material; it is also possible to homogenize a larger number of berries to get more representative data.

The effects of cultural and environmental stresses, e.g. effects of water deficit on berry cell division and enlargement is currently studied with this method.

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