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Department of Viticulture, Catholic University, Piacenza, Italy

Relationships between seed number, gibberellin and abscisic acid levels and ripening in Cabernet Sauvignon grape berries[1])

by

A. Scienza[2]), R. Miravalle, C. Visai[3]) and M. Fregoni

Beziehungen zwischen Anzahl der Samen sowie Gibberellin- und Abscisinsäuregehalt und Reifeverlauf der Traubenbeeren bei Cabernet Sauvignon

Zusammenfassung. — Bei den Trauben von Cabernet Sauvignon wurden das Beerenwachstum, der Anthocyangehalt und die Zusammensetzung der Phytohormone in Beziehung zur Anzahl der Samen untersucht.

Die Gibberellinaktivität erreichte 45 d nach der Anthese ihren höchsten Stand, während der Abscisinsäuregehalt (je Beere) zwei Maxima zeigte; das erste trat kurz nach dem Beerenansatz auf, das zweite fiel mit dem Beginn der Beerenreife zusammen.

Die Anzahl der Samen war positiv mit der Gibberellinaktivität und der Abscisinsäurekonzentration (je Beere) korreliert. Einsamige Beeren wiesen jedoch einen höheren Anthocyangehalt als zwei- oder dreisamige Beeren auf.

Introduction

Considerable research has proved a definite link between seed number, berry development and its sugar and acid content (Winkler and Williams 1936, Olmo 1946, Gärtel 1954, Pastena 1964, Schumann 1973, Rapp and Klenert 1974).

Such relationship has been attributed to hormones, such as auxins, gibberellins, cytokinins, formed in the seeds and spread into the pulp, thus promoting cell division and extension and changing pulp composition (Coombe 1960, Nitsch *et al.* 1960, Hale 1968, Iwahori *et al.* 1968, Allewelt and Hifny 1972, Hale and Coombe 1974, Allewelt *et al.* 1975, Inaba *et al.* 1976).

Berry growth pattern follows a double-sigmoid curve in which four phases were identified by Nitsch *et al.* (1960). This pattern may be divided in two separate sigmoid curves: the first one is associated with strong mitotic cell activity and development of berry seeds, and is assumed to be under growth promoter control, while, during the second part, cell extension takes place and ripening processes occur; in this phase abscisic acid and small amounts of ethylene were detected in grape berries.

Our knowledge about gibberellin-like substances in grape berries is not exhaustive, but the results of Coombe (1960) and Inaba *et al.* (1976) indicate that a maximum content is reached during phases I and II of berry growth. In addition, Ito *et al.* (1969) and Farmahan and Pandey (1976) found higher levels of gibberellin-like substances in seeded than in seedless berries.

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[1]) Research performed under C.N.R. programme "Biology of Reproduction".

[2]) Professor at Piacenza and Assistant at the University of Milano.

[3]) Assistant at the Institute of Pomology of Milano.

Abscisic acid attains a high level at the beginning of phase IV, which corresponds with the initiation of the ripening processes in the berries (DÜRING 1974, HALE and COOMBE 1974, COOMBE 1976, LILOV and ANGELOVA 1977). After ABA treatments HALE and COOMBE (1974) found an earlier maturation and a higher sugar content in the berries, while, phase II, ethrel application increased the ABA content with a corresponding hastening of maturation.

Abscisic acid is supposed to be produced not only in the seeds (LOTT 1968, ALLEWELDT *et al.* 1975, COOMBE 1976) but seems also to be transported from the leaves to the berries (DÜRING *et al.* 1978).

Material and methods

In Mr. ERNESTO VIGEVANI's vineyard at Ancarano di Rivergaro in the province of Piacenza berries were picked every 8—10 days starting at fruit set until ripeness, following the technique described by HUGLIN and JUILLIARD (1959), using Cabernet Sauvignon vines on Kober 5 BB rootstocks. Berries were classified in the laboratory according to their seed number. Berries containing 1, 2 or 3 seeds were analyzed as follows:

Determination of gibberellin-like substance activity by barley endosperm bioassay. Extraction, purification and chromatography were carried out following ECCHER *et al.* (1976). Chromatograms were tested as described by COOMBE *et al.* (1967), modified by RYUGO and INTRIERI (1972).

Free abscisic acid determination by high pressure liquid chromatography (HPLC). Extraction by methanol and ether was carried out following the method of DÜRING and SCIENZA (1975), while the identification and quantitative determination was performed as follows, modifying the method of DÜRING and BACHMANN (1975):

Column: Lisorb AMM (25 × 0.21 cm); mobile phase: buffer phosphate 0.02 M, pH 5; flow rate: 60 ml/h at 2100 psi; column temperature: 25 °C; detector: UV 262 nm; injection: 5 μl.

Sugar determination was carried out by refractometer and acidity expressed in tartaric acid titrated by 0.1 n NaOH, using centrifugated grape juice. Tartaric acid contents were determined by REBELEIN's method (1961) modified by LIPKA and TANNER (1974), while malic acid was determined by the enzymatic method described by BERGMEYER (1970).

The anthocyanin content was determined following the method of EWART and KLIEWER (1977).

All results were elaborated according to analysis of variance and L.S.D. calculation by TUKEY's test (1956).

Results

Gibberellin-like substances in berries

The gibberellin-like activity of extracted berries showed two peaks in phases II and III of the growth curve. The second peak showed particularly high levels (up to 0,14—1,15 μg/100 g fresh weight), according to seed number (Fig. 1).

During the final part of phase III a decline of gibberellin-like substances was detected, while, during phase IV, gibberellin activity was very low. In phase III,

three-seeded berries contained higher levels of gibberellin-like substances than one or two-seeded berries.

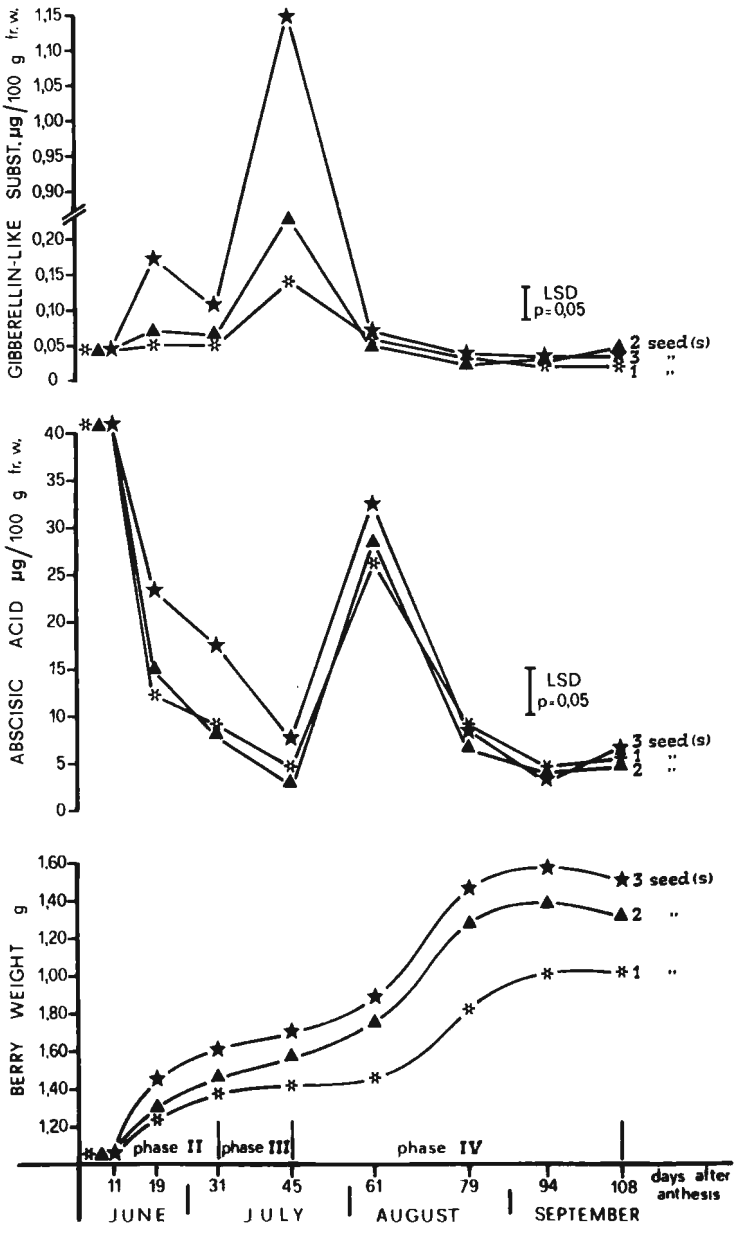

Fig. 1: Gibberellin-like substance and ABA contents (μg/100 g fresh weight) in berries with different number of seeds in relation to the development phases of the berries.

Gibberellin- und Abscisinsäuregehalt (μg/100 g Frischgewicht) von Beeren mit unterschiedlicher Anzahl von Samen in Beziehung zu den Entwicklungsphasen der Beeren.

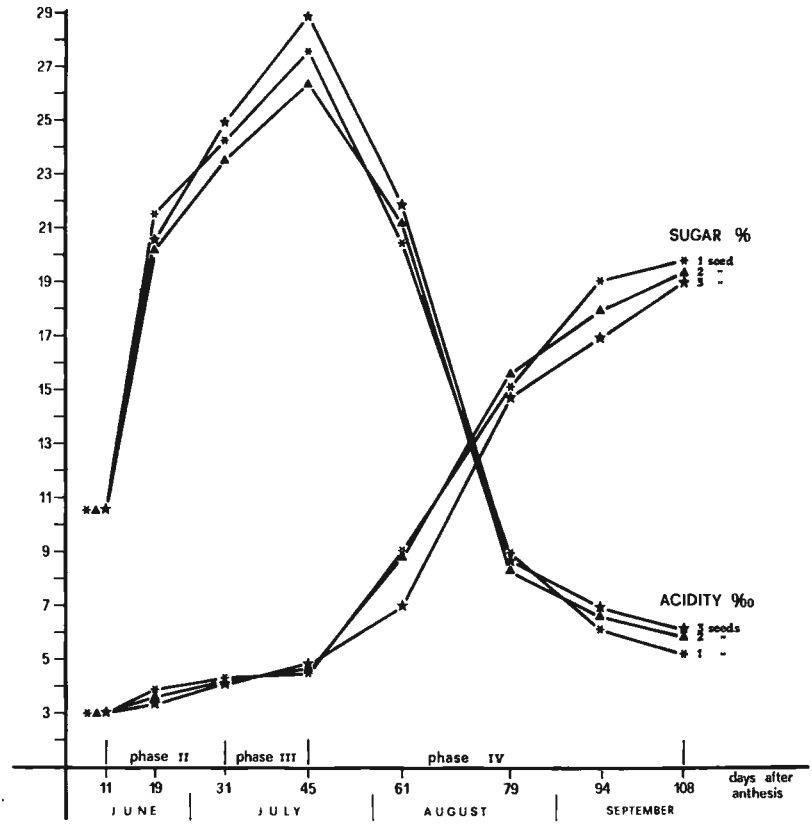

Fig. 2: Behaviour of sugar content and acids in the berries in relation to the number of seeds.
Zucker- und Säureverlauf in den Beeren in Beziehung zur Anzahl der Samen.

Abscisic acid content in berries

The highest levels of abscisic acid in berries were detected in the period immediately after fruit set (40,7 μg/100 g fresh weight) (Fig. 1). A decline of the abscisic acid level was noticed during phases II and III of the growth curve with a content of 3—7,6 μg/100 g fresh weight depending on seed number. In phase IV, the abscisic acid concentrations raised up to 26—32 μg/100 g fresh weight, and then declined to low values. While there were no significant differences in abscisic acid values to a fresh-weight basis detectable between the one, two and three-seeded berries except one date (31st d after anthesis), abscisic acid on a per-berry basis shows higher amounts in three-seeded than in two or one-seeded berries (Fig. 4).

Abscisic acid content in rachis

Abscisic acid levels in rachis developed independently from those of the berries; i.e. ABA concentration increased greatly during the first phases of rachis development. The level kept constant in the period of berry colour change and a second rise was observed during the final phase of ripening.

Sugar content, titrable acidity, tartaric and malic acid content

There is a small (but only at the 61st d after anthesis) significant difference in the increase of sugar percentage between the one, two and three-seeded berries showing a more rapid sugar increase in one and two-seeded berries (Fig. 2). The increase of sugars on a per-berry basis shows that this increase is more rapid in three-seeded than in two or one-seeded berries (Fig. 4).

The number of seeds per berry had no significant influence on the course of concentrations of titrable acids (Fig. 2).

Tartaric acid content decreased steadily from fruit set to ripeness, being always higher in one-seeded berries, but these differences are not significant. Malic acid increased and subsequently decreased with progressing maturity. The malic acid maximum of three-seeded berries is reached 16 d later than that of one or two-seeded berries.

Anthocyanin content

The appearance of anthocyanins was associated with berry colour change; they accumulate in the skin progressively during the ripening process, especially in August.

One-seeded berries had a statistically higher amount of anthocyanin than two or three-seeded berries, in particular during the final phase of ripening in September (Fig. 3).

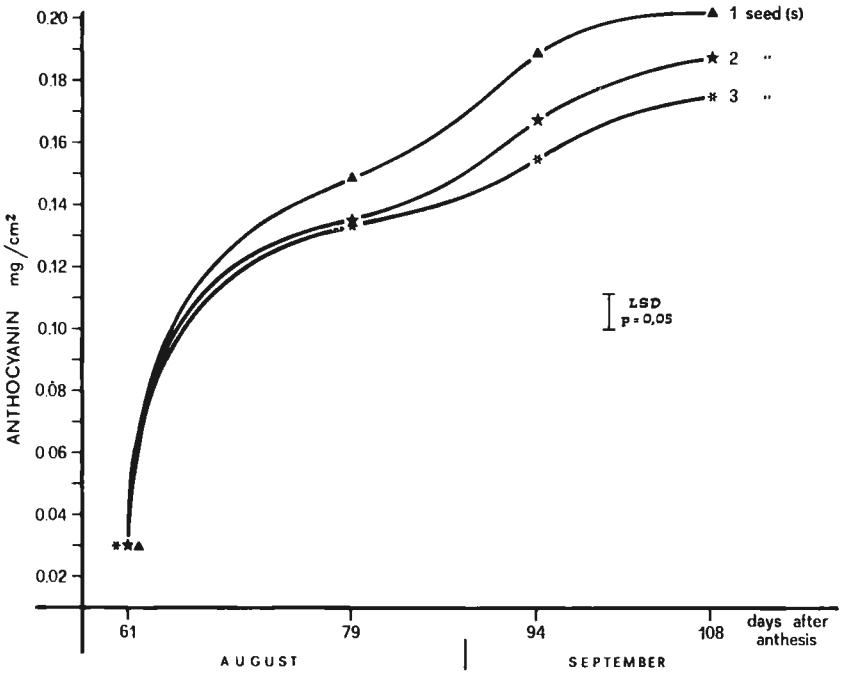

Fig. 3: Influence of seed number on the anthocyanins (mg/cm²) of the skins.
Einfluß der Samenzahl auf den Anthocyangehalt der Beerenhaut (mg/cm²).

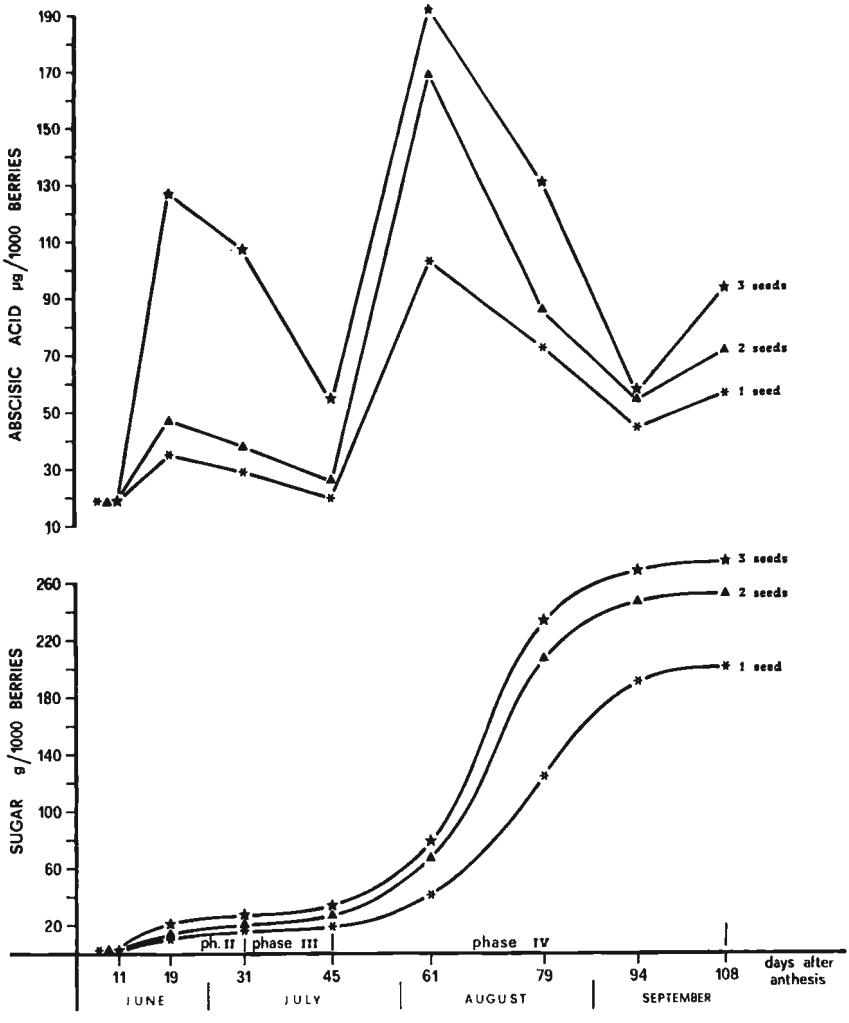

Fig. 4: Influence of seed number on ABA content (μg/1000 berries) and sugar content (g/1000 berries) in berries.

Einfluß der Samenzahl auf den Abscisinsäuregehalt (μg/1000 Beeren) und den Zuckergehalt (g/1000 Beeren) von Traubenbeeren.

Discussion

From the above mentioned results it can be concluded that grape-berry development and changes in their chemical composition are correlated to their number of seeds. The content of gibberellin-like substances was at its highest at the end of phase III. Especially in this period the results show a positive correlation between the content of gibberellin-like substances and the number of seeds, while no significant correlation was registered between the abscisic acid content of berries (to a

fresh weight basis) and the number of seeds. Therefore, the gibberellin : abscisic acid ratio is higher in the more developed, i.e. three-seeded berries. This observation agrees in some respect with the results of CONSIDINE and COOMBE (1972) who found that GA_3 application was inefficient to stimulate the berry growth of two to three-seeded berries, while application of CCC reduced berry size.

The course of the content of abscisic acid in the berries during their development (on a per-berry basis) agrees well with earlier results of COOMBE and HALE (1973), DÜRING *et al.* (1978) and INABA *et al.* (1976) who also found high amounts of abscisic acid in berries after anthesis and a second increase which was correlated to the inception of the ripening processes, e.g. sugar increase. In our results the content of abscisic acid as well as sugars increased more rapidly in three-seeded berries than in two or one-seeded berries.

Summary

In grapes of Cabernet Sauvignon, berry growth, content of anthocyanin and hormonal composition affected by seed number were investigated.

Gibberellin-like substance content showed highest values 45 d after anthesis, while abscisic acid content (on a per-berry basis) had two peaks, the first one just after berry set, the second coinciding with ripening phenomenon.

Seed number was positively correlated with the concentrations of gibberellin-like substances and abscisic acid (on a per-berry basis). One-seeded berries, however, had a higher amount of anthocyanin than two or three-seeded ones.

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Dr. A. Scienza
Istituto di Coltivazione Arboree
Sezione Viticoltura
Università Cattolica
29100 Piacenza
Italia