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Studies on the cytokinins in fruits
I. Occurrence and levels of cytokinin-like substances in grape berries at different developmental stages

by

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Untersuchungen über die Cytokinine in Früchten
I. Vorkommen und Gehalt cytokininartiger Substanzen in Traubenbeeren verschiedenen Entwicklungszustandes

Zusammenfassung. — In verschiedenen Entwicklungsstadien der Beeren von Bangalore Blue wurden das Auftreten und die Menge cytokininartiger Substanzen untersucht. Die Cytokininkonzentration war während der Anthese und der ersten Phase raschen Wachstums am höchsten. Während der anschließenden Phase verlangsamten Wachstums und der darauf folgenden Phase erneuten starken Wachstums fiel die Cytokininkonzentration deutlich ab. Mit Hilfe des Sojabohnenkallus-Tests wurden auf den Dünnschichtchromatogrammen von Beerenextrakten aller Entwicklungsstadien zwei wachstumsfördernde Zonen nachgewiesen. Obgleich die cytokininartigen Substanzen nicht identifiziert wurden, wird durch die vorliegende Untersuchung doch die Hypothese gestützt, daß neben Auxinen, Gibberellinen, Abscisinsäure und Äthylen auch Cytokinine in das Wachstums- und Entwicklungsgeschehen der Traubenbeeren eingeschaltet sind.

Introduction

All the five classes of growth regulators, viz. auxins, gibberellins, cytokinins, abscisic acid and ethylene have been found to exert varied growth responses in grape berry setting and development (Coombe 1973, Weaver 1973). Attempts have also been made to study the relationship between the endogenous growth substances like auxins, gibberellins, abscisic acid and ethylene and the different phases of growth and development of seeded and seedless grape berries (Coombe 1960, Nitsch *et al.* 1960, Iwahori *et al.* 1968, Allewelt and Hifny 1972, Coombe and Hale 1973, Düring 1974).

Cytokinins have been identified in the bleeding sap of grapevines (Skene 1972) and the importance of such compounds in the inflorescence and pistil development has been demonstrated by *in vitro* studies (Pool 1975). External application of synthetic cytokinin has also been found to affect sex, fruit set and development in grapes (Negi and Olmo 1966, Weaver *et al.* 1966, Weaver and van Overbeek 1973). The berry growth shows a double sigmoid pattern, where the first rapid growth phase involves cell division and cell enlargement (Harris *et al.* 1968, Coombe 1973), and attempts have been made to study the endogenous cell division factors like cyto-

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kinins in *Vitis vinifera* grapes by Alleweldt *et al.* (1975). The results of our studies on the cytokinin-like substances in grape berries of the variety Bangalore Blue *(Vitis vinifera* × *V. labrusca)* are presented in this communication.

Materials and Methods

Grape berries of the variety Bangalore Blue were obtained during the fruiting season from May to July of the year 1975. Samples were collected at weekly or biweekly intervals right from anthesis to maturity. Twenty berries were used to study the changes in length, breadth and fresh weight at each stage.

A known weight of berries including seeds was extracted with 80 per cent ethanol for a period of 24 hours at 5 °C. The ethanolic extract was then filtered through a Buchner funnel using Whatman No. 1 filter paper, with repeated washings using an excess of 80 per cent ethanol. The washings were pooled with filtrate and evaporated to the watery phase in a flash evaporator under reduced pressure at 40 °C. The flash evaporated aqueous phase was adjusted immediately to pH 3 and partitioned three times with equal volumes of distilled n-hexane (Hemberg and Westlin 1973). The aqueous fraction containing the cytokinins was collected for purification using Dowex 50 [H^+], 200 — 400 mesh, ion-exchange resin (Letham and Williams 1969). The cytokinin-like substances were eluated from the ion-exchange column with 2 n followed by 5 n ammonium hydroxide. The pooled eluate was completely dried under vacuum at 40 °C and was taken up in a known volume of 80 per cent ethanol. The cytokinin activity in the purified extracts corresponding to a known weight of grape berries was tested by soybean (var. Acme) callus bioassay (Miller 1961). Also, extracts were further purified by thin layer chromatography on

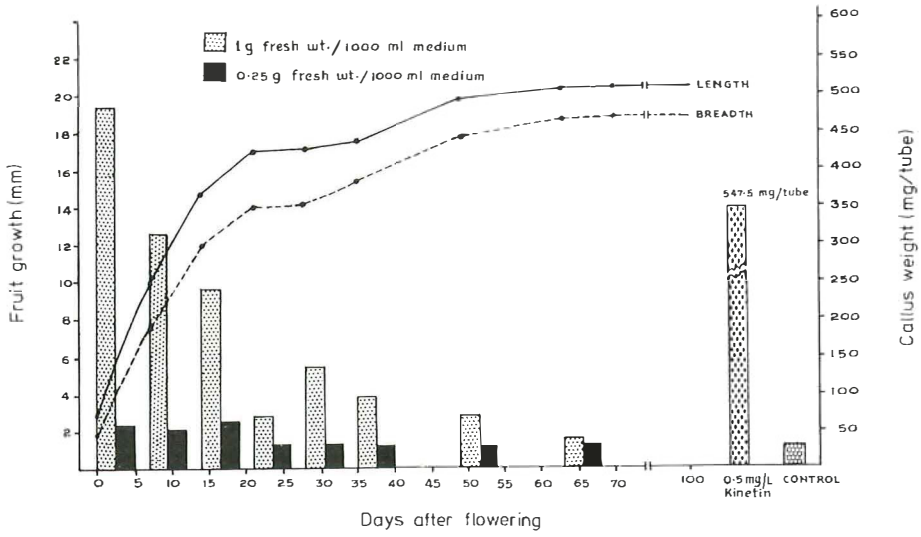

Fig. 1: Relationship between growth of Bangalore Blue berries and cytokinin activity (ion exchange purified extract) at different developmental stages.

Beziehung zwischen dem Beerenwachstum von Bangalore Blue und der Cytokininaktivität verschiedener Entwicklungsstadien (Ionenaustauscher-gereinigter Extrakt).

silica gel coated glass plates using isopropanol : ammonia : water (10 : 1 : 1) as a solvent system. The cytokinin activity in different Rf zones in the TLC plates was tested by the soybean callus bioassay.

Uniform callus explants of approximately 10 to 15 mg were planted in each of the five replicate culture tubes containing ion-exchange or TLC-purified extracts. The cultures were maintained at 25 ± 2 °C under diffuse fluorescent light. After a growth period of 30 days, fresh weights of the callus were recorded. As a check,

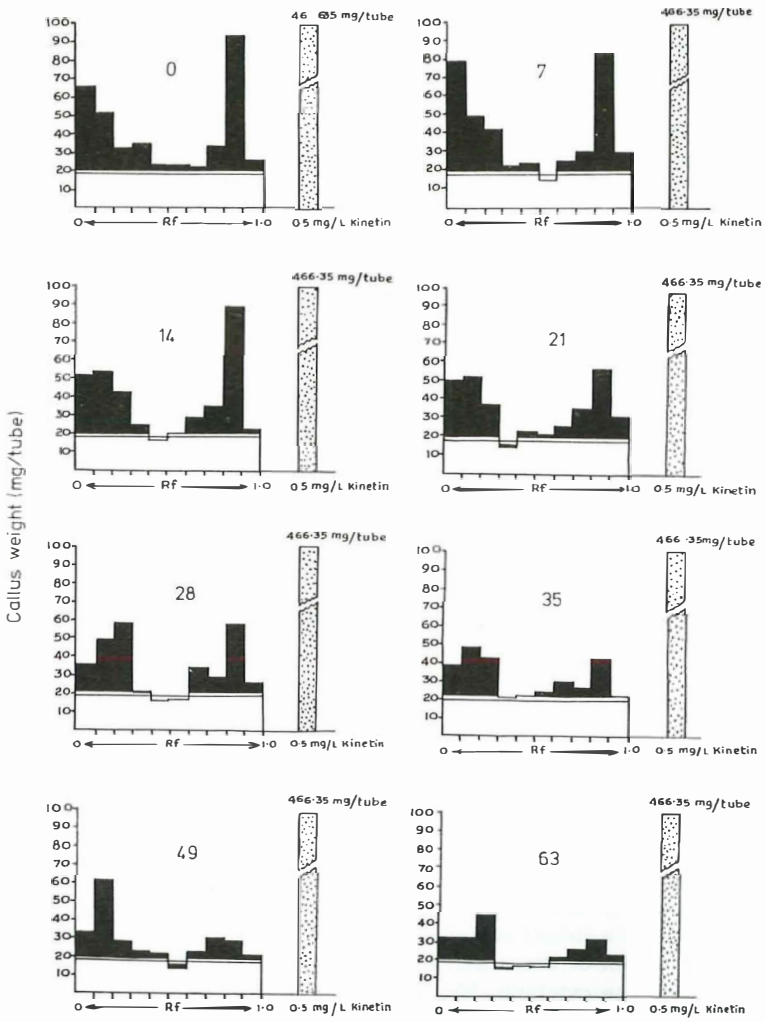

Fig. 2: Rf values (isopropanol : ammonia : water = 10 : 1 : 1) and the levels of cytokinin-like substances extracted from Bangalore Blue grape berries (500 mg fresh weight/l Miller's medium) sampled at different intervals.

Rf-Werte (Isopropanol : Ammoniak : Wasser = 10 : 1 : 1) und Konzentration cytokininartiger Substanzen aus Beerenextrakten von Bangalore Blue (500 mg Frischgewicht/l Miller-Medium) bei zeitlich gestaffelter Probenahme.

parallel bioassays were also run using a known amount of kinetin, along with a control without any cytokinin. The approximate amount of cytokinin-like substances present in the extracts was calculated in terms of kinetin equivalents from the standard growth response curve.

Results and Discussion

The growth of berries in the variety Bangalore Blue was found to be double sigmoid. This confirms earlier studies in grapes (Crane 1964). The activity of cytokinin-like substances in the grape berries at different stages of growth is graphically presented in Fig. 1. Maximum cytokinin activity was found in samples collected at anthesis (0.5 µg kinetin equivalents per g fresh weight) followed by 7 days (0.1 µg kinetin equivalents per g fresh weight) and 14 days after anthesis (0.025 µg kinetin equivalents per g fresh weight), the period at which berries were enlarging at a rapid rate. During the period of suspended growth (fourth week after anthesis), the concentration of cytokinin-like substances in berries decreased considerably and remained at a fairly low level even during the second phase of rapid berry growth. Studies by Coombe (1960) and Harris *et al.* (1968) in Thompson Seedless berries indicated that during the first rapid growth phase, extending to 25 days after anthesis, the berry develops both by cell division and cell enlargement, cell division starting even 5 to 10 days before anthesis.

In grapes, studies by Mullins (1968) on the effect of synthetic cytokinin in cuttings together with the evidence for the presence of cytokinins in xylem sap (Skene 1972) suggest that cytokinins originating in roots are involved in inflorescence growth. According to Alleweldt *et al.* (1975) cytokinin content increases during the first rapid enlargement of grape berries and reaches its maximum during the lag phase; thereafter it decreases rapidly. External application of cytokinins is also known to influence berry set and development in grapes (Weaver *et al.* 1966).

High concentrations of cytokinin-like substances have also been detected during the period of active cell division in a number of fruits like apple, watermelon, Japanese pear, Japanese persimmon and cotton, suggesting the involvement of such substances in the early stages of flower and fruit development (Letham 1966 a, b, Letham and Williams 1969, Prakash and Maheshwari 1970, Ohkawa 1973, Sandstedt 1974, Sobajima *et al.* 1974). The present results, showing high concentrations of cytokinin-like substances during anthesis and the first rapid growth phase, strongly suggest the possible involvement of such factors either alone or in combination with other natural plant growth substances like auxins and gibberellins in nutrient mobilisation as well as in the enlargement of grape berries.

Separation of the cytokinin-like substances in the extracts of grape berries using thin layer chromatography showed the presence o ftwo peaks in

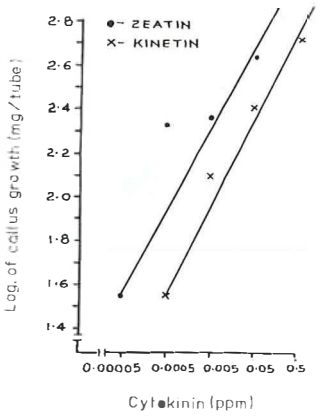

Fig. 3: Linear relationship between callus growth (log) and kinetin and zeatin concentrations.
Lineare Beziehung zwischen Kalluswachstum (log) und Kinetin- bzw. Zeatinkonzentration.

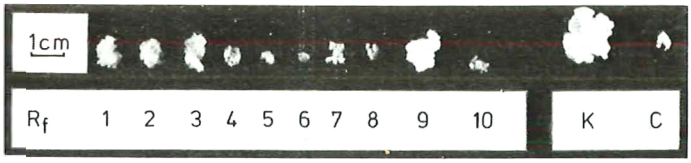

Fig. 4: Response of soybean callus to different Rf zones in TLC of Bangalore Blue extract developed with isopropanol : ammonia : water (10 : 1 : 1) solvent system. Samples were taken approximately two weeks after anthesis. C = Check without added cytokinin, K = kinetin 0.5 ml/l.

Reaktion von Sojabohnenkallus auf verschiedene Rf-Zonen aus DC von Beerenextrakt der Sorte Bangalore Blue (Fließmittel Isopropanol : Ammoniak : Wasser = 10 : 1 : 1). Probenahme etwa 2 Wochen nach der Anthese. C = Kontrolle ohne Cytokininzusatz, K = 0,5 ml Kinetin/l.

soybean callus growth when the different Rf zones were analysed (Figs. 2 and 3). The first peak was found at Rf 0.0 to 0.4 and the second at Rf 0.9 (Fig. 4). These two zones appeared at all stages of development, but their activities changed. Synthetic zeatin and the cytokinin-like substance(s) in the second peak from the berry extracts migrated to the same Rf (0.9) when chromatographed together in isopropanol : ammonia : water. Skene (1972) showed the presence of zeatin, zeatin riboside and ribotide in the xylem sap of grape vines. The identity of the cytokinin-like substances in the berries of Bangalore Blue is being presently studied using paper and thin layer chromatography in different solvent systems, sephadex gel flitration and precipitation with silver salts, the results of which will be communicated elsewhere.

Summary

The occurrence and levels of cytokinin-like substances in the berries of Bangalore Blue grapes were studied at different stages of growth. The concentration of cytokinins was maximum during anthesis and the first rapid growth period. The level of cytokinins declined markedly during the lag and subsequent rapid growth phase. Two growth promoting zones were detected by soybean callus bioassay in the thin layer chromatographs of berry extracts, at all stages of sampling. Although the identity of cytokinin-like substances has not been established, the present study supports the hypothesis that besides auxins, gibberellins, abscisic acid and ethylene, cytokinins are also involved in the growth and development of grape berries.

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