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Effects of cytokinins on elongation, proliferation and total mass of shoots derived from shoot apices of grapevine cultured in vitro[1])

by

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Der Einfluß von Cytokininen auf Längenwachstum, Vermehrung und Gesamtmasse der aus Rebentriebspitzen bei in-vitro-Kultur entstandenen Triebe

Zusammenfassung. — Triebspitzen der Rebe (*Vitis vinifera* L.) wurden auf einem Nährmedium kultiviert, das — einzeln und kombiniert — unterschiedliche Konzentrationen von 6-Benzylaminopurin (BAP) und Zeatinribosid (ZR) enthielt. Bei getrennter Anwendung der Wachstumsregulatoren förderten 2 mg BAP/l oder 10 mg ZR/l das Längenwachstum und die Triebvermehrung am meisten. Beide Vorgänge wurden durch 5 mg BAP/l unterdrückt. Diese Hemmung konnte auch nicht durch zusätzliche Anwendung von ZR, selbst nicht in der Konzentration von 10 mg/l, aufgehoben werden. Eine optimale Triebvermehrung wurde durch ZR in Verbindung mit BAP erzielt, falls die BAP-Konzentration 2 mg/l nicht überschritt.

Introduction

Clonal production of plants has become a major economic application of *in vitro* techniques (Murashige 1974, 1977, Sagawa 1976, Abbott 1978). Shoot apices of herbaceous plants are widely used for *in vitro* propagation, but those of woody species to a lesser degree (Murashige 1974, 1977, Abbott 1978).

Barlass and Skene (1978, 1979) reported that proliferation of adventitious buds from fragmented shoot apices of grapevine was greatly stimulated by 6-benzylaminopurine (BAP). Pool (1975) stated that zeatin riboside (ZR) is the main cytokinin in grapevine xylem exudate. He also found that ZR favoured the *in vitro* development of ovaries and filaments of excised Concord flowers (prior to bloom). Budburst and shoot elongation of *Vitis labruscana* B. (cv. Concord) cultured *in vitro* from nonflowering unrooted pieces of shoots increased dramatically in response to ZR treatments (Pool 1974).

Zeatin riboside apparently plays an important role in the physiology of the grapevine. The successful *in vitro* culture of small apices and apical meristems of shoots of grapevines have major advantages, hence the need to study the effects of ZR and BAP singly and in combination.

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Material and methods

1. Plant material

Dormant canes of *V. vinifera* L. (cv. Chenin blanc) were collected during winter from selected vines. Only vines visually free of symptoms induced by virus and virus-like diseases were used. The canes were cut into lengths of ca. 40 cm, treated with Captan (2 %) and stored in sealed plastic bags at 2—3 °C until use.

Upon removal from cold storage, the canes were sectioned into segments of 12—15 cm comprising 2 internodes with 3 buds. These segments were placed in 250 ml beakers with the bases in ca. 80 ml water. Activation of shoot primordia was enhanced at an ambient temperature of 28—30 °C under constant illumination. Budburst, followed by rapid shoot elongation, occurred after 5—8 d. Preliminary experiments indicated that most of the young shoot tips were free of fungi, which are the main contaminants in culture. Large quantities of elongating shoots could hereby be procured on a small bench area within a short period and virtually at any time of the year.

Shoot tips (15 mm) were removed from elongating shoots exceeding 40 mm in length. Following removal of some of the outer leaves, the tips were surface-sterilized in a 0,5 % sodium hypochlorite solution for 10 min and rinsed thrice in sterile distilled water. Shoot apices (0,75—1 mm) containing 2—3 leaf primordia were excised aseptically under a dissecting microscope fitted with an ocular micrometer. The explants were individually cultured in test tubes (25 × 250 mm) containing 20 ml of the test medium. The tubes were stoppered with cotton wool and covered with tin foil caps.

2. Culture medium

Explants were cultured on the full-strength medium of Murashige and Skoog (1962) supplemented with 30 g sucrose/l and 6,8 g Difco Bacto agar/l. The concentrations of BAP and ZR added are indicated in the table and in Fig. 1. The pH of the medium was adjusted to 5,7 with n NaOH prior to autoclaving.

3. Culture conditions

Explants were incubated in a walk-in temperature-controlled culture room, maintained at 27 °C during a 16 h light period and at 24—25 °C during an 8 h dark period. The light source consisted of "Atlas" cool-white fluorescent tubes providing ca. 3 000 lux at culture level.

4. Experimental design

A two-dimensional grid experiment was established with each treatment consisting of two replicates of 10 explants each (Fig. 1) with a culture period of 42 d. Recordings were made of (a) shoot elongation (length of primary shoot), (b) mass of shoots, (c) number of proliferated shoots exceeding 7 mm in length and (d) percentages of explants with proliferated shoots. Data were subjected to analysis of variance followed by the Student Newmann-Keuls plural comparison test (Snedecor and Cochran (1967).

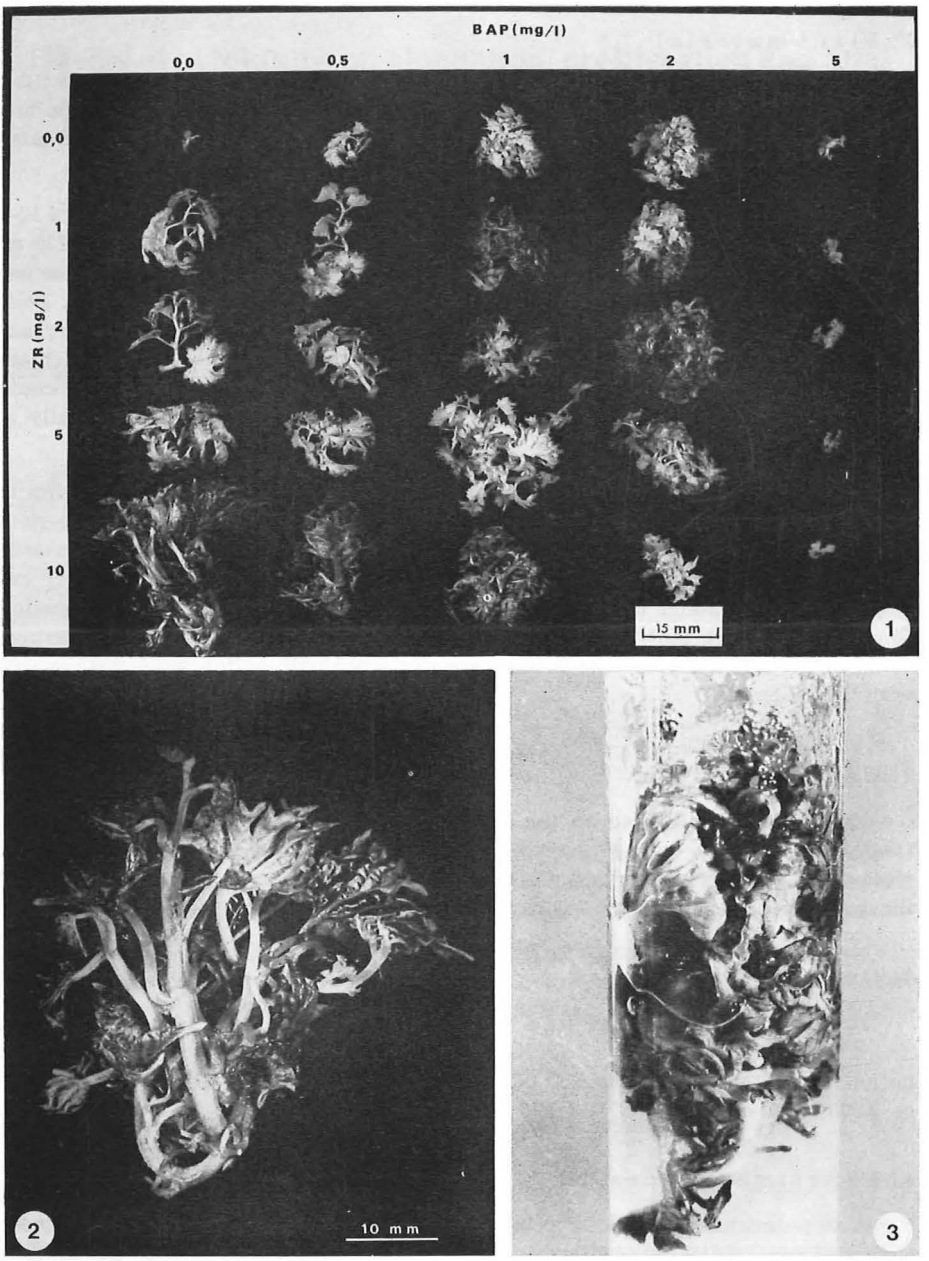

Fig. 1: Effects of 6-benzylaminopurine (BAP) and zeatin riboside (ZR) on elongation and shoot proliferation of shoot apices of *Vitis vinifera* L.

Fig. 2: Shoot elongation and proliferation.

Fig. 3: Shoot proliferation in a submerged culture.

Results

1. Elongation

Elongation increased considerably with concomitant leaf expansion in response to single ZR treatments (Table, Fig. 1). Maximum elongation was obtained with 10 mg ZR/l, which was significantly higher (5 % level) than that obtained with any other treatment (Fig. 2). With BAP used singly, maximum elongation was recorded at 2 mg/l; however, at 5 mg/l elongation was severely inhibited. Combined applications of BAP (0,5—2 mg/l and ZR (1—5 mg/l) resulted in a slight increase in elongation. In comparison with elongation obtained with ZR singly at 10 mg/l, a decrease was observed with ZR (10 mg/l) in combination with BAP (0,5—2 mg/l). The inhibitory effect of 5 mg BAP/l on elongation could not be cancelled by adding ZR (1—10 mg/l). There were no significant differences (5 % level) between elongation with BAP (5 mg/l) combined with ZR (1—10 mg/l) or the control.

2. Shoot mass

Maximum increase in mass was achieved with a combination of BAP (2 mg/l) and ZR (2 mg/l) (Table, Fig. 1). Although elongation was inhibited with BAP (5 mg/l) singly and in combination with ZR (1—10 mg/l), a small increase in mass was recorded.

3. Shoot proliferation

Singly ZR induced maximum shoot proliferation at the highest concentration tested (10 mg/l) (Table, Figs. 1—2). Although a higher level of proliferation was achieved with ZR (10 mg/) compared with BAP (2 mg/), the results do not differ significantly (5 % level). At 5 mg/l, BAP resulted in a complete inhibition of shoot proliferation. More shoot proliferation occurred with a combination of cytokinins (Table, Fig. 1). A combination of BAP and ZR (both at 2 mg/l) proved the most effective (significant at 5 % level). It was also evident that the agar medium (Fig. 3) did not inhibit submerged growth i.e. elongation and proliferation.

4. Percentages of explants with proliferated shoots

Only those explants treated with the highest concentrations (5 and 10 mg/l) of ZR singly reached the proliferation stage (Table, Fig. 1). BAP by itself was most effective at 2 mg/l. Proliferation could be induced with lower concentrations of cytokinins in combination rather than with each cytokinin applied separately.

Discussion

Elongation and proliferation of detached shoot apices of *Vitis* are dependant on the presence of an external source of cytokinin. This is in agreement with reports for other plant species (Boxus 1974, Adams 1975, Abbott and Whiteley 1976, Hussey 1977, Ma and Wang 1977, Mekers 1977, Preil and Engelhardt 1977).

Abb. 1: Einfluß von 6-Benzylaminopurin (BAP) und Zeatinribosid (ZR) auf Längenwachstum und Vermehrung der Triebe aus Triebspitzenkulturen der Rebe (*Vitis vinifera* L.).

Abb. 2: Längenwachstum und Vermehrung von Trieben.

Abb. 3: Triebvermehrung in einer Submerskultur.

The effect of different concentration ratios of 6-benzylaminopurine (BAP) and zeatin riboside (ZR) on elongation, proliferation and total mass of shoots derived from shoot apices of grapevine (*Vitis vinifera* L.) cultured *in vitro*.

Der Einfluß unterschiedlicher Konzentrationsverhältnisse von 6-Benzylaminopurin (BAP) und Zeatinribosid (ZR) auf Längenwachstum, Vermehrung und Gesamtmasse der Triebe, die aus *in vitro* kultivierten Triebspitzen der Rebe (*Vitis vinifera* L.) hervorgingen

Treatment (mg/l) BAP : ZR	Elongation (mm)	Shoot mass (mg)	Number of proliferated shoots	% explants with proliferated shoots
0 0	3,22 f	10,60 k	0,00 f	0 b
" 1	14,27 d	185,50 ijk	0,00 f	0 b
" 2	22,72 bc	245,80 hijk	0,00 f	0 b
" 5	23,02 bc	701,00 defg	1,20 ef	100 a
" 10	36,24 a	853,90 cde	3,40 def	100 a
0,5 0	8,95 e	77,80 jk	0,00 f	0 b
" 1	21,15 bc	344,20 hijk	2,00 def	90 a
" 2	23,83 bc	433,00 ghij	2,10 def	100 a
" 5	24,55 bc	881,70 cde	3,30 def	100 a
" 10	26,08 b	1 107,00 abc	6,20 cd	100 a
1 0	12,11 de	170,20 ijk	0,00 f	0 b
" 1	21,85 bc	382,60 ghijk	2,30 def	100 a
" 2	23,95 bc	587,40 efgh	4,40 cdef	100 a
" 5	25,00 bc	1 012,50 bcd	7,65 bc	100 a
" 10	23,85 bc	1 248,70 ab	9,65 b	100 a
2 0	19,14 c	430,50 ghij	2,30 def	90 a
" 1	22,00 bc	501,30 fghi	3,00 def	100 a
" 2	25,13 bc	1 343,50 a	14,30 a	100 a
" 5	23,95 bc	781,50 def	5,30 cde	100 a
" 10	13,33 de	248,30 hijk	2,55 def	100 a
5 0	3,29 f	25,20 k	0,00 f	0 b
" 1	3,33 f	27,90 k	0,00 f	0 b
" 2	3,60 f	30,50 k	0,00 f	0 b
" 5	3,70 f	32,10 k	0,00 f	0 b
" 10	3,80 f	32,62 k	0,00 f	0 b

Means accompanied by the same letter in a column do not differ statistically at the 5 % level of significance.

Low concentrations of BAP (0,5—2 mg/l) stimulated elongation of explants, whilst higher concentrations (5 mg/l) inhibited elongation; this agrees with the findings of ADAMS (1975) for hops, JONES (1967) for apple and MEKERS (1977) for ornamental bromeliad cultivars. The results also indicate that optimal single concentrations of BAP and ZR differ considerably. Stimulation of elongation obtained with combinations of BAP (0,5—2 mg/l) and ZR (1—5 mg/l) is probably due to the favourable effect of ZR at relatively high concentrations. However, this response was reduced in combinations of ZR (10 mg/l) with BAP (0,5—2 mg/l). Although elongation is reduced with combinations of BAP (0,5—1 mg/l) and ZR (10 mg/l), mass increased. This may be attributed to an increase in leaf expansion, proliferation of shoots and diameter increase of stem parts.

Stimulatory effects of BAP on shoot proliferation are in agreement with reports on other plant species (Boxus 1974, Abbott and Whiteley 1976, Hussey 1977, Mekers 1977, Yie and Liaw 1977). Although Preil and Engelhardt (1977) reported that shoot proliferation of azaleas was stimulated with increasing concentrations of BAP (2—5 mg/l), lower concentrations of BAP (0,1—1 mg/l) seem to be optimal for most species. The results of the present study clearly indicate that, although shoot proliferation was achieved with single applications of BAP (2 mg/l) and ZR (5 and 10 mg/l), interactions between the cytokinins exist, especially perceptible with a combination of BAP (2 mg/l) and ZR (2 mg/l) (Fig. 1). The inhibitory effect of BAP (5 mg/l) on shoot proliferation could however not be eliminated with combined ZR treatments (1—10 mg/l). Although single applications of BAP (2 mg/l) and ZR (5 and 10 mg/l), as well as in combination (0,5—2 mg/l and 1—10 mg/l), induced a 90—100 % proliferation of shoots the results varied considerably between different treatments.

Summary

Shoot apices of grapevine (*Vitis vinifera* L.) were cultured on a nutrient medium containing different concentrations singly and in combination of 6-benzylaminopurine (BAP) and zeatin riboside (ZR). If supplied separately, maximum elongation and shoot proliferation resulted with BAP at 2 mg/l or with ZR at 10 mg/l. Both processes were inhibited by BAP at 5 mg/l. Combined applications of ZR (even at 10 mg/l failed in cancelling this inhibition. Optimum shoot proliferation resulted with the cytokinins in combination, provided the concentration of BAP did not exceed 2 mg/l.

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