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Morphological responses of shoot apices of grapevine cultured *in vitro* Effects of cytokinins in routine subculturing [1])

by

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Die morphologischen Reaktionen *in vitro* kultivierter Rebentriebspitzen Der Einfluß von Cytokininen bei aufeinanderfolgenden Subkulturen

Zusammenfassung. — Aus Rebentriebspitzen gewonnene Triebe wurden wiederholt auf Nährmedien mit unterschiedlichen Konzentrationen von 6-Benzylaminopurin (BAP) und Zeatinribosid (ZR) übertragen. Eine optimale Triebvermehrung wurde mit 2 mg BAP/l in Verbindung mit 2 mg ZR/l erzielt. Ohne BAP, nur mit 10 mg ZR/l erzeugte Triebe wuchsen rasch in die Länge, waren schlank und aufrecht und besaßen nahezu normale Blätter. Mit 2 mg BAP/l, allein oder mit 2 mg ZR/l kombiniert, entstanden unterschiedlich große, gestauchte, oftmals verzweigte Triebe von gekrümmtem Wuchs und mit atypischen Blättern. Cytokinine dürften ein wertvolles Hilfsmittel darstellen, um Rebenklone in großer Zahl zu erzeugen.

Introduction

Stimulatory effects of cytokinins on *in vitro* shoot proliferation from shoot apices of woody plant species have been reported by ABBOTT and WHITELEY (1976), JONES *et al.* (1977), QUORIN and LEPOIVRE (1977), ABBOTT (1978), CHENG (1978).

Proliferation of adventitious buds from fragmented shoot apices of grapevine as well as subsequent shoot production in subcultures were greatly stimulated by 6-benzylaminopurine (BAP) (BARLASS and SKENE 1978, 1979). Non-fragmented shoot apices of *Vitis vinifera* L. (cv. Chenin blanc) responded with marked shoot elongation and proliferation to single and combined applications of zeatin riboside (ZR) and BAP (GOUSSARD 1981).

Objectives of the present study were the effects of BAP and ZR on the rate of proliferation and the morphology of proliferated shoots derived from shoot apices of grapevine cultured *in vitro*.

Material and methods

The procedure for the activation of shoot primordia, excision of shoot apices and subsequent culture conditions, except where specified in the text, were according to GOUSSARD (1981), using *V. vinifera* L. (cv. Chenin blanc). The basal medium (BM) consisted of the full-strength medium of MURASHIGE and SKOOG (1962) supplemented with 30 g sucrose/l and 6,8 g Difco-Bacto agar/l. For initial culturing 10 mg ZR/l was added.

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ZR was included at such high levels (initially and in subcultures) because of its previously observed marked stimulation of elongation and proliferation of shoots derived from grapevine shoot apices (GOUSSARD 1981).

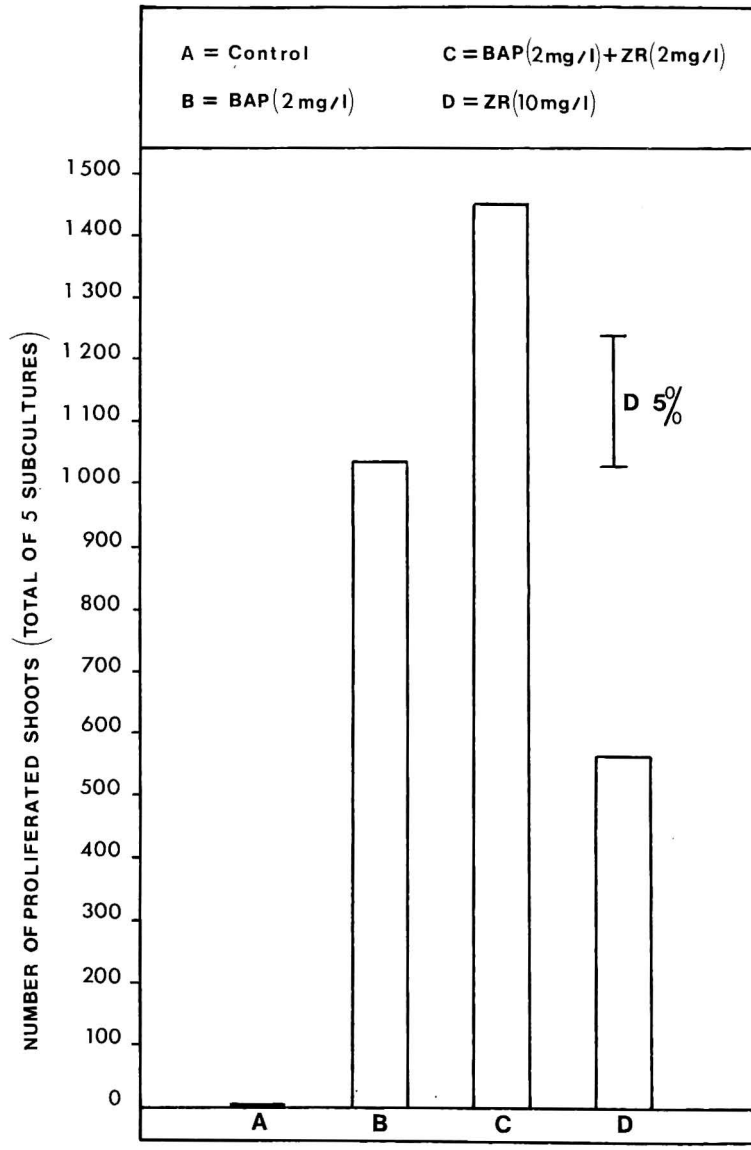


Fig. 1: Effect of different concentration ratios of 6-benzylaminopurine (BAP) and zeatin riboside (ZR) in subculture on proliferation of shoots derived from shoot apices of grapevine.

Der Einfluß unterschiedlicher Konzentrationsverhältnisse von 6-Benzylaminopurin (BAP) und Zeatinribosid (ZR) in Subkulturen auf die Vermehrung der aus Rebentriebspitzen entstandenen Triebe.

After 30 d single cultures with vigorous shoot elongation and proliferation (each having at least 5 shoots > 5 mm) were transferred from individual test tubes to 500 ml wide-mouth Erlenmeyer flasks containing 80 ml of medium. Subculture media included BM enriched with: (1) 2 mg BAP/l; (2) 2 mg BAP/l +2 mg ZR/l and (3) 10 mg ZR/l. Control flasks lacked cytokinins. Each treatment consisted of 5 flasks replicated twice. Cultures were repeatedly transferred to fresh media at 15 d intervals. At each subculture shoot clumps were subdivided into more or less equal parts after proliferated shoots (> 5 mm) had been excised. The experiment was terminated after 5 subcultures. Data were subjected to analysis of variance followed by Duncans multiple range test (SNEDECOR and COCHRAN 1967).

Results and discussion

Cytokinin application in subculture resulted in considerable shoot proliferation (Fig. 1). Maximum shoot production (significant at the 5 % level) was induced by a combination of BAP and ZR (both at 2 mg/l). The increased levels of shoot proliferation obtained with cytokinins in combination are in accordance with previous results (GOUSSARD 1981).

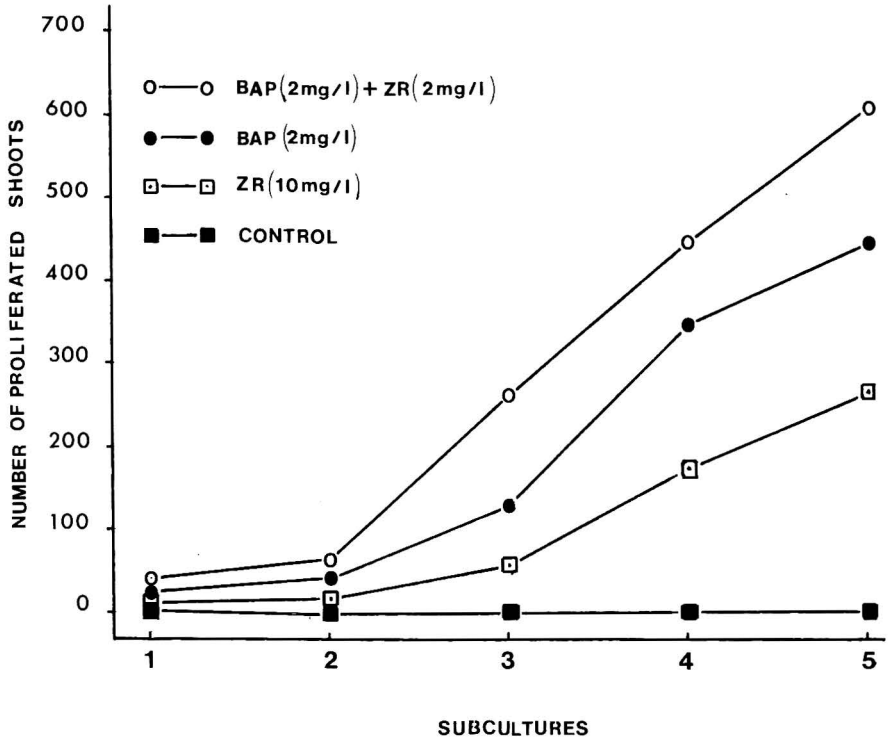
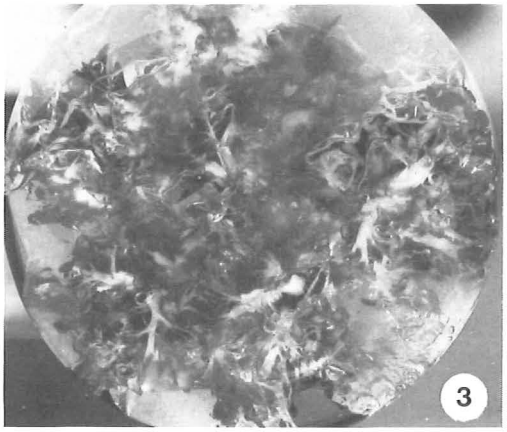


Fig. 2: Shoot production over 5 subcultures in response to different concentration ratios of cytokinins.

Die Triebvermehrung in 5 Subkulturen bei unterschiedlichen Konzentrationsverhältnissen der Cytokinine.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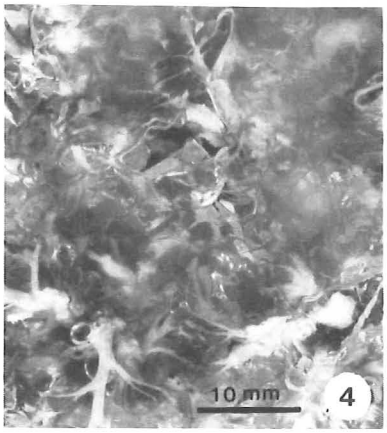
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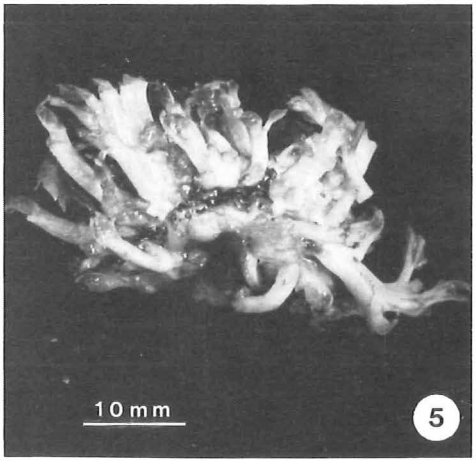
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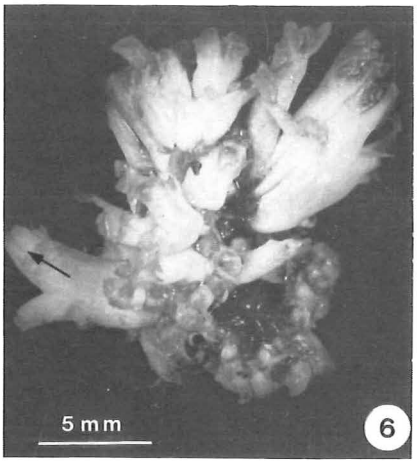
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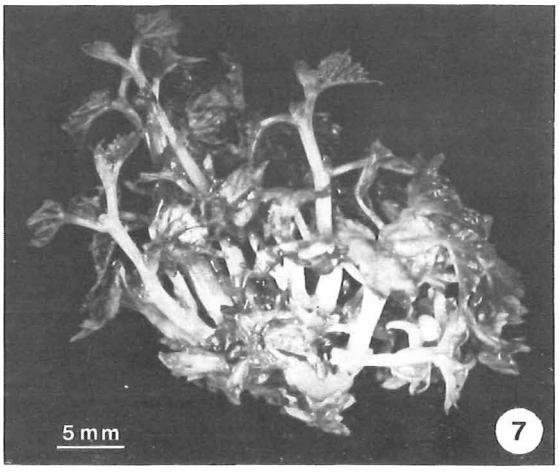
5 mm
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Up to the second subculture, shoot proliferation in response to cytokinin application was relatively limited. Subsequently, it increased almost linearly (Fig. 2). Treatments lacking cytokinins (control) produced no shoots in subsequent subcultures.

Extensive submerged shoot proliferation was achieved with BAP alone and in combination with ZR at the concentrations applied (Figs. 3 and 4). Submerged shoot proliferation may account for higher overall shoot frequency as well as for sharp increases in the number of shoots within 15 d intervals. With 10 mg ZR/l no submerged shoot proliferation occurred. In subsequent subcultures shoot clumps responded after 35—40 d to high levels of ZR (10 mg/l) with submerged callus initiation. A compact, nodular, greenish callus tissue resulted, but was completely absent with 2 mg BAP/l, either alone or in combination with low concentrations of ZR (2 mg/l).

Shoots produced with 2 mg BAP/l, alone and in combination with 2 mg ZR/l, lacked uniformity of size, appeared shorter and thicker and exhibited curved growth patterns. Leaves were conspiciously abnormal (Fig. 5). Dichotomous-like branching of proliferated shoots occured; it was especially discernible at 4—5 d following transfer to fresh media (Fig. 6). Although after 9—10 d the apical regions of many shoots were forked, subsequent elongation was limited. Continuous branching of proliferating shoots gave rise to the formation of dense shoot clusters with numerous protruding shoots. When applied in combination with BAP, low concentrations of ZR (2 mg/l) did not alter the morphological characteristics of proliferated shoots. It is not clear whether this response could be specifically attributed to BAP or whether it was caused by gross shoot proliferation. Shoots produced with 10 mg ZR/l were characterized by single axes, were slim, erect, more uniform in size, bore almost typical leaves and elongated rapidly (Fig. 7). Proliferated shoots with ZR at high concentrations lacked any branching and resulted in shoot clumps of lower density. Shoot production with the above treatment appeared to be mainly due to development and growth of axillary meristems.

It would appear therefore that although ZR at 10 mg/l resulted in lower quantities of proliferated shoots, the morphological characteristics resembled those of the normal condition. It was indicated, however, (BARLASS and SKENE 1978, 1979) that once rooted, shoots produced with BAP in the medium develop into normal plants following passage through a "juvenile" phase (i. e. spiral phyllotaxy and absence of tendrils). With rapid propagation of grapevine clones as a goal, cytokinin application in routine subculturing of shoot clumps derived from shoot apices would be an effective tool.

Figs. 3 and 4: Submerged shoot proliferation.

Fig. 5: Morphological characteristics of shoots proliferated with 2 mg BAP/l alone or in combination with 2 mg ZR/l.

Fig. 6: Enlarged part of shoot clump in Fig. 5 showing dichotomous-like branching (arrowed).

Fig. 7: Shoot proliferation with 10 mg ZR/l.

Abb. 3 und 4: Triebvermehrung in einer Submerskultur.

Abb. 5: Morphologisches Erscheinungsbild der Triebe bei Kultur mit 2 mg BAP/l allein oder in Kombination mit 2 mg ZR/l.

Abb. 6: Vergrößerte Partie der in Abb. 5 gezeigten Triebbüschel mit dichotomen Verzweigungen (Pfeil).

Abb. 7: Triebvermehrung mit 10 mg ZR/l.

Summary

Shoots derived from shoot apices of grapevine were repeatedly subcultured on nutrient media containing different concentrations of 6-benzylaminopurine (BAP) and zeatin riboside (ZR). Optimum shoot proliferation resulted with a combination of the cytokinins, each at 2 mg/l. Shoots produced with ZR only (10 mg/l) elongated rapidly, were slim and erect, with almost normal leaves. With BAP at 2 mg/l alone and in combination with low concentrations of ZR (2 mg/l) proliferated shoots were thicker, often branched, less elongated, curved and not uniform in size. Leaves were atypical. It was concluded that application of cytokinins should be useful in the production of large numbers of grapevine clones.

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