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In vitro propagation of grapevine (Vitis vinifera L.) from fragmented shoot apices

by

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In-vitro-Vermehrung der Rebe (Vitis vinifera L.) aus zerstückelten Triebspitzen

Zusammenfassung. — Eine Methode für die *in-vitro*-Vermehrung der Rebe aus zerstückelten Triebspitzen wird beschrieben. Zellklumpen der Triebspitze wurden auf einem flüssigen Medium, das Cytokinin, aber kein Auxin enthielt, kultiviert. Nach Übertragung der differenzierten Zellklumpen auf ein mit Agar verfestigtes, aber sonst gleich zusammengesetztes Medium entstanden Büschel von Trieben, die zerteilt und weiter vermehrt werden konnten. Explantierte Einzeltriebe bewurzelten sich auf einem hormonfreien Grundmedium rasch.

Introduction

The number of woody plant species which has been propagated *in vitro* by means of somatic embryos or adventitious buds is very small compared with herbaceous plant species (Murashige 1974). Induction of plantlets from embryogenic

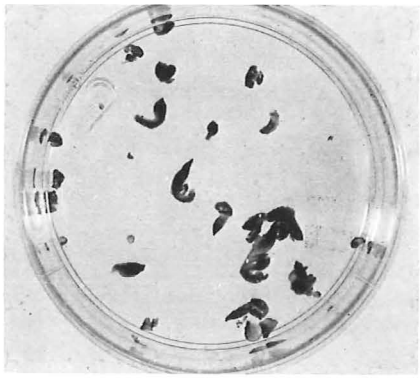

Fig. 1: Differentiation of leaf-like structures from a single fragmented apex of Cabernet Sauvignon grapevine after 1 month in liquid culture. × 0.8.

Differenzierung blattartiger Gebilde aus einer einzigen zerstückelten Triebspitze der Sorte Cabernet Sauvignon nach 1monatiger Flüssigkultur. 0,8 ×.

callus of *Vitis vinifera* (Mullins and Srinivasan 1976) and the French hybrid Seyval (Krul and Worley 1977) has recently been achieved. Adventitious buds have been induced in the hybrid *Vitis riparia* × *Vitis rupestris* (Favre 1977), but apart from a brief reference to the cultivar Prodigiosa in the above paper, the formation of adventitious buds has not been reported with *Vitis vinifera*.

This paper describes a simple, rapid method by which large numbers of adventitious buds were proliferated from a single fragmented grapevine shoot apex.

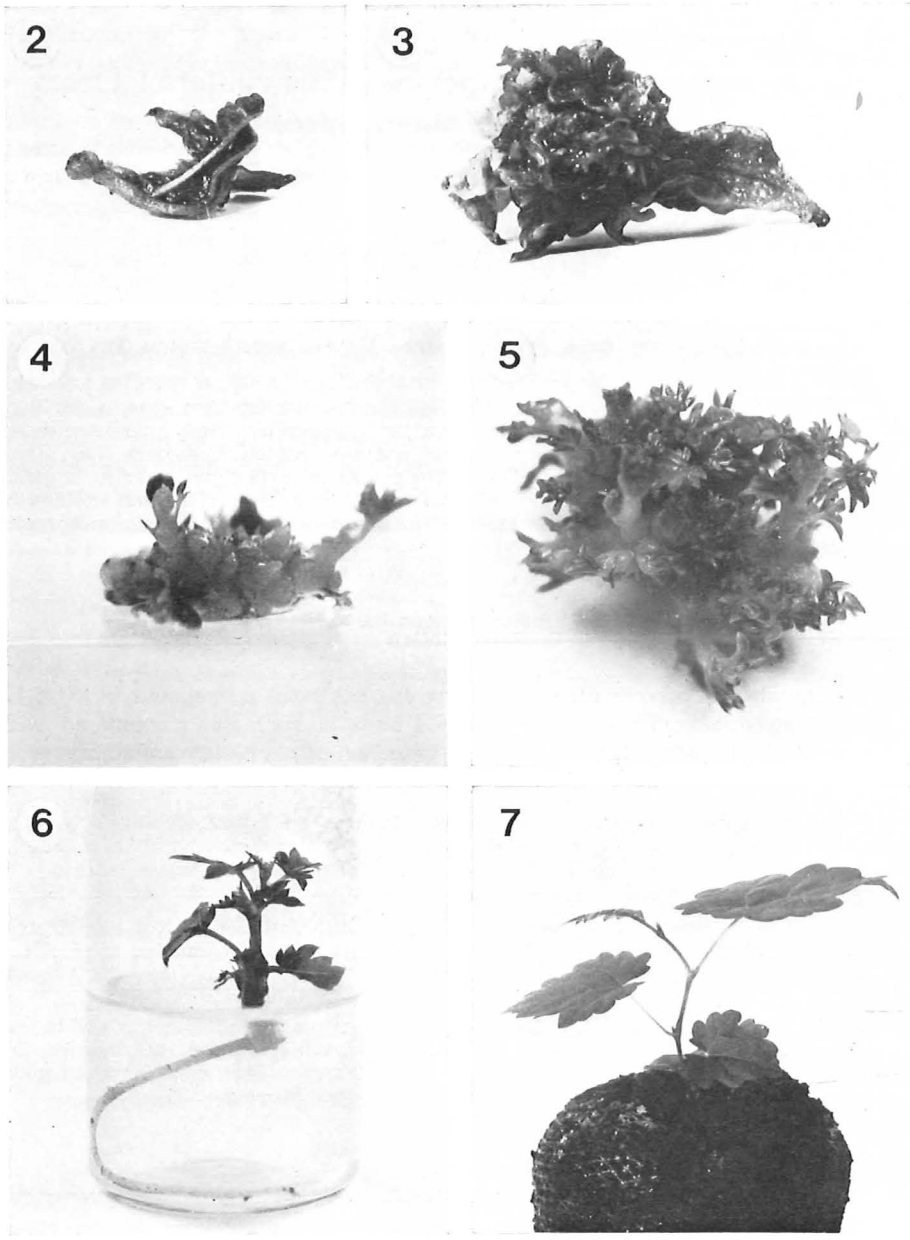

Fig. 2: Leaf-like structure with basal swelling at time of transfer to solid medium. × 1.4.

Fig. 3: Proliferation of shoots from basal swelling of leaf-like structure 1 month after transfer to solid medium. × 1.4.

Fig. 4: Cluster of adventitious buds after 2 subcultures (10—14 d each) of the proliferation area from the leaf-like structure. × 2.3.

Fig. 5: Growth of shoots from adventitious buds. × 1.7.

The regenerated shoots were removed and rooted to produce plants, and the system could form the basis of large-scale clonal propagation.

Materials and methods

Shoot tips (10 mm in length) were removed from glasshouse-grown vines (*Vitis vinifera* L., cv. Cabernet Sauvignon) raised from hardwood cuttings which had been rooted 4 months earlier. The tips were surface sterilized with 5 % w/v calcium hypochlorite solution (filtered) containing 0.01 % Tween 20 wetting agent for 15 min and rinsed three times in sterile distilled water. Shoot apices (ca. 1 mm in length) containing 2—3 leaf primordia, were then excised under aseptic conditions in a laminar flow cabinet. Individual apices were cut into several fragments with a scalpel on dry, pre-sterilized 50 mm plastic petri-dishes and then further teased apart in 5 ml liquid culture medium containing the basal medium of Murashige and Skoog (1962) supplemented with benzyladenine (2 mg/l). Each apex yielded approximately 20 separate cell clumps.

The petri-dishes were sealed with Parafilm and incubated in a walk-in, temperature controlled culture room maintained at 27 °C during a 15 h light period, and 20 °C during a 9 h dark period. The light source was cool-white fluorescent tubes providing approximately 50 $\mu E \cdot m^{-2} \cdot sec^{-1}$ at the culture level. Experimental details on subsequent culture stages will be given in "Results".

Plant material was prepared for light microscopy by fixation in glutaraldehyde and embedding in glycol methacrylate according to the methods of Cole and Sykes (1974). Sections, 2 μm thick, were stained with toluidine blue and periodic acid-Schiff.

Results

After 1 month, at least 90 % of the cell clumps in liquid medium had differentiated into leaf-like structures (ca. 10 mm in length) each with a basal swelling of the central vein (Figs. 1 and 2). At this time, the leaf-like structures were trans-

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Fig. 6: Root formation on excised shoot after transfer to hormone-free basal medium. × 1.4.

Fig. 7: A plantlet of Cabernet Sauvignon grapevine 4 months after fragmentation of shoot apex. × 0.8.

Abb. 2: Blattartiges Gebilde mit basaler Anschwellung zur Zeit der Übertragung auf das feste Medium. 1,4 ×.

Abb. 3: Sprossung von Trieben aus der basalen Anschwellung des blattartigen Gebildes; 1 Monat nach der Übertragung auf das feste Medium. 1,4 ×.

Abb. 4: Büschel von Adventivknospen nach zweimaliger Subkultur (jeweils 10—14 d) der Sprossungszone des blattartigen Gebildes. 2,3 ×.

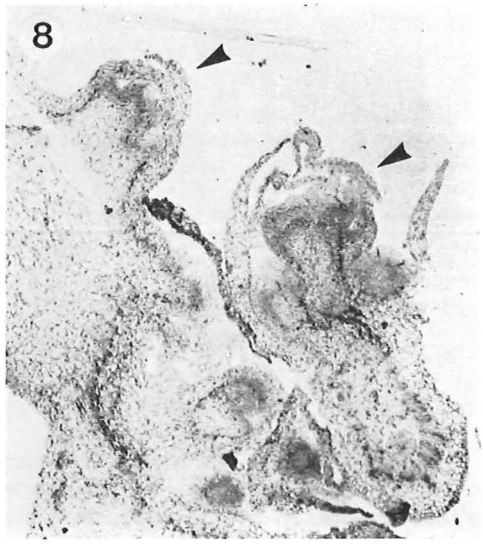
Abb. 5: Aus Adventivknospen hervorgegangene Triebe. 1,7 ×.

Abb. 6: Wurzelbildung an einem isolierten Trieb nach Übertragung auf hormonfreies Grundmedium. 1,4 ×.

Abb. 7: Pflänzchen von Cabernet Sauvignon 4 Monate nach der Zerstückelung der Triebspitze. 0,8 ×.

ferred to the same medium gelled with agar (6 g/l) in 125 ml Erlenmeyer flasks (25 ml per flask). Subsequent experiments showed that the differentiating cell clumps could be transferred to solid medium only 10 d after teasing apart of the apices. Culture conditions were as for liquid medium.

The leaf-like structures on solid medium increased in size to ca. 30 mm in length and after 1 month there was a proliferation of shoots arising from the basal swelling (Fig. 3). Excision of this area from the leaf-like structure, followed by division and subculturing to the same medium, resulted in prolific formation of adventitious buds (Figs. 4 and 8). The rate of growth of the shoot cultures was such that a subcultured 50 mm^2 area of shoots containing approximately 25 visible buds increased four-fold every 10—14 d and required further division and subculturing. Initially, shoots of varying sizes appeared, but after 2 subcultures the shoot size was more uniform (Fig. 5).



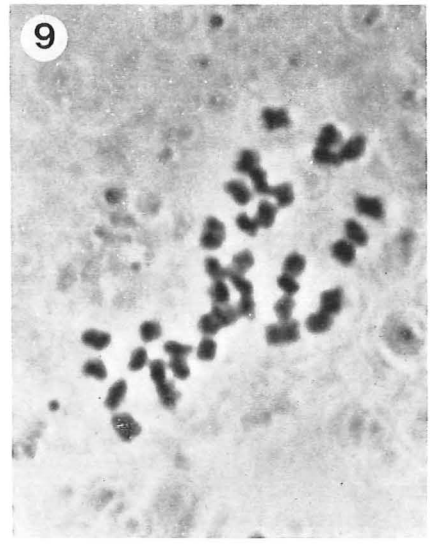


Fig. 8: Section through adventitious buds as in Fig. 4, showing at least 2 buds (arrowed) in longitudinal section. × 24.

Fig. 9: Metaphase plate from root tip squash of cultured plantlet. Phase contrast microscopy after pretreatment with 0.05 % Colchicine and Feulgen staining. × 2,880.

Abb. 8: Schnitt durch Adventivknospen des in Abb. 4 gezeigten Stadiums mit mindestens zwei längs getroffenen Knospen (Pfeile). 24 ×.

Abb. 9: Metaphaseplatte aus der Wurzelspitze eines in vitro kultivierten Pflänzchens. Quetschpräparat, Vorbehandlung mit 0,05 % Colchicin und Feulgenfärbung, Phasenkontrast. 2.880 ×.

Shoots (> 3 mm in length) were excised from cultures and transferred singly to autoclaved 80 × 25 mm screw-capped, clear, polycarbonate tubes containing 12 ml WHITE's basal medium (WHITE 1943, except that Fe was added in the chelated form). Root formation commenced after 7 d (Fig. 6). After a further 14 d in the rooting medium, the plantlets, showing active shoot and root elongation were transferred to Jiffy 7 peat blocks (Jiffy Products Ltd., Grorud, Norway). The plantlets were main-

tained, continuously moist, in covered beakers in the culture room for 9 d. Beaker lids were then removed. 3 weeks after plantlets were transferred to the Jiffy 7 peat blocks, shoot growth had increased from 20 mm to 50 mm in length (Fig. 7) and roots were beginning to appear from the peat. Plantlets were then potted up in a mixture of John Innes soil and perlite (40 : 60) and 4 months after fragmentation of apices the first regenerated plantlets were growing under glasshouse conditions.

Chromosome counts from root tip preparations of the plantlets (Fig. 9) have indicated the normal diploid condition (2n = 38).

Discussion

Previous attempts to obtain adventitious buds from cultured vegetative tissues of Cabernet Sauvignon have been unsuccessful (Mullins and Srinivasan 1976). We report here a preliminary account of the induction of adventitious buds from fragmented shoot apices of Cabernet Sauvignon. Subsequent experiments with Sultana (syn. Thompson Seedless) have given essentially similar results under the same conditions but cultures are not yet to the stage of plantlet formation.

The method described is simple, involving only 2 media, and very rapid, having the potential to produce approximately 8000 plantlets in 3—4 months (allowing only 2 subcultures) from one apex. Although the success rate of both shoot and root formation is very high (virtually 100 %), further work is in progress to refine the system. The unusual differentiation of the apical cell clumps into individual leaf-like structures and the origin of the adventitious buds from these structures is also under study. Chromosome counting, so far, has revealed no abnormal genetic conditions in the plantlets, but ploidy levels after continued subculture are still to be determined. Observation of the cultured vines under field conditions is required to establish the clonal value of the system. However, it is anticipated that this *in vitro* propagation method has commercial potential in the cloning of grapevine cultivars, and possibly also in the production of virus-free planting material.

Summary

A method is described for the *in vitro* propagation of grapevine from fragmented shoot apices, which has the potential to produce approximately 8000 plantlets from a single apex within 4 months. Apical cell clumps were grown in a liquid culture medium with cytokinin but in the absence of auxin. Transfer of the differentiated cell clumps to the same medium gelled with agar resulted in shoot masses which could be repeatedly subcultured. Excised shoots rooted readily on a hormone-free basal medium, and were successfully transferred to glasshouse conditions.

This method has potential value in commercial clonal grapevine propagation.

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