Vitis **20**, 218—227 (1981)

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Organogenesis in internode explants of grapevines

by

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Organogenese bei Internodienexplantaten von Reben

Zusammenfassung. - Bei Kalluskulturen aus Internodien verschiedener Rebsorten, -kreuzungen und -arten wurde die Organogenese untersucht. Adventivwurzeln bildeten sich auf zahlreichen Nährmedien und bei vielen Genotypen. Adventivknospen entstanden an Explantaten in Schüttelkulturen auf einem flüssigen Medium nach NITSCH mit einem Zusatz von Benzyladenin (1 μM) und 2,4-D (5 μM) oder einem Gemisch von 2,4-D und β-Naphthyloxyessigsäure (5 μM). Knospen bildeten sich nur in Kulturen aus Sämlingen von *Muscadinia rotundifolia*, Gloryvine (*Vitis vinifera L.* × *Vitis rupestris* SCHEELE) sowie *V. vinifera* × Gloryvine, nämlich (Grenache × Cabernet Sauvignon) × Gloryvine und (Sumoll × Cabernet Sauvignon) × Gloryvine. Aus ausgereiftem Holz von *V.-vinifera*-Sorten oder einem Gloryvine-Klon hergestellte Stecklinge ergaben Explantate, die keine Knospenbildung zeigten. Die Ergebnisse werden im Hinblick auf die Zusammensetzung der Nährmedien, den Ursprung des Explantats, den Reifezustand der Mutterpflanze und den Genotyp diskutiert.

Introduction

The formation *in vitro* of adventitious roots and adventitious buds has been described in many species but greatest success in regenerating plantlets by organogenesis has been with herbaceous plants. Root formation in explants of woody perennials is of common occurrence but formation of adventitious buds is rare (VASIL *et al.* 1979).

In the Vitaceae, callus or root formation *in vitro* was reported by MOREL (1944), FALLOT (1954), PELET *et al.* (1959) and GALZY (1969) but the first report of formation of adventitious buds was by FAVRE (1977). In this work plantlets were regenerated from leaf tissues of interspecific hybrid grapevines (*Vitis riparia* × *Vitis rupestris*) and from leaf tissues of a little-known cultivar of *V. vinifera*, Prodigiosa (Bicane × Poète Matabon). According to BARLASS and SKENE (1978) shoot formation occurs *de novo* in cultures derived from fragmented apices of *V. vinifera* grapes. The formation of adventitious buds by callus produced from internodal explants has not been described hitherto. The present paper is concerned with attempts to induce organogenesis in isolated internodes of *V. vinifera* L. cultivars, in interspecific hybrids of *Vitis* and in the muscadine grape, *Muscadinia rotundifolia* (syn. *Vitis rotundifolia* MICHAUX).

Materials and methods

Internode explants from a wide range of grapevine genotypes were obtained from plants grown from seed and from plants propagated by hardwood cuttings.

Seed of *M. rotundifolia* was provided by Prof. H. P. OLMO, University of California, Davis. Seedlings of Cabernet Sauvignon were raised from open-pollinated seed harvested from vines grown by the method of MULLINS and RAJASEKARAN (1981). Other seed-grown material was the product of controlled hybridization. Two female hybrid *V. vinifera* grapevines, Grenache × Cabernet Sauvignon and Sumoll × Cabernet Sauvignon, were crossed with Gloryvine, an interspecific hybrid ornamental grape (*V. vinifera* L. × *V. rupestris* SCHEELE) (ANTCLIFF 1980). Gloryvine is normally a male but it was converted into an hermaphrodite by the technique of NEGI and OLMO (1966) so as to produce a supply of selfed-seed. Seed of all genotypes was stratified for 8 weeks (4 °C) before sowing. Plants were propagated from hardwood cuttings of *Vitis* spp. and of *V. vinifera* cultivars by the method of MULLINS and RAJASEKARAN (1981) except that the emerging shoots were usually left unpruned.

Seedlings and cuttings were raised in a controlled environment cabinet illuminated (16 h) with a mixture of 16 cool white fluorescent (Sylvania, Seneca Falls, N. Y., U. S. A.) and 8 incandescent lamps (60 W). The irradiance at the levels of the shoot tips was 40 $W \cdot m^{-2}$ and the temperature regime was 27 °C at day and 22 °C at night. Extension shoots were harvested at 40 d from seedling emergence or when approximately 400 mm in length in the case of cuttings. Internode segments were collected from the upper portions of the shoots. The cut ends of these segments were flamed and sealed with paraffin wax. The segments were then surface-sterilized by shaking for 15 min with a solution of sodium hypochlorite (1 % avail-

Table 1

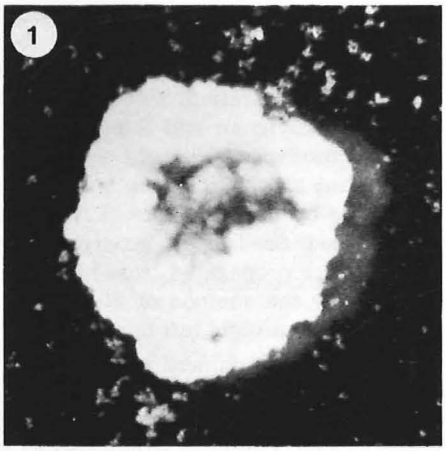
Effects of auxins on callus production by internode explants of a hybrid grapevine, (Grenache × Cabernet Sauvignon) × Gloryvine

Der Einfluß von Auxinen auf die Kallusbildung durch Internodienexplantate der Rebkreuzung (Grenache × Cabernet Sauvignon) × Gloryvine

Auxin supplement to NITSCH medium containing BA (1 µM)	No. explants producing callus (out of 30)		Consistency of callus		Mean callus fresh wt mg[1, 2]
	Auxin concentration		Auxin concentration		Auxin concentration
	5 µM	10 µM	5 µM	10 µM	5 µM
NAA	13	22	Loose	Loose	165 ± 6
NOA	18	24	Loose	Loose	180 ± 6
2,4-D	11	19	Compact	Compact	103 ± 4
2,4,5-T	0	0	—	—	—
4-CPA	8	12	Intermediate	Compact	123 ± 8
α-CPPA	17	21	Loose	Intermediate	150 ± 6
2,4-D + NOA	24	22	Compact	Intermediate	183 ± 7

[1]) Measured after 20 d of culture.
[2]) Means of 3 cultures each of 5 explants.

Abbreviations:
BA = benzyladenine (syn. 6-benzylaminopurine).
NAA = α-naphthaleneacetic acid.
NOA = β-naphthoxyacetic acid.
2,4-D = 2,4-dichlorophenoxyacetic acid.
2,4,5-T = 2,4,5-trichlorophenoxyacetic acid.
4-CPA = 4-chlorophenoxyacetic acid.
α-CPPA = α-(2-chlorophenoxy)-propionic acid.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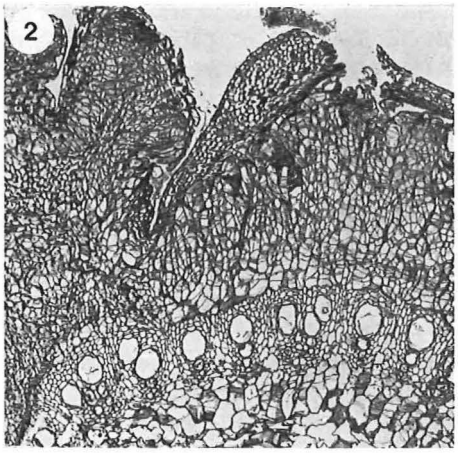
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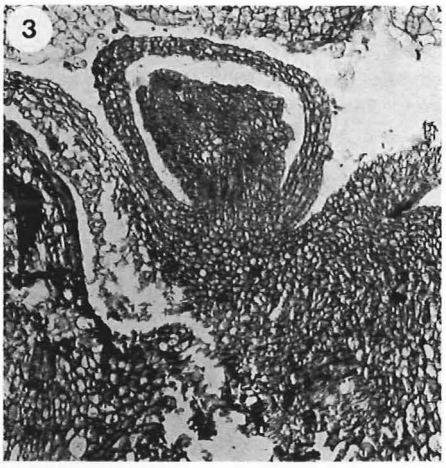
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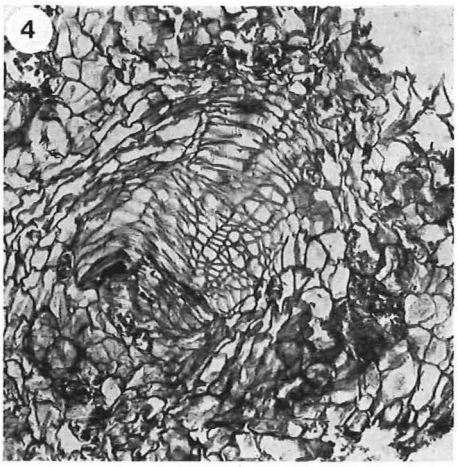
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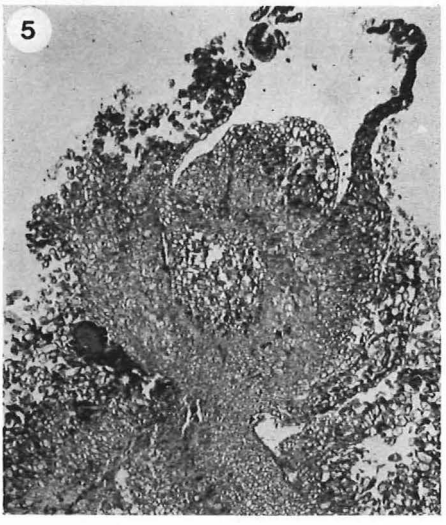
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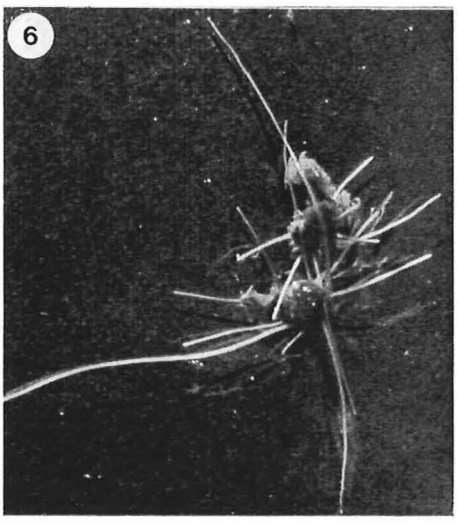
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able chlorine) containing Tween-20 (0.1 %) as a wetting agent. After several rinses with sterile distilled water the segments were blotted dry and the waxed ends were cut off and discarded. The remaining internodal segments were cut into uniform explants (2—3 mm in length, average fresh weight 8 mg).

Cultures were grown in 125 ml flasks and each flask contained 5 explants. The flasks contained 25 ml of liquid medium according to NITSCH and NITSCH (1969), except that $FeSO_4 \cdot 7H_2O$ was supplied at 5.57 g $\cdot$ l^{-1}. Further details of media constituents will be given with "Results". The cultures were subjected to continuous agitation on a New Brunswick gyratory shaker (80 osc. $\cdot$ min^{-1}, stroke amplitude 3 cm) and were grown either in the dark or with continuous illumination (2.5 W $\cdot$ m^{-2}) at 27 °C.

Results

1. Production of callus

In the initial stages of all experiments the internode explants were grown in darkness in media containing one or more synthetic auxins at different concentrations (Table 1; for abbreviations of growth regulators see also this table) and benzyladenine (BA) at a single concentration (1 μM). At concentrations in excess of 1 μM, BA caused the production of red anthocyanin pigments and was inhibitory to callus growth. Except in the presence of 2,4,5-T explants of all genotypes readily gave rise to callus and the pattern of production of this tissue was similar in all treatments (Tables 1 and 2). Callus cells first appeared at the cut surfaces of explants after 10 d of culture and the origin of the callus was the cambial zone (Fig. 2). After

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Fig. 1: Callus formation after 20 d of culture (× 7.5).

Fig. 2: Transverse section of internode explant after 10 d of culture showing the origin of callus from the cambial region (× 70).

Fig. 3: Adventitious bud formation in callus derived from an internode explant of (Grenache × Cabernet Sauvignon) × Gloryvine (× 60).

Fig. 4: Tracheids in callus grown with BA (10 μM) (× 120).

Fig. 5: A bud and a leaf formed in callus derived from internode explant of *Muscadinia rotundifolia*. Photographed after 50 d of culture (× 20).

Fig. 6: Adventitious root formation in callus from the muscadine grape. Photographed after 50 d of culture (× 1.5).

Abb. 1: Kallusbildung nach einer Kulturdauer von 20 d (7,5 ×).

Abb. 2: Querschnitt durch ein Internodienexplantat nach 10tägiger Kultur (70 ×). Der Kallus geht aus dem Kambiumbereich hervor.

Abb. 3: Bildung einer Adventivknospe an einem Kallus aus einem Internodienexplantat von (Grenache × Cabernet Sauvignon) × Gloryvine (60 ×).

Abb. 4: Tracheiden in einem mit BA (10 μM) kultivierten Kallus (120 ×).

Abb. 5: Knospe und Blatt an einem Kallus aus einem Internodienexplantat von *Muscadinia rotundifolia* nach einer Kulturdauer von 50 d (20 ×).

Abb. 6: Bildung von Adventivwurzeln an einem Kallus von *Muscadinia rotundifolia* nach einer Kulturdauer von 50 d (1,5 ×).

20 d the explants had become encased by a rapidly proliferating mass of white callus and their internal structure was disorganized (Fig. 1).

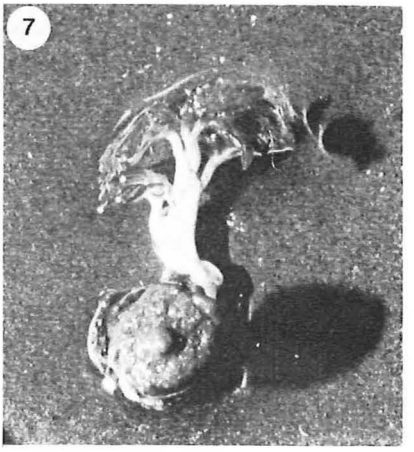
The character and growth rate of the callus varied with the identity and concentration of the auxin supplied to the medium. These effects are illustrated by results with the hybrid grapevine (Grenache × Cabernet Sauvignon) × Gloryvine (Table 1). With NAA and NOA large amount of callus of a very loose consistency was produced. This callus subsequently disintegrated to form a suspension of cells and clumps of cells. No organized structures were found in these suspensions. With 2,4-D the growth of callus was less prolific but it had a compact structure. When 2,4-D (5 μM) and NOA (5 μM) were supplied together a vigorously growing callus of intermediate consistency was produced. This combination of auxins was used in subsequent experiments to produce stocks of callus. Of the other chlorophenoxy

Table 2

Effects of auxin and cytokinin on growth and differentiation of stem explants of *Muscadinia rotundifolia* and a hybrid grapevine, (Grenache × Cabernet Sauvignon) × Gloryvine

Der Einfluß von Auxinen und Cytokinin auf Wachstum und Differenzierung von Sproßexplantaten von *Muscadinia rotundifolia* und der Rebkreuzung (Grenache × Cabernet Sauvignon) × Gloryvine

Growth regulator supplement to NITSCH basal medium	Particulars of growth and differentiation (unless stated otherwise descriptions refer to both the muscadine and the hybrid grape)
Basal medium (no supplement)	60% of the cultures formed roots.
BA 1 μM	No further growth of callus, 40% of the cultures formed roots.
BA 5 μM BA 10 μM	No further growth of callus. Root formation occurred only after prolonged culture. Callus developed many knob-like structure which on histological examination showed differentiation of tracheids (Fig. 4).
2,4-D 5 μM 2,4-D 10 μM	The callus ceased to grow and turned brown.
NOA 5 μM NOA 10 μM	The callus disintegrated into small pieces which subsequently formed roots. Root formation from the original callus was also observed in 60% of the cultures (Fig. 6).
NAA 5 μM NAA 10 μM	70% of the cultures formed roots. Root formation was abundant.
BA 1 μM + 2,4-D 5 μM	The callus disintegrated to form cellular aggregates which later formed roots. Leafy structures were formed in 10% of the muscadine cultures (Fig. 5). Shoot buds were formed in the hybrid callus.
BA 1μM + NOA 5 μM	The callus developed a loose consistency. Roots were formed in cultures.
BA 1 μM + 2,4-D 5 μM + + NOA 5 μM	Up to 20% of cultures of the hybrid grapevine formed adventitious buds (Fig. 3). Shoot formation (Fig. 7) was followed by formation of roots.



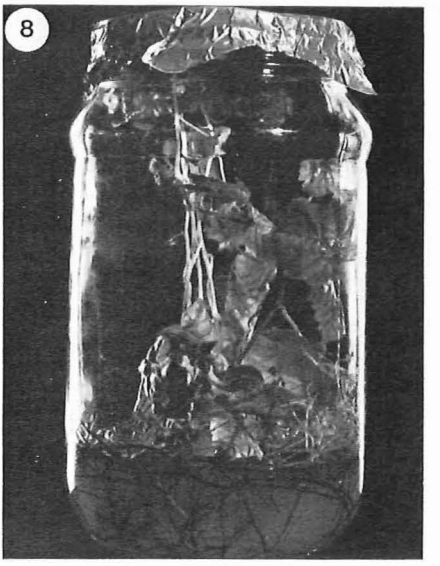


Fig. 7: Shoot production in callus of (Grenache × Cabernet Sauvignon) × Gloryvine. Photographed after 65 d of culture (× 3.5).

Fig. 8: Plantlets of (Grenache × Cabernet Sauvignon) × Gloryvine regenerated from internode explants (× 0.4).

Abb. 7: Sproßbildung an einem Kallus von (Grenache × Cabernet Sauvignon) × Gloryvine nach einer Kulturdauer von 65 d (3,5 ×).

Abb. 8: Aus Internodienexplantaten von (Grenache × Cabernet Sauvignon) × Gloryvine regenerierte Jungpflanzen (0,4 ×).

acids, 2,4,5-T was phytotoxic at 5—10 μM, 4-CPA gave a weakly-growing callus, and tissue produced by α-CPPA was similar in character and growth to that produced with NOA or NAA.

2. Organogenesis in explants of *Muscadinia rotundifolia* and an interspecific hybrid grapevine, (Grenache × Cabernet Sauvignon) × Gloryvine

Internode explants from the elongating shoots of 40-d-old seedlings of both genotypes were established *in vitro* on Nitsch medium supplemented with BA (1 μM) and a mixture of 2,4-D (5 μM) and NOA (5 μM). The explants were taken from shoots which exhibited the adult morphology, i.e. distichous phyllotaxy and presence of tendrils.

The cultures were kept in darkness for 30 d to produce a prolific callus. This material was the stock culture for subsequent work on induction of organogenesis. The explants were then transferred to 13 treatments consisting of media with differing growth regulator constituents and growing conditions were changed to continuous illumination. The results of this experiment are summarized in Table 2.

Adventitious roots were formed in all cultures except those supplied with 2,4-D alone. Adventitious buds and shoots were developed in cultures containing BA and 2,4-D or BA and a mixture of 2,4-D and NOA. The pattern of adventitious organ formation varied with the genotype. In *M. rotundifolia*, formation of roots preceded

the appearance of leafy structures but in the *Vitis* hybrid the formation of shoots came first and was followed by production of roots. With both genotypes organ formation commenced within 10 d of transfer to the new medium. Adventitious buds were found to arise near the surface of the callus. Plantlets were successfully established from cultures of the hybrid grapevine (2n = 38) (Fig. 8) but attempts to grow muscadine plantlets under glasshouse conditions were unsuccessful.

3. Effects of genotype and maturity of the mother plant on organogenesis in internode explants

Studies on organogenesis were made on cultures of a wide range of genotypes (Table 3) using the BA + 2,4-D + NOA medium. Cultures of most cultivars of *V. vinifera* formed adventitious roots *in vitro* as did clones of *V. rupestris* and the complex interspecific hybrid J.S. 23-416. A male clone of *V. longii* was highly

Table 3

Summary of the effects of genotype and mode of propagation of the mother plant on organogenesis in internode explants

Übersicht über den Einfluß von Genotyp und Vermehrungsmodus der Mutterpflanzen auf die Organogenese bei Internodienexplantaten

Genotype	Propagation		Organogenesis[1])		
	Seed	Cutting	Roots	Buds	Plantlets
Vitis vinifera					
cv. Grenache	–	+	+	–	–
Shiraz	–	+	+	–	–
Sultana	–	+	–	–	–
Cabernet Sauvignon	–	+	+	–	–
Traminer	–	+	–	–	–
Riesling	–	+	–	–	–
Muscat Gordo	–	+	–	–	–
Cabernet Sauvignon seedling	+	–	+	–	–
Vitis longii					
♀ clone	–	+	–	–	–
♂ clone	–	+	+	–	–
Vitis rupestris					
cv. Metallica Cape (♂)	–	+	+	–	–
Hybrids					
Johannes Seyve 23-416	–	+	+	–	–
Gloryvine (*V. vinifera* × *V. rupestris* (clone) ♂	–	+	+	–	–
Gloryvine seedling ♂	+	–	+	+	+
(Grenache × Cabernet Sauvignon) × Gloryvine	+	–	+	+	+
(Sumoll × Cabernet Sauvignon) × Gloryvine	+	–	+	+	+
Muscadine grape					
Muscadinia rotundifolia	+	–	+	+	–

[1]) Cultures grown in NITSCH medium containing BA (1 μM), 2,4-D (5 μM) and NOA (5 μM).

rhizogenic but a female clone of this species did not form roots. Adventitious buds were formed in seedlings of Gloryvine and in hybrid seedlings in which Gloryvine was a parent. Included among these hybrids were the *V. vinifera* crosses, Grenache × Cabernet Sauvignon and Sumoll × Cabernet Sauvignon. Explants from clonal Gloryvine, produced by hardwood cuttings, did not form buds and neither did explants from Cabernet Sauvignon seedlings.

Discussion

Earlier attempts to induce organogenesis in cultured internodes of grapevines were unsuccessful (Mullins and Srinivasan 1976, Jona and Webb 1978). The present results show that success in the induction of adventitious organs in explants of *Vitis* is dependent not only on the auxin and cytokinin content of the culture medium (Skoog and Miller 1957) but also on the state of maturity of the mother plant and the genotype.

The first step was to produce a rapidly growing callus of a consistency suitable for cultivation in agitated solutions. The requirement was for callus which retains a structure and which does not disintegrate to form a cell suspension. A medium containing BA (1 μM) and a mixture of 2,4-D (5 μM) and NOA (5 μM) was found to be suitable in this respect (Table 1). Moreover, this medium was found to favour the production of adventitious roots and adventitious buds (Tables 2 and 3).

In earlier work with agitated liquid cultures of grapevine ovules (Mullins and Srinivasan 1976) and anthers (Rajasekaran and Mullins 1979) a combination of BA, 2,4-D and NOA led to the formation of somatic embryos. No embryos were found in cultures derived from internodes.

The state of maturity of the mother plant is of major importance in determining the potential of explants to form adventitious buds. The formation of buds occurred only in internodes derived from seedlings. Explants from most cultivars which were propagated by cuttings formed roots but they did not form buds (Table 3). It is well known in horticulture that juvenile seedlings have a higher degree of regenerative competence than adult plants (Hartmann and Kester 1975, pp. 181—244). However, the seedlings used in the present experiments were not juvenile in the strict sense because they exhibited the adult morphology when used as the source of explants, i.e. phyllotaxy was distichous and the shoots were tendril-bearing (see Bugnon and Bessis 1968). The presence of tendrils is significant because tendrils and inflorescences arise from the same anlagen and are homologous organs (Srinivasan and Mullins 1976). Juvenile seedlings do not bear tendrils and have spiral (2/5) phyllotaxy. Nevertheless, the seedlings used here were juvenile in the sense that they had not yet formed flowers and fruits and it seems that this condition is of greater importance with respect to capacity for bud formation than the presence of morphological characters of the juvenile or adult phases.

Genotype was the over-riding factor in organogenesis in internode explants. Cultures of *V. vinifera*, whether of clonal or of seedling origin, did not form adventitious buds (Table 3). Seedlings of Gloryvine were organogenic and the capacity for adventitious bud formation was found in two *V. vinifera* × Gloryvine hybrid seedlings. These results suggest that ability to form adventitious buds *in vitro* is a heritable character.

Summary

Organogenesis was studied in cultured internodes of grapevine cultivars, hybrids and species. Adventitious root formation occurred with a wide range of media and genotypes. Adventitious buds were formed in explants grown in agitated liquid culture with NITSCH medium supplemented with benzyladenine (1 μm) and 2,4-D (5 μM) or a mixture of 2,4-D and β-naphthoxyacetic acid (5 μM). Bud formation occurred only in cultures derived from seedlings of *Muscadinia rotundifolia*, Gloryvine (*Vitis vinifera* L. × *Vitis rupestris* SCHEELE) and *V. vinifera* × Gloryvine, i.e. (Grenache × Cabernet Sauvignon) × Gloryvine and (Sumoll × Cabernet Sauvignon) × Gloryvine. Cuttings from mature vines of *V. vinifera* cultivars, or from clonal Gloryvine, gave explants which failed to produce buds. Results are discussed in relation to effects of composition of media, origin of explant, maturity of the mother plant and genotype.

Acknowledgement

This research was supported by the Rural Credits Development Fund, Reserve Bank of Australia.

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Eingegangen am 28. 4. 1981

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