ed on a national basis considerable financial support would be necessary. The present fvf schemes for fruit commenced in 1971/72 with an annual budget of $30,000 and this has reached $100,000 for 1981/82. It must be acknowledged that the true running costs would be more than double those figures as there are many inputs by State Departments for which no charge is made. The gross annual value of the crops which the existing scheme is intended to support is $400 to 450 million. The f.o.b. value of related exports is 25 to 30% of this figure.

Governments in Australia provide varying degrees of services to the ornamental horticultural industries. Whilst in most cases industry would like to see greater involvement, continued pressure could improve the situation.

Conclusion. There are other vegetatively propagated fruit crops and, indeed, plants which are not covered by fvf schemes and in considering development of a scheme for ornamentals, priorities would have to be considered. For example, funds are required for the maintenance of plant genetic resources which need to be balanced against any expansion of the fvf scheme into ornamentals would be favourable received by Governments considering their generally tight financial situation and growing acceptance of the principle that "the user pays." Perhaps an expanded activity in the production of disease-free propagation material of ornamentals seems justified, but the best means of achieving this needs close appraisal. Maybe low cost systems of maintaining and distributing this material, such as is happening in Victoria, is the best way to proceed.

The real questions which need to be answered I think are these:

- What are the threats to the commercial ornamental industries under the present system?
- What would be the benefits of an fvf scheme?
- Is there a real need?
- What is the demand?
- Will the user pay?

MICROPROPAGATION OF GRAPEVINE

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Abstract. A method is described for the *in vitro* propagation of grapevine (*Vitis vinifera* L.) from fragmented shoot apices, which has the potential of

producing many thousands of plants from a single apex between one growing season and the next Apical fragments were grown in a liquid culture medium with cytokinin but in the absence of auxin Transfer of the differentiated fragments to the same medium solidified with agar resulted in shoot masses which could be repeatedly subcultured Excised shoots readily initiated roots on a basal medium which, for most cultivars, was supplemented with low levels of auxin Plantlets were successfully transferred to the glasshouse and subsequently to the field The technique has also been used for a range of *Vitis* species and hybrids, and is considered to have promise for the commercial clonal propagation of grapevine

REVIEW OF LITERATURE

Although *in vitro* propagation has mainly been a technique applied to herbaceous species, the number of woody perennials that are now propagated by tissue culture, or offer promise of being multiplied in this way, is steadily increasing (e.g., 15). In the case of grapevine, Galzy (6) first described a technique for the growth *in vitro* of explants bearing terminal or lateral buds. However, her method was not designed specifically to stimulate multiple shoot formation. Limited multiplication of lateral buds has been achieved recently with *Vitis vinifera* 'Sylvaner' (7), and somatic embryos have been induced in *V. vinifera* 'Cabernet Sauvignon' (9) and the hybrid 'Seyval' (8). *In vitro* plantlets have also been produced from cultures of *V. riparia* × *V. rupestris* (5), apparently from embryos (13) Barlass and Skene (1) reported on a method that produced many shoots from a single fragmented apex of *V. vinifera* 'Cabernet Sauvignon', and suggested that it had potential for micropropagation of grapevines. They later extended their findings to other *V. vinifera* cultivars and other *Vitis* species (2,3,4). This paper describes the features of the phenomenon that are relevant to micropropagation.

MATERIALS AND METHODS

Grapevines raised in the glasshouse from hardwood cuttings provided the main source of experimental material. Results were essentially the same from glasshouse and field-grown vines, from vines in growth cabinets, and from vines raised *in vitro* from tip cuttings. Most experiments were carried out on *Vitis vinifera* 'Cabernet Sauvignon', although later work included other cultivars of *V. vinifera*, and several *Vitis* species and hybrids (Table 1). All of these cultivars are now being routinely cultured *in vitro* in our laboratory by the methods described below.

Shoot tips about 1 cm long were surface-sterilised for 15 min in a 5% w/v filtered solution of calcium hypochlorite containing 0.01% v/v Tween-20 and rinsed three times in sterile distilled water. Apices approximately 1 mm in length were excised under aseptic conditions and cut into several pieces on dry pre-sterilised 50 mm plastic dishes. These fragments were further teased

Table 1. Grapevine cultivars to which the fragmented shoot apex procedure has been applied

Cultivar	Parentage
Cabernet Sauvignon	*Vitis vinifera*
Cabernet Franc	*V vinifera*
Sultana (Syn Thompson Seedless)	*V vinifera*
Muscat Gordo Blanco (Syn Muscat of Alexandria)	*V vinifera*
Doradillo	*V vinifera*
Concord	*V. labrusca*
Ramsey (Synonym Salt Creek)	*V champini*
Dog Ridge	*V champini*
Rupestris St George (Syn Rupestris du Lot)	*V rupestris*
R-99	*V rupestris* x *V berlandieri*
1613	*V longii* x *[V vinifera* x *(V riparia* x *V labrusca)]*
Harmony	Dog Ridge x 1613

apart after addition of 5 ml of the basal culture medium of Murashige and Skoog (MS) (11) supplemented with 2 mg/l benzyladenine. In additional experiments, the basal culture medium was supplemented with varying concentrations of benzyladenine (BA, 1 to 5 mg/l), as well as kinetin (K, 2mg/l), zeatin (Z, 1mg/l), α-naphthaleneacetic acid (NAA, 1mg/l), and 2,4-dichlorophenoxyacetic acid (2,4-D, 1mg/l), alone or in combination. A comparison was also made between growth in liquid and solid media. Petri dishes were sealed with Parafilm and incubated in a temperature-controlled room maintained at 27°C during the light period (15 h) and 20°C during the dark period (9 h). Light was provided by cool white fluorescent tubes giving approx. 3,000 lux at the culture level. Early experiments on the effects of constant darkness were not continued, due to the unsatisfactory growth of the apical fragments under these conditions.

The leaf-like structures that developed during culture in liquid medium (see 1) were transferred to the same medium solidified with agar (6 g/l) between 2 and 4 weeks after the start of culture. Shoots, which eventually developed from swellings at the basal ends of the leaf-like structures after transfer to solid medium (1,2,3,4), were individually excised and transferred to rooting medium. The rooting medium, contained in autoclaved 80 x 25mm screw-capped clear polycarbonate tubes, consisted of either hormone-free White's medium (14— containing iron in the chelated form or basal MS medium (11) at half strength supplemented with 0.1 mg/l NAA. Both media contained 6 g/l agar.

Rooted plantlets (6 to 8 cm in height) were removed from the agar medium, and after washing the roots in distilled water, the plantlets were transferred to Jiffy 7 peat blocks (Jiffy Products Ltd., Grorud, Norway) and maintained, continuously moist, in

glass tanks in the temperature-controlled light room for approximately 9 days. The lid was then removed from the tank. After a further 2 weeks plants were placed in potting mix (initally a John Innes/perlite mixture, but more recently peat and sand in equal proportions) and transferred to the glasshouse.

RESULT AND DISCUSSION

Unless specified, the results refer to 'Cabernet Sauvignon'. Apical fragments began to grow within the first few days of

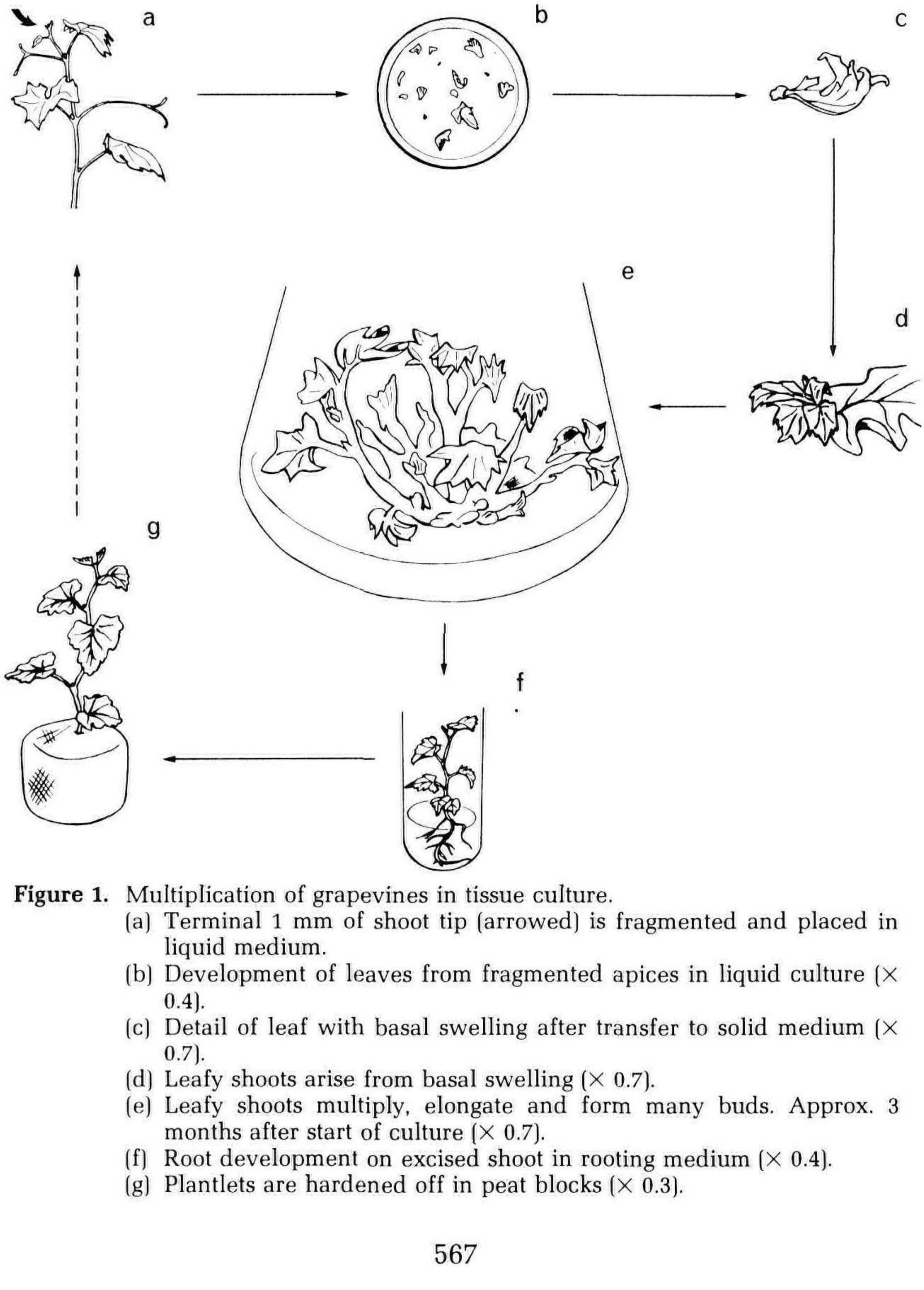

Figure 1. Multiplication of grapevines in tissue culture.
(a) Terminal 1 mm of shoot tip (arrowed) is fragmented and placed in liquid medium.
(b) Development of leaves from fragmented apices in liquid culture (× 0.4).
(c) Detail of leaf with basal swelling after transfer to solid medium (× 0.7).
(d) Leafy shoots arise from basal swelling (× 0.7).
(e) Leafy shoots multiply, elongate and form many buds. Approx. 3 months after start of culture (× 0.7).
(f) Root development on excised shoot in rooting medium (× 0.4).
(g) Plantlets are hardened off in peat blocks (× 0.3).

culture in liquid medium (MS + BA 2 mg/l), and by the third week up to 20 leaf-like structures, some 1 cm long, were evident in each dish (Figure 1b). These structures were, in fact, leaves. They have been shown to originate from pieces of leaf primordium (3), and heareafter will be referred to as *in vitro*-grown leaves. Fragmented apices cultured on solid media grew more slowly and erratically than did comparable material cultured in liquid. In later experiments, the initial stages of culture were always carried out in liquid. However, after the 2nd to 4th weeks, it was necessary to transfer *in vitro*-grown leaves to solid medium to stimulate subsequent development, the actual time of transfer depending on the cultivar and its rate of growth.

After transfer to solid medium, the *in vitro*-grown leaves, each with a basal swelling of the central vein (Figure 1c), increased to about 30 mm in length. During the next 4 weeks, leafy shoots began to appear from the basal sweling (Figure 1d). Excision of this area from the *in vitro*-grown leaves, followed by subdivision and subculturing to the same medium, resulted in a prolific formation of buds. There then followed a continuous process of bud multiplication and expansion to produce many elongating shoots in each culture (Figure 1e). These shoots could be repeatedly multiplied and subcultured to achieve the desired numbers.

The addition of various growth regulators to liquid media containing fragmented apices of either 'Cabernet Sauvignon' or 'Sultana' confirmed that 2 mg/l BA, without auxin, was optimal for the initial growth of apical fragments, and also for their subsequent ability to proliferate shoots when transferred to solid medium (4). Other cytokinins and auxins elicited only short-lived responses. The inclusion of NAA in the solid medium containing BA favored callus production rather than continued leaf growth and shoot proliferation.

Excised shoots from 'Cabernet Sauvignon' cultures rooted readily on hormone-free White's medium, with roots first appearing after about one week. All other cultivars, except 'Cabernet Franc', required auxin to stimulate root formation (0.1 mg/l NAA). Once root initiation occurred, shoots were transferred to hormone-free medium to prevent root distortion. Approximately 2 weeks after the onset of root initiation, plantlets showing active root and shoot elongation were transferred to peat blocks, and hardened-off, as described in Materials and Methods, for approximately 3 weeks, before putting into pots in the glasshouse. Losses during this critical early period out of culture were quite low.

So far, this technique has been attempted with a range of grapevine cultivars, including several major *Vitis* species (2, 4;

Table 1). Although there were minor differences in response, all material basically exhibited the same pattern of behaviour, except that *V. rupestris* and its hybrids gave only limited bud proliferation. It is not surprising that optimal cultural requirements differ among *Vitis* species, and the media may require some modification as occasion demands.

The growth habit of *in vitro*-grown shoots resembled seedlings with respect to the absence of tendrils, spiral phyllotaxy, and leaves lacking lateral sinuses. However, this apparent juvenility is a common feature of *in vitro* grapevine cultures (e.g., 12), and mature characteristics quickly appeared on transfer to the glasshouse (viz., production of tendrils, alternate phyllotaxy and characteristic leaf shape). Moreover, these same plants produced fruitful buds during their first season in the field, whereas seedlings would usually take several seasons to flower.

It can be calculated that the proliferating grape cultures have the potential of producing several thousand plants from a single shoot tip between one growing season and the next. This rapid multiplication would be of particular advantage when plants are in short supply as, for example, when new or introduced cultivars first become available for release. We consider that micropropagation is capable of producing many more plants in a given time from limited stocks than even methods such as the striking of green cuttings under mist.

Finally, there is the question of whether the resulting plants are true-to-type. Genetic instability is a problem more usually associated with long-term culture of callus (10), and as the grapevine system described here allows very little callus formation, it is felt that plantlet variation is likely to be minimal, particularly if individual cultures are not maintained for extended periods. One hundred 'Cabernet Sauvignon' vines propagated through tissue culture were planted in the Mildura region during December, 1979, as a first step towards answering this question. Outwardly the vines appear true-to-type, but further comment at this early stage would be premature. They will be observed during the next few years to assess whether any changes occurred during tissue culture.

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LITERATURE CITED

1 Barlass, M and K G M Skene 1978 *In vitro* propagation of grapevine (*Vitis vinifera* L) from fragmented shoot apices *Vitis 17* 335-340

2 Barlass, M and K G M Skene 1979 Clonal propagation through tissue culture *The Australian Grapegrower and Winemaker 191* 12-13

3 Barlass, M and K G M Skene 1980 Studies on the fragmented shoot apex of grapevine I The regenerative capacity of leaf primordial fragments *in vitro* *J exp Bot 31* 483-488

4 Barlass, M and K G M Skene 1980. Studies on the fragmented shoot apex of grapevine II Factors affecting growth and differentiation *in vitro J exp Bot. 31* 489-495.

5 Favre, J -M 1977 Premiers résultats concernant l'obtention *in vitro* de néoformaltions caulinaires chez la vigne *Ann Amélior. Plantes 27*· 151-169

6 Galzy, Rose 1964. Technique de thermothérapie des viroses de la vigne *Ann Epithyties 15*· 245-256

7 Jona, R and K J Webb 1978 Callus and axillary-bud culture of *Vitis vinifera* Sylvaner Riesling *Scientia Hortic 9* 55-60

8 Krul, W R and J F Worley 1977 Formation of adventitious embryos in callus cultures of Seyval, a French hybrid grape *J Amer Soc Hort. Sci 102* 360-363

9 Mullins, M G and C Srinivasan 1976 Somatic embryos and plantlets from an ancient clone of the grapevine (cv Cabernet Sauvignon) by apomixis *in vitro J exp Bot 27* 1022-1030

10. Murashige, T 1974 Plant propagation through tissue cultures *Ann Rev Plant Physiol 25* 135-166

11 Murashige, T and F Skog. 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures *Physiol Plant 15* 473-497.

12 Nozeran, R 1978 Multiple growth correlations in phanerogams. In· *Tropical trees as living systems* (Eds P B Tomlinson and Martin H Zimmermann) pp 423-443 Cambridge University Press

13. Sicard, Georges 1978. Etude ontogénétique des divers types de néoformaltions caulinaires obtenues chez la Vigne à partir de fragments de limbes cultivés in vitro *Rapport de stage, D E A d'amélioration des plantes,* Université de Paris-Sud, Centre d'Orsay

14. White, P R 1943 A handbook of plant tissue culture Ronald Press Co , New York

15 Winton, Lawson L 1978 Morphogenesis in clonal propagation of woody plants In *Frontiers of plant tissue culture 1978* (Ed Trevor A. Thorpe) pp 419-426 Calgary, Canada

PLANT BREEDING WITH A WOODY PERENNIAL — THE GRAPEVINE

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HISTORY

The idea of breeding grape cultivars specifically for Australia is almost as old as Australian viticulture itself. The Macarthurs, more famous for their activities with sheep, also grew grapes and believed that they should raise vines from seed to allow selection of types suited to local conditions. Busby (3) records that William Macarthur had 250 such seedlings, out of a much larger number raised from seed in 1824, under trial. None of these appear to have survived and this may be because they were not the result of deliberate crosses but raised from open-pollinated