

RAPID MICROPROPAGATION OF GRAPEVINE CV. AGIORGITIKO THROUGH LATERAL BUD DEVELOPMENT

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Abstract

Micropropagation has been a well-established methodology for *in vitro* regeneration of grapevine (*Vitis vinifera* L.), usually through shoot apical meristem culture or adventitious bud formation. However, recent studies have shown that micropropagated plants often show genetic discrepancy from the mother plant, due to high levels of PGRs applied. Here we present an efficient protocol for rapid *in vitro* regeneration of grapevine cv. Agiorgitiko genotypes through nodal culture. Development of a single main shoot was prominent on agar-solidified basal MS medium without any PGR or supplemented with relatively low levels (e.g., up to 2.5 μ M) of benzyladenine (BA). At higher BA levels axillary bud growth and shoot multiplication were enhanced, but hyperhydricity was also apparent. Relatively low concentrations of indole-3-butyric acid (IBA) promoted both the frequency of shoots forming roots and the number of roots per shoot. Although the overall yield is rather lower compared with shoot tip culture approaches, present protocol may serve as an alternative for true-to-type regeneration of selected clones of ‘Agiorgitiko’ and possibly of other grapevine cultivars.

Introduction

Micropropagation is a means to propagate selected genotypes by applying *in vitro* culture techniques. Depending on the plant species and cultural conditions, tissue

culture may enable the mass production of genetically homogeneous populations from elite (e.g., high-yielding or disease resistant) individuals. Micropropagation of selected *Vitis* genotypes can be carried out, among others, by the culture of intact or fragmented shoot apical meristems, axillary-bud microcuttings or through adventitious bud formation (Heloir et al. 1997; Barlass and Skene, 1980; Gray and Fischer, 1985; Monette 1988). However, most efficient protocols have been reported for muscadine or other than *V. vinifera* grapes (Thies and Graves, 1992; Qiu et al. 2004), while studies with cultivars of *V. vinifera* L. have met with less success (Chee and Pool, 1983; Zatiko and Molnar, 1985; Mhatre et al. 2000). The degree of competence is highly depended on the particular genotype, as various *Vitis* species, cultivars or hybrids respond differently to certain culture conditions (Chee and Pool 1983; Monette 1988, Qiu et al. 2004).

Despite years of investigation, the application of tissue culture techniques in the grape-growing industry is still limited. Among the major obstacles for the widespread utilization of this technology are the relatively high cost compared with the conventional methods (Chee et al. 1984, Monette 1988) and the possibility of genetic discrepancy in micropropagated plants, often called as somaclonal variation. Somaclonal variation is highly undesirable in applications aiming to reproduce selected elite genotypes, e.g., ancient and rare clones, pathogen-resistant or drought/salinity-tolerant genotypes and it is most likely caused by high levels of plant growth regulators (PGRs), mainly cytokinins, usually applied to promote shoot multiplication and thus increase the yield (Gray et al. 2005).

Present protocol is based on the development of main shoots from intact nodal explants. It is proposed as a simple and efficient alternative for producing genetically stable micropropagated genotypes of ‘Agiorgitiko’, an ancient Greek grapevine cultivar of highly local importance for wine production. To our knowledge, this is the first report on micropropagation of Greek grapevine germplasm.

Materials and methods

Initial explants comprised intact nodes, bearing dormant buds, on a 2-4 cm long section of internodes of a grapevine (*Vitis vinifera* L.) cv. Agiorgitiko shoot. Plants were grown in a natural environment at the TEI of Athens and explants were collected early in the growing season (April to May). Nodal segments were washed

thoroughly for 15 min under running tap water, dipped in 75% (v/v) ethanol for 1 min, immersed for 30 min in 25 % (v/v) commercial bleach (1.5 % sodium hypochlorite final concentration) containing 0.1% Tween-20, and rinsed three times in sterile, distilled water. The damaged tissues on both explant sides were aseptically cut off and the nodal segments were immersed base down into solid culture medium. Half-strength MS medium (Murashige and Skoog, 1962) supplemented with 30 g L⁻¹ sucrose was used as the basal medium for bud development. The same medium but with 20 g L⁻¹ sucrose was used for rooting of microshoots. Media were solidified with 0.25 % (w/v) Phytigel (Sigma) and the pH was adjusted to 5.7 prior to autoclaving. All cultures were maintained at 23 °C with a 16-h photoperiod under 50 μmol m⁻² s⁻¹ illumination provided by cool-white fluorescent lamps.

Different BA concentrations (2.5-20.0 μM) were tested in lateral bud development experiments. As a control a cytokinin-free medium was used. Each condition consisted of 20 replicate explants and each experiment was repeated twice (n= 60). The number of developed shoots was determined after four weeks of incubation. For rooting, main shoots were cut from the donor explant and soaked with the basal ends in solidified MS medium supplemented with two IBA concentrations (2.5 and 5.0 μM). An auxin-free medium was included as a control. The number of roots was determined after four weeks. There were 10 replicates per treatment and each experiment was repeated twice (n= 30). Treatment means and standard deviations (SD) were calculated. Acclimatisation of *in vitro* developed plantlets was accomplished in plastic cups containing a 1:1 mixture by volume of soil and perlite covered with a plastic wrap for four weeks. Finally, plantlets were potted to soil.

Results and discussion

The selection of suitable explants is a crucial step for efficient initiation of micropropagation of grapevine as well as other woody species (Grenan 1992). In this study, nodal segments comprising lateral buds were selected (Figure 1), in order to minimize the possibility for somaclonal variation often occurring during *in vitro* axillary bud multiplication or adventitious bud formation. Most of the regeneration protocols in *Vitis* species have been developed on explants derived directly from *in vitro* grown plants or from plants grown in the greenhouse. In this study explants were derived from field-grown grapevines, where surface sterilization is usually considered

to be a serious problem. Present sterilization procedure proved to be quite successful, since less than 10 % of the explants became infected. Nevertheless, the time period of explant collection was essential for efficient sterilization of explants, since in earlier experiments conducted at the end of the growing season the frequency of infected explants was much higher (data not shown).

Figure 1. Representative nodal explant used for culture initiation. Arrow indicates an undeveloped lateral bud. Bar = 1 cm.



Previous studies have clearly shown that BA is the most effective among other cytokinins for inducing shoot development in *Vitis* (Lee and Wetzstein, 1990; Heloir et al., 1997). In this study, development of lateral buds into shoots was also efficient at medium lacking BA. Only the main shoot developed while the growth of axillary buds was inhibited (Figure 2A-B). The presence of BA, even at relatively low levels (i.e., 2.5 and 5.0 μM), enhanced bud multiplication (Table 1). At relatively high BA concentrations ($>10.0 \mu\text{M}$) axillary bud proliferation was apparent, while at the same time the growth of the main shoot was suppressed.

Present results are in general accordance with previous studies showing optimum shoot multiplication rate at a concentration range of 5.0 - 10.0 μM BA (Harris and Stevenson, 1982; Chee and Pool, 1983, Mhatre et al. 2000). Importantly, at BA concentrations above 5.0 μM axillary shoots showed hyperhydricity; a phenomenon not detected at lower BA levels. Hyperhydricity (vitrification) has previously been repeatedly reported in grapevine tissue culture (Morini et al., 1985; Heloir et al. 1997). It is a serious problem because vitrified shoots may not be

micropropagated further. Although the mechanism for vitrification remains to be elucidated, it may occur during axillary bud proliferation phase and has been correlated, among others, with relatively high cytokinin levels (Heloir et al. 1997). Therefore, the results of the present study suggest the use of lower BA levels, i.e., up to 5.0 μM , for bud development. Furthermore, under these conditions shoots grew normally and within a month reached an appropriate size for rooting (Figure 2C).

Table 1. Bud development from cv. ‘Agiorgitiko’ nodal explants: influence of BA concentration on branching (explants with axillary bud proliferation) and multiplication rate. Data obtained from a total of 60 explants per treatment.

BA concentration (μM)	Branching (%)	Multiplication rate (mean \pm SD)
0	0	1
2.5	15.0	1.2 \pm 0.4
5.0	61.7	1.9 \pm 0.8
10.0	100.0	2.9 \pm 0.9
20.0	100.0	5.2 \pm 1.2

Shoots that developed as single entities or from bud proliferating cultures were excised and transferred into rooting media. Although spontaneous rooting at relatively high frequency (73.3 %) occurred in medium lacking IBA, the addition of auxin, particularly at 5.0 μM , facilitated rooting in terms of rooting percentage and the number of roots per shoot (Table 2). Similarly, in a previous study on *in vitro* rooting of cv. ‘Pinot noir’ microshoots (Helior et al. 1997) it was shown that IBA is a suitable auxin, while other types of auxins (e.g., NAA) may lead to callus formation. After acclimatization, plantlets grew normally when transferred to soil (Figure 2D).

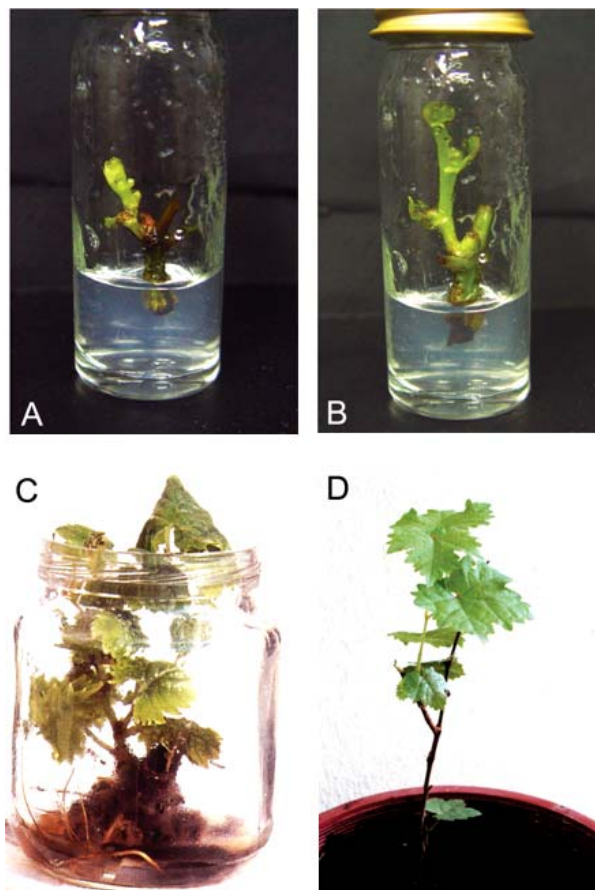


Figure 2. Stages of micropropagation through nodal culture of grapevine cv. Agiorgitiko. Development of a single lateral bud on $\frac{1}{2}$ MS medium supplemented with $2.5 \mu\text{M}$ BA, after two weeks in culture (A). The bud continues to grow as a single entity after four weeks in culture (B). Rooting and plantlet regeneration in $\frac{1}{2}$ MS medium supplemented with $2.5 \mu\text{M}$ IBA (C). A 1.5-year-old micropropagated plant grows normally in the soil (D).

In conclusion, present *in vitro* regeneration procedure through bud development from nodal culture provides certain advantages for rapid micropropagation of cv. Agiorgitiko and potentially for other grapevine cultivars. The development of a single later bud into shoot does not require the addition of high levels of cytokinins, as it is the case in most protocols implying shoot proliferation or adventitious bud formation. Although it may lower the overall yield, rooting of microshoots is quite efficient due to low levels of endogenous cytokinins that prevent possible vitrification. Importantly, by applying low amounts of both cytokinins and

auxins, present protocol may also minimise the serious problem of somaclonal variation often encountered in grapevine micropropagation.

Table 2. Influence of different IBA concentrations on the rooting of microshoots. Data obtained from 30 explants per treatment.

IBA concentration (μM)	Rooting (%)	Number of roots per shoot (mean \pm SD)
0	73.3	1.4 \pm 1.0
2.5	90.0	3.4 \pm 1.5
5.0	100.0	4.5 \pm 1.0

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