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Research Note

Micropropagation of *Vitis amurensis* Rupr.: An improved protocol

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Summary: An efficient micropropagation procedure of *V. amurensis* cv. Zuoshan 1 was established. NAA combined with BA resulted in callus formation and inhibition of shoot growth, whereas a combination of 0.3 µM IAA and 4.4 µM BA gave highest shoot growth and multiplication. IAA at 2.8 and 5.7 µM led to high root formation of shoots. 30 g l^{-1} sucrose was needed for high shoot growth, while high rooting was achieved with 0-20 g l^{-1} sucrose. Intact leaves are required for a high level of shoot rooting.

Key words: *in vitro* propagation, axillary bud, BA, auxin, sucrose.

Introduction: *Vitis amurensis* Rupr., a wild species native to Korea and northeastern China, is a useful resource of resistant germplasm in grape breeding programs as well as in viticulture (GUO *et al*. 1987; ALLEWELDT and POSSINGHAM 1988; WANG *et al*. 1998). To date, some *V. amurensis* varieties have been developed and cultivated for the production of high quality wine in China (SONG *et al*. 1998). Although *V. amurensis* is usually multiplied via seeds when used as cold-resistant rootstocks, cultivars must be subjected to vegetative propagation. Therefore, the establishment of a propagation system using an *in vitro* culture method must be developed for *V. amurensis* which is difficult to propagate by cuttings (GUO *et al*. 1987). Rapid *in vitro* propagation has been reported for many cultivars and interspecific hybrids of *Vitis* but not for the micropropagation of *V. amur-ensis*. In preliminary experiments, shoot cultures were established from axillary buds of *V. amurensis* cv. Zuoshan 1. This study aimed to establish a more efficient micropro-pagation system for this cultivar. Thus we investigated the effects of plant growth regulators and sucrose concentration on the development of axillary buds and the rooting of shoots, using *in vitro* material as initial explants.

Material and Methods: Nodal and shoot microcuttings from *in vitro* shoot cultures of *V. amurensis* cv. Zuoshan 1 were used in the present study. These shoot cultures were established one year before the onset of the present experiments. The sterilized softwood nodal cuttings (length: 1 cm) with a single axillary bud were placed on MS medium (MURASHIGE and SKOOG 1962) supplemented with 2.2 µM BA and 30 g l^{-1} sucrose. Shoots from the nodal cuttings were then subcultured on the same medium until sufficient plant material was available for the subsequent experiments described below.

We used MS media supplemented with a combination of BA (2.2 and 4.4 µM) and NAA (0.05-5.4 µM) or IAA (0.3-17 µM) to investigate the effect of BA and auxin on *in vitro* axillary bud development of microcuttings, and MS media supplemented with IAA (0-8.4 µM) to investigate the effect of auxin on the rooting of propagated shoots. Each treatment consisted of 3 replications each with 10 explants. The numbers of sprouted microcuttings and rooted shoots were recorded one month after culture.

To investigate the effect of sucrose concentration on *in vitro* axillary bud development of microcuttings and rooting of shoots, we used MS media supplemented with 2.2 µM BA and 0-40 g l^{-1} sucrose for culture of the microcuttings, and MS media with 0-30 g l^{-1} sucrose for the rooting of shoots. Each treatment consisted of 3 replications each with 10 explants.

Media were adjusted to pH 5.8 with 0.1 N NaOH before the addition of agar (7g l^{-1}), poured into each of the 100 ml Erlenmeyer flasks. Flasks were plugged using cotton and autoclaved for 10 min at 121°C under a pressure of 1.2 kg cm^{-2}. Cultures were maintained at 25 ºC in a 10-h photoperiod provided by white fluorescent lamps (50 µmol m^{-2} s^{-1}).

To investigate the formation of multiple shoots, samples were collected weekly, fixed with FAA solution (70 % ethanol:formalin:acetic acid = 90:5:5, v/v/v), dehydrated in a graded butanol series, and embedded in paraffin. Sections (10 µm in thickness) were stained with Delafield's hematoxylin, and then observed under a light microscope.

Results and Discussion: Effect of plant growth regulators: If combined with BA and auxins, 0.05-5.4 µM NAA led to a remarkable deterioration in bud sprouts, an inhibition of shoot growth and formation of calli, while IAA at low concentrations did not show these negative effects. The highest multiplication rate was obtained using a combination of 4.4 µM BA and 0.3 µM IAA (Figure). KUROI and SAWADA (1985) reported that combination of 4.4 µM BA and 0-0.5 µM NAA was suitable for shoot formation from the axillary buds of *V.* x *labruscana* 'Kyoho'. The present study showed that IAA was superior to NAA for the micropropagation of *V. amurensis* cv. 'Zuoshan 1'.

When shoots from axillary buds were transferred to rooting MS media containing different concentrations of IAA, adventitious roots appeared at the base of shoots 2 weeks after culture. A high percentage of rooted shoots (90-96 %) and a similar number of roots per shoot (2.4-2.8) were formed with MS medium containing 0-8.4 µM IAA. IAA at 2.8 and 5.6 µM gave more vigorous roots compared to other concentrations.

Effects of sucrose concentration: In the absence of sucrose, 30 % of axillary buds sprouted, but failed to show subsequent shoot growth. At 10-40 g l^{-1} sucrose, almost all axillary buds sprouted, and shoot growth increased with increasing sucrose concentrations from 10 to 30 g l^{-1}. At 40 g l^{-1} sucrose, shoot growth significantly de-

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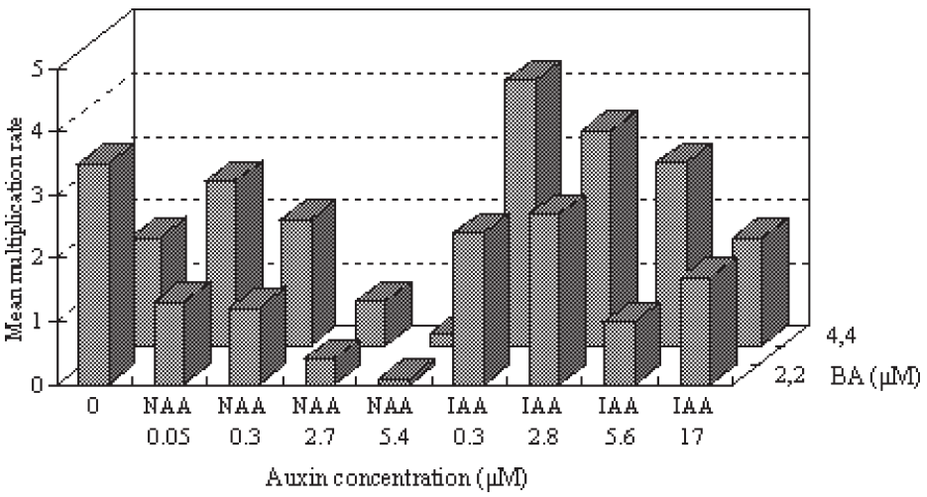

Figure: Effects of BA and auxins on multiplication of axillary buds cultured on MS medium containing 30 g l^{-1} sucrose. Data were recorded one month after culture.

creased compared with 30 g l^{-1} sucrose.

More than 90 % of shoots rooted when they were cultured in 0-30 g l^{-1} sucrose. Shoots cultured in 20 g l^{-1} sucrose formed a significantly greater number of roots than those with 30 g l^{-1} or without sucrose in the medium. No significant difference in the number of roots was detected in 5-20 g l^{-1} sucrose concentrations. Since high rooting was also achieved in the absence of sucrose, further treatments as shown in the Table were carried out to investigate effects of leaf and illumination on shoot rooting. In the absence of sucrose, about 90 % of shoots with leaves rooted in the light, similar to those cultured in the medium with 20 g l^{-1} sucrose. Shoots did not root when cultured in the dark or without leaves in the light. At 20 g l^{-1} sucrose, shoots without leaves showed remarkably less rooting than those with leaves. These results agree with previous reports showing that leaf removal had a negative effect on rooting of shoot or nodal microcutting of grapevines (NOVÁK and JÙVOVÁ 1983; THOMAS 1998). It has been confirmed that the role of leaves in the *in vitro* rooting is not only to supply IAA or an energy source but also to enable better utilization of sucrose (THOMAS 1998).

Subculture for shoot multiplication: When nodal or shoot microcuttings were transplanted to MS medium containing 4.4 µM BA, 0.3 µM IAA and 30 g l^{-1} sucrose, elongated shoots and short multiple shoots were obtained within 5 weeks. We observed a precocious development of axillary meristems in axillary buds, but no shoot regeneration from calluses in the base of stems, indicating that multiple shoots could derive from a developed axillary meristem. This approach could reduce the risk of genetic instability in the *in vitro* propagation of grapevines (NOVÁK and JÙVOVÁ 1983). For shoot multiplication, the elongated shoots were cut into microcuttings with buds, and the multiple shoots were pruned to just a single shoot, then transferred to a fresh medium in the subsequent subculture. In this way, 24 sampled shoots actually multiplied to 2094 after 3 subcultures in 3 months, for an average multiplication rate of 4.4 per month.

Rooting of *in vitro*-developed shoots and acclimatization: When propagated shoots were transferred to rooting MS medium containing 5.6 µM IAA and 2 % sucrose, almost all shoots formed adventitious roots from the base of shoots within 2 weeks. After 2-3 d with the culture vessels opened under daylight condition, rooted shoots were transplanted to greenhouse containers with sand and kept under very humid condition. Over 90 % of the plantlets having a well developed root system survived 3 weeks after transplanting.

Table

Effects of sucrose, shoot type and illumination on shoot rooting. Data were recorded 5 weeks after culture. Each treatment contains 3 replications each with 10 explants. Values represent mean ± SE

Sucrose concentration (g l^{-1})	Shoot type	Illumination	Rooted shoots, %
0	with leaves	light	88.9 ± 5.6
0	with leaves	dark	0
0	without leaves	light	0
20	with leaves	light	89.7 ± 5.8
20	without leaves	light	43.3 ± 8.1

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