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Research Note

In vitro propagation of *Vitis* x *Muscadinia* hybrids by microcuttings or axillary budding

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Muscadinia rotundifolia is known to be the best source for resistance to *Xiphinema index*, the nematode vector of grape fanleaf virus (GFLV). But this species is not suitable as rootstock because of poor rooting ability and graft incompatibility with *Vitis vinifera* (BOUQUET 1980 a). *V. vinifera* x *M. rotundifolia* hybrids (VRH) are known to have a high field resistance to virus transmission (WALKER *et al.* 1994). VR hybrids, like muscadine cultivars, are difficult to propagate by conventional methods (OLMO 1986). Micropropagation has been reported as an alternative for muscadine grape multiplication (GRAY and BENTON 1991) but not for *Vitis* x *Muscadinia* hybrids. Furthermore, micropropagation is a prerequisite to perform *in vitro* biotechnological studies and particularly genetic engineering experiments.

Materials and methods: Six F1 VR hybrids of different genetic origin (BOUQUET 1980 b) were studied and compared with a rootstock (cv. Fercal). Green stems were collected from actively growing plants and disinfected for 15 min in 7 % $Ca(ClO)_2$. Explants were developed in 250 x 25 mm culture tubes containing 25 ml of a modified MS/2 medium, maintained at 25 ± 1° C and a 15 h photoperiod (50 µmol photons m^{-2} s^{-1}) and subcultured every 4 months. The culture was considered to be stabilized after 3 subcultures. For experiments, explants were taken only from an intermediate part of 3-month-old plantlets. In the case of axillary propagation, single node cuttings were established in 100 mm petri dishes at 15 µmol photons m^{-2} s^{-1} light intensity (other conditions as above). After 15-30 d, axillary buds developed and formed multiple shoots structures. Single shoots excised from actively proliferating masses were used for subcultures. Axillary micropropagation was considered as stabilized after three subcultures. In each case, leaves were systematically removed from the explants. For rooting, shoots were transferred onto various media. These media and culture conditions were the same as for multiplication, except that growth regulators were : 1) omitted or 2) BAP at 1.1 µM or 3) IBA or NAA at 1 µM. Rooted plantlets were transferred in peat jiffy pots containing perlite and placed in humidity chambers. After acclimatization, hardened plants were maintained under greenhouse conditions.

Four macronutrient formulations were used for microcuttings culture : MS/2 (MURASHIGE and SKOOG 1962), CP/4 (CHEE and POOL 1987) and HLR (HELLER 1953). For all media, MS micronutrient formulation was used. Organic components ($mg.l^{-1}$) were myo-isonitol (50), nicotinic acid (1), pyridoxine-HCl (1), thiamine-HCl (1), Ca-panthotenate (1), biotin (0.01). Sucrose was added at 20 g l^{-1}. For axillary budding, MS/2 was used with two-fold Fe concentration. BAP was added at 1.1 and 4.4 µM (respectively B_1 and B_4) before autoclaving. All media were solidified with 7 g l^{-1} agar, adjusted to pH 5.8 with KOH and sterilized for 30 min at 115 °C. A complete randomized factorial design was used for all experiments. Data were analysed with SAS using Gabriel's procedure (p = 0.01).

Results and discussion: When cultured on growth regulator-free medium, VR hybrids showed great variability in their growth ability (Tab. 1).

Table 1

Effect of medium on *in vitro* growth of microcuttings

Genotype	Medium	Σ root length per explant (mm)	Stem height (mm)	Number of nodes per explant
VRH 8624	MS/2	151[a]	29[a]	3.7[a]
	HLR	87[ab]	22[ab]	3.3[a]
	CP/4	71[b]	24[b]	3.7[a]
VRH 8712	MS/2	231[a]	28[a]	3.4[a]
	HLR	158[b]	17[b]	3.3[a]
	CP/4	144[b]	18[b]	2.3[b]
VRH 8731	MS/2	180[a]	51[a]	6.3[a]
	HLR	157[a]	42[b]	5.0[b]
	CP/4	156[a]	45[b]	5.9[a]
VRH 8771	MS/2	149[a]	48[a]	5.1[a]
	HLR	136[a]	38[b]	4.5[a]
	CP/4	134[a]	38[b]	4.3[a]
VRH 8773	MS/2	123[a]	27[a]	4.2[a]
	HLR	51[b]	15[b]	2.6[b]
	CP/4	92[ab]	19[b]	3.0[b]
Fercal	MS/2	247[a]	70[a]	6.9[a]
	HLR	224[a]	53[b]	5.8[b]
	CP/4	210[a]	57[b]	5.8[b]

Data collected after 45 d in culture (values are means of 20 vitroplants). Means followed by the same letter are not significantly different. Gabriel's procedure on medium effect was made by genotype (p = 0.01).

VRH 8624 and 8773 presented poorest ability for both, root and stem development. VRH 8712 presented good root growth but its rate of stem elongation remained very low. In contrast, VRH 8731 and 8771 appeared to be more adapted to propagation by microcuttings. It is interesting to note that in field conditions, VRH 8731 and 8771 grow vigorously while VRH 8624 and 8773 grow very poorly (BOUQUET, unpublished). MS/2 proved to be more suitable than the other media, but the number of nodes did not allow high multiplication rates. Experiments were made to investigate if in vitro VHR growth ability would be improved by modifying culture conditions. Changing the culture recipient or the closing system to modify gas exchange proved to be inefficient. For instance, plantlets cultured in 250 ml Erlenmeyer vessels had high vigour characterized by large leaves, but stem height and number of nodes were not affected. Adding IAA to the medium to improve rhizogenesis also proved to be unsuitable.

When microcuttings were cultured on media containing BAP, buds produced shoots characterized by long

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Table 2

Effect of genotype and BAP on micropropagation

Genotype	Medium	Tissues formed per explant (mg)	Number of shoots per explant*	Σ root length per explant (mm)
VRH 8624	B0	222	0.6	95
	B1	135[a]	1.5[a]	73[c]
	B4	1040[b]	9.1[c]	4[a]
	B4[@]	291[a]	3.4[b]	44[ab]
VRH 8712	B0	237	0.8	124
	B1	78[a]	1.3[a]	47[a]
	B4	408[b]	5.0[b]	26[a]
	B4[@]	310[b]	4.2[b]	36[a]
VRH 8715	B0	256	0.9	116
	B1	193[a]	1.7[a]	100[b]
	B4	615[b]	8.9[c]	9[a]
	B4[@]	584[b]	7.2[b]	19[a]
VRH 8771	B0	345	0.9	105
	B1	219[a]	1.6[a]	41[b]
	B4	1073[b]	10.2[c]	12[a]
	B4[@]	943[b]	6.3[b]	14[a]
VRH 8773	B0	276	0.6	98
	B1	-	-	-
	B4	1096	15.4	0
	B4[@]	-	-	-
Fercal	B0	386	1.0	182
	B1	172[a]	5.1[a]	25[a]
	B4	436[b]	8.8[b]	0[a]
	B4[@]	330[ab]	8.0[b]	5[a]

B4[@] : Explants from plantlets cultured on growth regulator-free medium. For B4 and B1 treatments, explants came from established axillary proliferation (3 subcultures). * Shoots with length > 5 mm. Data were collected after 45 days in culture (values represent means of 22 to 28 explants). Means followed by the same letter are not significantly different. Gabriel's procedure on medium effect was made by genotype (p = 0.01).

internodes, during the first week. During the following week, other shoots presenting short basal internodes emerged from explant buds. Axillary proliferation became effective with the growth of the buds borne by these first shoots. To arrange a close contact between the explant and the medium for promoting bud proliferation was of advantage. When dishes were filled with 40 ml of medium, distance from dish top to the medium surface was about 5 mm. Shoot growth pushed back the explant into the medium enabling a continuous and favourable close contact. BAP inhibited strongly shoot elongation and rhizogenesis, but induced callusing on basal parts of the explant.

If effective axillary bud culture was observed on MS/2 medium containing 4.4 µM BAP, lower concentration (1.1 µM) proved unsuitable to induce proliferation (Tab. 2). Higher concentration (8.8 µM) increased total weight but decreased the number of shoots with length > 5 mm (data not shown). NOVAK and JUVOVA (1983) reported considerable differences in the multiplication rate of *Vitis* genotypes. Similarly, we noted a great variability of responses but, whatever the genotype, axillary budding appeared to be more efficient and adapted technique for micropropagation of VR hybrids than microcuttings. Compared to the best results obtained with microcuttings, increases of multiplication rate with axillary budding varied from 47 to 266 % for VRH 8712 and 8773. For Fercal, the increase was only 28 %. For Muscadine grapes, GRAY and BENTON (1991) suggested that the genotypic variability of response could be attenuated by a modification of culture conditions. For each variety, optimization of BAP concentration could be necessary to maximize shoot production. Explants excised from proliferating masses are more suitable for high multiplication rates than those excised from plantlets cultured on growth substance-free medium (B_4[@] treatment). This effect was more pronounced for VRH 8624 and 8771 than for VRH 8712 and 8715 but, all genotypes responded in the same way, suggesting that the axillary buds produced on proliferating masses could be more differentiated than those produced on rooted plantlets.
However, when maintained during more than 3 subcultures on proliferating medium, VR hybrids showed vitrification phenomena which reduced dramatically the competence of explants for tissue culture.

As reported by JONA and WEBB (1978) on *V. vinifera* we observed that shoots from axillary proliferation showed poor rooting abilities when transferred on BAP-free medium (10 to 40 % of successful rooting). When shoots were transferred on media supplemented with 1µM IBA or NAA, callogenesis and rhizogenesis were stimulated but responses were inconsistent, particularly with shoots produced on 4.4 µM BAP medium. Good results were obtained with a subculture of shoots on 1.1 µM BAP medium. In this case, percentage of successful rooting raised to 70 %.

In addition to its advantage to multiplicate a material potentially interesting for viticulture, micropropagation of VR hybrids by axillary bud proliferation appears to be an efficient method to supply great quantities of homogeneous explants for biotechnological studies and consequently represents an important step in the genetic improvement of the grapevine.

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