

## A study on organogenic potential in the *Vitis* genus

by

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**S u m m a r y :** In order to increase the number of grape genotypes adapted to *in vitro* manipulation studies, an investigation on the regenerative potentiality in the *Vitis* genus was conducted on cultivars of *Vitis vinifera* L., (Barbera, Cabernet franc, Cabernet Sauvignon, Canner Seedless bianco, Chardonnay, Enantio, Moscato bianco, Riesling, Sultana moscato bianco and Sultanina rouge), *V. vinifera silvestris* G., *V. amurensis* R., *V. armata* D. and G., *V. riparia* M., *V. simpsonii* M., as well as the rootstocks 110 Richter and Schwarzmann, and the interspecific variety Staufer. Regeneration was induced *via* direct organogenesis from young leaves, and a relevant genotypic influence on the morphogenic potentiality was observed. Among the 18 different genotypes tested, regeneration occurred with different efficiencies and precocity. Agreeable efficiencies were obtained for Sultana moscato, Riesling, Chardonnay and Cabernet franc, while Enantio and Cabernet Sauvignon were recalcitrant to shoot regeneration. *V. armata* was the most favourable to this regeneration system.

**K e y w o r d s :** *Vitis*, *in vitro*, regeneration, organogenesis.

**A b b r e v i a t i o n s :** BA = 6-benzyladenine; MS = MURASHIGE and SKOOG (1962); NN = NITSCH and NITSCH (1969); NAA = 2-naphthaleneacetic acid.

### Introduction

The adoption of molecular techniques for genetic improvement of grape results promising: recently, in fact, transgenic plants have been obtained in several laboratories with both *Agrobacterium*-mediated and direct transformation (LE GALL *et al.* 1994; MARTINELLI and MANDOLINO 1994; NAKANO *et al.* 1994; KRASTANOVA *et al.* 1995; SCORZA *et al.* 1995; KIKKERT *et al.* 1996). However, results are mostly restricted to the few genotypes with agreeable regenerative potential since genetic transformation is strictly related to *in vitro* plasticity. Thus, the genotype has been proved the most relevant component of morphogenic response within *Vitis* genus as well as other species (BOUQUET *et al.* 1982; MAURO *et al.* 1986; KOORNNEEF *et al.* 1987).

From different explants, somatic embryogenesis and organogenesis have been obtained in some *Vitis* species (MOSZAR and SÜLE, 1994; MARTINELLI *et al.* 1993; ROBACKER 1993) and hybrids (CLOG *et al.* 1990; HARST 1995), as well as rare *V. vinifera* cultivars (MAURO *et al.* 1986; STAMP *et al.* 1990 a and b; HARST and ALLEWELDT 1993; REUSTLE *et al.* 1994). Among the various regeneration approaches proposed, direct shoot organogenesis from young leaves (STAMP *et al.* 1990 a and b; COLBY *et al.* 1991) could be considered the most amenable because of its simplicity. Furthermore, this strategy represents a very interesting potential since regenerated leaflets proved to be juvenile tissues adapted to embryogenesis induction in species with difficult regeneration (JAMES *et al.* 1988; RUGINI and CARICATO 1995).

In our laboratory we have already conducted some research on grape tissue culture; however, our best results have been obtained on *V. rupestris* (GIANAZZA *et al.* 1992; MARTINELLI *et al.* 1993 a and b; MARTINELLI and MANDOLINO 1994). In order to increase the number of grape

genotypes adapted to our *in vitro* manipulation studies, we carried out an investigation on the regenerative potential in the *Vitis* genus, following a protocol derived from the „direct shoot organogenesis from leaves“ technique (STAMP *et al.* 1990 b). Cultivars of *V. vinifera*, species of *Vitis* genus and hybrids were analyzed. The results of this study are reported in the present paper.

### Materials and methods

**E x p l a n t s o u r c e :** The test was conducted on *V. vinifera* L. cultivars (Barbera, Cabernet franc, Cabernet Sauvignon, Canner Seedless bianco, Chardonnay, Enantio, Moscato bianco, Riesling, Sultana moscato bianco and Sultanina rouge), *V. vinifera silvestris* G., *V. amurensis* R., *V. armata* D. and G., *V. riparia* M., *V. simpsonii* M., as well as the rootstocks 110 Richter and Schwarzmann, and the interspecific variety Staufer (Bacchus x Villard blanc) - from the collections of our Institute - already established in *in vitro* cultures.

*In vitro* plants cultured on NN medium with 1.5 % sucrose and incubated at 25 °C with a 16 h photoperiod (70  $\mu\text{mol m}^{-2}\text{s}^{-1}$  cool white light) were sampled as starting material.

One-node segments were dissected and induced to adventitious shoot proliferation on a NN based medium with iron as in MS medium, 2 % sucrose and 2  $\text{mg l}^{-1}$  BA.

Cultures were incubated at 25 °C in the shade (10  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ). The number of segments cultured for each of the genotypes tested is summarized in the Table.

**O r g a n o g e n e s i s i n d u c t i o n :** Young leaves (3 - 4 mm) were separated by a cut across the petiole - according to STAMP *et al.* (1990 b) - from 40-day-old nodal cultures, and placed adaxial side down on half strength MS medium supplemented with 2 % sucrose, 2.25  $\text{mg l}^{-1}$  BA

Table

Efficiencies of regeneration (percentage of leaves presenting regeneration on the plated explants) among 18 *Vitis* genotypes during a culture period of 80 days

Genotype	node n	leaf n	day							
			20	30	40	50	60	70	80	
Cabernet Sauv.	157	141	0	0	0	0	0	0	0	
Enantio	170	143	0	0	0	0	0	0	0	
<i>V. riparia</i>	87	89	0	0	0	0	0	0	0	
110 Richter	99	164	0	0	2	4	4	4	4	
Staufer	121	131	0	1	1	5	8	8	8	
<i>V. amurensis</i>	84	116	0	0	0	3	3	4	8	
<i>V. simpsonii</i>	88	86	0	0	0	1	8	9	9	
<i>V. silvestris</i>	114	220	0	1	1	4	9	10	10	
Schwarzmann	206	286	1	3	9	12	15	15	15	
Barbera	95	264	0	5	5	12	14	14	16	
Moscato bianco	87	156	0	5	5	13	16	16	16	
Sultanina rouge	126	318	0	4	4	7	15	22	24	
Riesling	634	1141	10	20	26	27	28	28	28	
Chardonnay	541	776	5	31	38	41	42	43	43	
Cabernet franc	400	1075	9	37	44	46	47	48	48	
Sultana mosc. b.	251	393	10	17	31	42	49	53	53	
Canner Seedl. b.	62	67	0	0	0	6	31	46	57	
<i>V. armata</i>	160	182	0	0	0	0	1	49	59	

and 0.03 mg l<sup>-1</sup> NAA. Cultures were incubated at 25 °C in the dark for 10 d and then moved to the shade (10 µmol m<sup>-2</sup>s<sup>-1</sup>). The Table reports the number of leaves tested within the genotypes.

**Plantlet culture:** Regenerated shoots were dissected from regenerating leaf explants and grown at 25 °C with a 16 h photoperiod (70 µmol m<sup>-2</sup>s<sup>-1</sup> cool white light) in Magenta boxes containing 70 ml growth regulator-free NN-based medium with 1.5 % sucrose.

**Media completion and culture vessels:** Media were solidified with 0.9 % bacteriological Agar (Oxoid), and pH was adjusted with NaOH at 5.7 before a 20 min autoclaving at 121 °C and 1 bar.

Nodal cultures and regeneration from leaves have been conducted in plastic Petri dishes of 9 cm diameter sealed with Parafilm. Plant micropropagation has been carried out in Magenta boxes.

## Results and discussion

Within 40 d of culture, one-node explants produced adventitious bud formation with young leaves for the further regeneration induction. This first step yielded a homogeneous explant population within the tested genotypes as for age and development of the tissues for the subsequent morphogenic induction. The number of internode segments and leaves assayed for each genotype are reported in the Table.

Shoot regeneration from young leaves were followed during an 80-day culture period. Typically, shoot regeneration occurred *via* direct organogenesis without callus-

ing from the cut surface of the petiole, according to COLBY *et al.* (1991) (Figure, a). However, a different organogenic pathway was observed in *Vitis armata*, *V. simpsonii* and Sultana moscato where regeneration followed callusing (Figure, b and c). Regeneration occurred also from the central vein of the leaf without callusing in Sultana moscato (Figure, d).

Generally, more than one regeneration event occurred from each leaf, according to STAMP *et al.* (1990 a and b), and a considerable number of shoots (up to 30) was obtained when regeneration occurred after callusing.

Regeneration efficiencies were collected and expressed as percentage of leaves presenting the first regeneration event on the plated explants (Table). Among the 18 different genotypes tested, regeneration occurred with varying efficiencies and precocity.

As for *V. vinifera* cultivars, agreeable efficiencies have been obtained; furthermore, Sultana moscato, Riesling, Chardonnay and Cabernet franc showed also relevant precocity since regeneration was observed after only 20 d (Table). Only Enantio and Cabernet Sauvignon, at the opposite extreme, were recalcitrant to shoot regeneration. As for the other species, *V. armata* was the most favourable to this regeneration system. Roots were regenerated in Cabernet Sauvignon, Enantio and Schwarzmann.

The best plantlets, without deformations or vitrification, were produced from Riesling, Cabernet franc and Sultana moscato which demonstrated favourable adaptability to the *in vitro* techniques already during the nodal cultures with a great bud proliferation and the highest leaf number production (Table).

In this study, we were able to detect a great number of genotypes with relevant morphogenic capability for further *in vitro* studies. In particular, the regeneration of juvenile tissues for somatic embryogenesis induction (JAMES *et al.* 1988; RUGINI and CARICATO 1995) associated to this protocol, represents an interesting perspective since - according to our experience (MARTINELLI and MANDOLINO 1994) - somatic embryos resulted the best system for stable transgenic plants production in *Vitis* genus.

## Acknowledgements

Research supported by National Research Council of Italy, Special Project RAISA, Sub-project N.2, Paper N. 2777. Authors wish to thank Dott. L. DE MICHELI and Oen. I. RONCADOR for the plant material of the collections of our Institute, and Dott. G. MANDOLINO for useful discussions.

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Figure: Shoot organogenesis from the cut surface of the petiole - without (a) and in presence (b, c) of callusing - and from the middle vein of the leaf (d). R = regenerating shoots; C = callusing; V = middle vein of the leaf. For a, b and d: bar = 1 mm. Picture c has been obtained by scanning electron microscope (Hitachi S-2300 with tungsten filament).

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Received April 4, 1996