

Genotype effect on somatic embryogenesis and plant regeneration of *Vitis* spp.

by

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S u m m a r y : The effect of genotype on grapevine embryogenic culture was studied on eight *Vitis* spp. Cultures were obtained from excised anthers, isolated from flower buds. Somatic embryogenesis was achieved from 6 of the 8 varieties at various frequencies. Embryos were germinated either on solid or in liquid medium in the dark before being exposed to light. The regeneration rate ranged from 8 to 81 %. For induction of recurrent somatic embryogenesis, torpedoed and early cotyledonary stage somatic embryos were employed applying two plant growth regulator treatments. Results demonstrate that grape cultivars are highly variable in their response to induce somatic embryogenesis, recurrent somatic embryogenesis and germination of embryos.

K e y w o r d s : somatic embryogenesis, grapevine, genotype effect, germination.

Introduction

Although it has been relatively easy to achieve somatic embryogenesis from grapevine, the frequency of plant regeneration from these embryos usually is low. Several protocols have been developed to increase the regeneration rate, including chilling of embryos at 4 °C (RAJASEKARAN and MULLINS 1979; MARTINELLI *et al.* 1993), daily subculturing of embryos in a fresh medium (COUTOS-THEVENOT *et al.* 1992), removal of cotyledons from embryos (MAURO *et al.* 1986), application of plant growth regulators and dehydration of mature embryos (GRAY 1989), induction of adventitious buds on isolated hypocotyls (VILAPLANA and MULLINS 1989) and germination in liquid medium (MOZSÁR and SÜLE 1994). Grape somatic embryos were employed in *Agrobacterium*-mediated gene transfer (MARTINELLI and MANDOLINO 1994, SCORZA *et al.* 1995). In these experiments regeneration of transgenic plants occurred *via* recurrent embryogenesis. According to these results *in vitro* embryogenic culture with a high rate of plant recovery and recurrent somatic embryogenesis seems to be a prerequisite to successful genetic manipulation.

In this work 8 *Vitis* spp. were used to induce somatic embryogenesis and to obtain somatic embryos which were germinated or were subjected to the induction of recurrent somatic embryogenesis.

Materials and methods

Five *Vitis vinifera* L. cultivars, Chasselas, Szürkebarát, Ezerjő, Szlanka and EME (Ezeréves Magyarország Emléke, Chasselas Queen Victorian White x Calabrian White) and *Vitis amurensis* Rupr. (clone No. 66), *Vitis riparia* Michx. cv. Gloire de Montpellier and the interspecific rootstock G 28 (*V. berlandieri* x *V. riparia* x *V. vinifera*) were used in this study.

The initiation of embryogenic cultures from excised anthers has been described previously (MOZSÁR and SÜLE

1994). In the germination experiments globular and heart-shaped embryos were placed in 100 ml Erlenmeyer flasks in 25 ml liquid MS medium (MURASHIGE and SKOOG 1962) containing 20 g l⁻¹ sucrose and incubated in an orbital shaker (150 rpm at 28 °C in the dark). The medium was replenished weekly. In parallel with this experiment embryos were germinated on solid MS (0.8 % Difco Noble-agar) at the same temperature in darkness. After 3 weeks embryos with cotyledons and primary roots were placed individually in test tubes, which were filled with 10 ml induction medium (half-strength MS plus 10 g l⁻¹ sucrose) and incubated at 25–27 °C in permanent light. Two months later plantlets with well-defined root and shoot systems were registered.

For induction of repetitive somatic embryogenesis torpedoed (ca. 2–3 mm long) and early cotyledonary stage somatic embryos (ca. 5–8 mm long) were selected. They were cultured either on MSE medium containing MS plus 20 g l⁻¹ sucrose supplemented with 5 µM 2,4-dichlorophenoxyacetic acid (2,4D) and 0.9 µM 6-benzylamino-purine (BA) or MSE/2 medium containing MS plus 20 g l⁻¹ sucrose with 2.5 µM 2,4D and 0.45 µM BA. A solidifying agent (0.8 % Difco Noble-agar) was added. Plates were incubated at 28 °C in the dark. After 2 months explants that had produced somatic embryos or yellow embryogenic calli were registered. Calli were transferred to the induction medium to determine their embryogenic capacity.

Results

Induction of somatic embryogenesis: The embryogenic callus had a light-yellow color and was composed of an undifferentiated mass of cells and clusters of proembryo and globular, embryo-like structures. Primary somatic embryos appeared on these calli after 2 weeks of incubation on the induction medium.

We were not able to induce somatic embryogenesis on explants of Ezerjő and *V. amurensis*. Of the tested cultivars,

Chasselas was superior in somatic embryogenesis induction (5.5 %). Although its anthers yielded relatively few calli, almost all (91 %) were embryogenic. The efficiency of somatic embryogenesis induction of the other cultivars was quite low (less than 1 %), because only a low portion of calli were embryogenic (6-18 %).

Embryogenic calli formed numerous somatic embryos asynchronously on the induction medium, except for Szürkebarát. Somatic embryos of Szürkebarát were therefore exclusively employed in germination experiments.

Embryo germination: In previous experiments we found that the liquid MS medium with weekly replenishment greatly stimulated plant regeneration of *V. riparia* somatic embryos (MOZSÁR and SÜLE 1994) as compared to solid MS. Therefore we tested this method on embryos of other *Vitis* spp. Significant differences between the two germination methods were observed only with 3 out of the 5 cultivars (Tab. 1). Germination of somatic embryos of *V. riparia* and Chasselas increased in liquid medium while those of Szlanka decreased. Although the percentage of regenerated plants from Szürkebarát embryos was nearly doubled due to the liquid medium, statistically significant differences could not be demonstrated.

Table 1

Effects of different treatments of germination on plant regeneration

	Embryos germinated on solid medium / regenerated plantlets	Embryos germinated on liquid medium / regenerated plantlets
Chasselas*	62/24 38 % a	46/26 52 % a
EME	46/14 30 % a	78/22 28 % b
Szlanka*	30/14 46 % a	39/4 10 % c
Szürkebarát	16/2 12 % b	46/10 21 % bcd
G 28	69/24 35 % a	104/32 30 % bd
<i>Vitis riparia</i> *	45/4 8 % b	52/32 61 % a

Values with the same letter are not significantly different at $P = 0.05$ according to the Student's *t* test (by columns).

* Significant difference between the two germination methods.

Repetitive somatic embryogenesis: In most cases responsive embryos produced both somatic embryos and yellow embryogenic calli. Some explants became swollen, turned entirely or partly dark-brown and necrotic. In many cases from this kind of necrotic tissue an induction of secondary somatic embryogenesis or embryogenic calli could be observed.

The results shown in Tab. 2 indicate that repetitive somatic embryogenesis was affected by genotype, developmental stage of the somatic embryos and concentration of plant growth regulators. The best results were obtained with G 28 embryos, regardless of developmental stage and medium while Chasselas had the poorest capability for recurrent somatic embryogenesis. The cotyledonary stage embryos gave a higher frequency of embryogenesis than torpedo stage embryos except for *V. riparia* on MSE medium. In 4 cases significant differences were observed between the MSE and the MSE/2 medium. G 28 cotyledonary and *V. riparia* torpedo and cotyledonary stage embryos produced a significantly higher level of embryogenesis on MSE/2 than on MSE. However, 19 % of the Chasselas cotyledonary embryos showed a secondary embryo induction on MSE whereas only 2.4 % on MSE/2.

Table 2

Effect of developmental stages and different media (MSE, MSE/2) on the induction of recurrent somatic embryogenesis

	MSE Number of embryos / recurrent embryogenesis	MSE/2 Number of embryos / recurrent embryogenesis
Chasselas torpedo	76/1 (1.3 %) a	93/2 (2.1 %) a
Chasselas cotyledonary*	56/11 (19 %) bf	41/1 (2.4 %) a
EME torpedo	91/21 (23 %) bcf	94/49 (52 %) bd
EME cotyledonary	65/36 (55 %) d	38/25 (65 %) bd
Szlanka torpedo	40/27 (67 %) d	54/23 (42 %) bd
Szlanka cotyledonary	49/37 (75 %) d	40/32 (80 %) cd
G 28 torpedo	67/61 (91 %) e	53/50 (94 %) c
G 28 cotyledonary*	38/37 (97 %) e	42/42 (100 %)
Riparia torpedo*	35/11 (36 %) bcd	37/18 (48 %) b
Riparia cotyledonary*	39/5 (13 %) f	23/30 (76 %) d

Values with the same letter are not significantly different at $P = 0.05$ according to the Student's *t* test (by columns).

* Significant difference between the two plant growth regulator treatments.

Discussion

During induction of somatic embryogenesis, differences were observed among cultivars not only in the efficiency of embryogenesis but also in the behavior of embryogenic calli, e.g., calli of Szürkebarát produced few embryos.

Replenishment of liquid medium is supposed to remove inhibitory compounds (COUTOS-THEVENOT *et al.* 1992) and helps to promote normal shoot meristem. However, this technique was not clearly beneficial for all varieties tested. It promoted the conversion of Chasselas and *V. riparia* embryos to plants but reduced that of Szlanka and did not change significantly the conversion rate of EME, Szürkebarát and G 28. This indicates that the previously described method (MOZSÁR and SÜLE 1994) is appropriate only for certain cultivars. Other procedures published to improve regeneration frequency of grapevine somatic embryos had the same limitation (GRAY 1989, VILAPLANA and MULLINS 1989, COUTOS-THEVENOT *et al.* 1992).

For grape transformation recurrent cycles of embryogenesis seem to be the most promising way for selection of genetically transformed clones (MARTINELLI and MANDOLINO 1994). In our experiments its efficiency depended on genotype, developmental stage and growth regulator treatment. For the induction of repetitive embryogenesis early cotyledonary stage embryos generally were more efficient than embryos in the torpedo stage and the MSE/2 medium was more efficient than MSE.

Of the investigated genotypes, G 28 appears to be the best variety for a successful genetic transformation. Its anthers are adequate explants for the induction of somatic embryogenesis, it has an acceptable regeneration rate and an extremely good inclination for recurrent somatic embryogenesis. Suitable results were achieved with *V. riparia*, Szlanka and EME and Chasselas, if the optimum treatment was applied in each step. E.g., 76 % of the *V. riparia* cotyledonary embryos produced adventitious embryos on MSE/2, while only 13 % on MSE, 46 % of Szlanka embryos converted to plants when germinated on solid MS whereas only 10 % when germinated in liquid medium.

This study demonstrated that the behavior of grapevines in an *in vitro* embryogenic culture is highly influenced by the genotype. No correlation was observed between the degree of relatedness and the responses of grapevines. Closely related genotypes often gave divergent responses, while genetically different grapes behaved similarly.

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