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Somatic embryo germination and plant regeneration of three grapevine cvs: Effect of IAA, GA_3 and embryo morphology

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Summary

Plant regeneration was achieved using somatic embryos obtained from the *Vitis vinifera* cvs Sugraone, Crimson Seedless and Don Mariano. White somatic embryos, 1.5-3 mm in length, were cultured on embryo germination medium with or without IAA (10 µM) + GA3 (1 µM). Germinated embryos showed 5 different morphologies, (1) one cotyledon, (2) two cotyledons, (3) three cotyledons, (4) trumpet-like and (5) fused cotyledons before transfer to half strength MS medium to obtain plants. The conversion rate was higher when somatic embryos were germinated in culture medium with IAA and GA3. Embryo morphology also had an effect on plant regeneration, somatic embryos with developed cotyledons (one, two or three) showing a higher conversion rate (57.8-70.2 %) than those with abnormal or no cotyledons (36.2-36.3 %).

Key words: plant regeneration, somatic embryo morphology, indole-3-acetic acid, gibberellic acid.

Abbreviations: IAA = indole-3-acetic acid; GA_3 = gibberellic acid; BA = 6-benzyladenine; IBA = indole-3-butyric acid; ABA = abscisic acid

Introduction

Somatic embryogenesis has become a key tool for transformation strategies and embryogenic tissues have shown to be the best cell source for transgenic plant regeneration (Martinelli 1997). An efficient regeneration protocol requires high rates of embryogenesis induction and plant regeneration. While embryogenesis has been achieved in several grapevine cultivars, further germination and plant regeneration are a problem, in which dormancy and embryo morphology appear to play a major role (Martinelli and Gribaudo 2001). It has been described that germination only occurs in well shaped and polarised embryos with developed root and shoot axes, a hypocotyl and two cotyledons (Martinelli *et al*. 1993). Different strategies have focused on the release from dormancy to improve somatic embryo germination and plant regeneration; for example, chilling of the somatic embryos (Rajasekaran and Mullins 1979), cotyledon removal and chilling (Mauro *et al*. 1986), or the use of growth regulators (Goebel-Tourand *et al*. 1993, Faure *et al*. 1998, Jayasankar *et al*. 1999, Perrin *et al*. 2001). Previously, we reported somatic embryogenesis and plant regeneration in 4 table grape cultivars (López-Pérez *et al*. 2005). In this paper, we focus on the relation between IAA, GA_3 and the morphology of somatic embryos in an attempt to improve germination and conversion into plants.

Material and Methods

Plant material: Embryogenic callus, somatic embryos and plant regeneration from Crimson Seedless, Sugraone and Don Mariano were obtained from immature anthers or ovaries, as described by López-Pérez *et al*. (2005).

Effect of IAA, GA_3 and morphology on somatic embryo germination and plant regeneration: Somatic embryos, 1.5-3 mm in length, were cultivated on embryo germination medium (López-Pérez *et al*. 2005) with or without IAA (10 µM) + GA_3 (1 µM). For each condition and cultivar, 7 plates with 20 embryos per plate were used. After 15 d, green germinated embryos were classified according to their morphological characteristics and transferred to test tubes with 10 ml of plant regeneration medium (half strength MS, López-Pérez *et al*. 2005) for 30 d to obtain entire plants. Regeneration efficiency was calculated as the number of plants related to the total number of germinated embryos transferred to the plant regeneration medium.

Results and Discussion

High rates of somatic embryo germination were obtained (91.0-99.2 %), independently of the cv. and culture medium used (data not shown). However, differences in cotyledon development were observed and the germinated somatic embryos could be grouped and transferred to plant regeneration medium by reference to the following morphological characteristics: (1) one cotyledon, (2) two cotyledons, (3) three cotyledons, (4) trumpet-like and (5) fused cotyledons (Figure). As shown in the Table, regardless of embryo morphology, the total conversion rate was higher if somatic embryos were germinated in culture medium containing IAA and GA_3. In Crimson Seedless, for exam-

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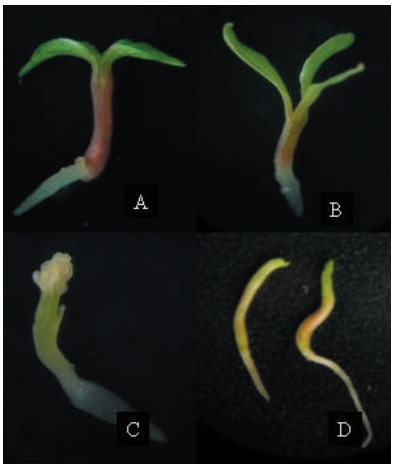

Figure: Morphology of the germinated somatic embryos before being transferred to plant regeneration medium: Two-cotyledon (**A**), Three-cotyledon (**B**), Trumpet-like (**C**) and Fused-cotyledon (**D**).

ple, the percentage of regenerated plants was 63.8 % for somatic embryos cultured in medium with IAA and GA_3 and 49.9 % for those cultured without growth regulators. For Sugraone, the corresponding percentages were 46.1 % and 42.7 % and for Don Mariano 55.3 % and 52.2 %, respectively.

Regarding embryo morphology, somatic embryos with developed cotyledons (one, two or three) showed a higher average conversion rate (57.8-70.2 %) than trumpet-like or fused-cotyledons embryos (36.2-36.3 %), regardless of the cvs and germination medium. In this study we demonstrate for the first time the relation between embryo morphology and plant conversion rate.

Although it was expected that two-cotyledon somatic embryos would show the highest conversion rates, surprisingly, the best rates (87 % Crimson Seedless, 75 % Sugraone, 80 % Don Mariano) were obtained with three-cotyledon somatic embryos cultured in EG-IAA+GA_3, although, the differences with respect to one- and two-cotyledon somatic embryos in Crimson Seedless and Don Mariano were not statistically significant.

Morphological alterations as well as dormancy proved to be crucial for the conversion of embryos into plants. Different strategies have been employed to increase the efficiency of plant regeneration, and a comprehensive review of the topic can be found in Martinelli and Gribaudo (2001). For the first time, Goebel-Tourand *et al*. (1993) classified 8 morphologically different classes of cotyledonary somatic embryos of the rootstock 41B but the conversion rates, regardless of classification, failed to exceed 20 %. In our case, the total conversion rate, regardless of embryo morphology, ranged from 42.7 % to 63.8 % depending on the cultivar. For Goebel-Tourand *et al*. (1993), abnormal 41B somatic embryos (about 97 %) were essentially characterized by their incomplete development or lack of an apical meristem, and very few embryos (about 3 %) had a normal meristem between two well-defined cotyledons, epicotyl ontogenesis only occurring when the two-cotyledon growth was achieved. In the present work, epicotyl development occurred not only in embryos with two well defined cotyledons (40.0-75.0 %) but also when somatic embryos were aberrant with one (42.7-77.7 %) or three cotyledons (51.2-87.0 %) or with trumpet-like (27.3-50.0 %) and fused cotyledons (13.3-55.5 %). Nevertheless, we do not know if plant development originated from an apical meristem or an adventitious bud as previously described by Goebel-Tourand *et al*. (1993). Rajasekaran and Mullins (1979) reported the use of GA_3 (1 µM) to induce normal plant development (47 %) although it was less effective than chilling. These authors report that anther-derived dormant embryos from the hybrid Gloryvine required chilling (4 ºC) for at least two weeks to induce normal germination

T a b l e

Conversion rate (%) of germinated somatic embryos of Crimson Seedless, Sugraone and Don Mariano as a function of the somatic embryo germination medium and morphology. One-cotyledon (1), Two-cotyledons (2), Three-cotyledons (3), Trumpet-like (4) and Fused-cotyledons (5)

Cultivar	Somatic embryo germination medium	Conversion rate (%) of the different morphological classes[a]					Total conversion rate[b] (%)
		1	2	3	4	5	
Crimson S.	EG-IAA+GA3	77.7a	75.0a	87.0a	38.0b	40.0b	63.8 a
	EG	67.8a	60.0a	53.3a	28.6b	37.5b	49.9 b
Sugraone	EG-IAA+GA3	51.5b	40.0b	75.0a	30.6c	33.3c	46.1 a
	EG	45.0a	53.3a	75.0a	27.3b	13.3b	42.7 a
Don Mariano	EG-IAA+GA3	62.2a	72.7a	80.0a	43.4b	37.5b	55.3 a
	EG	42.7a	54.4a	51.2a	50.0a	55.5a	52.2 a
	Average	57.8	59.2	70.2	36.3	36.2	51.7

[a] For each cultivar and culture medium the same letter means no statistical differences at $p < 0.05$ as determined by the Fisher LSD test.

[b] For each cultivar, the same letter means no statistical difference at $p < 0.05$ as determined by the Fisher LSD test.

and the production of normal plantlets (up to 97 %), while unchilled embryos mainly produced abnormal plants. The role of IAA in the release from dormancy and plant regeneration has been discussed by Faure *et al*. (1998). These authors suggested that grape somatic embryos do not accumulate ABA and/or IAA in sufficient concentrations to support normal plantlet development. Thus, the addition of IAA to the embryo germination medium could explain, in our case, the higher conversion rate of somatic embryos being cultured in medium with this growth regulator. Goebel-Tourand *et al*. (1993) described the way the application of ABA, zeatin or BAP led to differences in the growth rate and in the conversion rate of somatic embryos although none of the treatments led to high conversion rates.

This paper has focused on the effect of IAA, GA_3 and somatic embryo morphology on embryo conversion into plants. The results show that the combination of IAA (10 µM) and GA_3 (1 µM) increased the conversion rate of the different morphological classes of embryo in all three cultivars.

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