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Research Note

Effect of putrescine on embryo development in the stenospermocarpic grape cvs Emperatriz and Fantasy

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K e y w o r d s : *in ovulo* culture, embryo rescue, seedlessness, putrescine, polyamines.

Introduction: The grape embryo rescue allows to obtain plants from direct crossing between seedless cultivars (Bouquet and Davis 1989), but the proportion of normal plants obtained is low. In order to increase the number of plants obtained by this technique application of growth regulators provided some success (Agüero *et al.* 2000).

Polyamines are growth regulators present in all living organisms, essential for growth and development of tissues due to their role in cell reproduction, transduction of signals and protein synthesis (Tiburcio *et al.* 1993). In *Vitis vinifera*, the main polyamines are putrescine, spermidine and diaminpropane; their concentrations were determined for different organs and stages of development (Geny *et al.* 1997). Exogenous application of putrescine to grape clusters delayed maturity, whereas the application of a polyamine synthesis inhibitor accelerated it (Colin *et al.* 1998). The consequences of these applications on the development of grape embryos with or without seeds is unknown.

The goal of this investigation was to determine the effect of putrescine sprayed to clusters of stenospermocarpic grapes on the *in vitro* development of embryos.

Material and Methods: Experiments were carried out with the stenospermocarpic cvs Emperatriz and Fantasy, trained on pergola. For each cultivar, 18 vegetatively uniform plants were selected. The clusters of both cultivars were sprayed 5 d before anthesis with water solutions of 5.0 and 10.0 µM putrescine and water (control). In all cases Tween 20 (0.1 %) was added. Each treatment was carried out on 6 plants, spraying 5 clusters of each plant. Clusters were harvested 8 weeks after fruit set in Fantasy, and when ripe in the case of Emperatriz. Berries were sterilized with 20 % sodium hypochlorite (60 g l^{-1} active chlorine) for 20 min. Seeds were aseptically extracted from the berries and cultured in a medium according to Nitsch and Nitsch (1969), to which 20 g l^{-1} of saccharose and 2.8 g l^{-1} of phytagel had been added. Plates with 25 ml of medium and 30 seeds each were kept in a growth chamber at 25 ± 2 ºC with 16 h of fluorescent light (50 µmol m^{-2} s^{-1}). After three months, the germinated embryos, in which the radicle had emerged from inside the seed (direct germinations), were extracted. The remaining seeds were opened under a magnifying glass in order to excise the ungerminated embryos (rescued embryos). Plants from direct germination and rescued embryos were cultured according to Agüero *et al.* (2000) and treated like seeds.

Results: The development of embryos after application of putrescine differed depending on the cultivar. In Emperatriz, the 10 µM spray of putrescine statistically increased the percentage of total embryos, 5 µM putrescine did not affect growth of the embryos but had a greater effect, increasing the formation of polyembryos. In cv. Fantasy, the highest percentage of embryos and direct germinations was obtained with the lowest dose of putrescine, whereas with 10 µM no differences were found compared to control. Nonetheless, with 10 µM putrescine the highest frequency of polyembryos was obtained. The percentage of plants obtained, from both cultivars, was statistically higher if the clusters were sprayed with 10 µM putrescine. In Emperatriz, the highest number of plants is obtained with 10 µM putrescine whereas in Fantasy this treatment also led to the highest percentage of plants but did not differ from the control with regard to the percentage of total embryos (Table).

T a b l e

Effect of putrescine sprays on the *in vitro* development of embryos of stenospermocarpic berries cvs Emperatriz and Fantasy

	No. of seeds	Total embryos, %	Rescued embryos, %	Direct germination, %	Polyembryos %	Plants %
Emperatriz						
Control	1863	20.5 b	6.4 a	14.1 b	3.3 b	6.7 b
5 µM Putrescine	1350	18.8 b	5.9 a	12.9 b	5.5 a	7.5 b
10 µM Putrescine	2196	24.5 a	4.1 b	20.4 a	4.0 b	11.1 a
Fantasy						
Control	1545	18.6 b	5.2 b	13.5 b	7.2 b	2.1 b
5 µM Putrescine	1605	22.9 a	7.0 a	15.9 a	6.1 b	2.4 b
10 µM Putrescine	1374	18.3 b	6.7 a	11.6 b	9.9 a	3.5 a

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Percentages were analysed by χ^2 test. Differences at the 5 % level of significance were compared with the Arcsine test. Different letters in columns indicate significant differences between treatments for each cultivar.

Discussion and Conclusion: The effect of exogenous application of putrescine on embryo development depended on the cultivar. A similar response has already been described by AGÜERO *et al.* (2000) for growth retardants.

Polyamines have been shown to be growth stimulators in several species suggesting that the endogenous concentration of these substances may restrict growth; this would explain the fact that in both cultivars putrescine favored the development of more embryos and their subsequent growth allowing higher rates of direct germination.

In addition putrescine application affected secondary somatic embryogenesis and increased the frequency of polyembryos. This has also been described for eggplant (FOBERT and WEBB 1988).

The application of putrescine to clusters increased the proportion of normal plants developing *in vitro*. MARTIN-TANGUY and CARRE (1993) report better development of *in vitro* grape plants obtained from microcuttings in a growth medium containing putrescine.

FAURE *et al.* (1991) suggest that the inability of grape embryos to produce normal plants may be due to an inadequate composition of endogenous polyamines. This might be true for embryos obtained from Fantasy treated with 5 μM of putrescine, although it would be necessary to determine levels of endogenous polyamines to corroborate this hypothesis.

On the basis of the results obtained in this study, we conclude that spraying of stenospermocarpic grape clusters with putrescine increases yield in the embryo rescue technique.

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