BIOCELL
2002, 26(2): 263-266

ISSN 0327 - 9545
PRINTED IN ARGENTINA

Brief Note

Improved *in vitro* embryo development of stenospermic grape by putrescine

María Teresa Ponce, Mónica Elizabeth Guiñazú and Ricardo Tizio

Departamento de Biología. Cátedra de Fisiología Vegetal. Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza, Argentina.

* Prof. Eng. Ricardo M. Tizio died on April 16th, 2002.

Key words: putrescine, stenospermic grapes, embryo rescue, plant regeneration, *in vitro* culture, *Vitis vinifera*

ABSTRACT: The goal of this study was to determine the effect of putrescine, added to the culture medium, on the *in vitro* development of stenospermic grape (*Vitis vinifera L)* embryos. The cross breedings of Perlón x G.C88552 and Perlón x Argentina were used. 0 (control), 2 and 4 mM of putrescine were added to the immature seed's culture medium. In Perlón x Argentina, 2mM of putrescine statistically increased the percentage of total embryos, direct germination, polyembryos and normal plants. In Perlón x G.C88552, only 2 mM of putrescine increased all the variables considered, eventually tripling the percentage of normal plants obtained. The results suggest that the endogenous concentration of putrescine may be a growth limiting factor. Adding putrescine to the culture medium of immature grape seeds is a legitimate resource to significantly increase the results of this technique.

Abbreviations: put-putrescine, G.C.-Gargiulo's Crossing

Introduction

One of the main objectives of table grape breeding is to develop new seedless cultivars. In the so-called stenospermic grapes, fertilization takes place, but the embryo aborts (Stout, 1936). The causes for this abortion are not entirely known. Cain *et al.* (1983) hypothesized a hormonal imbalance in the maternal tissues during the first stages of the seeds ontogeny.

The embryo rescue technique in grape allows us to obtain plants from direct crossing between seedless cultivars (Cain *et al.*, 1983; Spiegel-Roy *et al.*, 1985; Bouquet and Davis, 1989; among others). The yield of this technique is conditioned by maternal genotype, harvest date, pollinator and culture medium, (Bouquet and Davis, 1989; Gribaudo *et al.*, 1993 and Ponce *et al.*, 2000). The proportion of normal plants obtained is relatively low: from 7 to 10% (Barlass *et al.*, 1988).

Efforts to increase the efficiency of the technique have had only relative success. Different combinations and concentrations of hormones (Ponce *et al.,* 1995), the effects of cold, gibberellic acid and manipulation of the chalazal (Agüero *et al.*, 1996), the use of light enriched in the long red wave lengths, and frequent changes to fresh medium, (Ponce *et al.*, 1997) have been studied.

On the basis of the results of some of the studies previously mentioned, it has been concluded that adding different auxins (indol acetic acid, indol butyric acid, naphtalen acetic acid) and gibberellic acid to the culture medium does not improve the proportion of embryos obtained.

Address correspondence to: Ing. María Teresa Ponce, Depto. de Biología, Cátedra de Fisiología Vegetal, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo. Alte. Brown 500, Chacras de Coria (5505) CC 7. Mendoza, ARGENTINA. Tel.: (+54-261) 496 0431; Fax: (+54-261) 496 0469; E-mail: mponce@fca.uncu.edu.ar
Received on August 8, 2001. Accepted on February 14, 2002.

Polyamines (putrescine, spermidine, spermine, among others) 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).

Putrescine added to the culture medium increased hairy root growth 2.3 times in *Chicorium intybus* (Bais *et al.*, 1999), remarkably increased the embryogenic capacity of leaf discs of *Solanum melongena* L. (Yadav and Rajam, 1998), stimulated the growth and development of microcuttings of *Fragaria x ananassa* (Tarenghi *et al.*, 1995), increased the growth of stems obtained from cotyledons of *Brassica campestris* (Chi *et al.,* 1994), and in *Vitis vinifera* increased micropropagation rate, number of roots, number of nodes, length of root and stem, and fresh and dry weight (Martin-Tanguy and Carre, 1993).

One proof of polyamines intervention in plant development is that when putrescine synthesis inhibitors are used on rice plantlets, their development is detained, whereas it will be reestablished when added exogenously (Bonneau *et al.*, 1994).

The goal of this study was to determine the effects of putrescine, added to the culture medium, on the *in vitro* development of stenospermic grape embryos.

Materials and Methods

The crossings of stenospermic cultivars Perlón x G.C. 88552 and Perlón x Argentina were used. The treatments consisted of adding 0 (control), 2 and 4 mM of putrescine to the culture medium, by sterile filtration (0.2 µ cellulosa acetate filter). The clusters were harvested the eighth week after set. The berries were sterilized with 20% sodium hypochlorite (60g.L^{-1} active chlorine) for 20 minutes. The immature seeds were aseptically extracted from the berries and put in 9 cm diameter Petri dishes (30 seeds per dish), with Nistch and Nistch (1969) medium, suplemented with 20 g.L^{-1} sucrose, 2.8 g.L^{-1} Phytagel (Sigma, St. Luis, USA) and different concentrations of putrescine. These dishes were taken to growth chambers at 25 ± 2ºC with 16 hours of fluorescent light (50 µmol.m^{-2}.s^{-1}).

After three months of growth under these conditions, the embryos that had germinated were extracted, considering germinated only those in which the radicule had emerged from the inside of the seed, and they were called direct germinations. The embryos that had not germinated were dissected under magnifying glass, and called rescued embryos. The sum of direct germinations and rescued embryos constituted the total embryos. The plants that originated from direct germinations and rescued embryos were put in test tubes with 15 ml of Murashige Skoog (1962) medium, with halfstrength macronutrients and without hormones, complemented with 20 g.L^{-1} sucrose and solidified with 2.8 g.L^{-1} Phytagel. Afterwards, they were put in growth chambers at 25 ± 2ºC and 50 µmoles.cm^{-2}.s^{-1}, with a 16 hour photoperiod. After two months, the number of plants obtained with each treatment was counted.

In the crossing of Perlón x G.C. 88552, 456, 508 and 463 immature seeds were planted *in vitro* for the control group, the 2 mM and 4mM putrescine respectively, and 1649, 2023 and 1269 in the crossing of Perlón x Argentina.

In the statistical analysis percentages were analysed by Chi squared test. When differences at the 5% level of significance were found they were compared with the Arcsine test, according to Capelletti (1982).

Results

The response to the inclusion of putrescine differed for each of the crossings studied. If we compare the control groups, in the crossing of Perlón x G.C. 88552, 26.8% total embryos were obtained, and in the crossing of Perlón x Argentina this quantity almost doubled to 48.8% (Table 1).

In the crossing of Perlón x G.C. 88552, adding 2mM of putrescine to the culture medium statistically increased the percentage of total embryos and the percentage of direct germinations, 49.7% and 28.4% respectively in relation to the number of seeds planted, whereas when the dose of putrescine was of 4 mM, there was no difference with the control group. This same relationship was observed in the percentage plants, when 2mM of putrescine were added to the culture medium 35.2% of the seeds originated normal plants, percentage that was markedly lower (13.1%) in the control group and the higher putrescine dose (4mM). (Table 1)

In the crossing of Perlón x Argentina, the 2mM treatment of putrescine statistically increased the percentage of total embryos (56.4%), the direct germinations, the frequency of polyembryos, and the percentage of plants. In this crossing, the higher dose of putrescine was detrimental to embryo development, producing lower values than the control group for all the measured variables. When 2mM of putrescine were added to the culture medium, 43% germinated directly

TABLE 1.

Effect of putrescine in the culture medium on the *in vitro* development of embryos of the crossings of Perlón x G.C. 88552 and Perlón x Argentina.

Treatment	% total embryos	% rescued embryos	% direct germination	% polyembryos	% plants
Crossing Perlón x G.C. 88552					
Control group	26.8 b	18.1 a	8.7 b	4.6 b	13.1 b
2mM Putrescine	49.7 a	21.3 a	28.4 a	20.0 a	35.2 a
4mM Putrescine	26.4 b	14.1 b	12.3 b	6.1 b	13.1 b
Crossing Perlón x Argentina					
Control group	48.8 b	24.5 a	24.3 b	9.1 b	12.6 b
2mM Putrescine	56.4 a	13.4 c	43.0 a	26.1 a	18.3 a
4mM Putrescine	36.9 c	19.6 b	17.3 c	3.9 c	6.7 b

Note: different letters in a column indicate significant differences between treatments for each crossing ($p \leq 0,05$)

and 18.3% produced normal plants, whereas in the control group 12.6% of the seeds originated plants and the higher dose of putrescine decreased this value to 6.7%. (Table 1)

Discussion

First, the effect of the pollinator is obvious, since comparing the response of the control groups of both crossings, the crossing of Perlón x Argentina had 100 to 200% more total embryos, direct germinations and polyembryos than the crossing of Perlón x G.C. 88552. This effect of the pollinator has already been described by Ponce *et al* (2000).

Polyamines have been shown to be stimulators of growth in several species (Tarenghi *et al.,* 1995). Our results suggest that the endogenous concentration of these substances may limit growth, and would explain the fact that in both crossings putrescine favored the development of a greater number of embryos and their subsequent growth, thus allowing a greater number of direct germinations and the development of a larger number of plants.

Both crossings had a spontaneous polyembryo frequency, which could be observed in the control group. This phenomena has already been reported by Valdez and Ulanovsky (1997) in the *in vitro* growth of immature seeds of the crossing of Superior x Ruby Seedless and its reciprocal. The presence of putrescine (2 mM) in the culture medium remarkably increased (200 to 300%) the frequency of polyembryos in both crossings. Yadav and Rajam (1998) have demonstrated that polyamines are involved in cell differentiation that occurs in somatic embryogenesis, and they propose the use of putrescine to improve this process in recalcitrant species.

On the basis of the results obtained, we conclude that including putrescine in the culture medium of immature grape seeds is a valid resource to considerably increase the yield of this technique.

Acknowledgements

This research was supported by a grant from SECYT, Universidad Nacional de Cuyo. The authors thank Professor Olga Fernandez for helpful suggestions on the manuscript and to Professor assistant Elisa Gustavson for the translation.

References

AGÜERO C, GREGORI MT, PONCE MT, IANDOLINO A, TIZIO R (1996). Improved germination of stenospermic ovules by low temperatures. Biocell 20(2): 123-126.

BAIS HP, GEORGE J, RAVISHANKAR GA (1999). Influence of polyamines on growth of hairy root cultures of witloof Chicory (*Cichorium intybus* L cv. Lucknow local) and formation of coumarins. J Plant Growth Regul 18: 33-37.

BARLASS M, RAMMING DW, DAVIS HP (1988). In ovulo embryo culture: a breeding technique to rescue seedless x seedless table grape crosses. Grapegrower Winemaker 292: 123-125.

BONNEAU L, CARRÉ M, DREUMONT C, MARTIN-TANGUY J (1994). Polyamines metabolism during seedling development in rice. Plant Growth Regul 15: 83-92.

BOUQUET A, DAVIS HP (1989). Culture *in vitro* d'ovules el d'embryons de vigne *Vitis vinifera* L appliquée à la sélection de variétés de raisins de table sans pépins. Agronomie 9: 565-574.

CAIN DW, EMERSHAD RL, TARAILO RE (1983). In-ovulo embryo culture and seedling development of seeded and seedless grapes (Vitis vinifera L.). Vitis 22: 9-14.

CAPELLETTI CA (1982). Elementos de estadística. Eds. Cesarino Hnos., Madrid, Chap. 10, pp 233-254.

CHI G, LIN W, LEE JEE, PUA E (1994). Role of polyamines on *de novo* shoot morphogenesis from cotyledons of *Brassica campestris* ssp. pekinensis (Lour) Olsson *in vitro*. Plant Cell Rep. 13: 323-329.

GRIBAUDO I, ZANETTI R, BOTTA R, VALLANIA R, EYNARD I (1993). In ovulo embryo culture of stenospermic grapes. Vitis 32: 9-14.

MARTIN-TANGUY J, CARRE M (1993). Polyamines in grapevine microcuttings cultivated *in vitro*. Effects of amines and inhibitors of polyamines biosynthesis on polyamines levels and microcutting growth and development. Plant Growth Regul 13: 269-280.

MURASHIGE T, SKOOG FM (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-479.

NISTCH JP, NISTCH C (1969). Haploid plants from pollen grains. Science 163: 85-87.

PONCE MT, AGÜERO C, GREGORI MT, IANDOLINO A, TIZIO R (1995). Factores que afectan el cultivo *in vitro* de óvulos de cultivares estenospérmicos de vid II. Ácido giberélico y Ácido indolacético. En resúmenes de la XV Jornadas de Investigación de la UNC, Mendoza, 7 al 11 de Agosto de 1995.

PONCE MT, AGÜERO C, TIZIO R (1997). Efecto de la calidad de la luz y del repique frecuente sobre el desarrollo *in vitro* de embriones de vides estenospérmicas. En resúmenes de la XVI Jornadas de Investigación de la UNC, Mendoza, 13 al 15 de Agosto de 1997.

PONCE MT, AGÜERO C, GREGORI MT, TIZIO R (2000). Factors affecting development of stenospermic grape *Vitis vinifera* L embryo cultured *in vitro*. Acta Horticulturae 528: 667-671.

SPIEGEL-ROY P, SAHAR N, BARON J, LAVI U (1985). *In vitro* culture and plant formation from grape cultivar Rubi (Italia Red). J Amer Soc Hort Sci 110: 109-1.

STOUT AB (1936). Seedlessness in grapes. New York Agricult. Exp Sta Tech Bull 238: 1-68.

TARENGHI E, CARRÉ M, MARTIN-TANGUY J (1995). Effects of inhibitors of polyamines biosynthesis and of polyamines on strawberry microcutting growth and development. Plant Cell Tiss Org Cul 42: 47-55.

TIBURCIO AF, CAMPOS JL, FIGUERAS X, BESFORD RT (1993). Recent advances in the understanding of polyamines function during plant development. Plant Growth Regul 12: 331-340.

VALDEZ JG, ULANOVSKY SM (1997). *In vitro* germination of stenospermic seeds from reciprocal crosses (Vitis vinifera L.) applying different techniques. Vitis 36(3): 105-107.

YADAV JS, RAJAM MV (1998). Temporal regulation of somatic embryogenesis by adjusting cellular polyamines content in eggplant. Plant Physiol 116: 617-625.