

# Effect of Seedcoat Manipulation on the Germination of Stenospermocarpic Grape Embryos Cultured in Ovulo

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The development of in-ovulo culture techniques has allowed grape breeders to perform seedless × seedless hybridizations using stenospermocarpic female parents (Emershad and Ramming, 1984). This technique requires culturing intact ovules in vitro for several months and then excising the embryos from the ovule. The excision is carried out in aseptic conditions and is quite tedious. Many embryos are lost from wounds or other mishaps.

Embryos can grow directly out of ruptured ovules (Gray et al., 1987) and from intact ovules (Spiegel-Roy et al., 1985). Methods that would reproduce the success of the techniques but eliminate complete embryo excision would streamline embryo rescue. We studied the effect of seedcoat manipulation on germination of stenospermocarpic grape embryos cultured in ovulo.

Ovules from five controlled cross pollinations, including as parents 'Saturn' and 'Venus' and breeding selections A-1905, A-1728, and A-1692 (Table 1) were cultured 8 weeks after bloom on a modified Murashige and Skoog (1962) medium as described in Gray et al. (1987). One of three culture regimes was administered to a group of ovules: 1) The procedure of Gray et al. (1987) involving culture of intact ovules on a solid medium for several weeks, followed by ex-

cision of the embryo. The embryo was then subcultured to fresh medium. 2) Ovules were initially excised from the fruit and sliced horizontally at the chalazal end but perpendicular to the embryo. About one-fourth of the seedcoat and adjacent endosperm was removed. The portion of the ovule containing the embryo was then placed with the cut surface in direct contact with the medium. After 3 weeks, ovules were repositioned and the cut end was no longer in direct contact with the medium. 3) We cultured intact ovules in vitro with no embryo excision or manipulation of the seedcoat during any part of the experiment.

For germination to occur, embryos must be removed from the remainder of the seed (Table 1). In all treatments, the highest percentage of both germinated embryos and established plants was obtained when the embryos were removed from the seed coat and endosperm. Removing part of the seed-

coat allowed some embryos to germinate; however, no established plants were obtained from this treatment. No embryos grew from intact ovules.

All female parents used in this study had large seed traces and were therefore considered to have a greater probability of producing viable embryos. 'Venus' and 'Saturn' had a higher percentage germination when embryos were excised than did other genotypes we used. Goldy and Amborn (1987) found that some stenospermocarpic genotypes are more successfully cultured in ovulo than others. Until alternative strategies can be developed involving culture manipulation of ovules, embryo excision continues to be the best method for obtaining plants from stenospermocarpic genotypes.

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Table 1. Effect of seedcoat manipulation on embryo germination and plants recovered from seedless × seedless crosses.

Cross	Treatment	No. ovules cultured	Germination (%)	Established plants (%)
Saturn × A-1905	1. Excise	60	37.0	15.0
	2. Cut	20	0.0	0.0
	3. Control	19	0.0	0.0
A-1728 × A-1692	1. Excise	18	17.0	0.0
	2. Cut	20	15.0	0.0
	3. Control	14	0.0	0.0
A-1692 × A-1728	1. Excise	20	10.0	5.0
	2. Cut	20	0.0	0.0
	3. Control	20	0.0	0.0
Saturn × Venus	1. Excise	69	22.0	3.0
	2. Cut	24	13.0	0.0
	3. Control	0	0.0	0.0
Venus × Saturn	1. Excise	39	67.0	33.0
	2. Cut	55	2.0	0.0
	3. Control	20	0.0	0.0

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