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In vitro germination of stenospermic seeds from reciprocal crosses (*Vitis vinifera* L.) applying different techniques

by

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Summary: The frequency of germination of stenospermic seeds cultured *in vitro* is analysed for two crosses of seedless varieties (*Vitis vinifera* L.) and their reciprocals. In the case of Superior x Dawn (SxD) the frequency of germination was not different to that of Superior x Ruby (SxR). Results of the reciprocal cross (SxD, DxS) show significantly better results with SxD. In the reciprocal cross SxR and RxS direct germination (DG) is compared with embryo excision (EE) and seed coat rupture (SCR). The majority of embryos of varieties with very soft seed coats, e.g. Superior, germinate directly: 18.8 % in DG, 18.4 % in EE and 23.2 % in SCR. Germination of varieties with harder seed coats (Ruby) could be mechanically affected: 17.6 % in DG, 62.4 % in EE and 58.8 % in SCR. There is no significant difference between EE and SCR. The results of SCR confirm that there is no inhibition of *in vitro* germination by chemical inhibitors in the seed tissues. The results of SCR and EE for SxR and RxS show a greater capacity of Ruby to generate viable embryos.

Key words: table grape, stenospermic seed, in ovulo culture, embryo rescue, direct germination, seed coat rupture.

Introduction

Seedlessness is one of the principal objectives of table grape improvement programs (EMERSHAD and RAMMING 1984; SPIEGEL-ROY *et al.* 1985). In these cultivars the development of the embryo and endosperm ceases after fertilization, leaving seed traces in different stages of formation, a phenomenon called stenospermocarpy (STOUT 1936).

The traditional method to obtain new seedless cultivars consists in the hybridization of a seeded cultivar with pollen of seedless varieties, resulting in a degree of seedlessness that depends on the parents used: 0 to 55 % (LOOMIS and WEINBERGER 1979).

The embryo rescue technique has been applied to grapevines, cultivating stenospermic seeds *in vitro* (CAIN *et al.* 1983; EMERSHAD and RAMMING 1984; SPIEGEL-ROY *et al.* 1985). By this method it is also possible to obtain offspring of early varieties (RAMMING *et al.* 1990 a) and interspecific hybrids of the *Vitis* genera (GOLDY *et al.* 1988; RAMMING *et al.* 1991).

In the F_1 of stenospermic progenitors, the frequency of seedlessness is between 44 and 94 % (SPIEGEL-ROY *et al.* 1990; RAMMING *et al.* 1990 b). All these advantages have made the technique preferable for a table grape improvement program.

In this work, direct germination (SPIEGEL-ROY *et al.* 1985; TSOLOVA 1990) was compared with embryo excision from seminal tissue remnants (EMERSHAD and RAMMING 1984; EMERSHAD *et al.* 1989; AGÜERO *et al.* 1995) and with seed coat rupture (Dr. A. PERL, Volcani Center, Israel).

AGÜERO *et al.* (1996 a) and FERNANDEZ *et al.* (1991) obtained better results by excising embryos, than by cutting seed traces in the chalazal region.

In this paper we analysed the percentage of direct germination of stenospermic seeds obtained from two reciprocal crosses of seedless varieties. In addition, the excised embryo, seed coat rupture and direct germination techniques were evaluated for their possible use in an improvement program.

Materials and methods

Direct germination: Crosses were made with the seedless varieties Superior Seedless (S), Dawn Seedless (D) and Ruby Seedless (R). The crosses analyzed were SxD, DxS, SxR and RxS. Berries were surface sterilized in 70 % ethanol (5 min) and sodium hypochlorite 1 % (10 min), then they were rinsed 3 x in sterilized deionized water.

The seed traces were rescued 60-70 d after pollination and were aseptically cultivated in 9 cm diameter petri dishes, 10 per dish in NITSCH and NITSCH (1969) medium to which was added 2.7 $g \cdot l^{-1}$ of activated charcoal (Dr. A. PERL, Volcani Center, Israel), 32 $g \cdot l^{-1}$ of sucrose and 2.4 $g \cdot l^{-1}$ of Phytagel (Sigma, St.Louis, USA). The medium was also supplemented with IAA (3 $mg \cdot l^{-1}$) and GA_3 (5 $mg \cdot l^{-1}$). The petri dishes were sealed with parafilm, and 25 dishes with each cross were chosen at random. The frequency of germination of the *in vitro* culture was analyzed after 110 and 180 d.

Optimization of germination: Dishes with the crosses SxR and RxS that had not germinated after 110 d were randomly sorted into 3 groups of 25 each. In one of them embryo excision (EE) was performed, in the other seed coat rupture (SCR) and the third was left as a control to evaluate direct germination (DG). To rupture the seed coats, the seeds were broken with tweezers in the

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chalazal region and the remnants were sown in the same media. The data were collected after 150 d (for the 3 treatments) and 180 d (for DG). The embryos and plantlets obtained were transplanted in a half diluted medium according to MURASHIGE and SKOOG (1962). The medium was supplemented with 25 $g \cdot l^{-1}$ of sucrose, 2.7 $g \cdot l^{-1}$ of activated charcoal and 2.4 $g \cdot l^{-1}$ of Phytagel. Multiple embryony and normal plantlets were registered.

The number of germinating plantlets were analysed by χ^2 test. When differences at the 5 % level of significance were found they were compared with the Arcsin test.

Results

Direct germination: There were no significant differences between crosses after 110 d of *in vitro* culture but there were differences after 180 d (Tab. 1). The Arcsin test applied to these data shows a significant difference in SxD *vs* DxS. A comparison between SxR *vs* RxS and SxD *vs* SxR shows no differences for the same level of significance.

Table 1

Direct germination evaluated after 110 and 180 d of *in vitro* culture, for the offspring of two crosses and their reciprocals. n = 250, except for SxR and RxS after 180 d with n = 170. When χ^2 test detected significant differences between crosses, percentages of germination were compared by the Arcsin test. Percentages followed by the same letter are not significantly different (P<0.05)

	Germination (%)	
Cross	after 110 d	after 180 d
SxD	6.80	24.80 a
DxS	2.40	4.00 c
SxR	5.20	18.82 ab
RxS	6.40	17.65 b
χ^2	6.005 (NS)	42.643*)

*) Significant for P=0.05, NS: not significant.

Optimization of germination: In the two crosses analysed by the χ^2 test, SxR and RxS were significantly different (34.28 and 271.42, respectively; df = 3). In both crosses the Arcsin test showed no differences between seed coat rupture (SCR) *vs* excised embryos (EE), but there were differences with SCR and EE *vs* DG (Tab. 2). In RxS, the number of germinated embryos from EE and SCR treatments were significantly higher than its reciprocal cross. Likewise the germination frequency was not different between DG from SxR and DG from RxS at the 5 % level of significance after 150 and 180 d of *in vitro* culture.

The percentage of normal plantlets was higher in the EE treatment and lower in direct germination (Tab. 3). In SxR the levels of polyembryony were about 54 %; they varied between 10 and 30 % in RxS.

Table 2

Germination percentage in SxR and RxS crosses. When the χ^2 test detected significant differences, percentages of germination were compared by the Arcsin test. Percentages followed by the same letter are not significantly different (P<0.05)

Cross	Treatment*)	Germination (%)
SxR	DG(150)	5.60 a
	DG(180)	12.40 b
	SCR	23.20 c
	EE	18.40 c
RxS	DG(150)	6.40 a
	DG(180)	11.67 b
	SCR	58.80 d
	EE	62.40 d

n = 250, except for RxS DG(180) with n = 180.
*) DG (direct germination) evaluated at 150 and 180 d *in vitro* culture. EE (embryo excision) and SCR (seed coat rupture) treatments performed at 110 d and evaluated at 150 d.

Table 3

Normal plantlets (Normal) and polyembryony (PE) percentage in SxR and RxS crosses

Cross	Treatment*)	Normal (%)	PE (%)
SxR	DG(150)	7.14	28.57
	DG(180)	6.45	54.84
	SCR	22.41	53.45
	EE	41.30	54.35
RxS	DG(150)	12.50	6.25
	DG(180)	23.81	28.57
	SCR	35.37	12.93
	EE	45.51	9.62

n = 250, except for RxS DG(180) with n = 180.
*) see Tab. 2.

Discussion and Conclusions

With Superior BOTTA *et al.* (1992) obtained better results when harvested 45 d after anthesis (56 % germination). Our results (18.4 %) lead us to suppose that 60-70 d after anthesis a high proportion of embryos dies. There was a significant difference between DG(180) and EE for SxR. However, it should be considered that in this trial we started with non-germinated petri dishes at 110 d. The percentage of embryos germinating directly, from the "Direct germination" trial (18.8 %), was similar to the percentage of excised embryos (18.4 %).

The cross RxS showed no difference with its reciprocal for DG in any case. Nevertheless, there were differences

when EE and SCR were performed, indicating that Ruby produced more embryos than Superior (Tab. 2).

The hardness of Superior seed coats is lower than that of Ruby. Thus, it can be assumed that the lack of germination is due to mechanical resistance of the seed coats and not to the inability of the embryos to germinate. AGÜERO *et al.* (1996 b) observed in the stenospermic cultivar Emperatriz deformed embryos that occupied all the seed in a direct germination control group indicating that germination had been prevented mechanically.

Therefore direct germination is not recommended for varieties with strong seed coats because they would prevent germination.

Pollen used for germination frequency did not appear to influence the percentages of germination of Superior seed traces in the crosses SxR and SxD. The reciprocal cross DxS indicated low germination for Dawn seeds making it advisable to use this variety only as a pollinator. The cross RxS did not show a difference with its reciprocal.

Tests to optimize germination showed that in SxR the percentage of seeds germinating directly approached the percentage germination after embryo excision. This may possibly be due to Superior having a sufficiently soft seed coat.

The reciprocal cross indicated that Ruby has a greater capacity than Superior for developing viable embryos at maturity and that the hardness of its seed coats prevents germination mechanically.

The main contribution of SCR was to show that the lack of germination was possibly due to the mechanical resistance of the seed coats.

AGÜERO *et al.* (1996 a) surmise that stenospermic seeds are in a state of dormancy during *in vitro* culture, making embryo excision necessary to avoid its contact with the seminal tissue. FERNANDEZ *et al.* (1991) observed that embryos must be removed from the remainder of the seed to germinate. Not having observed differences between the SCR and EE treatments for the two crosses analysed, we can surmise that all the viable embryos germinated in the presence of endosperm remnants and other structures.

These results allow us to bypass embryo excision using an intermediate method, i.e. by cutting the seed coats under a binocular and sowing the embryos together with seminal remnants.

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