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Embryo rescue from seedless grapevines (*Vitis vinifera* L.) treated with growth retardants

by

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S u m m a r y : The effects of two retardants (CCC and paclobutrazol) and the new compound XE 1019, applied before grapevine anthesis, were studied in order to increase the number of fertilised embryos and growing plantlets derived from *in ovulo* culture of seedless cultivars CG 102.011, Emperatriz and Malvinas. No significant differences were detected between treatments with CCC (400 and 800 $mg \cdot l^{-1}$, applied 2, 3 and 4 weeks before bloom), and the control in the cv. CG 102.011. The number of growing plantlets at 10 weeks after anthesis and at maturity was significantly higher than that from 8 weeks. In applications closer to bloom, CCC treatments increased the number of ovules per berry in the cultivars assayed. From all cultivars, only CG 102.011 showed a significant increase in plantlet production after CCC treatment when clusters were harvested the 10th week after bloom. It is believed that CCC would act through inhibition of endogenous gibberellin synthesis as the cause of ovule abortion. The idea is based on the fact that gibberellic acid can induce seedlessness in some seeded cultivars.

K e y w o r d s : in ovulo culture, embryo rescue, ovule abortion, seedlessness.

Introduction

The development of new cultivars of good quality, high yield and large berry size is the main purpose of grape breeding programs in order to satisfy the increasing interest in seedless grapes (Emershad and Ramming 1984; Bouquet and Davis 1989).

Conventional hybridization to obtain seedless progenies using seeded cultivars as female parents is of limited use due to a low proportion of seedless progeny, not higher than 10-15 % (Loomis and Weinberger 1979; Spiegel-Roy *et al.* 1990; Singh and Brar 1992), perhaps due to the fact that seedlessness appears to be controlled by single or few recessive genes (Stout 1936; Cain *et al.* 1983).

The proportion of seedless progeny increases in connection with the level of seedlessness in the parentage of the seeded female parent. This fact allows to postulate that seedless crosses would be more efficient (Barlass *et al.* 1988).

In seedless table grapes fertilization takes place but embryo and/or endospermic development stops soon after anthesis and the seed aborts in different stages of growth which mainly depends on the cultivar in question (Stout 1936; Nitsch *et al.* 1960; Winkler *et al.* 1974; Bouquet and Davis 1989).

The technique of embryo rescue from *in ovulo* culture has been adapted in grapevine. Results show that a mean of 85 % of the progeny from seedless x seedless crosses can be seedless (Cain *et al.* 1983; Emershad and Ramming 1984; Spiegel-Roy *et al.* 1985; Gray *et al.* 1987).

The development of growing embryos into plantlets is the basis to obtain new and promising genotypes of table grapes from crosses between seedless cultivars (Emershad and Ramming 1984; Bouquet and Davis 1989) but the proportion of growing normal plantlets is still very low, 7-10 % (Barlass *et al.* 1988).

The action and interaction of the following parameters have been studied: 1) culture date, since embryos become arrested and/or abort during different stages of ovule development; 2) genotype; 3) composition of culture media (Emershad and Ramming 1984; Emershad *et al.* 1989).

Differences in endogenous gibberellins between seeded and seedless grapes have been described in connection with early seed abortion (Coombe 1960; Nitsch *et al.* 1960; Bhullar and Dhillon 1977).

The aim of this work was to study the effects of two retardants (CCC: - 2-chloroethyl-trimethylammonium chloride, and paclobutrazol: β ((4-chlorophenyl)methyl)-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol and the new compound XE 1019 ((E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4 triazol-1-yl)pent-1-en-3-ol) applied before grapevine anthesis in order to increase the number of fertilised embryos derived from *in ovulo* culture. The use of those retardants is based on their capacity to inhibit gibberellin biosynthesis as a possible factor of seed abortion in seedless cultivars (Nitsch *et al.* 1960; Bhullar and Dhillon 1977; Hedden and Graebe 1985). XE 1019 significantly increases seed germination of the stenospermic cultivar C35-33 applied 35 d before bloom (Ledbetter and Shonnard 1990).

Materials and methods

F i r s t y e a r (1 9 9 1) : The stenospermic cultivar CG 102.011 was ground under forrow irrigation in a vineyard at San Rafael, province of Mendoza, Argentina. Vines were previously cane-pruned to 4 canes/vine.

Clusters were immersed for 1 h in a 400 or 800 $mg \cdot l^{-1}$ CCC aqueous solution 2, 3 or 4 weeks before bloom. A 4th treatment was performed with the same concentrations of the retardant applied 3 times (2, 3 and 4 weeks before anthesis). 10 clusters were selected for each treatment, harvested 8 and 10 weeks after bloom and at berry maturity.

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Berries of each cluster and treatment, were surface sterilised with 20 % sodium hypochlorite (80 g/l of active Cl) for 20 min. Fertilised ovules were excised and cultured in the solid medium of NITSCH and NITSCH (1969) supplemented with 10^{-6} M gibberellic acid (GA_3) and 10^{-5} M indolacetic acid (IAA) (SPIEGEL-ROY *et al.* 1985; GRIBAUDO 1993). 15 ovules were cultured in each 360 ml flasks. All treatments were placed at 24±1 °C day (16 h; 25 $\mu mol \cdot cm^{-2} \cdot s^{-1}$) and 20±1 °C night.

After 2 months the ovules were dissected for embryo rescue. The excised embryos were then cultured in the solid medium of MURASHIGE and SKOOG (MS, 1962) half diluted and supplemented with 1 µM benzylaminopurine (BAP). All treatments were placed on the same conditions of daylength, light intensity and temperatures as before, during 15 d. Then, the material was placed in the same environmental conditions (but light intensity 100 $\mu M \cdot cm^{-2} \cdot s^{-1}$).

Second year (1992): The cultivars were selected taking in account their *in vitro* capacity to produce growing embryos: high, medium and weak embryogenic rate (BOUQUET and DAVIS 1989) corresponding with cultivars CG 102.011, Emperatriz and Malvinas, resp., as determined in a previous work (AGÜERO *et al.* in press).

Two shoots of uniform length carrying cluster primordia were selected in 9 plants of cv. CG 102.011, and 12 plants of Emperatriz and Malvinas. Plants were treated with XE 1019 240 $mg \cdot l^{-1}$ and CCC 400 $mg \cdot l^{-1}$. Emperatriz and Malvinas were also treated with paclobutrazol 5 $mg \cdot l^{-1}$. Two applications were made, in November 8 and 18, with all regulators, which were sprinkled on foliage and clusters.

Blooming started from November 15 for cvs Malvinas and CG 102.011, and from November 18 for Emperatriz. Clusters of all treatments were harvested 8 and 10 weeks after anthesis.

Excised ovules were cultured as before, using 20 ovules per flask for Malvinas and 25 for CG 102.011 and Emperatriz. The flasks were placed at random into the growth chambers. Embryo rescue was made after 2 months of *in vitro* culture, and then cultured in MS medium half diluted and deprived of growth regulators.

Results and discussion

First year: The analysis of variance and Duncan's test, when the differences were significant, were employed to analyze the number of embryos (>0.7 mm in length) and the number of growing plantlets.

There were no significant differences between treatments with CCC 400 $mg \cdot l^{-1}$ and the control but 800 $mg \cdot l^{-1}$ gave some negative results. 3 consecutive applications of CCC (400 and 800 $mg \cdot l^{-1}$) exerted a strong phytotoxic effect on clusters inducing flower abortion. The number of growing plantlets obtained in week 8 was significantly lower than those from the week 10 and maturity (multifactorial analysis of variance).

Second year: Marked differences have been observed in berry size between treatments. Smallest berries were produced by plants treated with CCC, XE 1019 and paclobutrazol. Parallel to that, great differences in the mean number of ovules per berry related to that of the controls were observed (Figure).

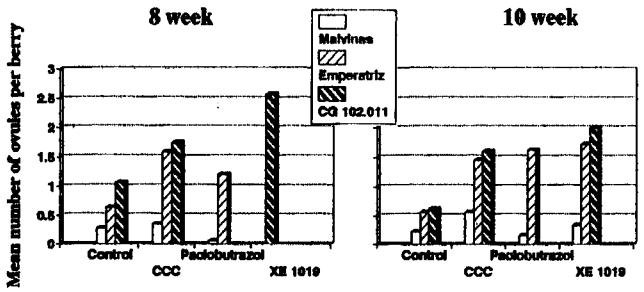

Figure: Mean number of ovules per berry of cultivars Malvinas, Emperatriz and CG 102.011, treated with CCC, XE 1019 and paclobutrazol, 8 and 10 weeks after bloom.

Concerning embryo rescue and plantlet production, the behaviour of the cultivars was very different:

CG 102.011 showed a marked effect of XE 1019 and CCC on ovule number in each berry (Figure). This behaviour could be attributed to a minor competition for nutrients and hormones between shoots and clusters, a consequence of CCC application as has been suggested by SKENE (1969). When treated with CCC, the number of developed plantlets significantly increased when the ovules were excised the 10th week, in contrast to data obtained the 8th week after bloom. These results would suggest that at the 10th week the embryos reached a suitable level of development as to continue their normal growth *in vitro*, as observed by SINGH and BRAR (1992) in other cultivars (Table). - XE 1019 did not exert the same effect as CCC did. It is possible that the concentration used was inadequate.

With Emperatriz CCC, XE 1019 and paclobutrazol treatments also produced an increase in ovule number per berry. Nevertheless, treatments with those regulators did not increase the number of growing embryos and plantlets from the 8th week after anthesis. On the contrary, CCC and XE 1019 significantly reduced the number of growing embryos and plantlets when the clusters were harvested during the 10th week (Table).

With Malvinas a slightly positive effect of CCC on ovule number per berry has also been observed. This behaviour is desirable, considering the extremely low number of ovules normally developed. Results can not be statistically analysed due to the inadequate number of repetitions which was a consequence of a very low number of berries per cluster (Table).

Conclusions

The effects of both growth regulators on cultivars CG 102.011 and Emperatriz were more marked when the material was harvested during the 10th week compared with those obtained from the 8th week after anthesis. This is probably due to the lower capacity of the younger embryos to survive under *in vitro* conditions. In Malvinas and

Table

Number of ovules, rescued embryos and plantlets from cultivars Malvinas, Emperatriz and CG 102.011, 8 and 10 weeks after anthesis

	8th week				10th week				
Treatment	Control	CCC	Paclo-butrazol		Control	CCC	Paclo-butrazol	XE 1019	
Malvinas									
Ovule number	48	100	21		81	80	56	80	
Embryo number	2	11	3		7	20	11	11	
Plantlet number	1	1	2		3	5	3	2	
Emperatriz				Pr>F					Pr>F
Ovule number	275	275	275		150	150	150	150	
Embryo number	9	7	2	0.336	34 a	14 b	25 ab	14 b	0.001
Plantlet number	5	3	0	0.079	19 a	6 b	11 a	5 b	0.022
CG 102.011									
Ovule number	275	275	—		150	150	—	150	
Embryo number	72	65	—	0.373	85	103	—	83	0.087
Plantlet number	44	37	—	0.446	40 b	66 a	—	42 b	0.044

References: Emperatriz and CG 102.011, 11 blocks at random. When ANOVA detected significative differences between treatments, mean values were compared with Duncan. Means followed by the same letter are not significantly different ($P<0.05$).

CG 102.011, CCC increased ovule number per berry. This fact is technically important because it allows a greater number of embryo rescue per cluster in these cultivars.

From all cultivars studied, only CG 102.011 showed a significant increase in plantlet production after CCC treatment, when the clusters were harvested 10 weeks after bloom. It is possible that CCC would act through inhibition of gibberellin synthesis at folliage and cluster level as the probable cause of ovule abortion. Moreover, the action of endogenous gibberellins coming from growing shoot tips cannot be discarded. Combinations of retardant treatments with periodic elimination of growing shoot tips could be important to make clear the role of endogenous gibberellins on ovule abortion in seedless grape cultivars. This is related to the fact that applications of GA_3 can induce seedlessness through ovule abortion in some treated cultivars of seeded grapevines (Pratt and Shaulis 1961; Clore 1965; Pharis and King 1985; Fellman *et al.* 1991).

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