EFFECT OF SHOOTING MEDIUM AND SOURCE OF MATERIAL ON GRAPEVINE (*Vitis vinifera* L.) SHOOT TIP RECOVERY AFTER CRYOPRESERVATION

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Abstract

In this work we investigated the effect of the physiological state of grapevine buds on their regrowth after liquid nitrogen exposure. In a first set of experiments, we tested the regrowth of cryopreserved buds sampled from microcuttings cultured on shooting medium containing benzylaminopurine or zeatin riboside for various durations. Regrowth of cryopreserved buds sampled from microcuttings was higher (30%), compared to buds sampled directly from *in vitro* plantlets (23%), for all culture durations of microcuttings on shooting medium tested (2-6 weeks). Addition of cytokinin in the shooting medium improved regrowth of cryopreserved buds compared to buds sampled from microcuttings cultured on medium devoid of growth regulators; however similar results were obtained with the two cytokinins tested. In a second set of experiments, we studied the regrowth after liquid nitrogen exposure of buds sampled from different positions on the stem of *in vitro* plantlets. Buds sampled on nodes 3-4 and 6-7 (from the top of the stem) displayed higher regrowth compared to shoot tips. No significant differences were noted in regrowth after cryopreservation between buds sampled from microcuttings produced from the terminal node, or nodes 3-4 and 6-7.

Keywords: droplet-vitrification, shoot tips, growth regulators, position of buds.

INTRODUCTION

Cryopreservation (liquid nitrogen [LN], -196°C) is the only method currently available for safe and cost-effective long-term conservation of vegetatively propagated plant species such as grapevine. A range of efficient vitrification-based cryopreservation techniques has been developed over the last 25 years and applied to numerous plant species (26, 22). Vitrification can be defined as the transition of water directly from the liquid phase into an amorphous phase or glass, whilst avoiding the formation of crystalline ice (4). Among the vitrification-based procedures established, encapsulation-dehydration, vitrification, encapsulation-vitrification and droplet-vitrification are the most commonly employed for bud cryopreservation (26).

In the case of grapevine, cryopreservation protocols have been established for shoot tips sampled from *in vitro* plantlets using encapsulation-dehydration (20, 21, 29, 31), vitrification (14, 15) and, more recently, droplet-vitrification (13).

Selecting experimental material at the optimal physiological stage is of paramount importance for successful cryopreservation (3). In the case of cold-tolerant species, mother-plants can be exposed to low temperature treatments to induce cold-acclimation processes. Shoot tips are generally excised from actively growing mother-plants, thus ensuring that they are composed of actively dividing meristematic cells, a characteristic that increases their tolerance to dehydration and LN exposure.

Buds located at different positions on a shoot are at different physiological stages (7), which may impact cryopreservation results. In the case of carnation, the lower the position of axillary buds on the stem (starting from the terminal bud) the lower their regrowth after LN exposure (2). By contrast, Plessis *et al.* (21) indicated that survival of grape shoot tips after cryopreservation was independent of their position on the stem. In order to reduce any potential heterogeneity in the physiological state of the buds employed for cryopreservation, Charoensub *et al.* (1) suggested cutting *in vitro* mother-plants into mononodal microcuttings and culturing those on fresh shooting medium for a given period of time prior to excision of homogenous and actively growing apices.

The composition of shooting medium, including notably the nature and concentration of growth regulators, can have a strong effect on the growth of microcuttings and thus on the physiological state of the buds. In the case of grapevine, benzylaminopurine (BAP) has been frequently used for multiplication (6, 10, 11, 16). However, hyperhydricity of cultures was noted when BAP was used over a threshold concentration (11). Zeatin riboside (ZR) has also been shown to stimulate bud proliferation in grapevine (8). In relation to cryopreservation, Wang *et al.* (30) investigated the effect of BAP addition to the recovery medium on regrowth of cryopreserved grapevine shoot tips. However, no study on the effect of growth regulator addition in the shooting medium has yet been performed with grapevine.

The objective of the present study was to investigate shoot regrowth from cryopreserved buds sampled from microcuttings cultured on shooting medium containing BAP or ZR at various concentrations and for different durations, and to study shoot regrowth after LN exposure of buds sampled at different levels on the stem of *in vitro* plantlets.

MATERIALS AND METHODS

Plant material

The plant material employed in this study consisted of 2-month old *in vitro* plants of grapevine (*Vitis vinifera* L.) cultivar Portan. These cultures were initially established from field-grown plants in the grape germplasm collection of Institut national de la recherche agronomique (INRA), Vassal (France).

Methods

In vitro culture

In vitro culture of mother-plants and of single node microcuttings

Grape *in vitro* plantlets were cultured on basal medium (BM) composed of half-strength MS (18) mineral elements with Morel's vitamins (17), 3% sucrose and 0.7% agar (Sigma) at pH 5.8. They were cultured at 24 ± 2 °C under a 12 h light/12 h dark photoperiod with a light intensity of 40 μE $m^{-2}s^{-1}$ provided by cool white fluorescent tubes. *In vitro* mother-plants were kept without subculture for 2 months to reach a length of approximately 12 cm before use for cryopreservation experiments. These *in vitro* mother-plants were cut into single node

microcuttings of approx. 1.5 cm in length, which were transferred to 9 cm Petri dishes (20 microcuttings/Petri dish) and placed on shooting medium consisting of ½ MS medium containing 20 g/l sucrose, 7 g/l microagar and 1 µMol ZR or BA or devoid of growth regulators. Petri dishes were placed in the environmental conditions described above. One-mm long shoot tips were excised from microcuttings after different durations and used for cryopreservation experiments.

Effect of growth regulators in shooting medium

Two experiments were performed to test the effect of growth regulators in shooting medium on growth recovery of cryopreserved shoot tips.

In the first experiment, microcuttings were placed on shooting medium with 1 µMol ZR and buds were excised after 2, 4 or 6 weeks. Control buds were excised directly from *in vitro* plantlets. This experiment was carried out in IRD Montpellier.

In the second experiment, microcuttings were cultured on shooting medium devoid of growth regulators or containing 1 µMol BAP or ZR; the buds were excised after 2 weeks. This experiment was carried out in Zagreb University.

Effect of source of material

Two experiments were performed in Zagreb University to test the effect of material source on growth recovery of cryopreserved shoot tips.

In the first experiment, buds were dissected from 2 month-old *in vitro* plantlets, bearing eight-nine buds. Buds were split in three groups depending on their position on the stem and used directly for cryopreservation experiments. The first group included terminal buds (node 1), the second group buds from nodes 3 and 4 from the top and the third one buds from nodes 6 and 7 from the top.

In the second experiment, *in vitro* plantlets were cut into single node microcuttings, which were split in the three groups described above. These microcuttings were cultured for 2 weeks on shooting medium with 1 µMol BAP before bud excision and their cryopreservation.

Control buds were dissected from nodes 1, 3-4, 6-7 and used directly for cryopreservation experiments.

Cryopreservation

Excised buds were cryopreserved using the droplet-vitrification technique developed by Markovic *et al.* (13). They were precultured on solid ½ MS medium with 0.1 M sucrose for 24 h. Precultured buds were then treated with a loading solution (LS) containing 2 M glycerol + 0.4 M sucrose in MS medium (25) for 20 min at room temperature. Buds were dehydrated with half-strength PVS2 at room temperature for 30 min, then with full strength PVS2 at 0°C for 25, 50 and 75 min, except in the second experiment on the effect of source of material, where only a 50 min PVS2 treatment was used. PVS2 contains 30% (w/v) glycerol, 15% (w/v) ethylene glycol (EG), 15% dimethylsulfoxide (DMSO) and 0.4 M sucrose in MS medium (24). Buds were then placed on aluminium foils in 5 µl droplets of PVS2 and immersed in LN for at least 1 h. For rewarming, the aluminium foils with the buds were immersed for 20 min in unloading solution containing 1.2 M sucrose (23) at room temperature, then transferred to recovery medium. Explants were post-cultured on medium containing 1µMol BAP, maintained in the dark at 26°C for 7 days and then transferred to the conditions described for stock cultures.

Assessment of survival and regrowth and statistical analyses

Survival was evaluated 2 weeks after cryopreservation by counting the number of shoots that showed any type of growth, while regrowth was defined as the development of apices into

shoots with expanded leaves 8 weeks after rewarming. Both survival and regrowth percentages were expressed relative to the total number of shoot tips treated. Experiments studying the effect of growth regulators in shooting medium were replicated thrice, with 10-15 shoot tips per experimental condition and in all other experiments 15-20 explants per experimental condition were used.

In the first experiment the effect of growth regulators in shooting medium, survival and regrowth data, presented as mean percentages with standard error of the mean (SD) were subjected to arcsine transformation. Statistical differences between mean values of all parameters were assessed by analysis of variance (ANOVA) and Duncan's Multiple Range Test for mean separation. Duncan's Multiple Range Test was performed for comparing percentages between conditions ($P \leq 0.05$), using the SAS software (27).

RESULTS

Effect of growth regulators in shooting medium

When buds were excised from microcuttings cultured on shooting medium with 1 µMol ZR, survival of LS and ½ PVS2 controls was very high (88-100%), for all times of exposure tested (Table 1); however, survival of non-cryopreserved explants decreased progressively with increasing PVS2 treatment durations. A similar pattern was observed with cryopreserved buds but survival values were generally lower compared to non-cryopreserved explants, especially for the shorter PVS2 treatment durations tested.

Table 1. Effect of time of exposure (weeks) of grapevine single node microcuttings on medium with 1 µMol ZR and of duration (min) of PVS2 treatment on survival (%) of non-cryopreserved (-LN) and cryopreserved (+LN) buds. Data followed by different letters are significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

	Survival (% ±SD)			
Time of exposure on shooting medium (weeks)	0	2	4	6
Control LS 20 min	100 a	97 ± 4 a	100 a	98 ± 4 a
Control ½ PVS2 30 min	97 ± 7 a	98± 3 a	95 ± 6 ab	88 ± 10 ab
PVS2 25 min (-LN)	71 ± 28 ab	74 ± 20 b	73± 20 bc	71 ± 13 bc
PVS2 25 min (+LN)	42 ± 16 bc	44 ± 1 cd	59 ± 28 cd	59 ± 8 c
PVS2 50 min (-LN)	51 ± 25 bc	82 ± 15 ab	51 ± 14 cde	63 ± 26 bc
PVS2 50 min (+LN)	32 ± 37 c	53 ± 13 c	51 ± 11 cde	51 ± 11 cd
PVS2 75 min (-LN)	42 ± 14 bc	42 ± 10 cd	36 ± 12 de	32 ± 12 de
PVS2 75 min (+LN)	31 ± 19 c	33 ± 16 d	27 ± 16 e	24 ± 15 e

Regrowth of LS and ½ PVS2 controls was high whatever the microcutting culture duration employed, and was between 61 and 82% (Table 2). Regrowth of non-cryopreserved buds decreased progressively in line with increased time of PVS2 treatment, although this decrease was more pronounced for longer times of exposure on shooting medium (4 and 6 weeks). After LN exposure, regrowth also decreased in line with increasing PVS2 treatment durations. Regrowth of buds cryopreserved directly after excision from *in vitro* plantlets was consistently lower, compared to buds sampled from microcuttings. The highest regrowth values, 48 and 41%, were achieved after a 25-min PVS2 treatment of buds sampled from 4 and 6-week old microcuttings, respectively.

Table 2. Effect of time of exposure (weeks) of grapevine single node microcuttings on medium with 1 µMol ZR and of duration (min) of PVS2 treatment on regrowth (%) of non-cryopreserved (-LN) and cryopreserved (+LN) buds. Data followed by different letters are significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

	Regrowth (% ±SD)			
Time of exposure on shooting medium (weeks)	0	2	4	6
Control LS 20 min	80 ± 20 a	73 ± 15 a	82 ± 14 a	77 ± 30 a
Control ½PVS2 30 min	63 ± 38 ab	61 ± 10 ab	72 ± 16 ab	62 ± 29 ab
PVS2 25 min (-LN)	54 ± 21 abc	44 ± 18 bc	57 ± 18 bc	49 ± 10 abc
PVS2 25 min (+LN)	25 ± 18 bc	32 ± 6 c	48 ± 16 cd	41 ± 12 bc
PVS2 50 min (-LN)	35 ± 26 bc	37 ± 16 c	43 ± 6 cde	45 ± 14 abc
PVS2 50 min (+LN)	23 ± 25 c	30 ± 20 c	35 ± 5 def	32 ± 9 bc
PVS2 75 min (-LN)	36 ± 17 bc	32 ± 7 c	23 ± 2 ef	22 ± 3 c
PVS2 75 min (+LN)	23 ± 17 c	24 ± 13 c	20 ± 21 f	24 ± 15 c

When comparing shooting media devoid of growth regulators and supplemented with ZR or BAP, it appeared, based on factorial ANOVA, that the treatment had a significant effect but that the shooting medium had no significant effect on bud survival and regrowth.

Survival of LS and ½ PVS2 controls was very high (90-100%) for all three shooting media tested (Table 3). Cryopreservation caused a decrease in survival for all times of PVS2 exposure tested, even though changes were statistically significant only in a few experimental conditions. In most experimental conditions, the hormonal content of the shooting medium had no effect on survival of non-cryopreserved buds. After cryopreservation, survival was significantly higher for buds sampled on shooting medium with BAP compared to ZR, for 25 and 75 min PVS2 treatments. After a 50 min exposure to PVS2, survival was similar for buds originating from ZR and BAP shooting media and significantly lower for shooting medium devoid of growth regulators.

Table 3. Effect of shooting medium containing half-strength MS without growth regulators, supplemented with benzyladenine (BAP) or zeatin-riboside (ZR) and of PVS2 treatment duration on survival (%) of control (-LN) and cryopreserved (+LN) grapevine buds.

Treatment	Survival (%)		
	No GR	BAP	ZR
Control LS 20 min	90 a/A	90 a/A	100 a/A
Control ½ PVS2 30 min	90 a/A	90 a/A	100 a/A
PVS2 25 min (-LN)	-	100 a/A	100 a/A
PVS2 25 min (+LN)	-	64 a/AB	20 b/C
PVS2 50 min (-LN)	62 a/B	80 a/A	70 a/AB
PVS2 50 min (+LN)	21 b/C	67 a/AB	60 a/B
PVS2 75 min (-LN)	-	100 a/A	58 b/B
PVS2 75 min (+LN)	-	40 a/B	20 b/C

Different lowercase letters indicate significant differences between media; different uppercase letters indicate significant differences between conditions of the cryopreservation protocol, according to Duncan's Multiple Range Test ($P < 0.05$). -: not tested.

Regrowth of LS and ½ PVS2 controls was high (90%) for all three shooting media tested, except for ½ PVS2 controls sampled from shooting medium without growth regulators, which was 60% (Table 4). Regrowth of non-cryopreserved controls decreased with increasing time of PVS2 treatment. After cryopreservation, regrowth was highest (40-44%) after 50 min PVS2 treatment (Table 4). The presence of ZR or BAP in the shooting medium had no significant effect on regrowth of non-cryopreserved and cryopreserved buds. Regrowth of control and cryopreserved buds sampled on medium without growth regulators was significantly lower compared to buds sampled on shooting medium containing BAP or ZR.

Table 4. Effect of shooting medium containing half-strength MS without growth regulators, supplemented with benzyladenine (BAP) or zeatin-riboside (ZR) and of PVS2 treatment duration on regrowth (%) of control (-LN) and cryopreserved (+LN) grapevine buds.

Treatment	Regrowth (%)		
	No GR	BAP	ZR
Control LS 20 min	90 a/A	90 a/A	90 a/A
Control ½ PVS2 30 min	60 a/B	90 a/A	90 a/A
PVS2 25 min (-LN)	-	100 a/A	90 a/A
PVS2 25 min (+LN)	-	19 a/B	20 a/B
PVS2 50 min (-LN)	10 b/C	30 a/B	40 a/B
PVS2 50 min (+LN)	11 b/C	44 a/B	40 a/B
PVS2 75 min (-LN)	-	50 a/B	8 b/B
PVS2 75 min (+LN)	-	20 a/B	10 a/B

Different lowercase letters indicate significant differences between media; different uppercase letters indicate significant differences between conditions of the cryopreservation protocol, according to Duncan's Multiple Range Test ($P < 0.05$). -: not tested.

Effect of source of material

Variance analysis showed that the treatment had a significant effect but that the position of buds had not a significant effect on bud survival and regrowth ($P<0.05$). Survival of LS and ½ PVS2 controls was between 57-100% for buds of the three groups studied (Table 5).

Table 5. Effect of bud position on the stem of *in vitro* mother-plants and of PVS2 treatment duration on survival (%) of control (-LN) and cryopreserved (+LN) grapevine buds.

Treatment	Survival (%)		
	Bud N°1	Buds N° 3-4	Buds N° 6-7
Control LS 20 min	100 a/A	57 b/BCD	100 a/A
Control ½ PVS2 30 min	100.a/A	88 a/AB	100 a/A
PVS2 25 min (-LN)	100 a/A	100 a/A	50 b/C
PVS2 25 min (+LN)	30 a/B	21 a/D	33 a/BC
PVS2 50 min (-LN)	30 a/B	50 a/CD	53 a/C
PVS2 50 min (+LN)	0 b/B	46 a/CD	42 a/C
PVS2 75 min (-LN)	20 b/B	82 a/ABC	86 a/AB
PVS2 75 min (+LN)	0 b/B	47 a/CD	50 a/C

Different lowercase letters indicate significant differences between bud position; different uppercase letters indicate significant differences between conditions of the cryopreservation protocol, according to Duncan's Multiple Range Test ($P < 0.05$).

Survival of non-cryopreserved buds N° 3-4 and 6-7 remained high after the three PVS2 treatment durations tested, whereas survival of terminal buds decreased to 30 and 20% after 50 and 75 min PVS2 treatment, respectively. After cryopreservation, no survival of terminal buds was achieved after 50 and 75 min of PVS2 exposure. In the case of buds N° 3-4 and 6-7, survival was between 21 (25 min PVS2 treatment) and 50% (75 min PVS2 treatment).
Regrowth of LS and ½ PVS2 controls was between 36-100% for buds of the three groups studied (Table 6). Regrowth of non-cryopreserved apical buds decreased drastically for longer PVS2 treatment durations, reaching 10% after 75 min. No regrowth of cryopreserved apical buds was achieved after 50 and 75 min PVS2 treatments. As regards buds N° 3-4 and 6-7, there was no significant effect of their origin, of the duration of the PVS2 treatment and of LN exposure on regrowth. Regrowth of non-cryopreserved N° 3-4 and 6-7 buds varied between 36 and 42% and regrowth of cryopreserved buds between 21 and 50%.

Table 6. Effect of position of bud on the stem of *in vitro* mother-plants and of PVS2 treatment duration on regrowth (%) of control (-LN) and cryopreserved (+LN) grapevine buds.

Conditions	Regrowth (%)		
	Bud N°1	Buds N° 3-4	Buds N° 6-7
Control LS 20 min	100 a/A	50 b/A	100 a/A
Control ½ PVS2 30 min	60 b/B	36 b/A	100 a/A
PVS2 25 min (-LN)	50 a/BC	36 a/A	36 a/B
PVS2 25 min (+LN)	30 a/BCD	21 a/A	33 a/B
PVS2 50 min (-LN)	30 a/D	42 a/A	40 a/B
PVS2 50 min (+LN)	0 b/D	42 a/A	25 ab/B
PVS2 75 min (-LN)	10 b/CD	41 a/A	36 a/B
PVS2 75 min (+LN)	0 b/D	40 a/A	50 a/B

Different lowercase letters indicate significant differences between positions of buds; different uppercase letters indicate significant differences between conditions of the cryopreservation protocol, according to Duncan's Multiple Range Test ($P < 0.05$).

Survival and regrowth of non-cryopreserved buds sampled directly from *in vitro* plantlets or from microcuttings was similar regardless of node position on the stem (Table 7). After cryopreservation, survival of shoot tips sampled directly from *in vitro* plantlets or from microcuttings was low (0 and 9%, respectively). Survival was between 39-47% for buds N° 3-4 and 6-7 sampled directly from *in vitro* plantlets or from microcuttings. Regrowth of buds N°3-4 was higher compared to buds N°6-7 and terminal buds. After cryopreservation, no regrowth was achieved with terminal buds sampled directly from *in vitro* plantlets. Regrowth was not significantly different in the other experimental conditions. The maximal regrowth (41.6%) was achieved with buds N°3-4 sampled directly from *in vitro* plantlets.

Table 7. Effect of bud position on grapevine *in vitro* plantlets, excised either directly from *in vitro* plantlets or from single-node microcuttings cultured for 2 weeks, on survival and regrowth (%) after cryopreservation.

Node N°	Survival (%)				Regrowth (%)			
	Directly		Microcuttings		Directly		Microcuttings	
	-LN	+LN	-LN	+LN	-LN	+LN	-LN	+LN
1	30 b	0 b	30 b	9 b	30 b	0 b	20 b	9 ab
3/4	50 a	46 a	77 a	40 a	42 ab	42 a	77 a	20 ab
6/7	53 a	42 a	64 a	39 a	40 b	25 ab	27 b	11 ab

Different lowercase letters indicate significant differences between different sources of material within columns, according to Duncan's Multiple Range Test ($P < 0.05$).

DISCUSSION

In this paper, we studied the effect of growth regulators (type and duration of exposure) in shooting medium on grapevine shoot tip recovery after cryopreservation and the effect of bud position on their response to our cryopreservation protocol.

We used a shooting medium in order to produce more homogenous and vigourously growing shoots. In our conditions, preliminary experiments had shown that explants developed more homogeneously and more rapidly when growth regulators were included in the shooting medium (data not shown). When comparing shooting media supplemented with ZR or BAP at the same concentration, no significant difference was observed on regrowth of cryopreserved buds after the optimal PVS2 treatment duration, which suggested that the cytokinin type did not influence explant response. However, with that same optimal PVS2 treatment, a significant difference in regrowth was observed between media with and without cytokinin, indicating that cytokinins, even at the low concentrations provided some specific protection against LN exposure. This is in contrast with results obtained on *Anisoganthos* shoot tips by Turner *et al.* (28) who demonstrated that cytokinins had not effect on cryopreservation success when added at the preculture stage, although they positively affected post-cryopreservation regrowth when combined to gibberellic acid (GA_3) in the recovery medium. On the other hand, Petijová *et al.* (19) stated that, whereas short-term pretreatment with BAP did not modify *Hypericum perforatum* shoot tip response to cryopreservation, its use prior to LN exposure in combination to abscised acid (ABA) decreased post-cryopreservation recovery.

In our experiments, the duration of the growth phase of microcuttings on shooting medium had little influence on explant survival and regrowth after LN exposure. To the best of our knowledge, no other report on the influence of microcutting culture duration before cryopreservation has been published. When comparing buds taken directly from grapevine *in vitro* plantlets and shoot tips sampled from microcuttings, buds displayed generally lower survival and regrowth before and after LN exposure.

Grapevine is a species which displays a strong apical dominance (5). In grapevine axillary buds are organized as bud complexes comprising prompt-buds and latent buds (12). Buds located at different levels on a stem are at different physiological stages (7). In our experiments, shoot tips completely failed to regrow after PVS2 exposure longer than 50 min. Buds N° 6-7 showed the highest regrowth after cryopreservation following a 75 min exposure to PVS2. For buds N° 3-4 and 6-7, no significant effect of their original position on survival and regrowth after PVS2 exposure was observed.

The effect of the bud position on the stem of donor *in vitro* plantlets on their recovery after cryopreservation has been tested in carnation, grape and potato (2, 9, 21). In the case of carnation, the lower the position of axillary buds on the stem (starting from the terminal bud)

the lower their regrowth after LN exposure, with regrowth ranging from 90% for the former to less than 10% for the latter (2). Similar results were obtained with potato (9). By contrast, Plessis *et al.* (21) did not observe any effect of bud position on the stem of grapevine *in vitro* plantlets on regrowth after cryopreservation. Our results are in accordance with those of Dereuddre *et al.* (2) and of Halmagyi *et al.* (9) and differ from those of Plessis *et al.* (21). The differences noted between our results and those of Plessis *et al.* (21), may be due to the fact that experiments were performed with different grapevine varieties, and possibly with plant materials which had been multiplied for different durations since their introduction *in vitro* and may thus be in a different physiological state.

In conclusion, the results obtained in this study underlined the paramount importance of the physiological state of the plant material for cryopreservation success. Actively growing buds sampled from microcuttings displayed higher regrowth compared to buds sampled directly on *in vitro* plantlets. The addition of growth regulators to the shooting medium had a positive effect on regrowth after LN exposure. We also confirmed that the position of buds on the stem of *in vitro* mother-plants affected regrowth after cryopreservation. Further experiments should include testing different grapevine varieties. An histological comparison of buds displaying different responses after cryopreservation, would provide a more accurate view of key factors involved in grapevine tolerance to cryopreservation.

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