Julia Gastmann[1]*, Amanda Pichani Primaz[1], Diego Brandão de Brito[1], Mara Cíntia Winhelmann[1], Sergio Echeverrigaray Laguna[2], Ana Paula Longaray Delamare[2], Elisete Maria de Freitas[1]

Vitis labrusca L. germination: influence of treatments to break dormancy, storage and ripening point of fruits

Affiliations
[1]Laboratory of Botany, University of Vale do Taquari – Univates, Lajeado, Brazil
[2]Laboratory of Enology and Applied Microbiology, Institute of Biotechnology, University of Caxias Do Sul, Caxias do Sul, Brazil

Correspondence
Julia Gastmann*: julia.gastmann@universo.univates.br, Amanda Pichani Primaz: amanda.primaz@universo.univates.br, Diego Brandão de Brito: dbrito@universo.univates.br, Mara Cíntia Winhelmann: marawinhelmann@gmail.com, Sergio Echeverrigaray Laguna: selaguna@ucs.br, Ana Paula Longaray Delamare: apldelam@ucs.br, Elisete Maria de Freitas: elicauf@univates.br

Summary

Grapevine seeds have morphophysiological dormancy, which complicates their germination, an important step in obtaining new plants after crossing during breeding. The objective of this work was to establish the ideal conditions for *Vitis labrusca* L. 'Isabella' seed storage conditions, the method for breaking seed dormancy, ripening of fruits at harvest, need for fungicide application before seed sowing and their germination. The seeds were submitted to three tests with (1 and 2) different treatments to break seed dormancy and storage in two conditions for two different times (Isabella), and (3) seeds from unripe and ripe fruits treated or untreated with fungicide. Germination was monitored two times per week. The percentage of germination (%G), germination mean time (GMT), and germination speed index (GSI) were calculated. Data were submitted to analysis of variance (ANOVA) and means were compared using the LSD-Fisher test ($p < 0.05$). The 12-month storage at a controlled temperature (25±2°C) provided the best germination results (up to 19.5% of G%). Stratification for 90 or 120 days on wet paper or sand at 5±2°C of seeds from mature fruits is the most suitable for the germination of Isabella seeds (65% to 72% of G%, and 1.73 and 1.95 of GSI for the treatments of stratification on wet sand for 120 days). It is also not necessary to apply fungicide on seeds before sowing, as long as disinfestation with 70% alcohol and sodium hypochlorite 1%, and triple washing with water is done (no statistical difference for seeds from ripe fruits treated or not with fungicide, 58% and 64.7% of %G, and 1.37 and 1.22 of GSI, respectively).

Keywords

grapevine, morphophysiological dormancy, seed propagation.

Introduction

Vitis spp. seeds show dormancy, a characteristic that hinders germination even under ideal environmental conditions for the species (Bewley *et al.* 2013, Taiz *et al.* 2017). Several studies (Ellis *et al.*, 1983, Pommer *et al.*, 1988, Val *et al.*, 2010, and Orsenigo *et al.*, 2017) have addressed grape seed dormancy and tested various dormancy breaking treatments including stratification or imbibition in gibberellic acid (GA_3) solution and other alternatives, such as cutting in the micropyle region and *in vitro* cultivation, using isolated or combined methods. These are an attempt of breaking the morphophysiological dormancy grapevine seeds have, because their embryos are underdeveloped, needing to grow and develop, while the seed needs to undergo a specific temperature treatment to break physiological dormancy (Baskin and Baskin, 2004, Baskin and Baskin, 2014). Ellis *et al.* (1983) obtained 96% germination in an unspecified *Vitis* cultivar treated with a conjunction of prechill (21 days at 3-5°C), imbibition in 1 M hydroxy-peroxide solution and 2000 ppm of GA_3 solution (both for 24 h), and Orsenigo *et al.* (2017), achieved up to 100% of germination in various *Vitis* species after stratification for five months at 5°C or stratification at alternate temperatures simulating autumn, winter, spring and summer for 52 weeks (both in 1% distilled water-agar Petri dishes). So, the results vary between species of the genus and even between cultivars of the same species. And also, there is a lack of comprehensive studies for certain cultivars, in special for those of *V. labrusca*.

Along with dormancy, several other elements may interfere with seed germination, such as temperature, availability of water, light, oxygen, nutrients, substrate, or type of soil where the seeds are found, in addition to the attack by microorganisms, storage time and maturation stage, both of fruits and seeds (Agrios, 2004; Bonner *et al.*, 2008; Carvalho and Nakagawa, 2012; Bewley *et al.*, 2013). Aside from dormancy, these other factors have received insufficient investigation in *Vitis* so far.

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Finally, efficient germination is essential in the process of genetic improvement. In addition to the need for work on flower emasculation and manual pollination, each of the branch plants derived from the crossing of grapes has the potential to carry the ideal genotype and phenotype for the development of a new cultivar or variety as it is a clonal reproduction plant (Reisch *et al.*, 2012, Bradshaw, 2016). 'Isabella' and 'Ives', for example, have potential for grapevine breeding because of their fruit characteristics for juice production (Rizzon and Meneguzzo, 2007).

Thus, the objective of this study was to establish the ideal conditions for *Vitis labrusca* L. germination, considering the storage condition, the method of breaking seed dormancy, the ripening of the fruits at collection, and the need for fungicide application to the seeds before sowing.

Material and Methods

Seed collection

At the same time (beginning of February, during South Hemisphere summer), 'Isabella' unripe and ripe fruits and the fruits obtained from the cross between 'Isabella' and 'Ives' (*V. labrusca*) cultivars were collected on a farm in the municipality of Garibaldi, state of Rio Grande do Sul, Brazil. In the laboratory, the seeds were removed from the fruits, washed, and kept drying on a paper towel on a bench at room temperature, after which they where stored in glass jars. The seeds from crosses between cultivars were kept in a cold chamber (5±2°C) (dry storage, in the dark) until the beginning of the tests. Half of the Isabella seeds were kept in a cold chamber (5±2°C) and the other in a controlled temperature of 25±2 °C (both dry storage and in the dark) until the beginning of the tests.

Determination of the seeds' moisture content

Over the storage period (three months after fruit removal) and before the establishment of the first experiment (five months of seed storage), the moisture content of the seeds of *V. labrusca*, 'Isabella', used in the first experiments in this study was determined. A sample with three replications of 1 g of seeds was used for each type of storage (controlled temperature and cold chamber), weighed on a scale (0.001 g) before and after drying in an oven at 105°C for 24 hours, according to the methodology of the Rules for Seed Analysis (Brasil, 2009).

Storage, dormancy breaking, and the germination of 'Isabella' seeds

'Isabella' seeds stored in both environments (controlled temperature and cold chamber) were subjected to 10 dormancy breaking methods, totaling 20 treatments, plus a control treatment for each storage type (total of 22 treatments) (Table 1), each consisting of four replications of 25 seeds. Before applying each treatment (and before sowing, in the case of controls), the seeds were disinfected through immersion in 70% ethyl alcohol, followed by 1% sodium hypochlorite (NaClO) for 10 minutes and triple washing with autoclaved reverse osmosis water.

For the evaluation of germination after storage (five months for C1, C2, T1, T2 and T11 to T20, and two months and one month for the treatment with stratification for 90 and 120, respectively) and treatment for breaking dormancy, the seeds were sown in plastic germination boxes (containing 30 g of a commercial substrate composed of peat moistened with 50% WRC) and kept in a controlled temperature of 25±2°C with a photoperiod of 16 h of light. The experimental design was completely randomized. The substrate was moistened once a week as water loss was observed. Germination was monitored twice a week, and the seed with a visible structure (radicle or calculus) at 3 mm above the substrate was considered to have germinated. Germination was monitored for about 90 days until reaching 14 days with no new germinations.

Dormancy breaking and germination of 'Isabella' seeds after 12 months in two storage conditions

After 12 months of storage at controlled temperature or in a cold chamber, another sample of 'Isabella' seeds were taken. They were disinfected as described in the previous test and subjected to imbibition in GA_3 solution for 24 h at concentrations of 0.1%, 0.2%, 0.3%, and 0.4% and water for 24 h, corresponding to 10 treatments. Two controls were added (controlled temperature and cold chamber), totaling 12 treatments.

The treated seeds were sown in plastic germination boxes, which were kept in the same substrate and under the same conditions as the previous test, with four replications of 25 seeds for each treatment. The experimental design and the counting of the emerged seeds followed the same procedures described for the previous tests. Seeds were monitored for about 150 days until reaching 14 days with no new germinations.

Germination of seeds from crosses between 'Isabella' and 'Ives'

Seeds from unripe and ripe fruits were cleaned following the description in the first test. Then, half of the seeds obtained from ripe fruits were treated with a solution of Captan® fungicide (dilution of 3 µL of the concentrated solution in 25 mL of reverse osmosis water). The other half was disinfected, however, without application of fungicide, as well as the seeds from unripe fruits. Next, three treatments were established: UF (unripe fruit seeds); RF (seeds of ripe fruits without fungicide treatment); RFF (seeds of ripe fruits treated with fungicide), each one with six replications of 25 seeds.

This third experiment was done because of (1) the non-uniform ripening of fruits and the necessity to collect them when most are ripe (so, also collecting unripe ones), (2) the contamination of fungi found in pilot germination tests that may negatively affect germination, and (3) 'Isabella' and 'Ives' are important cultivars of *V. labrusca* used in grapevine breeding.

Then, the seeds of the three treatments underwent stratification for 120 days in the sand (in a cold chamber at 5±2 °C and

Table 1: Storage condition (SC) before dormancy breaking (DB) treatments of *Vitis labrusca* L., 'Isabella' seeds

Code	SC	DB	Material used
C1	CS	NT	---
C2	CT	NT	
T1	CS	WS*	100 mL water **
T2	CT	WS*	
T3	CS	CSS 90 days	Plastic germination boxes (11 × 11 cm) with 200 g moistened sand at 30% WHC**
T4	CT	CSS 90 days	
T5	CS	CSS 120 days	
T6	CT	CSS 120 days	
T7	CS	CSP 90 days	Plastic germination boxes (11 × 11 cm) with moistened germinative paper (5.5 mL)**
T8	CT	CSP 90 days	
T9	CS	CSP 120 days	
T10	CT	CSP 120 days	
T11	CS	SS GA_3 0.1%*	100 mL of solution GA_3 using phosphate buffer solution**
T12	CT	SS GA_3 0.1%*	
T13	CS	SS GA_3 0.2%*	
T14	CT	SS GA_3 0.2%*	
T15	CS	SS GA_3 0.3%*	
T16	CT	SS GA_3 0.3%*	
T17	CS	SS GA_3 0.4%*	
T18	CT	SS GA_3 0.4%*	
T19	CS	CM	Tweezers and scalpel
T20	CT	CM	

CS – dry cold storage (5±2°C); CT – dry controlled temperature storage (25±2°C); NT – no treatment; WS – soaking in water; CSS – cold stratification in wet sand (5±2 °C); CSP – cold stratification in wet paper (5±2°C); CM – cut in the micropyle region; SS – soaking in solution (gibberellic acid); WHC – water-holding capacity; 0.1% = 1 $g \cdot L^{-1}$ or 1000 ppm.
* Seed imbibition for 24 hours.
** Autoclaved reverse osmosis water was used and sand was autoclaved and dried in a stove at 105°C for 24 h before moistening.

humidified at 30% of the WHC) when they were sown in plastic germination boxes and kept under the same conditions as in the previous tests. The experimental design and the counting of the germinated seeds also followed the description in the previous tests, but the monitoring of the seeds ended after 75 days, corresponding to 14 days without new germinations.

Data analysis

For the three tests, the variables of the germination percentage (%G), germination mean time (GMT) (Labouriau 1983) and germination speed index (GSI) (Maguire 1962) were calculated. Data were submitted to normality tests (Bartlett or Shapiro-Wilk), and to one-way or two-way analysis of variance (ANOVA) and means were compared using the LSD-Fisher test ($p < 0.05$). In the case of the two-way ANOVA, the interaction between storage type and dormancy breaking method was tested. The analyses were performed using the statistical programs CoStat 6.451 and Sigmaplot 14.5.

For the first test, the %G variable met the Shapiro-Wilk test normality assumption ($p = 0.082$), but GSI ($p < 0.001$) did not. Thus, GSI was transformed to log/10. In this test, we opted to not analyze GMT. The comparison between storage condition and the treatments for breaking dormancy for seeds with 12 months of storage showed that %G, GMT, and GSI did not pass the normality assumption of the Shapiro-Wilk test ($p = 0.05$ for the three variables) and were transformed into √x (%G and GSI) and inverse of √x (GMT). And for the test with 'Isabella' × 'Ives' crossed seeds, the variables %G, GMT, and GSI met the normality assumption of the Bertlett test ($p = 0.3433$, $p = 0.1192$ e $p = 0.1022$, respectively).

Results

Determination of the seeds' water content

The average moisture content of 'Isabella' seeds before establishment of the first experiment and stored in a cold chamber (5±2°C) was 10.33%. On the other hand, those stored at controlled temperature were 10.48% (25±2°C).

Storage, dormancy breaking and germination of 'Isabella' seeds

No interaction was observed between storage and treatment to break dormancy between %G ($p < 0.001$) (Figure 1a) and GSI ($p = 0.001$) (Figure 1b). The highest %G means were from treatments with stratification in both conditions of storage.

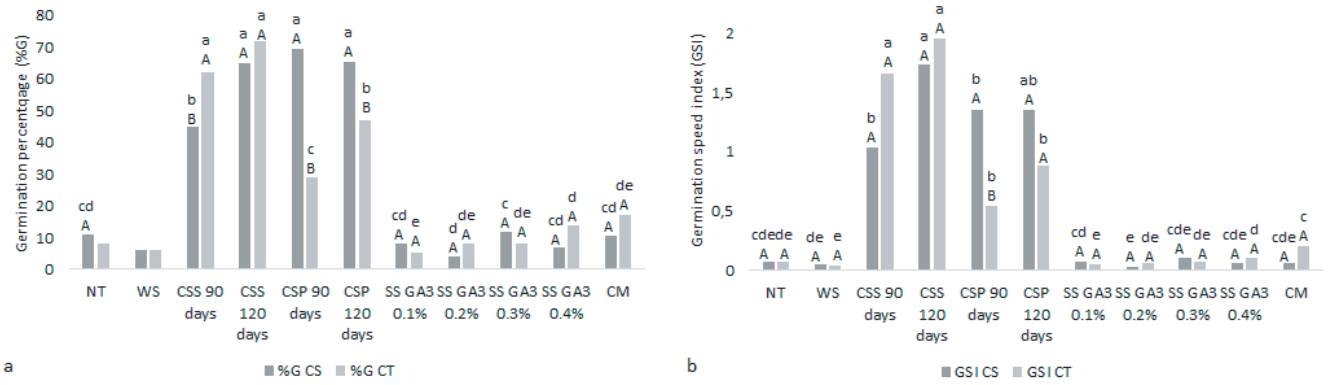

Figure 1: Means of germination percentage (%G) and germination speed index (GSI) of seeds of *Vitis labrusca* L., 'Isabella', stored at a controlled (CT) temperature or cold storage (CS) and further exposed to treatments to break dormancy.

CS – dry cold storage (5±2 °C); CT – dry controlled temperature storage (25±2°C); NT – no treatment; WS – soaking in water; CSS – cold stratification in wet sand (5±2°C); CSP – cold stratification in wet paper (5±2°C); CM – cut in the micropyle region; SS – soaking in solution (gibberellic acid); WHC – water-holding capacity; 0.1% = 1 g·L^{-1} or 1000 ppm.

* Means followed by different lowercase letters in the dormancy breaking methods and uppercase letters in the storage conditions are not different from each other by the LSD-Fisher test ($p < 0.05$).

The treatments without stratification did not differ from each other, including the controls, with no variation between the two storage conditions for the same treatment. While the storage condition was only significant to two dormancy breaking methods (T7/T8 and T9/T10, cold stratification in paper for 90 and 120 days).

For GSI, the treatments with stratification differed from the others, reaching the highest values (Figure 1b). The treatments without stratification, including controls, did not differ, except for T20 (controlled temperature storage and micropyle cut). Like %G, the storage mode also did not influence the GSI, except in T19 and T20 (micropyle cut).

'Isabella' dormancy breaking and germination at 12 months after storage

There was no interaction between the storage condition and the treatments to break dormancy for the variables %G ($p = 0.233$) and GSI ($p = 0.259$). The controlled temperature was shown as being the best storage condition for both variables (Figures 2a and 2b), while for treatments to break dormancy there was no statistical difference for both storage conditions compared to controls (no treatment). It should be noted, however, that the treatment with GA_3 (T17/T18) differed from the control for %G, despite being equal to other treatments that were also statistically equal to the control.

As for GMT, there was an interaction between storage and treatment to break dormancy ($p = 0.002$). Again, all other treatments did not differ from the controls (no treatment) (Figure 2c) in both storage conditions, while the controlled temperature was better for half of the treatments tested (no treatment to break dormancy, water soaking, soaking in gibberellic acid solution at a concentration of 0.3% GA_3); for the others, there was no difference regarding storage.

Germination of seeds from a cross between 'Isabella' and 'Ives'

For %G, treatments RFF (seeds from mature fruits with fungicide application) and RF (seeds from mature fruits without fungicide application) had statistically equal means (RFF = 64.7% and RF = 58%) and different from the UF average (seeds derived from unripe fruits, average of 14.7%). Likewise, the RFF and RF ESI means were statistically equal (RFF = 1.37 and RF = 1.22) and different from the UF mean (0.3). However, for GMT, the means were statistically equal for the three treatments (RFF = 12.11, RF = 12.47 and UF = 12.25 days). Thus, seeds from mature fruits, regardless of the fungicide treatment, germinated in greater quantity and with greater speed than those removed from unripe fruits.

Discussion

The results obtained in this work indicate that the *V. labrusca*, 'Isabella' seeds only had their morphophysiological dormancy broken with cold stratification for periods of 90 and 120 days. No studies tested methods to break seed dormancy aside from stratification for *V. labrusca* seeds, and our work proved that other methods, such as imbibition in water or GA_3 solution, had little to no effect in breaking dormancy. According to the literature, *V. labrusca* seeds have a dormancy of the deep complex morphophysiological type, presenting an underdeveloped embryo (Baskin and Baskin, 2004, Baskin and Baskin, 2014), so we can assume only cold stratification could make the embryo develop.

Chohan and Dhillon (1976) demonstrated that the increase in gibberellins and the decrease in ABA in *V. vinifera* seeds in cold stratification coincided with their highest germination point, while fresh seeds (not stratified) had the highest constitution of ABA and lower germination. Stratification at low temperatures would simulate the "winter climate" that occurs naturally in the environment of origin of these species, enabling the growth and development of the embryo,

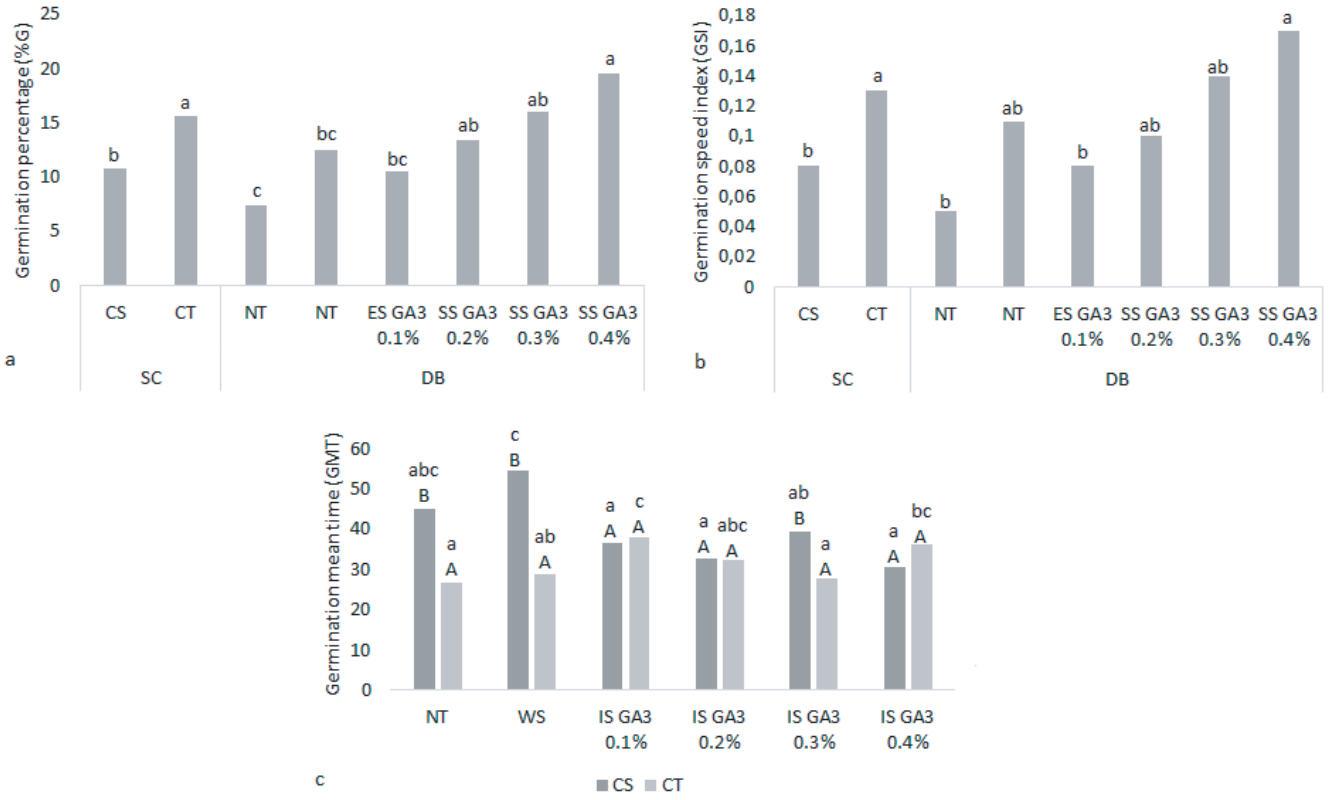

Figure 2: Means of the percentage of germination (%G), germination speed rate (GSI), and germination mean time (GMT) of *Vitis labrusca* L. seeds, 'Isabella', stored (SC) at a controlled temperature (CT) or cold storage (CS) for 12 months and subsequently treated to break dormancy (SD).

SC – storage condition; DB – method for breaking dormancy; CSS – cold stratification in wet sand (5±2°C); IS – imbibition in solution; CSP – cold stratification in wet paper (5±2°C); SS – imbibition in solution (gibberellic acid); CM – cut in the micropyle region; 0,1% = 1 g·L^{-1} or 1000 ppm.

* Means followed by different lowercase letters differ from each other for the same evaluated parameter, SC or SD, by the LSD-Fisher test ($p < 0.05$) (a and b).

** Means followed by different lowercase letters in the dormancy breaking methods and uppercase letters in the storage conditions are not different from each other by the LSD-Fisher test ($p < 0.05$) (c).

probably through the leaching of inhibitory substances such as ABA. Consequently, the content of gibberellins increases, stimulating germination (Baskin and Baskin, 2014, Kochhar and Gujral, 2020).

For comparison, Ellis *et al.* (1983) achieved germination of 75%, 76%, and 80% in three unidentified *Vitis* cultivars, using only imbibition in a GA_3 solution at a concentration of 2000 ppm (0.2%), which are very distant results from those reached in this work in the treatments with GA_3.

Works using other species with a deep complex morphophysiological dormancy have indicated that they are not able to break dormancy using only this treatment (characteristic of this type of dormancy), but require some type of cold stratification for their dormancy to break (Zardari *et al.*, 2019, Chen *et al.*, 2021, Zhang *et al.*, 2021) – as seen here with *V. labrusca*. In addition, cultivars of North American *Vitis* and their hybrids, due to the geographical conditions of origin, have deeper dormancy and require more than six months of stratification, compared to the time of approximately two months for species of European and Asian origin – varying degrees of dormancy according to provenience and adaptation to the environment (Gan *et al.*, 2008). It is therefore possible that some cultivars show different degrees of dormancies from the rest of their species, as with some interspecific cultivars of *V. vinifera* or interspecific hybrids (Nikolaeva *et al.*, 1985, Baskin and Baskin, 2004, Baskin and Baskin, 2014, Atak and Sen, 2021). For 'Isabella', our study confirms that it is of the deep complex morphophysiological kind, unlike it was described by other cultivars of *V. labrusca*.

Muscadinia rotundifolia (Michx.) Small (≡ *Vitis rotundifolia* Michx.), an American species, whose seeds were subjected to stratification in vermiculite at 4°C for 0, 30, 60, and 90 days, showed higher germination in the treatment with longer stratification time (90 days) (Conner, 2008), coinciding with the treatments of 90 days of stratification (T3, T4, T7, and T8) that were used in this work and resulted in higher values for %G (69.3% in T7).

Celik (2001) reported that the stratification of seeds of 'Isabella' for 60 days in the sand at 4-5°C followed by imbibition in different GA_3 concentrations for 24 h did not differ from treatments by stratification only in the sand in most treatments. Those with stratification only in three different substrates (perlite + silt, sphagnum, and soil mixture), had 58%, 53.33%, and 54.67% germination, respectively (Celik, 2001).

Such results are close to the treatments T3 and T4 (62%) of this work, which corresponds to treatments with stratification in cold sand of seeds stored at a controlled temperature and in a cold chamber.

As shown by the dormancy exhibited by 'Isabella', only the micropyle cut (T19 and T20) did not favor the germination variables analyzed. It was also observed that in these treatments a proliferation of microorganisms occurred in the seeds, which may have penetrated the internal tissues, facilitated by the cut, unlike the other treatments, in which the seeds were found intact. Val *et al.* (2010) achieved 52.5% germination in an average time of 9.75 days in *V. labrusca* 'Niagara Red' by performing the same procedure on the seeds, cultivating them *in vitro*. The same authors highlighted that the medium was essential for seed germination, due to contamination control and the greater availability of water and nutrients during the process. In addition, they managed to achieve up to 77% (*in vitro*) and 50% (*ex vitro*) germination by combining cutting in the micropyle and imbibition in a GA_3 solution at 4000 ppm (or 0.4%) (Val *et al.*, 2010). The results differ completely from those obtained in this work with 'Isabella' seeds (less than 20%), both with cutting in the micropyle and when soaking the seeds in GA_3 at the different concentrations tested, for both storage times (five and 12 months).

At five months of storage, treatments with imbibition in GA_3 showed lower results than those of stratification for %G and GSI, and equal to controls, while for 12 months of storage, the results were still lower than 20% for the three analyzed variables. The controlled temperature was more suitable for seed storage for a period of 12 months, a result similar to that found by Maeda *et al.* (1985) for seeds of 'Patricia' (hybrid *Vitis*) at 20°C and a degree of humidity similar to the present study. Furthermore, Wheal *et al.* (2003) found that *Vitis berlandieri* Planch. × *Vitis vinifera* seeds remained viable (up to 8.3% emergence after stratification) after cold storage (4°C) for 16 years.

As for the moisture content of the 'Isabella' seeds, despite the difference between the two storage types, both are within storage standards for orthodox seeds, as suggested by Bonner (2008). Because they are orthodox, their metabolism is reduced when refrigerated, with a deceleration of deterioration and preservation of seed integrity (Bonner *et al.*, 2008; Carvalho and Nakagawa, 2012). Another factor that may have contributed to reducing seed metabolism during storage is the very presence of dormancy in the seeds. Thus, it is suggested that the controlled temperature was more efficient in some treatments of this work, probably as a result of a small effect on breaking dormancy provided by the combination of storage time and temperature, with emphasis on soaking at 0.4 % GA_3 with 19.5% %E.

As for the ripening point of the fruits of the 'Isabella' × 'Ives' cross, the lower performance for %G and GSI for UF, seeds from unripe fruits, suggests that the seeds were mostly immature. Immature seeds still do not have their endosperm reserves fully developed (Carvalho and Nakagawa, 2012), and have a greater accumulation of ABA than the mature seeds (Yan and Chen, 2017), factors that inhibit or hinder germination. Thus, the lack of adequate reserves and with a higher amount of ABA in relation to the seeds obtained from mature fruits, they had more difficulty in emerging and did so for a longer time. Maeda *et al.* (1984) also found that 'Patricia' seeds from immature fruits had a germination percentage (2.5 – 6%) and ESI (0.42 – 1.74) lower than seeds from mature and medium mature fruits, in addition to lower viability (33 – 54%) and greater dormancy (86.7 – 92% of dormant seeds) for the three times tested (0, 6 and 12 months). Cruz-Tejada *et al.* (2018), when studying seeds of six different species, found that *Chamaedorea linearis* (Ruiz & Pav.) Mart., *Chamaedorea pinnatifrons* (Jacq.) Oerst. e *Stylogyne longifolia* (Mart. ex Miq.) Mez had a lower percentage of dormant seeds in fruits harvested at intermediate or ripe maturity compared to those from immature fruits. A similar phenomenon may have happened with the seeds of *V. labrusca* in this study.

The seed maturation state is directly related to the fruit maturation state. Therefore, the fruit should be collected preferably when the seeds are fully mature, but this maturity is difficult to be defined, varying according to the species (Bonner *et al.*, 2008; Carvalho and Nakagawa, 2012). Thus, for *V. labrusca*, according to the results obtained, it is recommended to use seeds from mature fruits, which in this case were those with a dark purplish color, considering the use of grapes with red fruits.

Finally, it is common to treat seeds with sodium hypochlorite or fungicides to eliminate pathogens or microorganisms that may affect germination through the production of toxins or the release of enzymes and other metabolites that degrade and cause deterioration in the seeds or the seedling in formation (Agrios, 2004, Carvalho and Nakagawa, 2012, Baskin and Baskin, 2014). In this study, only disinfestation with sodium hypochlorite and alcohol were sufficient to avoid harmful contamination of the seeds, evidenced by similar values of %E, EMT, and ESI between seeds of mature fruits treated or untreated with fungicide. Similar methods with 70% alcohol and sodium hypochlorite were previously used in studies with seeds of *Ilex paraguariensis* A.St.-Hil. and *Feijoa sellowiana* (O.Berg) O.Berg, being able to reduce the incidence of different species of fungi, most to less than 10% incidence (Fantinel *et al.*, 2017, Souza *et al.*, 2020).

Conclusion

The best treatment for breaking dormancy and promoting the germination of *V. labrusca* 'Isabella' is stratification of 90 or 120 days, in paper or cold sand (5±2°C), without previous treatment with fungicide on the seeds and these originated of ripe fruits (dark purplish color for red cultivars). For storage, in periods of up to 5 months, there are no evident differences in the germination behavior of seeds stored in a cold chamber and with controlled temperature, but the 12-month storage with a controlled temperature (25±2°C) provides better germination results, indicating the break of the morphological part of the seed's morphophysiological dormancy.

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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