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Domestic grape germination behaviour: the 'Chardonnay' and 'Syrah' international cultivars's study case[1]

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ABSTRACT - The domestic grape germination eco-physiology is a little studied aspect since reproduction occurs predominantly agamically, despite the sexual reproduction remains the main form for the selection of new cultivars. In this study, two international cultivars grown all over the world -Chardonnay and Syrah- were chosen as models of the domestic grape for the experiments in the eco-physiology of germination. The experimental design consisted of chemical, mechanical pre-treatments and combination of them as the bird ingestion pre-treatment that simulates the transit through the digestive tract of birds. Furthermore, seeds were submitted to different periods of cold stratification - 0, 15, 30, 60 and 90 days - to simulate the winter effect. Seeds were placed to germinate at different incubation temperatures, to find the optimal germination protocol. The results showed that domestic grapes retain the need for cold stratification, and the best germination temperature is represented by the fluctuating temperature that simulates spring conditions. Our results help to understand what the best germination conditions of domestic grapes are and offer a contribution to extend the knowledge on how the process of domestication may have affected the biology of *Vitis vinifera* L. subsp. *vinifera*.

Key words: Vitaceae. *Vitis*. Crops. Plant Physiology. Dormancy release.

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INTRODUCTION

The domestic grape represents one of the oldest crops in the world, with remarkable importance both from a cultural and economic point of view (ORRÙ *et al*., 2013; UCCHESU *et al*., 2015). Grapes are arguably the most important horticultural crop in the world, with 7.1 million hectares producing 77.4 million tons of fruit globally (MIGICOVSKY *et al*., 2017; OIV, 2015). It has been shown that *Vitis vinifera* L. subsp. *sylvestris* (CCGmel.) Hegi. is the ancestor of the domestic grape. It is worth pointing out that artificial selection acts continuously during the stages of domestication and this selection can be conscious or unconscious. Conscious selection leads to actively selecting forms that show interest such as larger and sweeter fruits (SMÝKAL *et al*., 2018). Conversely, unconscious selection is a consequence of humans changing the conditions in which a species is cultivated, without emphasizing a particular trait or predetermined goal (SMÝKAL *et al*., 2018). The selection of particular phenotypes has been at the basis of the domestication process of wild grapes, involving, over the years, radical changes in morphology and phenology (OLSEN; JONATHAN, 2013), and in particular in their reproductive system, allowing a high production by each individual (GRASSI *et al.*, 2003). The differences between the wild and domestic forms are collectively known as the "domestication syndrome" (ZHOU; MUYLE; GAUT, 2019; ZOHARI; HOPF; WEISS, 2012). Furthermore, genetic diversity or variation between different populations belonging to the same genus resulted from the evolution of crops through the history, in response to different environments and husbandry practices (FOWLER, 2008).

In the genus *Vitis*, the seeds immediately after ripening are physiologically dormant, and germination requires long periods of cold stratification for it to occur (CONNER, 2008; ELLIS; HONG; ROBERTS, 1985; ORRÙ *et al*., 2012; WANG *et al*., 2011). Seed germination optimum occurs at 20-30 °C and suggests that in nature this will occur in spring after winter cooling (BASKIN; BASKIN, 1998). The studies by Wang *et al*. (2011) on dormancy in Beichun grape seeds (crossing of *V. vinifera* × *V. amurensis* Rupr.), found that dormancy was consistently released with a prolonged stratification time at temperatures < 15 °C (with optimal approx. 10 °C), while stratification at 25 °C induced a secondary dormancy. Alternatively, hot and dry post-ripening can interrupt dormancy in grape seeds (ELLIS; HONG; ROBERTS, 1985) For example, in *V. amurensis* seeds, dormancy was released with an stratification for 1–2 months at low temperature (5 °C) (WANG *et al*., 2011). Investigations on wild grapes *V. vinifera* subsp. *sylvestris* have shown that germination necessarily takes place after a cold stratification period (5 °C) of 60/90 days (ORRÙ *et al*., 2012). The experiments developed by Orrù *et al*. (2012) showed that the minimum germination temperature is 15 °C, while the optimum germination occurs with the fluctuating temperature incubation (25/10 °C), both after three months of cold stratification at 5 °C. However, all the studies carried out with the wild grape, to our knowledge, no information is available on dormancy release and seed germination for the domestic grape (*V. vinifera* subsp. *vinifera*).

The aims of this study are to investigate what are the best germination conditions for domestic grape, using two international cultivars as models (Chardonnay and Syrah), and attest to if the combination of several pre-treatments increase the germination.

MATERIAL AND METHODS

Grape cultivars investigated

Two domestic grape cultivars of *V. vinifera* subsp. *vinifera* (Chardonnay and Syrah) were chosen in this study because of their widespread use. On one hand, Chardonnay is a white grape cultivar that has small to medium, cylindrical, compact clusters, small to medium berries, spherical or sometimes slightly oblong, rather thin skin, amber in the sun. It has early budding and late ripening of the fruits, this cultivar is quite vigorous and very productive depending on the clones (ROBINSON; HARDING; VOUILLAMOZ, 2012). Moderately sensitive to powdery mildew, but mostly to gray rot. Chardonnay is a cultivar sensitive to rains and sudden changes in temperature at the blooming time - that lead to flowers fall - as well as being intolerant to drought (ROBINSON; HARDING; VOUILLAMOZ, 2012). The historical area of cultivation of Chardonnay, are the regions of central-eastern France, ranging from Burgundy to Champagne, mainly in the Côte d'Or, in the Saone and Loire and the Marne (ROBINSON; HARDING; VOUILLAMOZ, 2012). DNA kinship analysis unexpectedly demonstrated that Chardonnay is a natural cross between Pinot and Gouais Blanc (SCIENZA; IMAZIO, 2018).

On the other hand, Syrah is a black berry cultivar with clusters with medium or oversized peduncles, elongated, sometimes winged, more or less compact, long and a little slender, sometimes bent and quickly lignified. The medium or sub-medium berries, from ovoid to ellipsoid, thin but quite resistant skin of bluish black with abundant flowering, firm and juicy pulp very sweet and spicy with a pleasant taste, long pedicels. It is late budding vine with medium-good vigor and productive (ROBINSON; HARDING; VOUILLAMOZ, 2012).

The most historic places of Syrah's are located in the vineyards of the northern Rhône, such as Hermitage and Côte Rôtie. Parental DNA analysis unexpectedly showed that Syrah is a natural cross between Mondeuse Blanche and Dureza (SCIENZA; IMAZIO, 2018). Genetic analyses have placed Syrah in the Pinot family (ROBINSON; HARDING; VOUILLAMOZ, 2012).

Chardonnay and Syrah cultivars are widespread and used in all wine-growing areas of the world, thanks to their adaptability, quality, and consistency of production. Furthermore, their genealogy is well known through genetic analysis (SCIENZA; IMAZIO, 2018), making them an ideal model for germination ecophysiology experiments.

Seed collection

Seed samples were collected from Sardinia (Italy); as regards Chardonnay, it was collected from the countryside in the municipality of Senorbì (WGS84: 39.331278 N - 9.092877 E), while Syrah samples were collected from the countryside in the municipality of Serdiana (WGS84: 39.231937 N - 9.092877 E). For each cultivar, 5 kg of fresh fruits from different plants were collected and seeds were manually cleaned and separated from the fleshy part of the berries at the Banca del Germoplasma della Sardegna (BG-SAR) laboratories.

Germination test

Germination tests were performed in the laboratories of BG-SAR. Three replicates of 25 seeds each were sown under a laminar flow hood (Faster KB) on 1% water agar substrate, which provided a solid, non-sterile medium for germination, in plastic Petri dishes of 90 mm diameter.

Before starting the tests, seeds were floated for 10 minutes in distilled water. The floating seeds fraction has been eliminated to separate empty and/or dead seeds, thus excluding low quality seeds.

The experimental tests included mechanical, chemical pre-treatments and combination of them. The following pre-treatments were applied to seeds: (1) control, without any pre-treatment, incubated directly at germination conditions; (2) mechanical scarification, seeds were treated through superficial abrasion with sandpaper grit size n. 80 for five minutes; (3) chemical scarification, seeds were immersed for 10 minutes in hydrochloric acid (HCl 37%), diluted 1:1 with distilled water, after which, they were washed in running water and rinsed off with distilled water; (4) simulation of bird ingestion, seeds were mechanically scarified with sandpaper n. 80 for five minutes, then treated with HCl (37%) and distilled water (1:1) for 10 min then washed with distilled water, and finally placed in warm conditions (42 °C oven) for two hours, this pre-treatment simulates the passage of seeds in the digestive tract of birds (KLEYHEEG; CLAESSENS; SOONS, 2018; TRAVESET; RIERA; MAS, 2001; TRAVESET; VERDÙ, 2002).

After all pre-treatments, the effect of different duration of cold period from two weeks to three months were evaluated, denominated as follow [0 (0), 15 (A), 30 (B), 60 (C) and 90 (D) days]. During the cold period, seeds were exposed at temperature of 5 °C in moist conditions in a growth chamber (MLR-351, SANYO, Moriguchi, Japan).

Once the pre-treatments finished, seeds were incubated at a range of constant (20, 25 °C) and fluctuating temperatures (25/10 °C), in a cycle light of 12/12 h. In this latter temperature regime, the higher temperature coincided with the light period. Light in each growth chamber was provided by nine fluorescent lamps with white light (Mitsubishi OSRAM 40; 53 W, photosynthetic photon flux density of 40 μmol m−2s−1).

The criterion for germination was the visible radicle protrusion (≥ 1 mm). Seeds were scored twice a week, and germinated seeds discarded. After two consecutive weeks without additional germination under control conditions, non-germinated seeds were checked by a cut test with scalpel and subsequent observation of the seed endosperm under a binocular microscope (ISTA, 2008).

Data analysis

Final germination percentages (FGPs) were calculated as the mean of three replicates (± SD). For all data, the normality values were verified by the Shapiro-Wilk test. Arcsine-transformed germination percentages were analysed by ANOVA to detect differences among different cultivars, temperatures and pre-treatments and consequent Fisher's Least Significant Differences (LSD) post hoc test. All graphs were made using the Sigmaplot 11.0 software (Systat Software Inc., London, UK), while all the statistical analyses were performed using the statistical software SPSS.

RESULTS AND DISCUSSION

Effect of temperatures and cultivar type on seed germination

For both cultivars examined, the duration of stratification period ($p < 0.005$) and the incubation temperature ($p < 0.005$) were the main factors controlling germination, while the pre-treatments effect did not show a significant effect on the germination of both cultivars ($p > 0.005$).

The comparison between the control and the different cold stratification periods in both cultivars

showed that germination had a significant increase with the number of days of cold exposure ($p < 0.001$). The interval between 60 (C) and 90 (D) days of stratification showed the highest percentages of germination (Figure 1 and 2).

The analysis of the incubation temperatures showed that the percentage of germination with fluctuating temperatures 25/10 °C were higher than in a constant temperature regime. In particular, the temperature at 20 °C was the condition with the lowest percentage of germination in both cultivars examined (Figure 1 and 2).

In the multivariate analysis, the two cultivars showed a different germination response ($p < 0.001$). The Chardonnay had significantly higher germination rates compared to Syrah, independently of the experimental conditions tested (Figure 1 and 2).

The seeds of both tested cultivars of domestic grape had poor germinability without any pre-treatment, in accordance with the results of Orrù *et al*. (2012) which were also observed in the wild grape. The percentages of germination improved with the increase of the cold period duration, reaching maximum percentages between 60 and 90 days at 5 °C, in both cases.

Solely chemical, mechanical and bird ingestion pre-treatments were not decisive in promoting and/or increasing the rate of germination. However, they had improved the germination rate after cold stratification in both cultivars tested. The two cultivars examined showed significant differences in the percentage of germination in all cases considered. The Chardonnay had germination percentages higher than 80% after 60/90 days of cold stratification and at fluctuating temperature incubation 25/10 °C. The Syrah cultivar showed a lower germinability, with optimal germination percentages after 60/90 days at fluctuating temperature of 25/10 °C, however in contrast to the Chardonnay, the germination percentage slightly exceeded 40% of the seeds germinated only in the best condition and remaining around 30%. This behaviour could be attributable to an inbreeding depression effect, this is supported by some studies, which have shown self-pollinating effects in *Vitis* sp. and how this significantly reduces germination rates (MULLINS; BOUQUET; WILLIAMS, 1992). Formal experiments that evaluate the inbreeding depression in grapes are poorly described in the literature and knowledge on the effect of inbreeding derive primarily by the empirical experience of the breeders (*pers. comm.*).

Figure 1 - Final germination percentage (FGP %) of Chardonnay seeds exposed to different pre-treatments (0 no cold stratification, A indicates 15 days cold stratification; B 30 days cold stratification; C 60 days cold stratification; D 90 days cold stratification) and incubation temperatures (constant temperature 20 and 25 °C, fluctuating temperatures 25/10 °C)

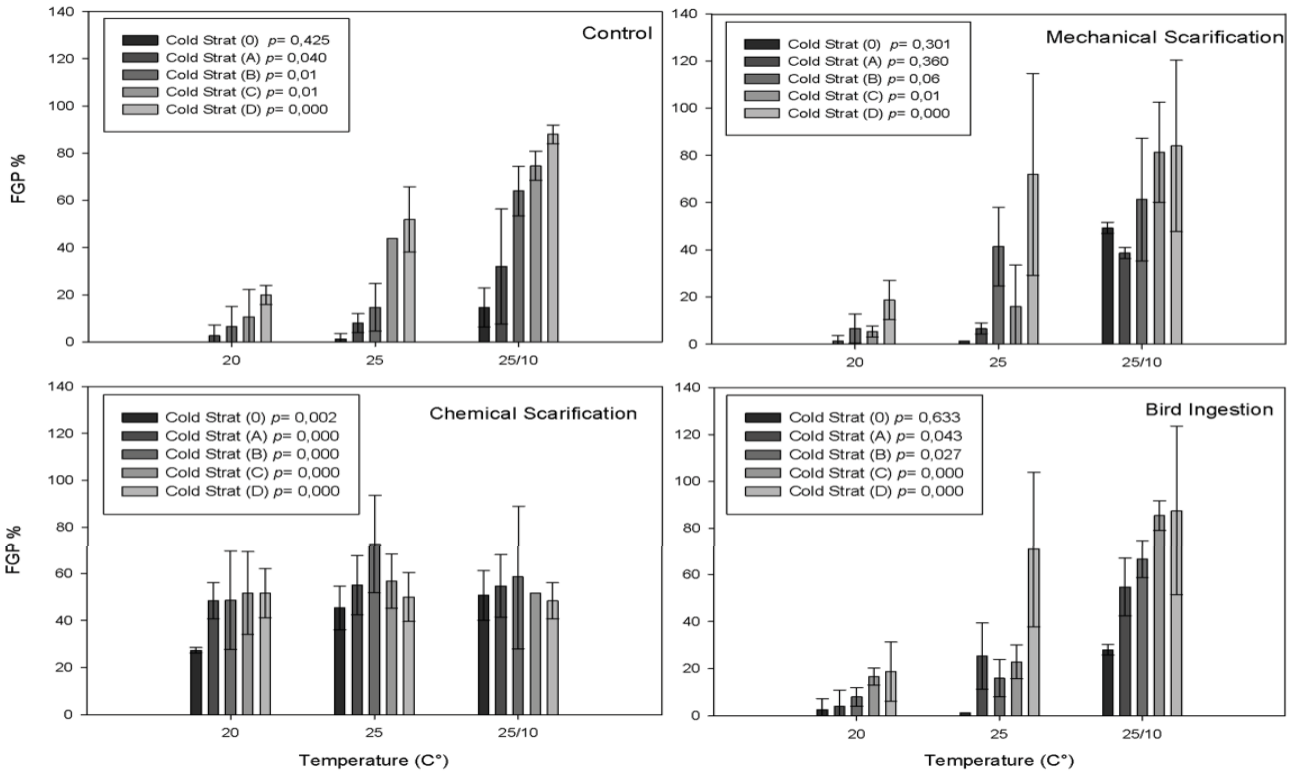
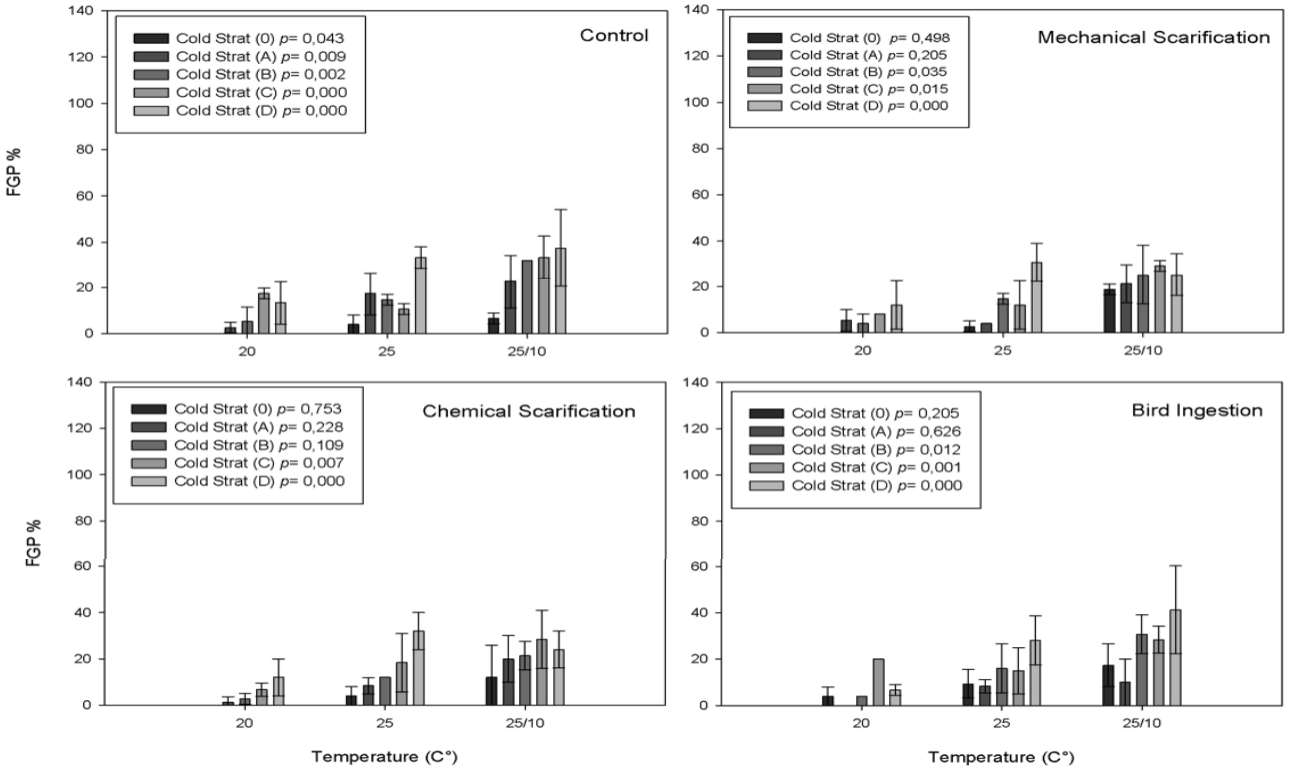


Figure 2 - Final germination percentage (FGP %) of Syrah seeds exposed to different pre-treatments (0 no cold stratification, A indicates 15 days cold stratification; B 30 days cold stratification; C 60 days cold stratification; D 90 days cold stratification) and incubation temperatures (constant temperature 20 and 25 °C, fluctuating temperatures 25/10 °C)



Effect of pre-treatments on seed germination

In Chardonnay cultivar, the chemical scarification was able to significantly increase the germination even in the absence of cold stratification ($p = 0.002$) and after the cold stratification showed high germination rates at all incubation temperatures. However, the pre-treatments of mechanical scarification and bird ingestion, had shown significant germination rates only after 60 days of cold stratification ($p > 0.005$).

The Syrah cultivar had low germination rates compared to Chardonnay. For this cultivar the germination depends mainly on the cold stratification, with significance after 60 days of cold stratification ($p > 0.005$) and with the fluctuating incubation temperature of 25/10 °C. However, the bird ingestion pre-treatment achieved higher germination rates than the others, reaching 41.3% in the condition of fluctuating temperatures 25/10 °C (Figure 2).

Considering the temperatures of incubation, we observed that at constant temperature (20° and 25 °C) there was an increase in the final germination percentage with increasing temperature. However, the best responses were verified with the fluctuating temperature condition of 25/10 °C in both cultivars.

These results confirmed that the optimal germination protocol for the domestic grape, consisted of a cold stratification period of 60/90 days at 5 °C, and then incubated at a fluctuating temperature of 25/10 °C. However, even the constant temperature of 25 °C presents good results. Pre-treatments alone were not significant in promoting germination, but they significantly improved the final germination after cold stratification, in particular the chemical pre-treatment. The interaction with the digestive tract of birds, is not an essential condition for the interruption of dormancy, but as stated by previous studies (ATAK; ŞEN, 2021; BIAGINI *et al*., 2016; KELLER, 2020), birds act as the primary drivers for grape seeds dispersion. Once on the ground, the seeds spend the winter, and thanks to the cold break dormancy and germinate during the following spring. These characteristics have remained unmodified in the domestic form which therefore remains linked by its reproductive biology to the climatic conditions of the Mediterranean and temperate-warm zones.

The domestication process operated by humans that changed the morphology and phenology of domestic grapes compared to wild grapes (ZOHARI;

HOPF; WEISS, 2012), did not alter the ecophysiology of germination (ORRÙ *et al*., 2012). However, this grape aspect has never been subject to domestication selective pressures because reproduction takes place mainly with agamic ways (VANNOZZI *et al*., 2021). If we determined a change in the germination behaviour, this would be due to unconscious selection occurred randomly during the process of domestication.

CONCLUSION

1. This work provided information on domestic grapes - Chardonnay and Syrah - germination behaviour and their responses to different environmental conditions. This information can be useful in the selection processes of new cultivars to identify the best germination protocols;
2. Finally, through this work it was possible to affirm that the domestication process that has strongly modified the morphology and phenology of the domestic grape from as compared to the wild form but has not affected the germination ecophysiology of the domestic cultivars under examination.

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