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Research Paper

Comparison of pollen quality in *Vitis vinifera* L. cultivars

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ABSTRACT

Pollen quality of 15 cultivars of *Vitis vinifera* L. was studied in this work. Pollen viability was tested by the fluorochromatic reaction and germination was analyzed by *in vitro* assays, using two different media. Differences among cultivars in the number of pollen apertures were observed under light microscope. All the cultivars studied showed a higher percentage of tricolporated pollen, however, pollen grains containing one, two or four apertures were also observed. The cultivar Loureiro was the one with the higher percentage of pollen grains with four apertures (3.8%) and Touriga Nacional presented 100% of tricolporated pollen grains. The viability analysis showed that 13 cultivars presented values higher than 50%, with 8 cultivars reaching values above 75%. The pollen germination rates vary greatly for the grapevine cultivars studied, three cultivars show low values of germination (under 14%) in the two media tested, which were Touriga Nacional, Cabernet Franc, and Cabernet Sauvignon while others presented high values of germination like Castelão, Loureiro, Malbec and Petit Verdot. No significant statistical differences between the percentages of germination in the two media studied were found for the majority of cultivars analyzed.

1. Introduction

Grapevine is one of the most cultivated and economically important fruit crops. It is the number one perennial crop, with more than seven million hectares planted, ranging from 50° N, through the tropics, to 43° S, in all continents except Antarctica. Although grapevines grow from temperate to tropical regions, most vineyards are planted in temperate climates regions, with the most concentrated vineyard area occurring in Europe (OIV, 2014). Grapes can be used for multiple purposes but winemaking has the highest economic value.

Grape production is related to pollen fertility which depends on the viability and germination potential of pollen (Lombardo et al., 1978). Pollen contains the male gametophyte and it is produced in the anthers of the higher plants, being its main role the sexual reproduction of spermatophytes (Pérez et al., 2007).

Pollen polymorphism is a widespread phenomenon among the higher plants including different grapevine species and cultivars (Cargnello et al., 1980; Dzyuba et al., 2006; Gallardo et al., 2009; Maria et al., 1994). Generally, the pollen grains of *Vitis vinifera* L. present a sub-spherical to triangular shape due to the presence of three furrows with three apertures (furrows with pores − tricolporated form) (Abreu et al., 2006; Alva et al., 2015). The irregular productivity presented by some grapevine cultivars may be related to the presence of atypical pollen with bicolporated, acolporate, collapsed or shriveled morphology (Abreu et al., 2006; Caporali et al., 2003; Lombardo et al., 1978). However it can also be due to other factors such as adverse environmental conditions, to incompatible pollen–pistil interaction (recognition system), to anomalous development of the ovule, to the life period of the embryo sac, and to several other physiological factors of the plants such as nutritional and phytopathological conditions (Carraro et al., 1979; Keller and Koblet, 1994; Keller et al., 2003). The presence of hermaphrodite flowers, one of the most important traits developed during the grapevine domestication, has been suggested to be the result of a mutation, and the development of acolporate pollen could be some reminiscence of their earlier dioecious lineages (Alva et al., 2015).

Pollen quality, often designated as pollen fertility, is the result of a combination of different traits such as the viability of the mature pollen and the germination ability through the formation and growth of the pollen tube *in vitro* conditions (Stanley and Linskens, 1974). They can be influenced by genetic, environmental (temperature and humidity) and agronomic factors.

The evaluation of pollen quality is important in several aspects, namely in the study of the storage potential of pollen grains for controlled pollination, in the evaluation of intra- and inter-cultivar incompatibility, in the clonal selection and genetic breeding trials (Dafni

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and Firmage, 2000) as well as to define some agronomic practices to improve pollen fertility (Caliskan et al., 2017; Novara et al., 2017; Sabir, 2015). Furthermore, some studies show that if pollen germination ratio is equal or superior to 30% the cultivars could be used as a pollinator (Fidan, 1975), which may be important in the selection of cultivars for vineyard plots.

This study aims to increase the knowledge on pollen quality of several grapevine cultivars with a different number of pollen apertures by the evaluation of their viability and germination rates in two nutritional media. This information can be used to support sustainable agronomic decisions in order to improve pollen quality providing appropriate nutrients as well as the selection of grape cultivars avoiding irregular productivity.

2. Materials and methods

2.1. Plant material

The pollen was collected from the ampelographic collection of Quinta dos Almoínha (39°01′46″N, 9°01′35″W, 99 m a.s.l) belonging to the 'Instituto Nacional de Investigação Agrária e Veterinária' (INIAV), located in the Lisbon wine region, Dois Portos, Torres Vedras. The vineyard has 2 ha and 724 accessions or botanical clones, the vines are trained on a vertical trellis at a plant density of about 3200 vines per hectare and grated onto SO4 rootstock. The soil is classified as calcic fluvisol (eutric) (FAO, 2006). Fifteen grapevine cultivars were studied in this work, some are mainly cultivated in Portugal (white cultivars: Fernão Pires and Loureiro and red cultivars: Castelão, Touriga Franca e Touriga Nacional) and others are spread worldwide (white cultivars: Colombard, Sauvignon, Sémilllon and Ugni Blanc and red cultivars: Cárménère, Cabernet Franc, Cabernet Sauvignon, Malbec, Merlot, Petit Verdot) (Anderson and Aryal, 2013). All the cultivars present androgynous flowers, produce seeded berries and are used for winemaking. The cultivars Castelão, Petit Verdot, Touriga Franca, Touriga Nacional are reported to present problems in fruit set (Dupraz and Spring, 2011; Eiras-Dias and Loureiro, 2016; Kerridge and Antcliff, 1999; Reynier, 2016; Sousa et al., 2007).

For each cultivar, several inflorescences of different vine stocks were randomly picked to Petri dishes at the stage 'EL 65 Full flowering: 50% of flowerhoods fallen' according to the Eichhorn and Lorenz (E-L) system (Lorenz et al., 1995). The collected samples were promptly isolated to avoid possible contamination with pollen of other cultivars and dried at 27 °C. After two days, the anthers were gently crushed and the pollen thus released was passed through different grades of sieves to obtain pure pollen. Pollen samples were stored at −20° C.

2.2. Pollen apertures

The number of pollen apertures was studied on acetolyzed pollen samples (Erdtman, 1969). This method had as purpose the destruction of the cytoplasmic content facilitating the later observation.

The microscopy observations were based on five fields per sample (each one containing 100 pollen grains) and the pollen grains were counted separately according to the number of apertures. These results were expressed in percentage.

2.3. Pollen viability

The pollen viability was tested by a fluorochromatic reaction with fluorescein diacetate (FDA) using a Leica microscope equipped with a mercury lamp of 50 W. The pollen grains were suspended in FDA 2%, during 5 min in obscurity. The FDA after entering the pollen grain is subsequently converted, by intracellular enzymes, into fluorescein which is a polar and fluorescent molecule. If the pollen vegetative cell membrane is intact, the fluorescein accumulates temporarily inside the pollen inducing fluorescence (Shivanna and Heslop-Harrison, 1981). So, pollen grains that appear with strong fluorescence are viable, while non-fluorescence pollen grains are non-viable (Heslop-Harrison, 1992). The viability was calculated counting three fields per sample, each one containing 100 pollen grains and the results expressed in percentage.

2.4. Pollen germination

In vitro pollen germination rate was assessed using two germination media. The medium 1 was composed by 100 ppm of boric acid, 20% of sucrose, and 0.6% of agar. The medium 2 was supplemented with 300 ppm of calcium nitrate. Pollen samples were germinated at 25 °C in the dark for 48 h. In order to calculate the germination rate, three fields per sample (each one containing 100 pollen grains) were counted in two moments – after 24 h and 48 h – using a light microscope (Leica DMLB). The pollen grains were considered as germinated when the length of their tube is greater than the pollen diameter (Nepi and Franchi, 2000).

2.5. Statistical analysis

Statistical analysis included a *t*-test to determine the effects of the both germination media studied on the percentage of pollen germination for each grape cultivar.

A one-way ANOVA followed by a *post-hoc* Duncan test was performed in order to determine the significant statistical differences between the percentage of viability and of germination among grape cultivars. A P value < 0.05 was considered to be significant.

3. Results

3.1. Pollen apertures

Fig. 1 presents the diversity in the number of pollen apertures observed among the studied grapevine cultivars. All the cultivars studied showed more than 95% of tricolporated pollen being of 100% in the Touriga Nacional cultivar (Table 1). In all 15 cultivars analyzed, none of them presented acolporated pollen grains and only 2 cultivars (Loureiro and Cabernet Sauvignon) contain pollen grains with only one aperture. Loureiro is the cultivar with the major percentage of pollen grains with four apertures (3.8%). The highest frequency (4.4%) of

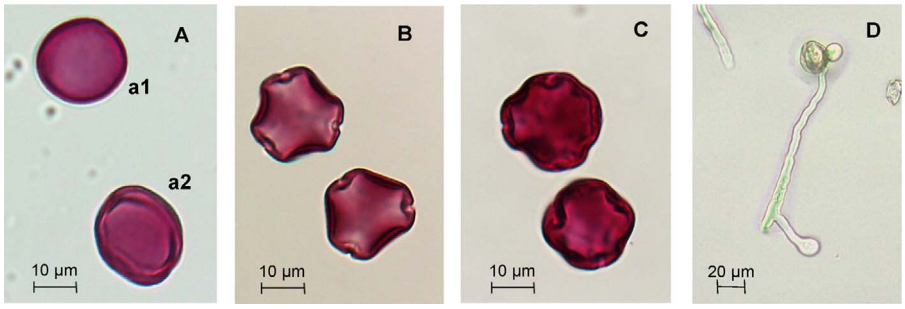

Fig. 1. A,B,C – Acetolyzed pollen of Vitis vinifera L. cultivars analyzed under light microscopy: A – Cabernet Sauvignon with one aperture (a1) and with two apertures (a2); B – Tricolporated pollen of Petit Verdot; C – Carménére with four apertures. D – *in vitro* germination of pollen analyzed under light microscopy.