Adv. Hort. Sci., 2016 30(1):

Characterization and evaluation of *Berberis microphylla* G. Forst pollen grains

S. Radice (*), M. Arena
CONICET, Facultad de Agronomía y Ciencias Agroalimentarias, Universidad de Morón, Machado 914, B17108EOH Morón, Buenos Aires, Argentina.

Key words: barberry, germination, Patagonia, size, viability.

Abstract: *Berberis microphylla,* commonly known as "calafate", is a non-timber forest product native from Patagonia, and its berries possess highlighted nutraceutical value. The objective of this research was to describe the morphology and anatomy of pollen grains of *Berberis microphylla* G. Forst genotypes growing spontaneously on the island of Tierra del Fuego (Argentina), and evaluate their vitality and germination. Pollen grain diameter varied from 40 to 47.26 µm, the pollen grains of 124 and 201 genotypes being significantly smaller than the others. Vitality measured by DAPI methodology was also variable among genotypes, although always about 50%. *In vitro* germination of pollen grains measured one day after the flowers were collected was very high for some genotypes (near 80%), and then decreased after 21 days of storage, except for genotype 123 whose germination value increased from 44.34 to 69%. The significant variability found in pollen performance (size, viability and germination) among *B. microphylla* genotypes from a natural population could be interpreted as an enhanced survival strategy to maximize reproduction fitness, with a marked capacity of response to environmental changes. High viable pollen frequency together with germination percentages observed in all the genotypes tested could indicate a good fertilization process. The correlation observed between size and germination percentage could be used as markers of pollen grain performance, paving the way for possible *B. microphylla* breeding.

1. Introduction

In spite of the well-known importance of wild flora as a source of food and medicinal substances, more studies on the diversity and agronomic value of these plant species are still needed (Arena and Vater, 2005). Areas with indigenous flora offer non-domesticated plants (Monge *et al.*, 2000), like *Berberis* genus in Patagonia (Orsi, 1984) for these purposes. *Berberidaceae* in the widest sense is a small family, consisting of 10 to 12 genera and about 600 species, with as many as 500 of these belonging to *Berberis* L., widely distributed in both the Old World and New World (Nowicke and Skvarla, 1981). In Patagonia, *Berberis* genus is well represented by 16 species of native shrubs and they are distributed from Neuquén to Tierra del Fuego (Arena and Curvetto, 2008). However, according to a later classification of the genus (Landrum, 1999), the number of species is less than previous studies cited by Orsi (1984), as Landrum groups the species *B. buxifolia*, *B. microphylla* and *B. heterophylla* under *B. microphylla* G. Forst, postulating that the differences among them may fluctuate to retain its range of species.

In particular, *B. microphylla* (ex *B. buxifolia* Lam.), commonly known as "calafate", is an evergreen shrub that is present throughout the region mentioned, prevalent on the island of Tierra del Fuego over other species of *Berberis*. *B. microphylla* has growing economic potential due to the production of fruits as a non-timber forest product (Tacón Clavaín, 2004). In fact, its dark blue berries are consumed fresh, as jams and preserves, and are used for the production of soft drinks and ice cream. Moreover, the fruits have a high content of phenols and antioxidants (Arena and Curvetto, 2008; Arena *et al.*, 2012). Some characteristics of its phenological phases (Arena *et al.*, 2013 a; Arena and Radice, 2014), fruit composition and production (Arena and Curvetto, 2008; Arena *et al.*, 2003; 2011; 2013 b) have already been studied in natural populations of this species.

Characterization of pollen grains is an important step for programs of genetic resource conservation and improvement, complementing basic studies of biological data that characterize genotypes (de Castro Nunes *et al.*, 2012). Selection on male game-

(*) Corresponding author: siradice@yahoo.com
Received for publication 31 October 2015
Accepted for publication 4 February 2016

tophytes (pollen) to alter the genetic constitution of the subsequent sporophytic generation has been suggested as an interesting tool in plant breeding programs (Hormaza and Herrero, 1992). Pollen competitive ability describes the reproductive success of a pollen grain and can therefore be considered as equivalent to pollen fitness (Sari-Gorla and Frova, 2005). The variability in pollen performance (size, viability and germination) among genotypes of a natural population could be interpreted as a survival strategy to maximize reproduction fitness (Tejaswini, 2002), while enabling a capacity of response to environmental changes (Hedly *et al.*, 2005). Nevertheless, aspects related to fertility and reproductive organs have not yet been studied on *Berberis microphylla.*

The objective of this research was to describe the morphology and anatomy of pollen grains of *Berberis microphylla* G. Forst genotypes growing spontaneously on the island of Tierra del Fuego (Argentina), and evaluate their vitality and germination.

2. Materials and Methods

Plant material

Flowers (*n=20*) in phase E (before anthesis according to Arena *et al*., 2011) were collected from each *B. microphylla* genotype grown near Ushuaia city, Tierra del Fuego (54° 48' SL, 68° 19' WL and 30 m asl) (Table 1), in October 2013. The flowers were immediately placed in Petri dishes with wet paper at 5°C for viability and germination studies.

Table 1 - *Berberis microphylla* genotype number and its satelital position in Tierra del Fuego (Argentina)

Genotype number	SL	WL
107	54 49 43 0	68 19 01 7
108	54 49 42 9	68 19 02 1
111	54 49 43 5	68 19 00 1
122	54 49 40 9	68 19 04 1
123	54 49 42 4	68 19 07 1
124	54 49 42 8	68 19 04 2
125	54 49 46 1	68 19 00 6
126	54 49 45 4	68 18 58 7
177	54 49 42 9	68 19 27 9
201	54 49 50 7	68 19 21 7
202	54 49 51 2	68 19 20 1

Pollen grain description and size

Equatorial and polar diameters of the pollen grains (*n=50*, randomly selected) were measured for each studied genotype using a Leica DM 2500 microscope. The average of the two parameters for each pollen grain was then calculated.

Light microscopy

Button flowers were dehydrated in an ethanol series and embedded in Spurr's resin. Thin sections (75-90 nm thick) were stained with uranyl acetate and lead citrate.

Scanning electron microscopy

Button flowers were dehydrated in an ethanol series and a critical point drying technique was employed. Samples were sputter coated with 20 nm gold and observed with a Philips XL 30 SEM.

Transmission electron microscopy

Anthers were pre-fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) for 2 h and then post-fixed in OsO_4 at 2°C in the same buffer for 3 h. They were then dehydrated in ethanol series and embedded in Spurr's resin. Thin sections (75-90 nm thick) were made on a Sorval ultramicrotome, stained with uranyl acetate and lead citrate (O'Brien and Mc Cully, 1981). Sections were observed with a Jeol-Jem 1200 EXII TEM at 85.0 kv.

Pollen grain viability

Pollen grains were hydrated with sucrose solution (15%) and treated with fluorescein diacetate (10%) and propidium iodine (2%) (Greissl, 1989). The number of viable and not viable pollen grains was recorded under optic microscope, with a minimum of 300 pollen grains per genotype.

Pollen grain germination

Pollen grains were put on micro drops of a saline solution composed of 2 x 10^{-3} M H_3BO_3 and 6 x10^{-3} M Ca $(NO_3)_2$ added with sucrose 30% (Dafni, 1992). Micro drops were placed on the inside of the lid of a petri dish in which 3 ml of water were added in the base to create a humid chamber. Incubation was at 21±2°C. The number of germinated and aborted pollen grains was recorded under optic microscope 24 h after the test started and performed with anthers conserved for 1, 10 and 21 days at 5°C.

Data analysis

Measurements were analyzed by ANOVA and Tukey's test and chi-square test was employed to evaluate pollen vitality.

3. Results

Pollen grain description and size

Flowers collected in phase E had five stamens; the anthers were not yet dehiscent (Fig. 1A) although the pollen grains were already mature (Fig. 1B, 2A-B). A mature pollen grain is formed of a vegetative cell with a dense cytoplasm with numerous starch grains (Fig. 1B). *B. microphylla* pollen grains are spherical with a psilate punctate surface interrupted by mild cracks (Fig 2. A-B), resembling a tennis ball.

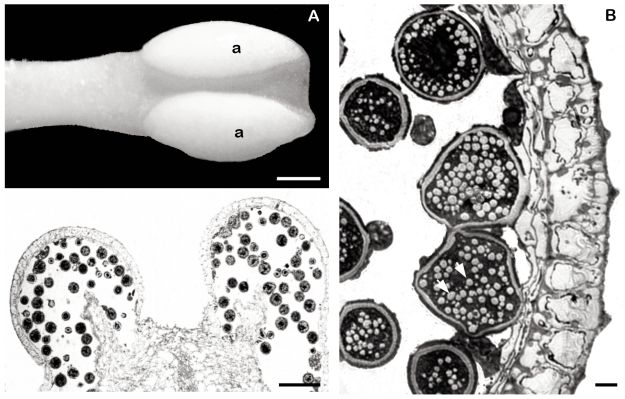

Fig. 1 - Androecium and pollen grain of *B. microphylla* flower in phase E. A) view of androecium with anther (a) no dehiscent; B-C) microphotograph of cross-section of anther and mature pollen grain with starch grains (arrowhead). Bars: A = 1mm; B = 10 µm; C = 100 µm.

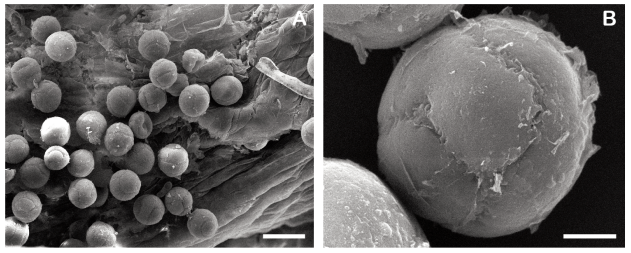

Fig. 2 - SEM micrograph of mature pollen grain of *B. microphylla*. A) view of microsporangium with pollen grain; B) detail of a pollen grain. Bars: A = 10,000 nm; B = 50 µm.

The pollen wall is formed by an exine and intine of considerable thickness (Fig. 3A). Transmission electron micrographs make it possible to identify an exine with two different layers, the ektexine and the endexine (Fig. 3A). Ektexine is nearly amorphous and not organized into typical foot layer, columellae, and tectum units. Conversely, ektexine appears as an external irregular cover with channels and small enclosed areas which are electron translucent (Fig. 3). Endexine has greater electro-density than ektexine. Immediately below this, the intine is present, at least four times thinner than the exine and with a

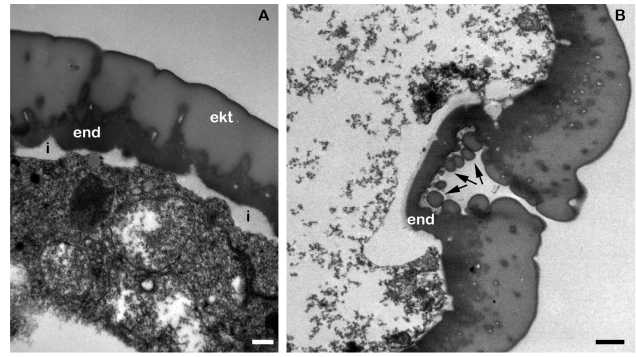

Fig. 3 - TEM micrograph of pollen grain wall of *B. microphylla.* A) Detail of different parts of exine: endexine (end), ektexine (ekt) and intine (i); B) Detail of pore zone with ektexine fragmented (arrows). Barras = A-B = 1 µm.

very low electro-density. Pollen grain wall appears different on the apertures or pore zone (Fig. 3B). Ektexine is less structured and is represented by nodules above the endexine.

The pollen grain diameters varied significantly among genotypes, from 40 to 47.26 µm ($p \leq 0.001$). The maximum values were observed for genotype 107, which was significantly higher than genotypes 124, 126, 177, and 201 (Fig. 4). Pollen grains of genotypes 124 and 201 were significantly smaller than genotypes 107, 111, 123 and 202 (Fig. 4).

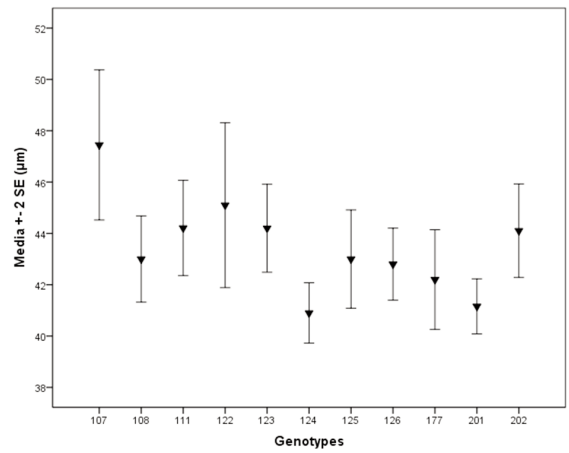

Fig. 4 - Mean diameter of *B. microphylla* pollen grains of the studied genotypes.

Pollen grain viability

Pollen grains stained with fluorescein diacetate and propidium iodine showed very different colors depending on whether they were vital or non-vital.

Vital pollen grains were bright green while non vital ones stained red (Fig. 5). In this latter case, another category was evaluated, the sub-vital pollen grains, those that can germinate but it is uncertain whether they can be efficient in fertilization. Pollen viability of different genotypes gave very different results ($p \leq 0.001$). For genotypes 111, 123, 124 and 202, values of vital pollen grains were above 70%, while for genotype 108 the value was 51.47% (Fig. 6). In coincidence, the 108 genotype shows a 36.80% of sub-vital pollen grains, value significantly greater than that observed for the 111, 123, 124, 126, and 202 genotypes.

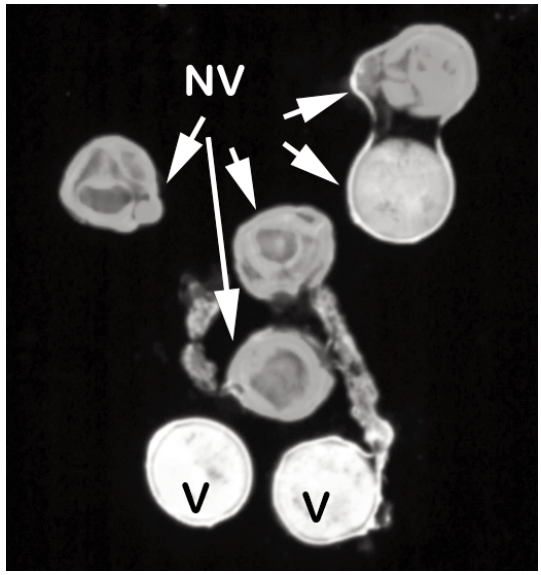

Fig. 5 - Viable (V), and non-viable (NV) pollen grains observed by fluorescence microscopy.

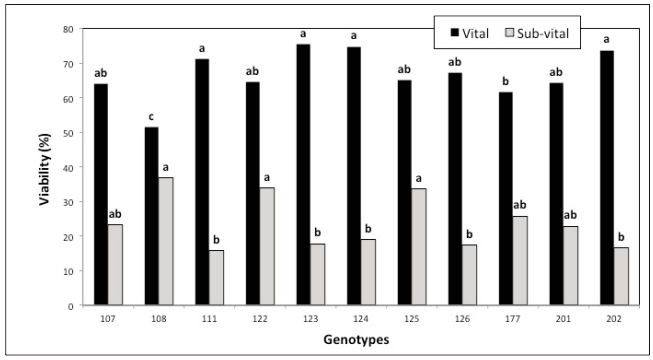

Fig. 6 - Viability of *B. microphylla* pollen grains from the assessed genotypes. Columns with different letters indicate significant differences between genotypes and date assessed (X^2 $p<0.05$).

Pollen grain germination

The pollen grain germination of *B. microphylla* was significantly different among genotypes ($p \leq 0.001$) and days of conservation ($p \leq 0.001$), and the interaction between the two factors studied ($p \leq 0.001$) was significant (Fig. 7). Germination of the pollen grain was at maximal level after one day of collection in most genotypes, except for genotype 123 which showed a maximum value after 21 days of storage, although without significant differences between 10 and 21 days (Fig. 7). Genotypes 124 and 125 showed percentages of pollen grain germination up to 70%, while genotype 201 presented a maximum value of 87.03% after one day of the collected flowers (Fig. 7). The pollen germination rate remained unchanged among the three tested dates, except for genotypes 122, 126 and 177. In addition, the values were significantly lower after 21 days of conservation for genotypes 122, 126 and 177 (Fig. 7). This decrease in the percentage of pollen germination is in accordance with the percentage of aborted pollen grains (data not shown). ANOVA values were 0.001 for both date and genotype factor and their

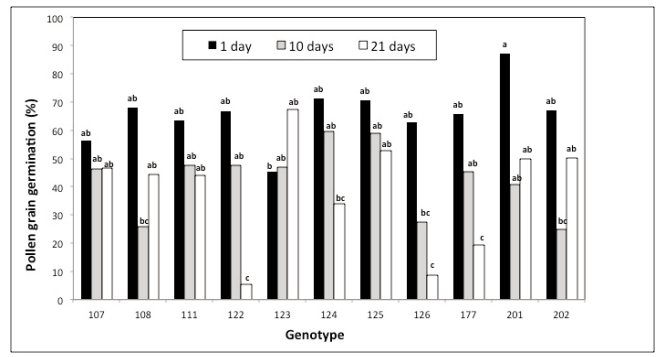

Fig. 7 - Germination percentage of *B. microphylla* pollen grains from the assessed genotypes. Columns with different letters indicate significant differences between genotypes and date assessed (Tukey $p<0.05$).

interaction. Furthermore, the genotypes 108 and 202 showed an increase in the percentage of germinated pollen grains between the second and third test, i.e. the values obtained after 21 days of flower conservation were higher than those obtained with 10 days of storage but these differences were not significant (Fig. 7).

Finally, a significant negative correlation between the pollen grain germination and the pollen grain size was found ($r = -0.252$; $p \leq 0.001$).

4. Discussion and Conclusions

It was observed that flower differentiation on *B. microphylla* started 12 weeks after bud break in coincidence with the end of the first fruit growth phase

(Arena and Radice, 2014). Nevertheless, final development of male gametes occurs the following spring when the flower bud elongates (data not shown). Mature pollen grains can be found in the stage of lower emergence code 59 according to the BBCH scale (Arena *et al*., 2013 a). There are few published studies on the pollen of *Berberis* species. Erdtman (1952) and Heusser (1971) described the pollen grains of the *Berberis* genus and they determined that the pollen grains measured an average of 30 to 65 µm with an exine 2-3 µm thick. Nowicke and Skvarla (1981) emphasized the presence of irregular apertures, a psilate surface and an unstratified exine. In adittion, the exine of *B. micropylla* shows an endexine as a prominent fibrous-granular layer and the ektexine with cavities and channels which suggest the endexine.

Pollen performance traits are often genetically based (Hedly *et al.*, 2005; Hove and Mazer, 2013), however they could be also affected by differences in the nutritive status of the developing pollen grains as well as by the environmental conditions (Hedly *et al.*, 2005). The size of pollen grains is considered to be one indicator of their viability (i.e. germinability and pollen tube growth rate), while the proportion of large pollen grains has been used to estimate pollen performance. Variation in pollen grain size among plants has been documented for several species (Varis *et al.*, 2011). Larger pollen grains are thought to contain more resources for germination and, thus, have greater viability than smaller grains (Dufaÿ *et al.*, 2008). However, in *B. microphylla* a negative correlation between pollen size and germination percentage was observed, as was also found in *Pinus sylvestris* (Varis *et al.*, 2011), as the growth of the pollen tube may be more dependent on pollen storage than pollen size. Good fertilization is directly related to very good pollen viability. It is estimated that the value of viability should be above 70% for fruit production (Urquieta, 2010). On the other hand, pollen grains which exceed 50% viability would be the only one that can be selected for use as male parents (Urquieta, 2010). All genotypes of *B. microphylla* tested presented viability values above 50%, in other words having very good prospects for fruit production. Urquieta (2010) found similar results when pollen grains of *B. bidentata*, *B. darwinii, B. parodii* and *B. trigona* were tested. Pollen grain viability of these species was variable between 59.6 and 74.1%.

It has been found that high percentages of pollen viability are due to high degrees of adaptability of the species to different environmental conditions (Kelly *et al*., 2002). In effect, high frequency of viable pollen reflects the adaptability of the species, since environmental plasticity submitted by pollen allows the genotype a satisfactory performance to different environmental conditions (Paupière *et al.*, 2014). On the other hand, pollen viability was influenced by relative humidity, temperature, atmospheric composition, and oxygen pressure after release from the anthers (Bots and Mariani, 2005), so it is expected that the experimental values obtained for *B. microphylla* are less than true values.

Pollen germination under *in vitro* conditions always produces lower values than those obtained with the viability test (Ontivero *et al.*, 2006). In the present study, this premise is true for genotypes 107, 111, 123, 124, and 202 (Figs. 6 and 7). On the contrary, all other genotypes showed viability values higher than those obtained by germination viability test. Note that after 21 days of harvested flowers, pollen germination values obtained were only 10% lower than viability values for genotypes 108 and 123. These results could be due to the protective effect that antioxidants have on pollen. In effect, it is well known that secondary metabolites produced in the tapetum, such as phenolic compounds, can spread to the pollen and play a role in pollen colour, in the attraction of pollinators, in pollen tube germination, and in protection against abiotic stress of pollen (Paupière *et al.*, 2014). Pollen germination and tube growth is largely due to the presence of flavonols in mature pollen grains (Yistra *et al.*, 1992). Although the content of flavonols was not measured in the anthers of *B. microphylla*, it is well known that flavonols are present in *Berberis* species (Končić *et al.*, 2010). In effect, accumulation patterns of phenolic compounds during fruit growth and ripening in *B. buxifolia* (*B. microphylla*) was studied by Arena *et al*. (2012). It is likely that high values obtained from *in vitro* germination of pollen grains could be explained by the protective effect that these compounds perform.

The variability found in pollen performance (size, viability and germination) among genotypes of the natural population of *B. microphylla,* and its correlations, suggest the existence of pollen competition leading to unequal reproductive success in this species, as was observed for *Camellia sinensis* by Muoki *et al.* (2007).

The significant variability found in pollen performance (size, viability and germination) among *B. microphylla* genotypes from a natural population could be interpreted as a highlighted survival strategy

to maximize reproduction fitness, with a marked capacity of response to environmental changes. High viable pollen frequency, together with germination percentages observed in all the genotypes tested, could indicate a good fertilization process. The correlation observed between size and germination percentage could be used as a marker of pollen grain performance, with these findings representing the first antecedents useful for *B. microphylla* breeding.

Acknowledgements

The authors thank the Prefectura Naval Argentina and Mrs. Isabel Farías for her assistance with the histology. This research was supported by grants PIP 314 subsidized by CONICET.

References

ARENA M.E., CURVETTO N., 2008 - Berberis buxifolia *fruiting: Kinetic growth behavior and evolution of chemical properties during the fruiting period and different growing seasons*. - Scientia Horticulturae, 118(2): 120-127.

ARENA M.E., GIORDANI E., RADICE S., 2011 - *Flowering, fruiting and leaf and seed variability in* Berberis buxifolia*, a Patagonian native fruit species*, pp. 117-136. - MARIN L., and D. KOVAÈ (eds.) *Native species: Identification, conservation and restoration*, Nova Sciencia Publisher Inc., Hauppauge, NY, USA, pp. 176.

ARENA M.E., GIORDANI E., RADICE S., 2013 a - *Phenological growth and development stages of the native Patagonian fruit species* Berberis buxifolia *Lam*. - J. Food, Agric. Environ., 11(3-4): 1323-1327.

ARENA M.E., POSTEMSKY P., CURVETTO N.R., 2012 - *Accumulation patterns of phenolic compounds during fruit growth and ripening of* Berberis buxifolia*, a native Patagonian species*. - New Zealand Journal of Botany, 50(1): 15-28.

ARENA M.E., RADICE S., 2014 - *Shoot growth and development of* Berberis buxifolia *Lam. in Tierra del Fuego (Patagonia)*. - Scientia Horticulturae, 165: 5-12.

ARENA M.E., VATER G., 2005 - *Native and introduced species of small fruits in Austral Patagonia, Tierra del Fuego*, pp. 96-110. - In: DRIS R. (ed.) *Fruits: Growth, nutrition, and quality*. WFL Publisher, Helsinki, Finlandia, pp. 220.

ARENA M.E., VATER G., PERI P., 2003 - *Fruit production of* Berberis buxifolia *Lam. in Tierra del Fuego.* - HortScience, 38(2): 200-202.

ARENA M.E., ZULETA A., DYNER L., CONSTENLA D., CECI M., CURVETTO N.R., 2013 b - Berberis buxifolia *fruit growth and ripening: evolution in carbohydrate and organic acid contents*. - Scientia Horticulturae, 158: 52-58.

BOTS M., MARIANI C., 2005 - *Pollen viability in the field*. - Research Reports No. CGM5.

DAFNI A., 1992 - *Pollination ecology: A practical approach*. - Oxford University Press, Oxford, UK, pp. 250.

DE CASTRO NUNES R., OLIVEIRA BUSTAMANTE F., TECHIO V.H., MITTELMAN A., 2012 - *Morphology and pollen viability of* Lolium multiflorum *Lam.* - Ciência e Agrotecnologia, 36(2): 180-188.

DUFAŸ M., VAUDEY V., DE CAUWER I., TOUZET P., CUGUEN J., ARNAUD J.F., 2008 - *Variation in pollen production and pollen viability in natural populations of gynodioecious* Beta vulgaris *ssp. maritima: evidence for a cost of restoration of male function?* - J. Evol. Biol., 21: 202-212.

ERDTMAN G., 1952 - *Pollen morphology and plant taxonomy*. - GFF, 74(4): 526-527.

GREISSL R., 1989 - *Vitality analysis of monadic and polyadic pollen grains using optical contrast-fluorescence microscopy*. - Scientific and Technical Information, IX(5): 180-184.

HEDLY A., HORMAZA J.I., HERRERO M., 2005 - *Influence of genotype-temperature interaction on pollen performance.* - J. Evol. Biol., 1(8): 1494-1502.

HEUSSER C.J., 1971 - *Pollen and spores of Chile: modern types of the* Pteridophyta*,* Gymnospermae*,* Angiospermae. - University of Arizona Press, Tucson, Arizona, USA, pp. 167.

HORMAZA J.I., HERRERO M., 1992 - *Pollen selection*. - Theoretical and Applied Genetics, 83**:** 663-672.

HOVE A.A., MAZER S.J., 2013 - *Pollen performance in* Clarkia *taxa with contrasting mating systems: Implications for male gametophytic evolution in selfers and outcrossers*. - Plants, 2: 248-278.

KELLY J.K., RASCH A., KALISZ S., 2002 - *A method to estimate pollen viability from pollen size variation*. - Am. J. Bot., 89(6): 1021-1023.

KONČIĆ M.Z., KREMER D., KARLOVIĆ K., KOSALEC I., 2010 - *Evaluation of antioxidant activities and phenolic content of* Berberis vulgaris *L. and* Berberis croatica *Horvat*. - Food and chemical toxicology**,** 48(8): 2176-2180.

LANDRUM L.R., 1999 - *Revision of* Berberis *(Berberidaceae) in Chile and adjacent Southern Argentina.* - Ann. Missouri Bot. Gard., 86(4): 793-834.

MONGE A., CHORGADE M., ERHARDT P.W., GENELLIN C.R., KOGA N., LINDBERG P., PERUN T.J., TOPLISS J.G., TRIVEDI B.K., WERMUTH C.G., 2000 - *Medicinal chemistry in the development of societies: biodiversity and natural products*. - Eur. J. Med. Chem.**,** 35(12)*:* 1121-1125.

MUOKI R.C., WACHIRA F.N., PATHAK R., KAMUNYA S.M., 2007 - *Potential male gametophyte competition among* Camellia sinensis *genotypes in isolated biclonal seed orchards*. - African Crop Science Journal, 15(2): 59-66.

NOWICKE J.W., SKVARLA J.J., 1981 - *Pollen morphology and phylogenetic relationships of the* Berberidaceae*.* -

Smithsonian Contributions to Botany, 50: 1-83.

O'BRIEN T.P., Mc CULLY M.E., 1981 - *The study of plant structure: principles and selected methods*. - Termarcarphy Pty, Melburne, Australia, pp. 357.

ONTIVERO M., RADICE S., GIORDANI E., BELLINI E., 2006 - *Effects of different pollination treatments in genotypes of* Prunus salicina *Lindl*. - International Journal of Horticultural Science, 12(2): 141-146.

ORSI M.C., 1984 - Berberidaceae, pp. 325-348. - In: CORREA M.N. (ed.) *Flora Patagónica Sección 4ª*. INTA, Buenos Aires, Argentina.

PAUPIÈRE M.J., VAN HEUSDEN A.W., BOVY A.G., 2014 - *The metabolic basis of pollen thermo-tolerance: perspectives for breeding*. - Metabolites, 4(4): 889-920.

SARI-GORLA M., FROVA C., 2005 - *Pollen tube growth and pollen selection*, pp. 333-351. - In: SHIVANA K.R., and V.K. SAWHNEY (eds.) *Pollen biotechnology for crop production and improvement*. Cambridge University Press, NY, USA, pp. 468.

TACÓN CLAVAÍN A., 2004 - *Manual de productos forestales no madereros.* - CIPMA, Valdivia, Chile, pp. 22.

TEJASWINI P., 2002 - *Variability of pollen grain features: a plant strategy to maximize reproductive fitness in two species of* Dianthus*?* - Sexual Plant Reproduction, 14: 347-353.

URQUIETA C.A.P., 2010 - *Evaluación de la viabilidad polínica de cuatro especies pertenecientes al género* Berberis *L. (*Berberidaceae*)*. - Thesis, Chile University, pp. 85.

VARIS S., REINIHARJU J., SANTANEN A., RANTA H., PULKKINEN P., 2011 - *The size and germinability of Scots pine pollen in different temperatures* in vitro*.* - Grana, 50(2): 129-135.

YISTRA B., TOURAEV A., MORENO R.M.B., STÖGER E., VAN TUNEN A.J., VICENTE O., HEBERLE-BORS E., 1992 - *Flavonols stimulate development, germination, and tube growth of tobacco pollen*. - Plant Physiology, 100(2): 902-907.