Histological changes of *Berberis mikuna* pollen grains in relation to viability and germinability

Silvia Radice (Conceptualization) (Methodology) (Validation) (Formal analysis) (Investigation) (Resources) (Visualization) (Supervision) (Project administration) (Funding acquisition), Beatriz G. Galati (Methodology) (Validation) (Investigation) (Resources) (Visualization) (Supervision), Gabriela Zarlavsky (Methodology) (Visualization) (Supervision), Miriam E Arena (Methodology) (Investigation) (Visualization) (Supervision)

PII: S0367-2530(20)30123-7

DOI: https://doi.org/10.1016/j.flora.2020.151623

Reference: FLORA 151623

To appear in: *Flora*

Received Date: 12 December 2019

Revised Date: 6 May 2020

Accepted Date: 13 May 2020

Please cite this article as: Radice S, Galati BG, Zarlavsky G, Arena ME, Histological changes of *Berberis mikuna* pollen grains in relation to viability and germinability, *Flora* (2020), doi: https://doi.org/10.1016/j.flora.2020.151623

Histological changes of *Berberis mikuna* pollen grains in relation to viability and germinability.

Silvia Radice

Laboratorio de Fisiología Vegetal. Facultad de Agronomía y Ciencias Agroalimentarias UM – CONICET. Machado 914, Lab. 501, B1708EOH. Morón. Buenos Aires. Argentina. E-mail: siradice@yahoo.com

Beatriz G. Galati

Cátedra de Botánica General. Facultad de Agronomía. Universidad de Buenos Aires. Av. San MartÌn 4453. C1417DSE. E-mail: galati@agro.uba.ar

Gabriela Zarlavsky

Cátedra de Botánica General. Facultad de Agronomía. Universidad de Buenos Aires. Av. San Martín 4453. C1417DSE. E-mail: gzarlavs@agro.uba.ar

Miriam E Arena*

Laboratorio de Fisiología Vegetal.Facultad de Agronomía y Ciencias Agroalimentarias UM – CONICET. Machado 914, Lab. 501, B1708EOH. Morón. Buenos Aires. Argentina. E-mail: miriamearena@gmail.com.

*Author for Correspondence:

M. E. Arena.

E-mail address: *miriamearena@gmail.com*; *miarena@unimoron.edu.ar*

Highlights

- *Berberis mikuna* is a spontaneous shrub grown in the Tucuman-Bolivian subtropical area.
- Pollen grain germination and viability decreased after 45 days of storage.
- Germination and viability decreased with increasing temperature.
- Ultrastructure study revealed differences in the fibrillar structure of the intine.
- A decrease in starch reserve in grains at 5°C and at room temperature was observed.

Abstract

Berberis mikuna is a spontaneous shrub grown in the Tucuman-Bolivian subtropical jungle area and its fruits were highly prized as food by diaguitas and calchaquies who

were local populations. Fruits are rich in phenolic compounds which would be responsible for the beneficial properties for health. Pollen preservation in gene banks should be implemented as standard procedures since pollen is a useful source of diverse alleles within a gene pool. Therefore, the aim of this work was to study the histological and ultrastructural changes of pollen grains after 45 days of conservation at different temperatures. *Berberis mikuna* pollen grain germination and viability decreased significantly after 45 days of its collection. Germination and viability decreased with increasing storage temperature, although the viability in T45 Ref and T45 Fr was similar. Ultrastructure studies revealed differences in the fibrillar structure of the intine, as well as the decrease in starch reserve in pollen grains stored at 5°C and at room temperature.

Keywords: ultrastructure; spontaneous shrub; small fruit

Introduction

Berberis L. (barberry) is the largest genus in Berberidaceae (Kim *et al.*, 2004) and it is the only genus of the family that is found in Argentina, counting with 18-26 species (Orsi, 1984; Zuloaga *et al*., 2008; Landrum, 1999). There are two major biogeographic distribution centers of *Berberis* in Argentina: the Tucuman-Bolivian subtropical jungle where *B. mikuna* is quite common (Job, 1942), and the Patagonian steppe and subantarctic forest. Others native *Berberis* are dispersed in different zones of Argentina as *B. ruscifolia* that is distributed on the Gallery Forests of the Paranaense Province (Arambarri *et al*., 2006), the Pampa region (Polop, 1989) and *B. hieronymis* only localized on the Córdoba mountains (Cabido *et al*., 1990). *Berberis* flowers are solitary or in inflorescences, ranging in color from yellow to orange, while its fruits are purplish berries. Some species are used as ornamental plants (Brickell, 1989), due to their leaves and quality and abundance of flowers. Fruits of *B. mikuna* and *B. ruscifolia* were highly prized as food by diaguitas and calchaquies who were local populations that still have descendants in the province of Tucumán. Similarly, *B. microphylla* fruits were an important contribution to the food and health of Patagonian native cultures (Domínguez Díaz, 2010) and, since a few years, these fruits have taken great economic value (Arena, 2016). The consumption of locally grown, wild or semi wild edible plants is important for most human cultures and they often contain higher amount of nutrients and bioactive compounds than many cultivated species (Ruiz-Rodríguez *et al*., 2011). In addition, most of the species of the *Berberis* genus have assigned medicinal properties due to the presence of the alkaloids called berberine and berbamine (Shaffer, 1985;Orsi, 1984; Fajardo Morales *et al*., 1986; Fajardo Morales, 1987; Mokhber-Dezfuli *et al*., 2014). In addition, a native group of Argentina called Warmi Pura is currently intensively using

B. mikuna wood for the extraction of berberine to dye wool, which endangers this species (https://www.dagri.unifi.it/vp-480-warmi-pura-esp.html).

Little is known about the species *B. mikuna*. However, some studies carried out in Tucumán contributed with some data of the phenological behavior and the characterization of the fruits of this species (Ceribelli, 2018). Pollen, as a male gametophyte, plays a fundamental role in the reproduction of plants since through pollination and fertilization it allows the formation of seeds that will perpetuate the species. Therefore, it is important to know how to preserve it. Although the quantity and quality of pollen produced by a flower is one of the most important aspects in evolutionary research, and in the adaptation of genotypes, very few studies were conducted on pollen of *Berberis* genus. Some of them are related to the pollen morphology of different *Berberis* species of the northern hemisphere (King-Tang and Li, 1983; Li *et al*., 2017; Ur *et al.*, 2019). There are some contributions on *Berberis* species of South America. Urquieta (2010) developed different pollen evaluation techniques in *B. bidentata, B. darwinii, B. parodii* and *B. trigona*. Radice and Arena (2016) carried out a complete study of *B. microphylla*, and Ceribelli (2018) recorded measures and germinability of *B. mikuna* pollen grains.

Genetic conservation through pollen storage is desirable for a variety of plant species, since pollen is known to transmit important genetically heritable characters. Pollen conservation is linked to viability which in turn depends on the physiology of the pollen grains and the presence or absence of specific structural modifications (Bots, 2005). So, the establishment of a method of pollen grain conservation of *B. mikuna* is an important issue that will contribute to the knowledge of this species and with decisive information on the processes of plant breeding. Due to the arguments presented, the aim of this work was to study the histological and ultrastructural changes of the pollen grain after 45 days of conservation at different temperatures and their relationship with the pollen grain viability and germination.

Materials and Methods.

Plant material. Flowers of *B. mikuna* in stage 59 according to Arena *et al.* (2013) were collected at the site called Chacra Dantur in the locality of Alto de Medina (Tucumán, Argentina) according to satellite position: 26° 25’ 49’’ 9 SL. 65°03’ 32’’ 2WL, 1498 m.a.s.l. Button flowers were randomly collected, in the amount of 30 buds per plant from a total of 10 plants (*n = 300*). Stamens were separated from the rest of the floral pieces immediately after the collection and placed in labeled and opened Eppendorfs. Dry anthers were preserved in three different temperature conditions: room temperature (RT)

(21±3 °C); 5 °C (Ref) and -20 °C (Fr). The evaluation was made 24 hs after the collection (T0) and 45 days after the conservation (T45) for the three different temperature conditions (T45 RT; T45 Ref andT45 Fr).

Light and Electron Microscopy. Anthers subjected to the different treatments of preservation were pre-fixed in 1% glutaraldehyde, 4% formaldehyde in phosphate buffer (pH 7.2) for 48 h and then post-fixed in OsO4 at 2ºC in the same buffer for 3 h. Following dehydration in an ethanol series, the material was embedded in Spurr's resin. Sections were made on a Reichert-Jung ultramicrotome. Sections of 1 µm were stained with toluidine blue and observed and photographed in a Labomed optical microscope equipped with a digital camera. Ultrathin sections (750- 900 nm) were stained with uranyl acetate and lead citrate (Zarlavsky, 2014). The ultrathin sections were observed and photographed with a Philips TEM 301 (Eindhoven, North Brabant, The Netherlands) at 60.0 Kv.

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%. 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 (Radice and Arena, 2016).

Pollen grain viability. Pollen grains were hydrated with sucrose solution (15%) and treated with fluoresce in diacetate (10%) and propidium iodine (2%) (Greissl, 1989). The number of viable and not viable pollen grains through the different colored (*n= 300/treatment*) was recorded under optic microscope Leica DM 2500 with BP 340 – 380 filter.

Data analysis. Measurements were analyzed by χ^2 test.

Results

Pollen grain germination. Twenty-four hours after pollen collection (T0 RT) pollen grain germination was 60.5% ± 10% (Table 1). After a conservation period, pollen grain germination significantly decreased, and results were quite different according to the treatment. Conservation at room temperature (T45 RT) was lethal for pollen grains since only 3.0% of the total pollen grains could germinate, while the decrease in temperature at 5°C (T45 Ref) allowed a 30.0% of germination and 49.0% (T45 Fr) at -20 ºC (Table 1).

Pollen grain viability. Viable pollen grains were observed bright green while non-viableones stained red (Fig. 1A-B). Results obtained with this method were very similar to germination test, with a significant decrease after a conservation period and in both

cases, values obtained for pollen grains conserved at room temperature (21±3 °C) were significantly lower than the other two treatments (Table 1).

Pollen grain histology. The cytoplasm of the vegetative cell is the one that presents higher modifications in the pollen grains subject to the different treatments. The vegetative cell has a dense cytoplasm with abundant amyloplasts in the initial stage (T0 RT) which are colored pink with toluidine blue (Fig. 2A). The number of amyloplasts and their size decreased in the vegetative cell cytoplasm of the pollen grains preserved for 45 days at different temperatures (Fig. 2B-D). These amyloplasts are composed of numerous starch grains (Fig.3A). The amount of starch grains by amyloplast is lower mainly in the pollen grains preserved at 21±3 °C and 5 ºC, and in these two cases, the cytoplasm of the vegetative cell also shows signs of degradation (Fig. 3B-C). On the other hand, presence of lipids can be observed in the cytoplasm of this cell in the treatments at 21±3 °C, 5 ºC and-20 °C (Fig. 3B-D).

The pollen wall did not show large differences in all treatments with respect to the initial stage (T0 RT). In this last one the exine presents high electron-density and the intine is observed very fibrillar (Fig. 3E). The intine loses some of its fibrillar appearance in all treatments (T45 RT, T45 Ref and T 45 Fr) (Fig. 3F-H). The lowest electron-density of the exine is presented by the pollen preserved at 5ºC (Fig. 3G). The electron-density of the exine is only conserved in the pollen storage at -20ºC (T45 Fr) (Fig. 3H).

Discussion

Pollen grains of *Berberis* species are characterized by spherical morphology, between 30 and 65 µm, and the thickness of the exine is 2-3 µm (Urquieta, 2010). In fact, pollen grains taken from twenty different genotypes of *B. mikuna*, measured between 38 and 45 µm (Ceribelli, 2018). It is well known that larger pollen grains are thought to contain more resources for germination and, thus, have greater viability than smaller grains (Dufaÿ *et al.*, 2008).

There are different methods to evaluate pollen viability. Fluorescein diacetate is commonly used to stain pollen grain to test membrane integrity. This technique was employed in *B. microphylla* pollen grains and viability results varied between 51.47 and 70% (Radice and Arena, 2016). Pollen grains of *B. mikuna* were tested 24 hs after flower collection and the viability was 52.33%. These values are like those obtained with *B. microphylla* and *B. bidentata*, *B. darwinii*, *B. parodii* and *B. trigona* for the pollen viability (Unquieta, 2010). In addition, 50% viability would be an acceptable value for genotype selection to use as a parent (Urquieta, 2010). However, the loss of pollen viability is rapid and progressive, depending on, among other factors, the age of the individual and exposure to various environmental agents (Stone *et al.*, 1995). Moreover, at high

temperature, the loss of viability is a continuous process, which involves a series of enzymes that are degraded with different rhythms and progressively (Dafni and Firmage, 2000). This reaction causes the progressive loss of the ability of the cell membranes of the vegetative cell to regain its normal structure for rehydration (Shivanna and Heslop-Harrison, 1980).

There are no data on the correlation between pollen size and germination in *B. mikuna*, but it was observed a negative correlation in *B. microphylla,* that is, smaller pollen, higher germination rate (Radice and Arena, 2016). Furthermore, Varis *et al.* (2011) observed that growth of the pollen tube of pollen grains of *Pinus sylvestris*, was more conditioned by pollen storage than pollen size. In effect, pollen grains of transgenic tobacco stored at different temperatures (room temperature, 4°C, -20°C, - 70°C, and -196°C) exhibited significantly highest germination rate at -20°C than pollen stored at any other temperature (Lim *et al.*, 2014). These results are consistent with the data obtained in the treatments applied to *B. mikuna* pollen in this investigation. In our case, the highest pollen germination rate was obtained for pollen stored at -20 ºC, but no significant differences were observed between this treatment and pollen stored at 5ºC which could mean that the temperature required for a long shelf life should be even lower.

The resistance of the exine to destruction is one of the greatest characteristics within the plant kingdom, since it supports the action of acids and concentrated bases, as well as heating at 300 °C. On the other hand, the intine, is easily destroyed due to its chemical composition (Saenz de Rivas, 1976). Fang *et al.* (2008) showed that exine is not absolutely necessary for pollen germination and pointed out that the characteristics and content of pectin, the types of hemicelluloses and the abundance of cellulose can be correlated with the rigidity, strength and elasticity of the pollen intine (Fang *et al.*, 2008). In addition, temperature changes affect the content of cell wall pectins as it happens in maize leaves (Bilska-Kos *et al.*, 2017). The intine has a microfibrillar component that is the cellulose and an outer pectic stratum that plays an important part in the early stages of germination in many pollen types (Heslop-Harrison, 1978). In our study, the fibrillar character of the intine is less pronounced in pollen grains subjected to the different conservation treatments. Therefore, this could be one of the reasons why the pollen germination rate in pollen stored at room temperature, 5°C and -20°C decreases with respect to the pollen germination rate twenty-four hours after its collection.

The carbohydrate and lipid reserves of the pollen grain are used principally for the synthesis of the pollen-tube wall (Heslop-Harrison, 1978). In *B. mikuna*, the starch reserve decreases considerably in pollen grains stored at 5°C and at room temperature. Pollen grains stored at -20°C retain a higher proportion of amyloplasts, although smaller

compared to those of the initial state, because they have fewer starch grains per plastid. These observations are consistent with the results of germination and pollen viability, since the highest values were obtained with pollen preserved at -20°C. Moreover, not significant differences on the germination and pollen viability were observed between pollen stored at -20ºC and fresh pollen grains.

On the other hand, the germinability is determined principally by the state of the membranes of the vegetative cell. When pollen grains are stored by long time, it is necessary to remove the water so plasmalemma can be altered during the period of dehydration. Recovery takes place on rehydration, but only if favorable structural relationships have been preserved in the modified membrane during the period of desiccation (Shivanna and Heslop-Harrison, 1980). Previous treatments in relation to pollen water content should be considered because there may be ice crystallization during the freezing process (Alba *et al.*, 2011). Franchi *et al.* (2002) classified pollen grains as ‘‘partially hydrated pollen’’, with a water content greater than 30%, and ‘‘partially dehydrated pollen’’, with a water content of less than 30%. Most of pollen grains of angiosperms have been found partially dehydrated, so, pollen of *B. mikuna* probably belongs to this type. In this case pollen resists hostile environments better because it can maintain its water content by mobilizing carbohydrate reserves (Guarnieri *et al.*, 2006). This would explain the decrease in starch reserves in all pollen storage treatments of *B. mikuna*. Due to its water content, partially hydrated pollen lacks mechanisms to prevent formation of ice crystals, and therefore it is difficult to store at low temperatures, unlike partially dehydrated pollen (Franchi *et al.*, 2002). *B. mikuna* pollen responded better, that is, it maintained a higher rate of viability at low temperatures (-20ºC) which reaffirms that it is partially dehydrated pollen.

This is the first contribution on the cytological and ultrastructural changes in pollen subjected to different storage conditions. Pollen preservation in gene banks should be implemented as standard procedures since pollen is a useful source of diverse alleles within a gene pool. Therefore, this research contributes with useful data for pollen preservation of *B. mikuna*, a species with high potential economic value.

Conclusions

Pollen grain germination and viability of *Berberis mikuna* decreased significantly after 45 days of its collection and the obtained results varied according to the temperature treatment. Germinability and viability decreased with increasing temperature, although viability in T45 Ref and T45 Fr were which could mean that the temperature required for a long shelf life should be even lower. These responses could be explained by the different fibrillar character of the intine, as well as by decrease in starch reserve in pollen

grains stored at 5°C and at room temperature. These results are relevant for the knowing of the pollen grain physiology and particularly when focusing with the breeding programs.

CRediT author statement

Silvia Radice: conceptualization, methodology, validation, formal analysis, investigation, resources, writing, visualization, supervision, project administration, funding acquisition; **Beatriz Galati:** methodology, validation, investigation, resources, writing, visualization, supervision; **Gabriela Zarlavsky:** methodology, writing, visualization, supervision; **Miriam Arena:** methodology, investigation, writing, visualization, supervision.

Acknowledgments

This work was founded by the Projects PIO/17-06-SR-008(University of Morón). The authors thank to Dr. Marina Gotelli for her english revision.

References

Alba, V., Bisignano, V., Alba, E., De Stradis, A., &Polignano, G. B. 2011. Effects of cryopreservation on germinability of olive (*Olea europaea* L.) pollen. Genetic Resources and Crop Evolution,58(7): 977-982.

Arambarri, A.M., Freire, S.E., Colares, M.N., Bayón, N.D., Novoa, M.c., Monti, C., Stenglein, S.A. 2006. Leaf anatomy of Medicinal Shrubs and Trees from Gallery Forests of the Paranaense Province (Argentina). Part 1. Bol. Soc. Argent. Bot. 41 (3-4): 233 – 268.

Arena, M.E., Giordani, E., Radice, S**.** 2013. Phenological growth and development stages of the native Patagonian fruit species *Berberis buxifolia* Lam. Journal of Food, Agriculture & Environment. Vol. 11 (3-4): 1323-1327.

Arena, M.E. 2016. Estudio de algunos fenómenos morfofisiológicos y cambios bioquímicos en *Berberis microphylla* G. Forst. (sinónimo*B. buxifolia*) asociados a la formación y maduración de frutos en Tierra del Fuego y su relación con la producción de metabolitos útiles. PhD Thesis, Universidad Nacional del Sur, 232 p.

Bilska-Kos, A., Solecka, D., Dziewulska, A., Ochodzki, P., Jończyk, M., Bilski, H., & Sowiński, P. 2017. Low temperature caused modifications in the arrangement of cell wall pectins due to changes of osmotic potential of cells of maize leaves (Zea mays L.). Protoplasma, 254(2): 713-724.

Bots, M., & Mariani, C. 2005. Pollen viability in the field. Research Reports No. CGM, 5.

Brickell. C.H. 1989. The Royal Horticultural Society gardeners encyclopedia of plants and flowers. Dorling Kindersley Publishers.

Cabido, M., Acosta, A., Díaz, S. 1990. The vascular flora and vegetation of granitic outcrops in the upper Córdoba mountains, Argentina. Phytoconenologia19 (2): 267-281.

Ceribelli A. 2018. *Berberis mikuna* Job, specie endemica argentina potenziale fonte alimentare di antiossidanti. Thesis. Florence University. 47pp.

Dafni, A. and Firmage, D. 2000. Pollen viability and longevity: Practical, ecological and evolutionary implications. In: Dafni, A., Hesse, M. y Pacini, E. (eds). Pollen and Pollination. Viena, Austria. Springer-Verlage. 113-132.

Dominguez Diaz, E, 2010. Flora de interés etnobotánico usada por los pueblos originarios: Aónikenk, Selk'nam, Kawésqar, Yagan y Haush en la Patagonia Austral. Dominguezia26(2), 19-29.

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.

Fajardo Morales, V, Podestá, F, Urzúa, A. 1986. Reseña de los alcaloides encontrados en el género *Berberis*de Chile. Rev. Lat. Quím. : 16: 141-156.

Fajardo Morales, V. 1987. Estudio químico de las especies chilenas del género *Berberis*. Rev. Lat Quím: 18: 46-50.

Fang, K., Wang, Y., Yu, T., Zhang, L., Baluška, F., Šamaj, J., & Lin, J. 2008. Isolation of de-exined pollen and cytological studies of the pollen intines of *Pinus bungeana* Zucc. Ex Endl. and*Picea wilsonii* Mast. Flora-Morphology, Distribution, Functional Ecology of Plants, 203 (4): 332-340.

Franchi, G. G., Nepi, M., Dafni, A., &Pacini, E. 2002. Partially hydrated pollen: taxonomic distribution, ecological and evolutionary significance. Plant Systematics and Evolution,234 (1-4), 211-227.

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

Guarnieri, M., Speranza, A., Nepi, M., Artese, D., Pacini, E. 2006. Ripe pollen carbohydrate changes in *Trachycarpus fortunei*: the effect of relative humidity. Sexual Plant Reproduction,19(3), 117.

Job, M.M. 1942. Los *Berberis*de la región del Nahuel-Huapi. Rev del Museo de la Plata (Sección Botánica) 5: 21-72.

Heslop-Harrison, J. 1987. Pollen Germination and Pollen-Tube Growth. International Review and Cytology 107: 1-78.

Kim, Y.-D., Kim, S.-H., Landrum, L.R. 2004. Taxonomic and phytogeographic implications from ITS phylogeny in *Berberis* (Berberidaceae). J Plant Res 117:175–182.

King-Tang, C., & Ping-Li, W. 1983. Study on the pollen morphology of the family Berberidaceae. Journal of Systematics and Evolution,21(2), 130-142.

Li, X., Zhang, L., Li, W., Yin, X., & Yuan, S. 2017. New taxa of *Berberis* (Berberidaceae) with greenish flowers from a biodiversity hotspot in Sichuan Province, China. Plant diversity,39(2): 94-103.

Lim, C. Y., Kim, D. S., Lee, K. J., Hwang, K. A., Choo, Y. K., Ko, K. 2014. Optimization of storage temperature for the pollen viability of transgenic plants that express the anti-breast cancer monoclonal antibody mAb BR55. Plant Omics, 7(5): 403-409

Mokhber-Dezfuli, N., Saeidnia, S., Reza Gohari, A. and Kurepaz-Mahmoodabadi, M. 2014. Phytochemistry and Pharmacology of *Berberis* species.Pharmacogn Rev 8(15): 8–15.

Orsi, M.C., 1984. Berberidaceae, in: Correa, M.N. (Ed.), Flora Patagónica. INTA, Buenos Aires, pp. 325-348.

Polop, J.J. 1989. Distribution and ecological observations of wild rodents in Pampa de Achala, Córdoba, Argentina, 1986. Studies on Neotropical Fauna and Environment 24 (2): 53-59.

Radice, S., Arena, M.E. 2016. Characterization and evaluation of *Berberis microphylla* G. Forst pollen grains. Adv. Hort. Sc. 30 (1): 31-37.

Ruiz-Rodríguez, B.M., Morales, P, Fernández-Ruiz, V., Sánchez-Mata, M.C., Cámara, M., Díez-Marqués, C., Pardo-de-Santayana, M., Molina, M., Tardío, J. 2011. Valorization of wild strawberry-tree fruits (*Arbutus unedo* L.) through nutritional assessment and natural production data. Food Res Int 44: 1244-1253.

Sáenz de Rivas, C. 1976. Sobre la nomenclatura palinológica: la esporodermis. In Anales del Instituto Botanico A. J. Cavanilles. Madrid, España 33(1): 159-177.

Shaffer, J.E. 1985. Inotropic and chronotropic activity of berberine on isolated Guinea pig Atria. J Cardiovascular Pharm 7: 307- 315.

Shivanna, K. R.2019.Pollen biology and biotechnology. CRC Press. 301 pp.

Stone, J., Thomson, J. y Dent-Acosta, S. 1995. Assessment of pollen viability in hand pollination experiments: A review. American Journal of Botany 82(9): 1186-1197.

Ur, S. R., Khan, S. M., Zafar, M., Ahmad, M., Khan, R., Hussain, S. & Kayani, S. I. 2019. Pollen morphological variation of *Berberis* L. from Pakistan and its systematic importance. Microscopy research and technique,82(9), 1593-1600.

Urquieta, C. A. P. 2010. Evaluación de la viabilidad polínica de cuatro especies pertenecientes al género *Berberis* L. (Berberidaceae), Doctoral Tesis Facultad Austral de Chile. 75pp.

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.

Zarlavsky G. E. 2014. Histología Vegetal: Técnicas Simples y Complejas. Soc. Argen.Bot., Buenos Aires, Argentina.

Zuloaga, F.O., Morrone, O., Belgrano, M.J. 2008. Catalogue of the vascular plants of the southern cone (Argentina, southern Brazil, Chile, Paraguay and Uruguay). Volume 2:*Dicotyledoneae: Acanthaceae-Fabaceae (*Abarema-Schizolobium*)*. Missouri Botanical Garden Press.

Figure 1. Viability and germinability of *Berberis mikuna* pollen grains. **A**, pollen grain viable (green) and not viable (red), **B**, pollen grains germinated. Bars =100µm.

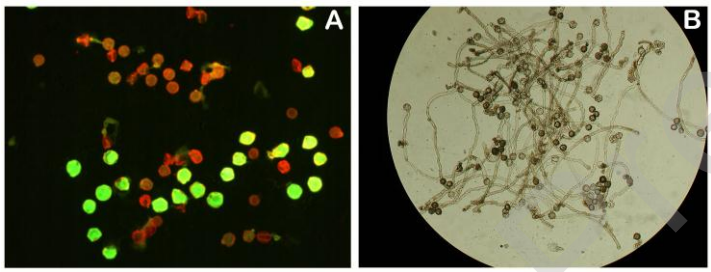

Figure 2. Anther of *Berberis mikuna* (light micrographs). **A**, Section of anther with pollen grains at initial time (**T0 RT**); **B**, pollen grains of **T45 RT**; **C**, pollen grains of **T45 Ref**; **D**, Pollen grains of **T45 Fr.,** epidermis (**ep**), endothecium (**en**), middle layers (**ml**), pollen grain (**pg**), nucleus (**n**). Bars =50µm.

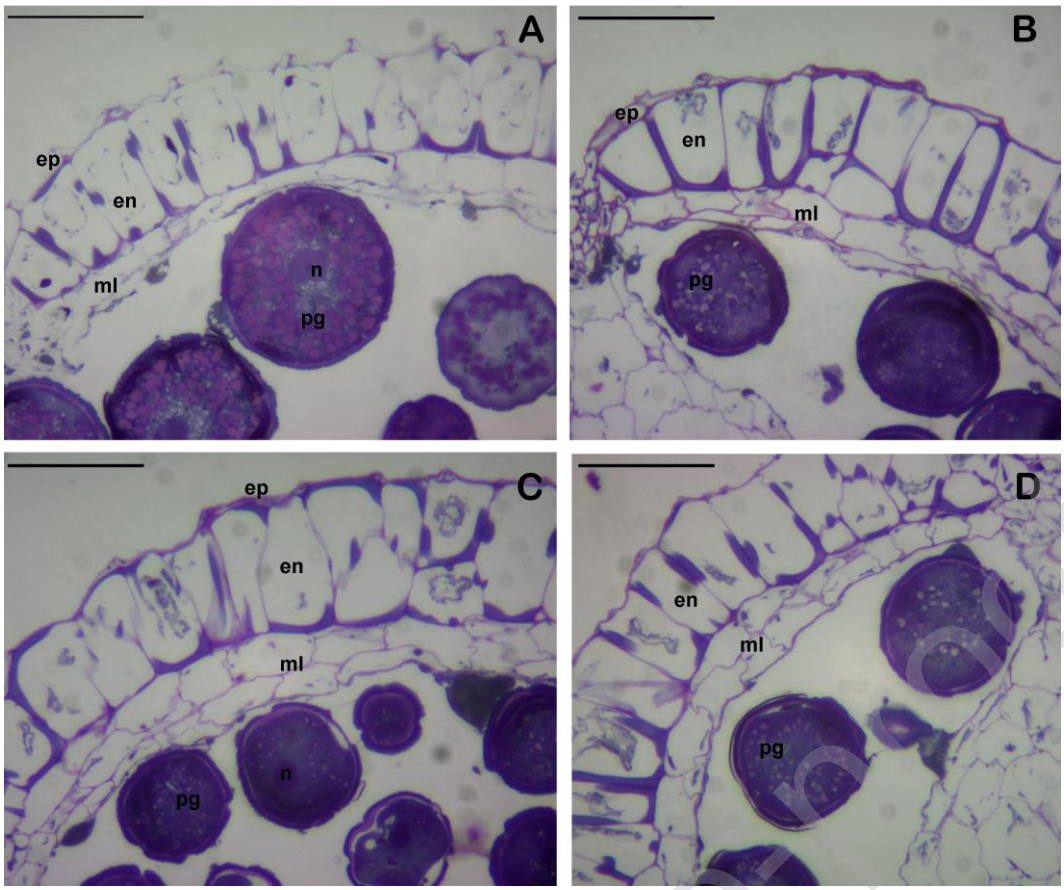

Figure 3. Anther of *Berberis mikuna* (TEM micrographs). **A-B**, treatment **T0 RT**; **C-D**, treatment **T45 RT**; **E-F**, treatment **T45 Ref**; **G-H**, treatment **T45 Fr A, C, E, G**, detail of the cytoplasmic content of the vegetative cell; amyloplast (a); lipidic globules (arrow head); **B, D, F, H,** detail of pollen grain wall; exine (e); intine (i); **B**, treatment Bars: **A, F, G, H** = 1 µm; **B-E** = 2 µm.

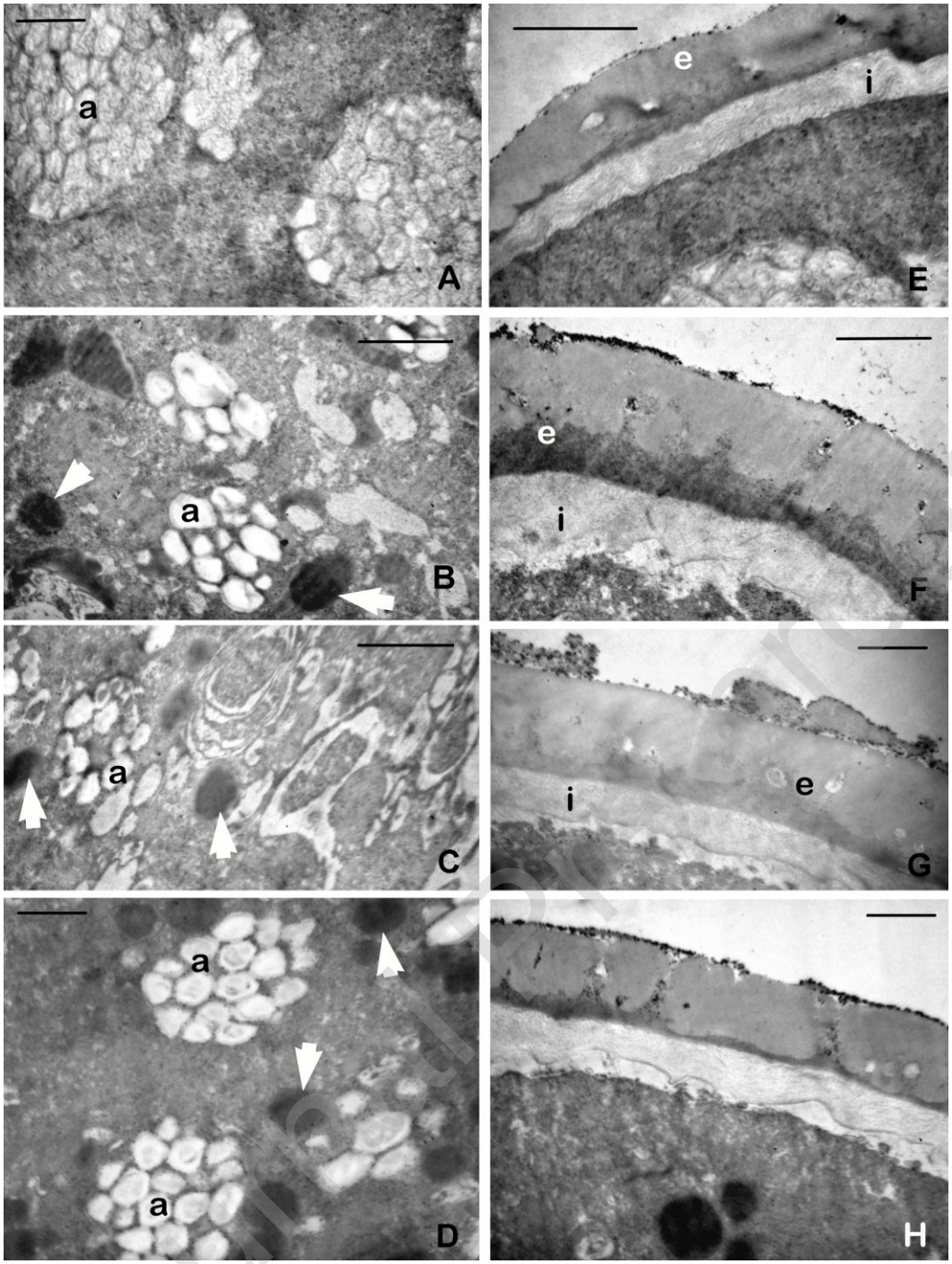

Table 1. Pollen grain germination and viability of *Berberis mikuna* evaluated after 24 hours (**T0 RT**) of collection and 45 days after storage at21±3 °C (**T45 RT**); 5 °C (**T45 Ref**) and -20 °C (**T45 Fr**).

Germinability (%)			
T0 RT	**T45 RT**	**T45 Ref**	**T45 Fr**
60.5a	1.0c	30.0b	49.0b
Viability (%)			

52.33a	3.0c	38.6b	30.0b

Different letters in each line indicate significant differences according to the Tukey test at $p \leq 0.05$