Internal morphology and histology of blueberry *Vaccinium corymbosum* L. (Ericaceae) in Lima, Peru

Morfología interna e histología de arándano *Vaccinium corymbosum* L. (Ericaceae) en Lima, Perú

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ABSTRACT

Although there is a lot of information about cultivation, use and medicinal properties of blueberry *Vaccinium corymbosum*, there is still little information about its internal morphology and histology. Therefore, we proposed to know more of those aspects and to understand the low seed germination. The material used was composed of seeds and mature plants obtained from a farm located in Trujillo, Peru. All histological work was made in the Laboratory of Plant Anatomy and Pharmacognosy belongs to Faculty of Biological Science in Universidad Nacional Mayor de San Marcos in Lima, Peru. Each organ was analyzed in cross and longitudinal sections, as well as in external or superficial view. Lugol and Sudan IV were used for seed sections, Safranin for stem and root sections, and Lugol for leaf sections. We found some variation in seed size and color, being assigned to the category of oil seeds. Germination was limited by the embryos viability, as well as thickness of the seed coat. Stems and roots have punctuated xylem vessels, which facilitate the lateral water transport. The radical system is highly branched, apparently due to mycorrhizal symbiosis. Leaves are bifacial, with all the stomata on the abaxial side, and features that are characteristic of C_3 plants.

Key words: leaf anatomy, root anatomy, seed anatomy, stem anatomy.

RESUMEN

Aunque existe mucha información sobre el cultivo, uso y propiedades medicinales de *Vaccinium corymbosum* “arándano” hay poca información básica sobre su morfología interna e histología. Por lo tanto se propuso conocer más sobre estos aspectos y entender la baja germinación de semillas. Se trabajó con semillas y planta madura obtenidas de una hacienda ubicada en Trujillo, Perú y todo el trabajo histológico se realizó en el Laboratorio de Anatomía y Farmacognosia Vegetal de la Facultad de Ciencias Biológicas de la Universidad Nacional Mayor de San Marcos en Lima, Perú; se hicieron cortes transversales, superficiales y longitudinales a mano alzada de cada órgano; se le agregaron lugol y sudan IV para los cortes de semilla, safranina para los cortes de tallo y raíz, lugol para los cortes de hoja. Se observaron diferencias en el tamaño forma y color de las semillas, se determinó que son semillas oleaginosas y que la germinación está limitada por la viabilidad de los embriones así como por el grosor de la testa. Los tallos y raíces presentan vasos xilemáticos punteados, lo que facilita el transporte lateral del agua. También se observó que el sistema radical está muy ramificado debido a simbiosis con micorrizas. Las hojas son bifaciales y con fotosíntesis C3, con la totalidad de los estomas en la cara abaxial.

Palabras clave: anatomía de la hoja, anatomía de la raíz, anatomía de la semilla, anatomía del tallo.

Introduction

The blueberry *Vaccinium corymbosum* L., native from Eastern North America (Missouri Botanical Garden, 2017), is widely accepted in the international market, mainly because of its several antioxidant agents (Dastmalchi *et al*., 2011; Namiesnik *et al*., 2014), which currently have a significant impact on human health, whether due to its bactericidal effects (Pervin *et al*., 2013), hepatoprotective effects (Madrigal *et al*., 2014), as reducer of hyperglycemia (Roopchand *et al*., 2013; Aktan *et al*., 2014), or as reducer of adipose tissue inflammation, thus reducing insulin resistance (De Furia *et al*., 2009), among some examples.

For these reasons, Peruvian blueberry exports, with the United States, Hong Kong and Holland reached $11 millions US dollars from January to September 2014 (Yaipén, 2014); this activity increased by 230% compared to 2013 (Agronegocios, 2014).

However, it is important to consider that large-scale production of this species depends on its phytosanitary status,

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as blueberry is affected by fungi, such as *Calonectria colhounii* (Beom *et al*., 2010), or nematodes, such as *Meloidogyne carolinensis* (Eisenback, 1982), *Xiphinema americanum* (Clark and Robbins, 1994), and *Mesocriconema ornatum* (Jagdale *et al*., 2013).

Despite the information available on the cultivation and properties of blueberries in general, very little information is available on their histology, except for the publication of Rabaey *et al*. (2006), who studied the micro morphology of xylem vessel walls in several species of the order Ericales, and the work of Konarska (2015), who made a histological study of blueberry fruits. Both studies used optical microscopy, as well as scanning electron microscopy. The objectives of our study were to reveal the internal anatomy and histology of seeds and vegetative organs of *V. corymbosum* and to give knowledge about low germination of seeds.

Materials and methods

The work was done using different organs from a healthy mature plant and seeds from mature fruits obtained from a farm located in Trujillo, Peru, with an average of annual temperature of 23°C and an average of relative humidity of 84%. All histological work was made in the Laboratory of Plant Anatomy and Pharmacognosy of the Faculty of Biological Science in Universidad Nacional Mayor de San Marcos in Lima, Peru.

When fruits were opened the seeds were selected by size and color (seed coat remaining with the largest and brown). Cross, longitudinal, and superficial sections were made for each organ. The stains Lugol and Sudan IV were used for seed sections, in order to determine the type of reserve substance present in the endosperm. Seeds contained in mature fruits were used for measurements and were kept cold storage until needed. Three seeds were used per section type and measurements were done in ten fields per section.

For stem and root sections, we used Safranin, in order to determine types of xylem vessels, since these characters allow us to understand the dynamics of water transport in the plant. Leaf sections were stained with Lugol and water, in order to identify the probable type of photosynthetic syndrome, as well as to determine the type of stomata present, and their distribution on both sides of the leaf.

A Leica DM 750 microscope (Leica, Wetzlar, Germany), with built-in camera Leica ICC50HD (Leica, Wetzlar, Germany), was used for the micrographs. Shots were made at 400X.

A seed viability test was also run, using the Tetrazolium reagent (TZ) (Ruiz, 2009), for which 12 seeds were first placed in water for 48 hours. After that time, small cuts were made on the seed coat so that the reagent could penetrate the seed and reach the embryo. Then, seeds were placed in a vial, submerged in the TZ for 24 h in darkness. Finally, seeds were opened to verify if embryos acquired the red color, indicative of viability. Hundred seeds were placed on moist filter paper, on a Petri dish, in order to see their germination.

Results and discussion

The fruits contain many seeds, but these vary in size, coloration and in state of maturity, the mature ones having dark brown seed coat, as reported by Kloet and Cabilio (2010) and Chaparro (1999) for *Vaccinium floribundum*, and Castro (2012) for *Vaccinium meridionale*.

Seeds have an ornamented episperm, and a dark brown color when ripen (Fig. 1). We also observed a difference in size among mature seeds, varying from 0.49 to 1.02 mm long, and 0.44 to 0.61 mm wide.

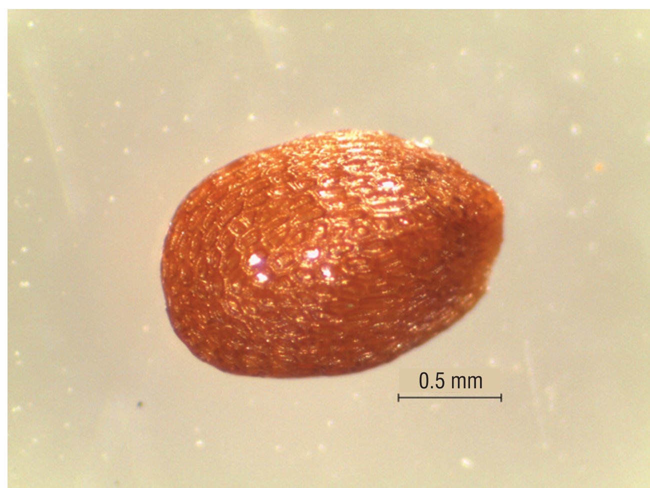

FIGURE. 1. Surface view of *V. corymbosum* seed, showing ornamentations in the seed coat.

In making seed cross sections, we observed that the seed coat is made by sclerenchymatic tissue composed of cells with very thick walls of 13.41 µm thick on average (Fig. 2). Towards the interior is the endosperm, which tested positive for lipids when reagents Lugol and Sudan IV were added (Fig. 3).

Regarding the Tetrazolium test, we found that out of 12 seeds tested with the reagent, only 6 responded positively with the change of color to red (Fig. 4). From 100 seeds that were plated, only eleven germinated, which indicates that of the 50% of viable embryos only a few germinate, this probably due to the presence of inhibitors of germination.

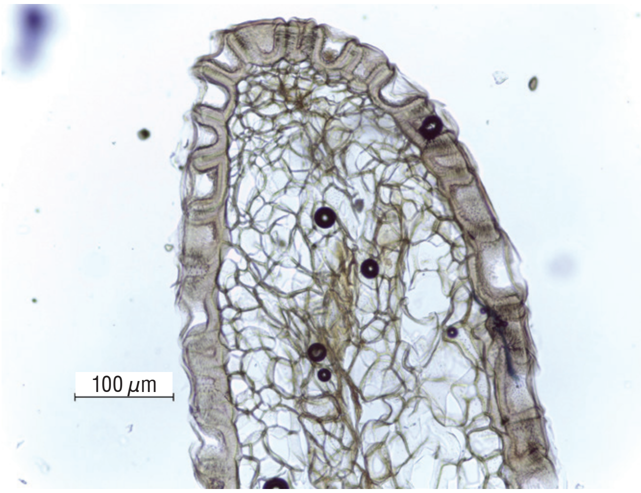

FIGURE 2. Cross section of seeds of *V. corymbosum* showing the seed coat made of sclerenchymatic tissue, with very thick walls.

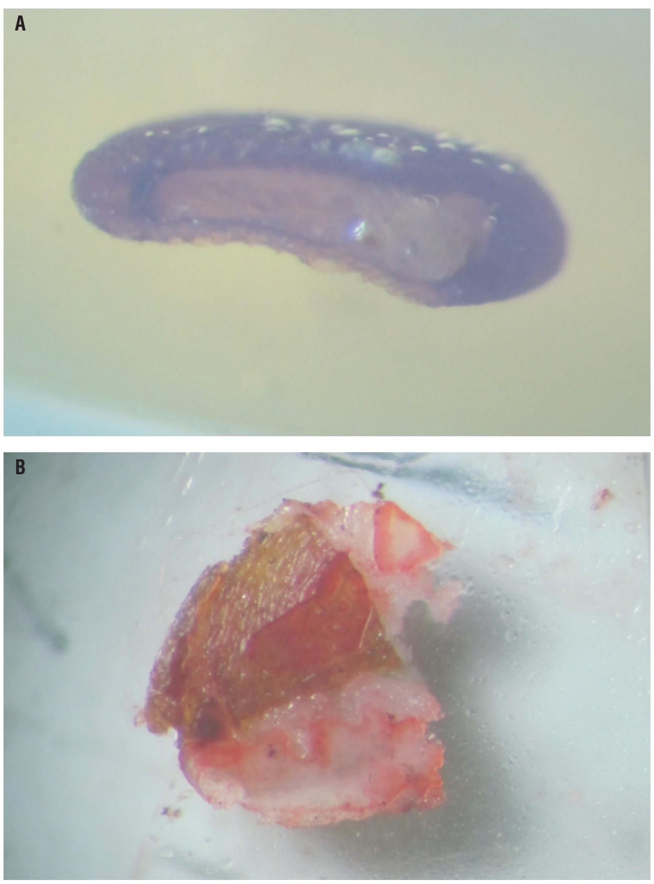

FIGURE 3. Sections of seeds after Lugol (A) and Sudan IV (B) were added.

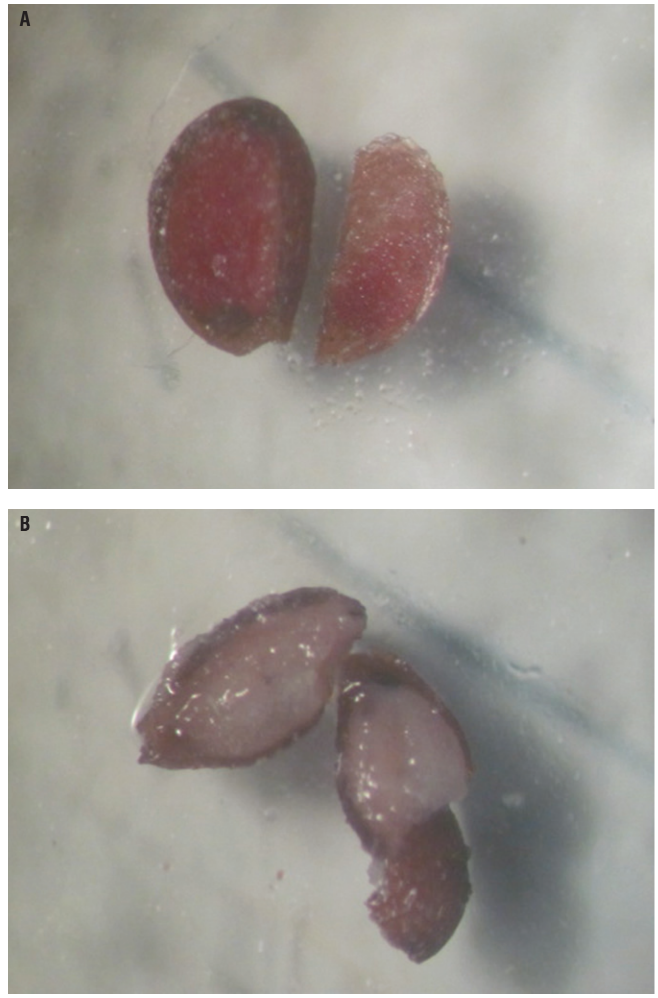

FIGURE 4. Seed sections of *V. corymbosum* (at 50X), after being treated with Tetrazolium. A. Positive reaction; B. Negative reaction.

Mature seeds present an episperm made of cells with thickened walls (Fig. 2) but endosperm reserves lipids (Fig. 3). The Tetrazolium test showed 50% viability of the embryos (Fig. 4) and germination was very low (11%), agreeing with Kloet and Cabilio (2010), who divided plants of *V. corymbosum* in three populations, one of them (the artico-alpino-boreal taxa), characterized by seeds with thick episperm, what gives them the possibility of remaining several years in the soil without germinating. In contrast, Castro (2012) obtained more than 60% germination rate in seeds of *Vaccinium meridionale*.

Thus, vegetative propagation is the alternative used by the vast majority of farmers, although this system of propagation also has its disadvantages, as it is slow, very expensive, and generates few plants from one, so there is low yield and spread of diseases (Victorian, 2010), although tissue culture techniques are presented as very good alternatives (Rache and Pacheco, 2010).

Cross sections of the stem showed a secondary structure typical of any dicotyledonous plant, although with a very thin peridermis, which allows the entrance of light. For that reason chloroplasts are found in the cortical parenchyma. The vascular bundles are opened collateral type IN the presence of a vascular cambium, and what stands out is the large amount of sclerenchyma between the phloem and xylem vessels, which gives rigidity to this organ (Fig. 5).

FIGURA 5. Cross section of stem of *V. corymbosum*, showing thin peridermis and parenchyma with chloroplast and abundant sclerenchyma, at 400X.

The longitudinal section of the stem shows the presence of two types of metaxilematic vessels, dotted and scalariform (Fig. 6).

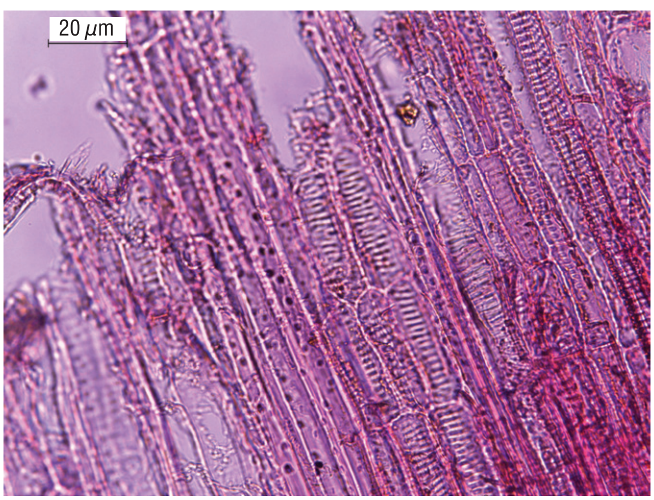

FIGURE 6. Longitudinal section of stem of *V. corymbosum*, showing two kinds of xylematic vessels (dotted and scalariform), at 400X.

The root system of this species is characterized by being of the axonomorphic type, and profusely branched, reason why we could only make sections of the thicker branches with secondary structure. The rootlets in the primary structure are very thin and could not be extracted completely from the substrate.

The cross section of the main root shows a peridermis size larger than the stem. The cortical parenchyma is not very large, and it is followed by a phloem full of reserve products, which are also observed along the medullary radius in the xylem. Otherwise, the area where the xylem is located is the widest with a large amount of sclerenchyma, which gives great rigidity to this organ (Fig. 7).

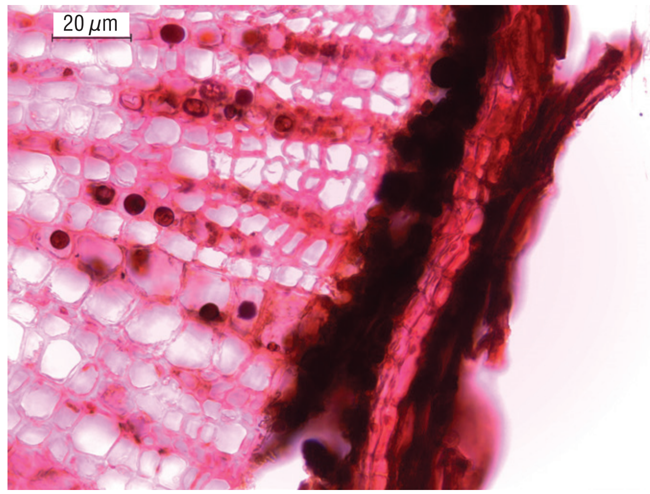

FIGURE 7. Cross section of roots of *V. corymbosum*, showing thicker peridermis, many reserve products and sclerenchymal tissue, at 400X.

In the longitudinal section of the root, we can observe the presence of xylematic vessels of the dotted type, as well as the scalariform type, as found in the stem (Fig. 8).

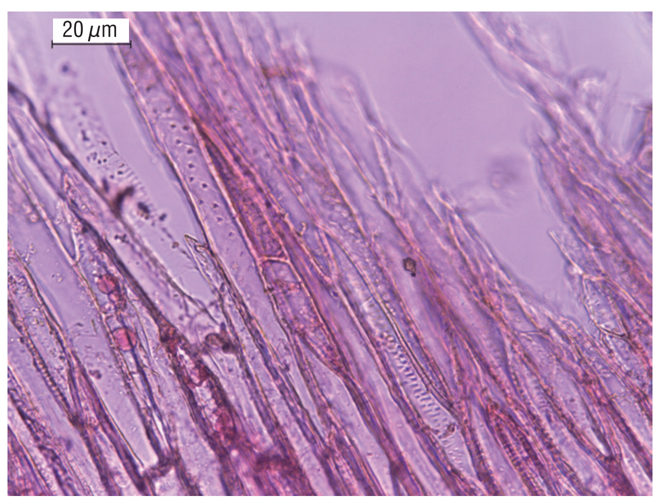

FIGURE 8. Longitudinal section of root of *V. corymbosum*, showing also dotted and scalariform vessels, at 400X.

The radical system of *V. corymbosum* is very branched due to the fact that the species has symbiosis with mycorrhizal fungi (Kloet and Cabilio, 2010) and these are the ones inducing, through the substances that secrete, the response in the root system (Bellini *et al*., 2014). This symbiotic association results in roots that adapt their internal anatomy by presenting small diameter xylem vessels, which gives the organ less risk of cavitation and embolism (Valenzuela *et al*., 2008), but also have xylematic vessels that are punctured in both the root and the stem (Figs. 6 and 8), giving the plant the advantage of a better lateral transport of water (Rabaey *et al*., 2006).

The cross section of the leaf shows a bifacial anatomy, with palisade and spongy parenchyma, with thin cuticle on both sides of the leaf, also presenting little sclerenchyma and more cells of the mesophyll, characteristics of leaves C3 (Basso and Barbero, 2015). By adding Lugol to the cross sections of leaves (Fig. 9), all cells of the mesophyll give a positive reaction to the presence of starch, which is a typical response of plants with C3 photosynthesis and confirm anatomic observations.

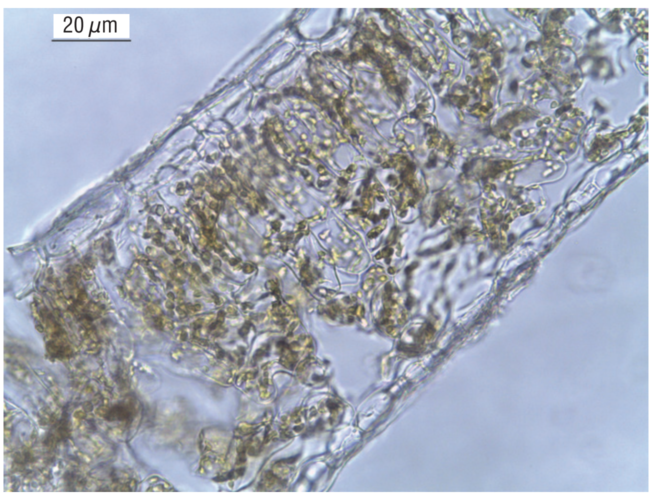

FIGURE 9. Cross section of the leaf of *V. corymbosum,* showing palisade and spongy parenchyma, thin cuticles, and stomata on abaxial side, at 400X.

In the superficial view, the stomas are distributed in their entirety on the abaxial side of the leaf (hypoestomatic), these stomata are of the paracytic type. The epidermal cells were characterized by sinuous and irregular borders, and no trichomes were observed on either side of the leaf (Fig. 10), indicating that leaves are always in a horizontal position. However, having paracytic stomata indicates that this species is of the mesophytic type, i.e. plant that requires intermediate conditions of humidity, not very resistant to drought.

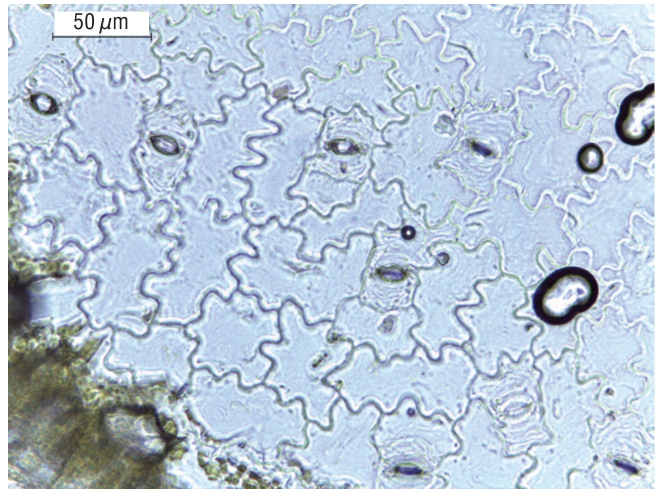

FIGURE 10. Superficial view of abaxial epidermis of the leaf of *V. corymbosum*, showing paracytic stomata, at 400X.

Conclusions

The difficult germination of seeds is mainly due to low viability of embryos as well as to hardness of seed coats and presence of metabolites inhibitors of germination. Furthermore and because of being a mesophytic plant, the selected material not resists well drought conditions so this species presents xylematic vessels that distribute better the water in a radial way, improving transpiration dynamics with all the stomata on the abaxial side. Finally, portions of stem and roots wouldn´t good explants for plant tissue culture system because of presence of high quantity of schlerenquima.

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