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Variability in floral traits and reproductive success among and within populations of *Berberis microphylla* G. Forst., an underutilized fruit species

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ABSTRACT

Berberis microphylla G. Forst. (calafate) is an evergreen and spiny shrub considered as a non-timber patagonian forest product, that is relevant for diversification of agrifood production, particularly interesting since its black–blue fruits are rich in phenolic compounds. The objective of this research is to analyze the variability in floral traits and reproductive success of *Berberis microphylla* G. Forst. among and within three populations of Tierra del Fuego along three growing seasons. The presence of variability in some floral traits as well as in the reproductive success of *B. microphylla* among and within three Tierra del Fuego populations was observed, in agreement with the environmental conditions i.e. mean daily temperatures and accumulated rainfall for the three populations and the three growing seasons. Flower dry weight and gynoecium area are good indicators of flower quality (i.e. ovule number), with positive and significant correlations between them (flower dry weight with gynoecium area, $r = 0.551$; $p = < 0.001$, and flower dry weight with ovule number, $r = 0.407$, $p < 0.001$). Pollen/ovule, seed/ovule, fruit/flower and fecundity indices are also good indicators not only of flower quality but also of the reproductive success. The multivariate analysis allowed to analyze jointly the whole measured variables, and explored the influence of annual climatic variability in the response of plants and populations. The variables with great changes among years were those representing quantities (the numbers of ovules and pollen grains) as well as some of the related with size (gynoecium elongation and pollen grain size). Likewise, the influence of each variable in the population split was highlighted at each growing season, which helps to understand the drivers of the differences among them. Plants with a highlight performance were detected and could be selected for their clonal propagation and ex-situ evaluation for the beginning of a breeding program.

1. Introduction

Presence of plant phenotypic variability leads to plant phenotypic plasticity, that is the capacity of a single genotype to change its phenotype in response to the environment, determines the range of conditions under which an individual can survive and reproduce (Atlan et al., 2015). Phenotypic plasticity can be present in different plant organs and functions and could be observed through its changes in phenology, morphology, anatomy, composition, and the three major functions that are basic for plant growth and development like photosynthesis, respiration and transpiration. Phenotypic plasticity in plant functional traits is thought to assist rapid adaptation to new living conditions and provide a buffer against rapid environmental changes (Dai et al., 2017). However, morphological traits are useful for preliminary assessment because they facilitate fast and simple evaluation and can be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions. Morphological characterization combined with multivariate statistical methods, such as principal component analysis (PCA), the most commonly applied, are useful tools for screening accessions (Čolić et al., 2012).

Recently, plasticity in plant reproductive traits has received substantial attention in the context of climate change. These studies have indicated that floral traits such as flowering phenology and duration, floral size as well as mating pattern, could shift in accordance with changes in environmental conditions (Dai et al., 2017). However, relatively few studies have investigated how geography, environmental factors, and genetics affect floral trait variation (Lankinen et al., 2017), as was observed for *Polygala vayredae* (Castro et al., 2008) and *Vaccinium meridinale* (Chamorro and Nates-Parra, 2015). Exploring plasticity of reproductive traits in perennial woody species is difficult because it

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requires the growth of sexually mature plants, which can take several years, and necessitates also long-term monitoring of potentially large individuals in controlled environments (Atlan et al., 2015).

Berberis microphylla G. Forst. (calafate) often grows in differentiated environments in Tierra del Fuego such as coastal scrubs, *Nothofagus* forest margins and clearings, moister areas in grass steppes, and along streams and rivers (Moore, 1983). It is an evergreen, spiny and erect medium size shrub, with a reproductive pattern based on both seedling recruitment and clonal development by rhizomes (Arena and Radice, 2014), that belongs to the so-called group of minor or underutilized fruit tree species that are relevant for diversification of agrifood production. It is classified as a non-timber forest product (Tacon Clavaín, 2004), particularly interesting since its black–blue fruits are rich in phenolic compounds (Arena et al., 2012; Ruiz et al., 2013, 2014; Ramirez et al., 2015; Reyes-Farias et al., 2015) and can be consumed fresh and processed in marmalades and jams, in non-alcoholic beverages and in ice creams. Also, *B. microphylla* is considered an excellent ornamental shrub for their foliage, quality of its flowers and abundant flowering, ideal for protecting gardens and orchards (Bottini, 2000). A recent research indicated that it is appreciated by local rural populations also as fuelwood (Cardoso et al., 2015). At present, commercial barberry orchards are being planned due to its economic potential related to flavour, taste and nutraceutical properties of the fruits. Some aspects of the phenological phases, flower anatomy, fruit composition, postharvest and production, and the annual cycle together with the vegetative morphological variation were already studied in natural populations of this species (Arena et al., 2003; Arena and Curvetto, 2008; Arena et al., 2011, 2013a, 2013b, 2017; Arena and Radice, 2014; Radice and Arena, 2017; Rodoni et al., 2014; Giordani et al., 2016). The objective of this research is to analyze the variability in floral traits and reproductive success of *Berberis microphylla* G. Forst. among and within three populations of Tierra del Fuego along three growing seasons.

2. Materials and methods

2.1. Plant material and growing conditions

Plants growing near Ushuaia city (*US*) ($n = 12$), bordering Fagnano lake (*FL*) ($n = 12$) and central area of the Tierra del Fuego island (*CI*) ($n = 10$) were selected and the height, maximum diameter, shape (domed, rounded, broadly rounded, semi rounded and cushion-like, according to Lenard, 2008), reproductive area, proximity to another plants (0 = low proximity, > 3 m); 1 = medium proximity, between 1 and 3 m; 2 = high proximity, < 1 m), shading (0–100%) and geographical position were registered (Table 1). The mean air daily temperatures, mean environmental relative humidity and cumulative rainfall were also registered for every situation since October to March for the 2014–2015, 2015–2016 and 2016–2017 growing seasons (Table 2). The soil nitrogen (N) was determined using the Kjeldahl technique using a Bϋchi K350 (Bϋchi, Flawil, Switzerland), while carbon (C) and phosphorus (P) soil concentration were determined with a plasma emission spectrometry (ICPS 1000 III, Shimadzu, Kyoto, Japan).

2.2. Sampling and determinations

Yellow flower buttons on phase 59 according to Arena et al. (2013a) ($n = 50$) were collected from the North, East, South and West sectors of each plant and were kept refrigerated until their use for the following determinations:

Flower dry weight: yellow flower buttons ($n = 10$) were dried in an oven at 50 °C for 7–10 days until constant weight was reached.

Gynoecium measurements: pistils were taken from the yellow flower buttons ($n = 10$) and then they were scanned to obtained the gynoecium area, gynoecium perimeter and gynoecium elongation (ratio of the length of the major axis to the length of the minor axis) using the UTHSCSA Image Tool software (San Antonio, TX, USA) (Giordani et al., 2016).

Ovule number: number of ovules in each scanned pistil ($n = 10$) was counted.

Pollen grain size: equatorial and polar diameters of the pollen grains ($n = 20$, 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, according to Radice and Arena (2016a).

Pollen grain germination: the pollen grain germination was registered ($n = 500$) according to Radice and Arena (2016a), during 2015 and 2016 springs. Pollen grains were put on micro drops of a saline solution composed of 2×10^{-3} M H_3BO_3 and 6×10^{-3} M Ca $(NO_3)_2$ added with sucrose 30 g L. 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.

Pollen grain number: the Neubauer hemocytometer was used to count the pollen grains following Godini (1981) ($n = 3$). Briefly, 6 anthers per flower were macerated with 1 ml of water and centrifuged at 2000 rpm during 10 min. Then, 10 μl of the supernatant were introduced into the Neubauer camera.

Pollen grain number/ ovule number (pollen/ovule): this ratio was calculated ($n = 3$) using the pollen grain number per flower and the mean ovule number per ovary.

Seed number/ ovule number (seed/ovule): this ratio was calculated using the mean number of seeds per fruit when the fruits were harvested and the mean number of ovules per ovary.

Fruit number/ flower number (fruit/flower): one-year-old shoots ($n = 8$) were chosen taking into account the plant and shoot orientation (North, South, West or East), according to Arena et al. (2011).

Fecundity rate: this value is the product of two ratios according to Cruden (1972): seed/ ovule and fruit/ flower (Silva and Pinheiro, 2009).

2.3. Statistical analysis

The results were analyzed for each population and each year by ANOVA and Tukey Test ($p < 0.05$). Correlations between pairs of variables were also made. Principal Component Analysis (PCA) was performed to explore multivariate relations between populations and plants at the three growing seasons, evaluating the influence of seven measured variables (flower dry weight, gynoecium area, gynoecium perimeter, gynoecium elongation, ovule number, pollen grain size and pollen grain number) over the whole sample distribution in an ordination space. PCA analysis included Monte Carlo permutation test ($n = 999$) to assess the significance of each axes. We selected correlation coefficients among columns to obtain the cross-products matrix. PCA was conducted in PCORD version 5.01 (McCune and Mefford, 1999).

3. Results

3.1. Plant material and growing conditions

Size of *FL* plants (1.9 m height and 4.8 m maximum diameter) were highest than *CI* (1.4 m height and 2.9 m maximum diameter) and *US* (1.3 m height and 4.8 m maximum diameter) plants (Table 1). Indeed, productive area was maxima in *CI* plants (69%) than in *US* (52%) and *FL* (41%) plants. In accordance, 58% of *US* plants presented the shrub shapes typical of small shrubs like semi rounded and cushion-like shapes, while only showed the semi rounded shape the 25 and 20% of *FL* and *CI* plants, respectively.

Mean temperatures in *FL* among October to March of 2014–2015 (8.6°C) and 2015–2016 (8.1°C) were higher than in *CI* (8.0°C for