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Genetic diversity and distribution patterns of Ecuadorian capuli (*Prunus serotina*)

Juan J. Guadalupe, Bernardo Gutiérrez, Dámaris P. Intriago-Baldeón, Venancio Arahana, José Tobar, Andrés F. Torres, María de Lourdes Torres*

Laboratorio de Biotecnología Vegetal, Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Campus Cumbayá, Diego de Robles y Vía Interoceánica, l, 17-1200-841 Quito, Ecuador

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ABSTRACT

Prunus serotina subsp. capuli (Cav.) is an arboreal species with promising economic prospects in the timber, health-food and neutraceutical markets. Despite its cultural and commercial significance, limited information exists with regards to the degree of genetic variation and ecological history of *P. serotina* in Ecuador. The main objective of this study was to evaluate the degree of genetic diversity and population structure of Ecuadorian *P. serotina*, as a preliminary step towards understanding the distribution history of this species in Ecuador and establishing germplasm conservation programs. *P. serotina* samples (217, representing 8 provinces from the Ecuadorian highlands) were characterized using 8 heterologous SSR markers derived from related *Prunus* species. Expected heterozygosity across samples ($H_e = 0.71$) reveals a moderate level of genetic diversity for Ecuadorian *P. serotina*. Nevertheless, simple allele-count analysis indicates that Ecuadorian capuli has a narrower degree of allelic richness relative to collections from the species' center of origin in North America. Furthermore, pairwise *F* statistics ($0.0069 \leq Fst \leq 0.0319$) and Nei genetic distance estimates ($0.02 \leq Ds \leq 0.09$) indicate minimal population differentiation within Ecuadorian capuli. However, Bayesian population structure analysis suggests a subtle genetic contrast between germplasm from the Northern and Southern highlands. Certainly, it is of interest to analyze whether this underlying genetic differentiation between the Northern- and Southern-Highland groups is also manifested in morphological, agronomic or other phenotypic characters that could indicate adaptive differences to divergent agro-ecosystems.

1. Introduction

Prunus serotina subsp. capuli (Cav.) is a wild arboreal tetraploid species (2n = 32) native to North America, but widely distributed throughout Central and South America (Fresnedo-Ramírez et al., 2011). Historical records indicate that this species was dispersed from Mexico to Central and South America following the Spanish conquest (Mille, 1942). In Ecuador, *P. serotina* (commonly known as "capuli") is only found along the Andean alley, which begins at the province of Carchi in the North and ends in the province of Loja in the South (Mille, 1942).

* Corresponding author. Tel.: +593 2 297 1700x1746.
E-mail address: ltorres@usfq.edu.ec (M.L. Torres).

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P. serotina has an important commercial value, particularly in North America where it remains an important source for high-quality timber (FIA, 2013). In Central and South America, however, *P. serotina* berries are a valued product and capuli trees commonly serve as windbreakers in agricultural fields (Palacios, 2011). More recently, it has been reported that the leaves and berries of *P. serotina* possess phenolic conjugates with antimicrobial and antioxidant properties which can be used to treat health disorders (Jiménez et al., 2011). Initial surveys indicate that the presence of these compounds in a balanced diet can help reduce the incidence of allergies, cardiovascular disease and other metabolic syndromes related to aging (Vasco et al., 2009). Certainly, opportunities exist to transform this arboreal species into a relevant commodity with national and international projections in the timber, health-food and neutraceutical markets.

Despite its cultural and commercial significance, limited information exists with regards to the degree of genetic diversity, population structure, domestication patterns and ecological history of *P. serotina* in Ecuador. To date, investigations analyzing the extent and geographic distribution of the genetic diversity of this species have primarily concentrated on North American and European germplasm (Downey and Iezzoni, 2000; Pairon et al., 2008, 2010). Amongst these studies, Downey and Iezzoni (2000) included a limited collection of Ecuadorian capuli accessions in their analyses, and have confirmed that Ecuadorian germplasm derives from Mexican ancestry. While this information provides insights into the origin of Ecuadorian capuli, it proves insufficient to accurately describe Ecuadorian *P. serotina* germplasm.

In view of the aforementioned limitations, the main objective of this study was to characterize the degree of genetic diversity and population structure of *P. serotina* in the Ecuadorian highlands, as a preliminary step towards understanding the distribution history of this species in Ecuador and establishing germplasm conservation programs. This information could also prove useful in the development of crop improvement strategies aimed at generating novel capuli varieties which promote their use in Andean agro-ecosystems.

2. Materials and methods

2.1. Plant material: geographic distribution and collection

A total of 217 *P. serotina* samples were collected at individual sites from 8 provinces across the Ecuadorian highlands (Fig. 1). From North to South, the sampling region covered a linear distance of 362 km. Throughout the sampling region, average daily temperatures ranged from 12 °C to 18 °C, and annual precipitation rates ranged between 500 mm and 2000 mm (INAMHI, 2013). Sampling sites were selected based on prior knowledge of the presence of capuli trees, which tend to group in small isolated clusters rather than in established cultivation fields. Collected samples were georeferenced using a Garmin E-Trex Legend HCx GPS system (Garmin International Inc., USA). For each identified sample, young leaves were collected for subsequent laboratory analyses.

2.2. Genetic diversity analysis via heterologous SSR markers

Total genomic DNA was isolated from young leaves using the CTAB procedure described by Kieleczawa (2006). Following extraction, DNA quality and concentration were assessed using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, USA), and samples were diluted to a final concentration of 20 ng/μl.

Twelve nuclear heterologous SSR primer pairs were tested for their applicability in the molecular characterization of *P. serotina* germplasm. These primers were originally designed to amplify microsatellite sequences for species closely related to capuli, including peach (*Prunus persica* L.), sweet cherry (*Prunus avium* L.) and sour cherry (*Prunus cerasus* L.) (Pairon et al., 2008; Downey and Iezzoni, 2000; Dirlewanger et al., 2002; Testolin et al., 2000). Genomic DNA (20 ng) was PCR amplified in a 10 μl reaction containing 50 mM KCl, 20 mM Tris–HCl (pH 8.4), 1.5 mM of $MgCl_2$, 0.2 mM Primer Mix (Forward and Reverse), 0.5 mM dNTPs, and 0.2 U *Taq* Polymerase (Life Technologies, Carlsbad, California). PCR amplification conditions consisted of an initial denaturation at 95 °C for 5 min; followed by 35 cycles of a 45 s denaturation at 94 °C, annealing of 1 min at 48–63 °C depending on the primer pair employed (Table 1), an extension at 72 °C for 45 s; and a final extension period of 8 min at 72 °C. All reactions were performed in a T-Personal Series Thermocycler (Biometra, Gottingen, Germany).

After amplification, PCR products were electrophoresed on 6% (w/v) denaturing polyacrylamide gels at 85 W for 2.5 h, using a Bio-RAD GT Sequencing Cell (Bio-RAD, Hercules, CA) (38 cm × 50 cm). Gel band patterns were visualized after silver staining following the protocol described by Benbouza et al. (2006) and approximate band sizes were estimated using a 10 bp DNA ladder (Life Technologies, Carlsbad, California). Band scoring was performed visually based on allele-size difference.

2.3. Data analyses

A co-dominant allelic matrix (derived from gel banding patterns) was used for the estimation of diversity indexes, genetic distances and population structure. Expected heterozygosity (H_e), used as a measure of genetic diversity, was determined using the TETRASAT software (Markwith et al., 2006). Ten independent runs for the calculation of H_e were performed using randomized population sub-samples ($n = 100$); each including representatives from all sampled provinces. H_e estimates from each iterative run were used to calculate an average H_e value for the entire data set as described by Hanson et al. (2008).

Analyses were performed to explore whether Ecuadorian capuli genetic diversity was structured geographically according to provinces. To this end, Wright's pairwise *Fst* values between provinces were calculated based on allelic frequency