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Genetic Variation in Needle Epicuticular Wax Characteristics in *Pinus Pinceana* Seedlings

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Abstract

Seedlings from each of 12 *Pinus pinceana* populations from throughout the species' range in Mexico were evaluated in a common-garden test to (1) determine the level of genetic variation and genetic structure of epicuticular needle wax quantity, (2) examine differences in wax chemical composition, and (3) seek evidence for an adaptive response in wax composition and quantity across environmental and geographic gradients. Regions and populations within regions showed high variation (38.2% and 10.5%, respectively, of the total variation) in wax quantity. Epicuticular wax recovered from primary needles of *P. pinceana* comprised eight classes. Secondary alcohols (71.7%) were the major homologs identified by gas chromatography. Seedlings from the northern region were separated based on wax composition from seedlings from the central and southern regions by canonical discriminant analysis. A strong differentiation among regions (Q_{STR} = 0.571) and populations within regions ($Q_{STP(R)}$ = 0.384) was observed for wax quantity. Data on wax quantity and chemical composition indicate that physicochemical characteristics of epicuticular wax may show adaptation of *P. pinceana* to local environments.

Key words: environmental adaptation, epicuticular wax characteristics, genetic variation, physicochemical characteristics, *Pinus pinceana*, selection.

Introduction

Pinus pinceana Gordon is a piñon pine occurring in small, isolated populations in three regions along the Sierra Madre Oriental of Mexico, where annual precipitation ranges from about 360 to 800 mm (PERRY, 1991). Despite its rarity, the species is significant because it provides food and shelter for wildlife and people in semiarid woodland ecosystems (PERRY, 1991). Its edible seeds are collected for human consumption, and trees or branches are harvested for firewood and rural construction. It is sometimes called weeping piñon because of its unusual and attractive form and this, along with its variation in needle color and ability to withstand extreme drought, holds appeal for urban planting.

Genetic variation and population genetic structure of epicuticular wax (EW) traits have been little studied in conifers. Quantification of additive genetic variation in wax quantity and the identification of its role in the adaptation of *P. pinceana* to xeric locations may provide useful information to support the development of a conservation strategy. This study's objective was to determine: (1) the level of genetic variation and population genetic structure for wax quantity in *P. pinceana* and (2) the extent to which the variation is adaptive.

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Materials and Methods

Seedlings from six half-sib families in each of 12 populations in the three regions of Mexico where *P. pinceana* occurs naturally were included in this study (*Table 1*). Rainfall average was 30% lower in the northern region than in the southern region (*Table 1*). Thirty seeds from each half-sib family were germinated following the procedure described by RAMIREZ-HERRERA et al. (2008) and seedlings were planted in 3.8 x 21 cm Ray Leach containers (Stuewe & Sons, Inc., Corvallis, OR, USA), filled using a 2:1 peat:vermiculite mix.

Containers were arranged in a randomized block design with regions, populations, and families randomized in each of six blocks. Five seedlings per family were planted in row plots in each block. Seedlings in two blocks were planted 23 d after seedlings in the first four blocks. Seedlings were grown in a greenhouse under the following conditions: 22 °C, 12-h photoperiod, 60% relative humidity; 18 °C, 12-h night, 50% relative humidity. They were watered and fertilized with a 20-08-20 fertilizer solution twice weekly. After 1 year, the seedlings were transplanted to 15 x 15 x 20 cm propagating pots (ROPAK CAN-AM Ltd., Springhill, NS, Canada) filled using a 2:1 peat moss:perlite mix.

Epicuticular wax amount and chemical analysis

Seedlings from each of six families from each of the 12 populations were included in wax analysis. Each family was represented by eight to 19 seedlings. Twenty primary needles were removed from the stem of each seedling at the end of the first year of growth. Wax was extracted according to the procedure described by TURUNEN et al. (1997). Six samples of *P. pinceana* from each of the three regions were analyzed for wax chemical composition using previously published techniques (TURUNEN et al., 1997).

Statistical analysis

Wax quantity residual data were analyzed for fit to the normal distribution using UNIVARIATE/SAS procedure for Windows version 9.1 (SAS Institute Inc., 2002). Data were transformed to their arcsine (θ = arcsine;