Flora 209 (2014) 504–512

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Genetic relationships between wild progenitor pear (*Pyrus* L.) species and local cultivars native to Georgia, South Caucasus

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ARTICLE INFO

Article history:
Received 19 December 2013
Accepted 27 June 2014
Edited by R. Lösch
Available online 6 August 2014

Keywords:
Caucasus
Diversity
SSR markers
Pear cultivars
Pyrus communis subsp. *caucasica*
Pyrus spp.

ABSTRACT

The genetic diversity of 108 individuals of wild pear species (*Pyrus communis* subsp. *caucasica*, *P. balansae*, *P. salicifolia*, *P. syriaca*, *P. demetrii*, *P. bulgarica*, *P. ketzkhovelii*, *P. sachokiana*) and 35 samples of local and introduced cultivated pears from the country of Georgia were compared to 73 individuals of wild *P. communis* subsp. *caucasica* and *P. communis* subsp. *pyraster* in the collection of USDA-ARS National Plant Germplasm System (NPGS). *Pyrus communis* subsp. *caucasica* from both Georgia and the NPGS, *P. communis* subsp. *pyraster* from the NPGS, and *P. salicifolia* from Georgia were differentiated, based on analysis of eleven microsatellite markers. In addition, accessions of *P. communis* subsp. *caucasica* from Georgia were genetically distinct from accessions of the same subspecies in the NPGS collection that originated from other European and Middle Eastern Asian countries. Local pear cultivars in Georgia were genetically similar to *P. communis* subsp. *caucasica* and *P. balansae* growing wild in Georgia suggesting that they may have originated from native pear trees that could serve as unique genetic resources for pear breeding programmes.

Introduction

Asia Minor (South Caucasus, Iran, and Turkey) represents a centre of diversity for wild pear species (Vavilov, 1994). Wild *Pyrus communis* s. l. is native to many Eastern and Central European countries, including Georgia. *P. communis* L. subsp. *caucasica* (Fed.) Browicz is endemic to the Caucasus Mountains while *P. communis* L. subsp. *pyraster* (L.) Ehrh. is found in Central and Eastern European countries, but not in Georgia (Browicz, 1993). Some authors identify *P. communis* subsp. *caucasica* and *P. communis* subsp. *pyraster* as *P. caucasica* and *P. pyraster*, respectively (Grossheim, 1952; Fedorov, 1954). Although there are phenotypic and morphological differences between these subspecies, the phenotypic level – comparing morphological characters based on leaf, shoot and fruit traits – and molecular findings confirm the taxonomic distinction. Nevertheless, the two taxa may not be sufficient different in order to rank them as distinct species (Asanidze et al., 2011).

Eleven species of wild pears are native to the South Caucasus country of Georgia (Kuthatheladze, 1980). Two wild pears, *P. communis* subsp. *caucasica* and *P. balansae* Decne. are mesophilous, and the other nine species, *P. salicifolia* Pall., *P. eldarica* Grossh., *P. kezkhovelii* Kuth., *P. oxyprion* Woronow, *P. fedorovii* Kuth., *P. takhtadzianii* Fed., *P. demetrii* Kuth., *P. sachokiana* Kuth., and *P. georgica* Kuth. are native to semi-arid regions of the country. Georgian *Pyrus* species often hybridize amongst themselves and with other European and Middle Eastern pear species, including *P. eleagnifolia*, *P. communis* subsp. *pyraster*, and *P. syriaca*, which can make accurate species identification a challenge (Asanidze et al., 2011; Browicz, 1993; Ercisli, 2004; Zamani et al., 2012). In addition, introduced cultivars have been planted as windbreak rows to prevent erosion over the last 100 years, and they too may have hybridized with native species.

Wild *P. communis* is believed to be ancestral to the cultivated European pear (Volk et al., 2006; Yamamoto and Chevreau, 2009; Zohary and Hopf, 2000). Phenotypically, *P. communis* subsp. *caucasica* and *P. communis* subsp. *pyraster* are rather similar; however, the leaf margins are considered entire in *P. communis* subsp. *caucasica* and serrate in *P. communis* subsp. *pyraster* (Fedorov, 1954; Grossheim, 1952). They are mostly geographically distinct, with wild *P. communis* subsp. *pyraster* found in eastern and southern European countries and wild *P. communis* subsp. *caucasica* endemic

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http://dx.doi.org/10.1016/j.flora.2014.06.013
0367-2530/

to the Caucasus ecoregion (Fedorov, 1943; Tuz, 1974; Volk et al., 2006; Yamamoto and Chevreau, 2009). Although also similar genetically, the two subspecies can be differentiated using microsatellite markers (Volk et al., 2006; Wolko et al., 2010).

Asanidze et al. (2011) identified a population of the wild species *P. balansae* of sect. *Pyrus* in Western Georgia that is related to the "Communis" group and was known before only as a TBI herbarium specimen, originally sampled in Northern Turkey and on Greek Islands (Chouliaras et al., 2003; Gladkova and Sveschnikova, 1990). *P. balansae* has serrate upper parts of the leaf margins (Kuthatheladze, 1947) and appears to be a transitional form between *P. communis* subsp. *caucasica*, with entire margin leaf blades and *P. communis* subsp. *pyraster* leaves, which are serrate. This form of the wild pear was described by French botanist J. Decaisne in the1920s and appeared in the "Flora of the Caucasus" as a separate species: *P. balansae* (Grossheim, 1952). In our opinion, existing data about the morphological characteristics and distribution of *P. balansae* were not sufficient to differentiate it as a separate species.

In Georgia, pears have a long-standing importance as a food source, both for fresh eating, dried fruit, and alcoholic beverage. It is likely that local cultivars originated in breeding and selection programmes in historical Georgian breeding stations. Local Georgian pear cultivars and wild Georgian *P. communis* subsp. *caucasica* have high levels of similarity based on leaf morphology (Asanidze et al., 2011). An understanding of the genetic relationships among wild European and Middle Eastern *Pyrus* species and local cultivars will provide insights into possible breeding opportunities for improved pear cultivars, since wild *P. communis* subsp. *caucasica* and *P. communis* subsp. *pyraster* are likely primary progenitors to European pear cultivars (Volk et al., 2006; Yamamoto and Chevreau, 2009; Zohary and Hopf, 2000).

In this work, we use microsatellite markers to evaluate the genetic differentiation between several *Pyrus* taxa that are native to Georgia. We determined genotypes for Georgian cultivars as well as wild species, and compared results to those previously obtained for *P. communis* accessions maintained in the USDA-ARS National Plant Germplasm System (NPGS). First we contrast *P. salicifolia*, *P. communis* subsp. *pyraster* and *P. communis* subsp. *caucasica*. Next, we compare the wild Georgian *P. communis* subsp. *caucasica* to NPGS accessions of *P. communis* subsp. *caucasica* collected from other countries. Finally, we compare the genetic diversity of local Georgian pear cultivars to that of the Georgian wild *P. communis* subsp. *caucasica* to determine the relatedness among local cultivars and a likely progenitor subspecies.

Materials and methods

Plant material

A total of 143 individuals of cultivated and wild species of pears from Georgia were compared to 73 individuals of wild *P. communis* subsp. *caucasica* and *P. communis* subsp. *pyraster* in the NPGS. The Georgian wild pear species were identified according to Kuthatheladze (1947) and collected in their natural habitats (Table 1; Fig. 1). Georgian *P. communis* subsp. *caucasica* ($n = 73$) was sampled from 35 sites in 9 different administrative regions of Georgia (Fig. 1). *P. communis* subsp. *caucasica* occurred in 4 different habitats: (1) oak-hornbeam forests in East and South Georgia (234–1387 m) with *Quercus iberica*, *Carpinus caucasica*, *C. orientalis*, *Acer campestre*, *A. laetum*, *Cerasus avium*, *Fraxinus excelsior*, *Malus orientalis*, *Mespilus germanica*, *Prunus divaricata*, *Tilia begoniifolia*, *Cornus mas*, *Corylus avellana*, *Salix caprea*, *Clematis vitalba*, etc.; (2) pine forests with Colchic understory of *Rhododendron luteum*, in Svaneti (1212–1887 m): *Pinus kochiana*, *Picea orientalis*, *Quercus iberica*, *Carpinus caucasica*, *Crataegus kyrtostyla*, *Cornus mas*, *Daphne pontica*, *Frangula alnus*, *Rhododendron luteum*, *Ruscus ponticus*, *Sorbus torminalis*, *Tilia begoniifolia*, *Vaccinium arctostaphylos*, *V. vitis-idaea*; (3) beech forests with Colchic understory (Fageta fruticosa colchica) in Imereti, Racha, Samegrelo and Adjara (600–1890 m) with *Abies nordmanniana*, *Picea orientalis*, *Pinus kochiana*, *Quercus imeretina*, *Q. hartwissiana*, *Acer laetum*, *Carpinus caucasica*, *Tilia begoniifolia*, *Ficus carica*, *Malus orientalis*, *Staphylea colchica*, *S. pinnata* and evergreen understory with *Laurocerasus officinalis*, *Rhododendron ponticum*, *R. ungernii*, *Ruscus ponticus*, *R. colchicus*, *Ilex colchica*, *Daphne pontica*, *Epigaea gaultherioides*, *Vaccinium arctostaphylos*, *Viburnum orientale* and *Buxus colchica*; (4) wild Caucasian pear occurs among vegetation of urban and rural areas in different regions of Georgia together with *Prunus divaricata*, *Punica granatum*, *Juglans regia*, *Cornus mas*, *Corylus avellana*, etc.

Pyrus balansae ($n = 10$) was sampled on 4 sites in the forest areas of the Samtskhe-Javakseti, Lechkhumi and Adjara regions of Georgia at elevations that ranged from 210 to 1600 m (Table 1; Fig. 1).

Samples of wild pear group: *P. salicifolia* ($n = 15$), *P. bulgarica* ($n = 1$), *P. demetrii* ($n = 4$), *P. ketzkhovelii* ($n = 1$), *P. sachokiana* ($n = 1$) and *P. syriaca* ($n = 3$) were collected from four locations in dry open woodlands with *Pistacia mutica*, *Ulmus carpinifolia*, *Rhamnus pallasii*, *Juniperus foetidissima*, *J. oblonga*, *J. polycarpos*, *J. rufescens*, *Ephedra procera*, *Cotinus coggygria*, *Celtis australis*, *Paliurus spina-christi*, etc. in East Georgia at elevations between 423 and 992 m (Table 1; Fig. 1).

The genetic diversity of the Georgian wild pear species and cultivars was compared to 42 *P. communis* subsp. *caucasica* and 31 *P. communis* subsp. *pyraster* accessions from the NPGS, maintained at the National Clonal Germplasm Repository in Corvallis, OR, USA (Volk et al., 2006; Table 1; Fig. 2).

Samples of 35 individuals of cultivated pear trees were analyzed (Table 1; Fig. 1). Nineteen local cultivars (Akhalkatsi et al., 2012; Asanidze et al., 2011) were collected in high mountain villages of Georgian regions – Adjara, Samtskhe-Javakheti, Svaneti, Lechkhumi and Racha (Table 1). In addition, other local and hybrid cultivars were obtained from lowland villages of Georgian regions – Shida Kartli, Samegrelo, Guria, Kakheti and Mtskheta-Mtianeti. The living collection of the Institute of Horticulture, Viticulture and Oenology (IHVO), v. Skra, Gori distr., Georgia, was used for collection of introduced cultivars, and at the living collection of Biological Farming Association "ELKANA" in v. Tsnisi, Akhaltsikhe distr., Georgia, material of local cultivars were sampled (Table 1). Two introduced cultivars, 'Beurre Bosque' and 'Virgla', were also included in this study, sampled from village occurrences. Sampling locations were mapped using the DIVA-GIS software package (Hijmans et al., 2012, Fig. 1). Leaf samples were labelled and dried in plastic bags packed with silica gel and stored at −20 °C. The materials collected in Georgia were sent to the Plant Germplasm Preservation Research Unit at the USDA-ARS National Center for Genetic Resources Preservation in Fort Collins, Colorado, USA, for genetic analyses.

Molecular analyses

Eleven microsatellite markers (GD96, GD142, GD147, CH01D08, CH01D09, CH01F07A, CH02B10, CH02D12, CH01h01, NH015a) according to literature (Bao et al., 2007; Hemmat et al., 2003; Liebhard et al., 2002) were analyzed (Table 2). Duplicate samples of genomic DNA were isolated from young leaf tissue and allele amplification and detection from replicate samples at each locus were carried out according to the method described by Volk et al. (2006). Only accessions for which there were no more than two missing loci were included in the final dataset.