

Analysis of the genetic diversity of local apple cultivars from mountainous areas from Aragon (Northeastern Spain)



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ARTICLE INFO

Article history:

Received 4 February 2014

Received in revised form 23 April 2014

Accepted 29 April 2014

Available online 25 May 2014

Keywords:

Apple germplasm

Biodiversity

Genetic structure

Identification

Malus x domestica Borkh

SSR markers

ABSTRACT

A set of 183 apple accessions prospected in mountainous areas in Northeastern Spain (Pyrenees and Iberian Cordillera) was studied using 20 simple sequence repeats (SSR markers) in order to estimate its genetic diversity and to identify the genetic structure and relationships among the accessions. Additionally, a set of 23 international as well as traditional Spanish cultivars were included as a reference for comparative purposes. The average number of alleles per locus was 12.8 using the 20 SSR markers, with an expected heterozygosity ranging from 0.60 to 0.90, and a mean of 0.80. Genotypes were considered to be duplicated if they matched at all alleles across all 20 SSR markers and, based on molecular data, a 29% of duplication was observed. Genetic analyses performed by a model-based Bayesian procedure, principal coordinate analysis and analysis of molecular variance supported the existence of four sub-groups among the accessions, with moderate but significant differentiation ($F_{ST} = 0.07$; $P < 0.001$). In this study, more than 60% of local accessions were included within sub-groups containing nearly no foreign cultivars, which suggests the uniqueness and potential interest of this type of material for conservation purposes and for apple breeding programs.

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1. Introduction

Northeastern Spain has a long tradition of apple production, and a wide diversity of cultivars was grown in this major area since ancient times, either for the fresh market as dessert apples, or for cider production. For many centuries, Spanish mountains played an important social and economic role, producing various resources that supported a high population density and enabled the development of dynamic economic activities, including diversified agriculture (Lasanta et al., 2005). The selection processes performed by farmers in their orchards and home gardens for generations gave rise to a wide diversity of quality fruit plant material that constitutes an extraordinary genetic heritage of germplasm. Nevertheless, since the 1950s, the mountainous areas underwent a process of population decline, and many villages and a large part of the traditional farming areas were abandoned (García-Ruiz et al., 1996). As a consequence, many of the traditional cultivars, well adapted to both local needs and environmental conditions, and upon which the production was based in the past, went into a dramatic danger of extinction. This severe situation encouraged

the establishment of systematic prospecting missions in order to recover this traditional material and to preserve it in germplasm collections.

An efficient management of the material held in a germplasm collection implies the unequivocal identification of the accessions that compose it. In this context, molecular markers have become indispensable tools for collection management, and its use is widespread for characterization to assist and complement phenotypic assessments (Bretting and Widrlechner, 1995). Molecular characterization of the accessions accelerates and optimizes the identification process, allowing the fingerprinting of each genotype at any stage of development, independently of the environmental factors that may influence its phenotype (Wünsch and Hormaza, 2002). Microsatellites (Simple Sequence Repeats; SSRs markers), due to their properties (Schlötterer, 2004; Weising et al., 2005), have become the marker of choice for an array of applications in plants (Kalia et al., 2011), including the examination of genetic variation at molecular level in germplasm collections, and also for diversity and population genetics studies. In apple, several hundreds of SSRs have been developed and positioned on the genetic linkage map of this species (Guilford et al., 1997; Gianfranceschi et al., 1998; Hokanson et al., 1998; Liebhard et al., 2002; Silfverberg-Dilworth et al., 2006). Due to the high number of SSR markers available for *Malus*, the European Cooperative

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Programme for Plant Genetic Resources (ECPGR) has proposed in the last years several lists of recommended SSR markers in order to standardize international protocols, facilitating the cross-comparison among different apple germplasm collections (Laurens et al., 2004; Evans et al., 2007; Lateur et al., 2013).

The Spanish apple genetic resources are curated at seven apple collections integrated in the “Network of Collection of the National Program of Plant Genetics Resources” (INIA, 2013). The genetic diversity and identification of several of these collections have been already analyzed, both in Northeastern (Itoiz and Royo, 2003; Urrestarazu et al., 2011, 2012) and Northwestern Spain (Ramos-Cabrer et al., 2007; Pereira-Lorenzo et al., 2007, 2008). However, those studies did not extensively include local cultivars from mountainous areas of Aragon. This local apple germplasm, nowadays highly threatened and in danger of extinction, could preserve potential agronomic and genetic value due to its adaptation to severe environmental conditions since long time ago. The main purposes of this study were: (i) to determine the genetic identity of the apple material collected in two mountainous areas (Pyrenees and Iberian Cordillera) in Aragon (NE Spain) using SSR markers; (ii) to assess the genetic diversity of this germplasm and relationship between these accessions and a set of international reference cultivars, and (iii) to elucidate the genetic structure in the studied germplasm. The analysis and identification of local apple accessions prospected in these areas will contribute to the knowledge of

the genetic identity and variability of this material, as well as to the enrichment of the genetic heritage of the Spanish apple germplasm collections.

2. Material and methods

2.1. Plant material

The apple material analyzed in this study was collected in mountain areas of Aragon (Spain) with different eco-geographic features (Fig. 1). One hundred and eighty-three apple accessions were collected from 49 different localities from the three provinces of Aragon (Huesca, Zaragoza and Teruel). All accessions were prospected from abandoned old trees or small farms, and correspond to different material to that included in earlier works performed in that area (Urrestarazu et al., 2012), since it belongs to other prospecting missions. When the local denomination could not be known, the accessions were named after the village where they had been collected. In addition, old Spanish cultivars described in different guidebooks of autochthonous cultivars (Herrero, 1964; Carrera, 1998), as well as international cultivars grown in Spain since at least 1950s and modern international ones were included as reference cultivars for comparison (Table 1).

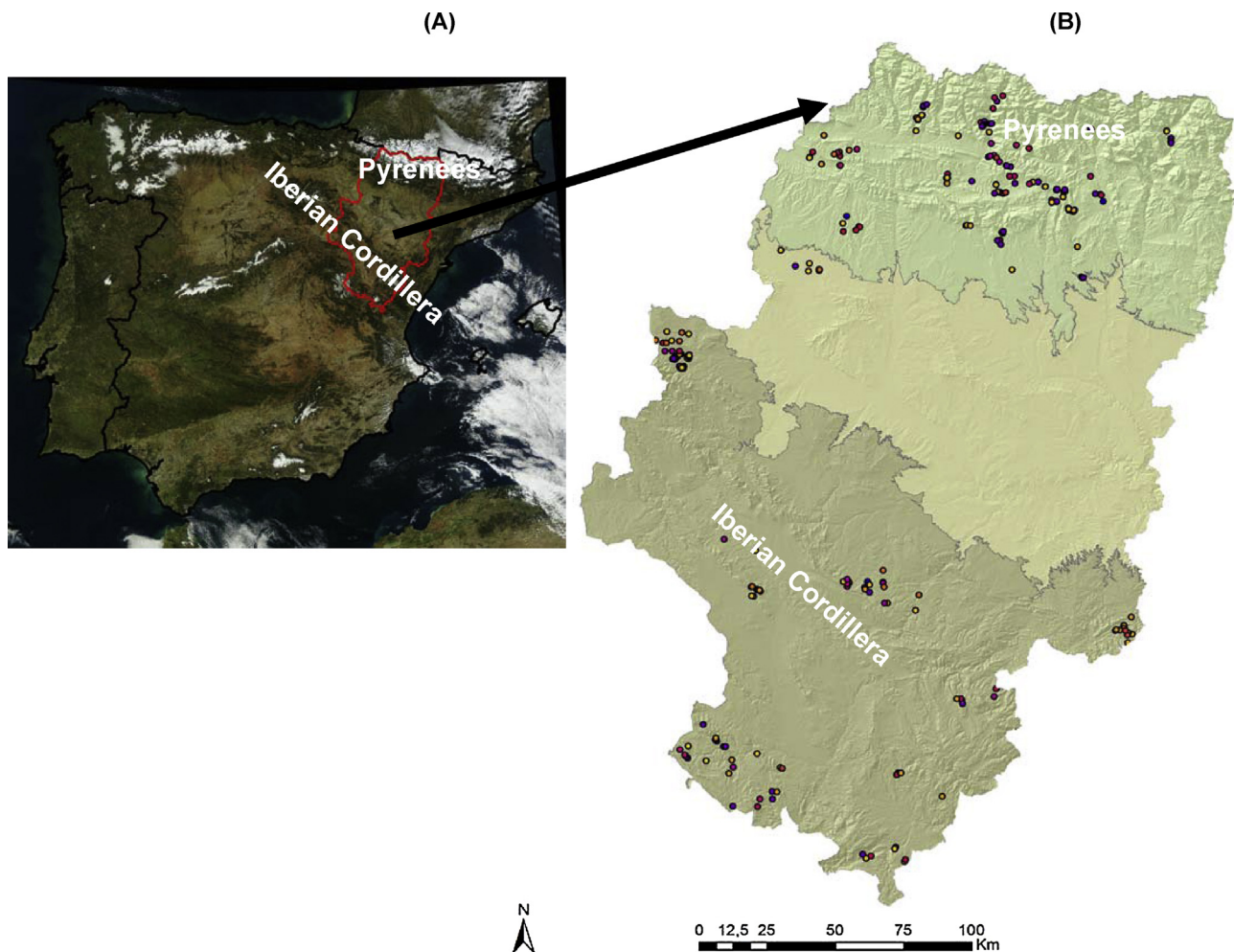


Fig. 1. Schematic illustration of the studied area in Northeastern Spain where 183 apple local accessions were prospected (A). The collection sites of mountain areas, Pyrenees and Iberian cordillera are showed with spots (B).

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