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Molecular markers for conservation genetic resources of four *Passiflora* species

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ABSTRACT

The aim of this study was to use microsatellite markers (SSR) for the characterization of the *Passiflora* species and to verify the effect of random selection of individuals in parameters that characterize the genetic variability of germplasm for conservation purposes. Four species, *Passiflora edulis* f. *flavicarpa* Degener, *P. cincinnata* Mast., *P. alata* Curtis and *P. setacea* D.C., were evaluated. For each species two random samples were evaluated, one consisting of 60 plants (S60) and the other of 10 plants (S10) randomly selected from the S60. Initially, the S10 and S60 were used to calculate the genetic parameters of number of alleles, expected and observed heterozygosity, effective population size, inbreeding and polymorphic information content based on 40 microsatellite markers developed for *P. edulis* and 20 for *P. alata*. Further bootstrap analysis was performed to identify the minimum number of individuals needed to represent the variability of each *Passiflora* species from a range of 2 to 59. The number of polymorphic microsatellites was 15, 9, 6 and 2 on *P. edulis* f. *flavicarpa*, *P. cincinnata*, *P. alata* and *P. setacea*, respectively. The allelic loss due to the under-representation of the samples was 19 (30%), 16 (43%) and nine (39%) alleles, respectively, for *P. edulis* f. *flavicarpa*, *P. cincinnata* and *P. alata*. No allelic loss was observed for *P. setacea*, probably because only two polymorphic microsatellites were identified. In general, there are differences between S10 and S60 because of lost genetic variability on S10, indicating that the use of these 10 individuals to represent the *Passiflora* species is insufficient for long-term preservation. In contrast, the bootstrap analysis revealed that the stability of the genetic parameters due to the increase in sample size was close to 30, 23, 25 and 24 individuals for *P. cincinnata*, *P. edulis* f. *flavicarpa*, *P. setacea* and *P. alata*, respectively. The difference of genetic estimates between samples S10 and S60 demonstrated that 23–30 individuals are the minimum range of population to represent the *Passiflora* species studied. This study may optimize the strategies for conservation the *Passiflora* germplasm avoiding the under-representation of samples and consequent loss of genetic variability during sexual propagation.

1. Introduction

The Brazilian passionfruit market has grown substantially in recent years, as reflected in the production of up to 823,000 tons of fruit in 2014, which were grown on 57,000 ha (14.48 t ha^{-1}), almost entirely in the Brazilian northeast (IBGE, 2015). Brazil stands out in the international market as the world's largest producer of yellow passionfruit (*Passiflora edulis* f. *flavicarpa* Degener), which is the most cultivated species in Brazil (95% of planted area and sales) because it presents a quick early harvest and good appreciation both for the *in natura* market and industrialization (Malacrida and Jorge, 2012; Janzantti and Monteiro, 2014).

Passiflora is the largest genus of the Passifloraceae family, with about 500 species of wide inter- and intraspecific genetic variability (Meletti et al., 2005; Yockteng et al., 2011). The most variability is dispersed in Colombia and Brazil with more than 140 *Passiflora* species (Ocampo et al., 2010; Bernacci et al., 2014). However, human activities resulting from the urbanization process, expansion of agricultural activities, forest clearing and changes in climatic conditions have threatened the survival of this diversity, which could lead to genetic erosion of many species of economic interest (Alcázar, 2005; Govindaraj et al., 2015). In contrast, in recent years

Abbreviations: SSR, simple sequence repeat; Ho, observed heterozygosity; He, expected heterozygosity; PIC, polymorphic information content.

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many ex situ conservation studies have been carried out in order to enlarge and maintain genetic variability in many plant species (Silva et al., 2011; Barbieri et al., 2014; Gentili et al., 2015).

Most of the conservation of the genus *Passiflora* is carried out ex situ by germplasm banks, which aim to preserve genetic resources for immediate or future use in breeding programs. Embrapa Cassava & Fruits has one of the largest *Passiflora* germplasm bank (Passionfruit Germplasm Bank—"PGB-Passionfruit"), with approximately 372 accessions and 20 species that are periodically planted in the field for agronomic characterization and evaluations, and is simultaneously used to produce seeds to preserve the germplasm. On the other hand, the number of accessions stored in collections and germplasm banks is not considered fully representative of the genus *Passiflora* (Ferreira, 2005; Wetzel et al., 2011) because technical and financial difficulties faced by institutions keep the *Passiflora* species in gene banks. In addition to the limit on the number of accessions to be preserved, there are also restrictions on the number of plants of each accession for seed production in the field. Currently, each accession of *Passiflora* of the PGB-Passionfruit is represented in the field by 10 plants, which are used to restore the vigor and germination power for new storage in cold chambers. However, the passionfruit is an allogamous species and the most are self-incompatibility, which prevents inbreeding and even the crossing of different plants with the same incompatible alleles (Santos et al., 2011; Madureira et al., 2014; Soares et al., 2015; Ocampo et al., 2016). Therefore, wide intra-accession variability can be expected, and thus 10 plants could be insufficient to represent the allelic variation of this germplasm throughout the cycles of seed regeneration/multiplication. As a result of this process, there could be a narrowing of the genetic population and loss of important genes, which could reduce the ability of species to respond to environmental adversities in future generations (Raposo et al., 2007).

In addition, allogamy and self-incompatibility need to be considered in the conservation of passionfruit germplasm, since selection and crossing of a few plants can result in inbreeding depression, as well as loss of allelic variability in incompatible microsatellites. Therefore, it is important to monitor the conservation strategy adopted, in order to maintain the maximum genetic variability of different *Passiflora* accessions (Bernal-Parra et al., 2014). If intra-accession variability, as well as inter- and intraspecific diversity in *Passiflora* germplasm, is not preserved over time, the storage and future use of this genetic resource as a source of genes of interest for breeding could be seriously compromised. The management of *Passiflora* germplasm requires a fairly complete genetic characterization to facilitate the most efficient conservation strategies (Cerqueira-Silva et al., 2014b; Cerqueira-Silva et al., 2014a). In general, this characterization and evaluation of accessions can be done based on different methods, starting with morphological characterization (Castro et al., 2012; Lawinscky et al., 2014). However, these characterizations can benefit from the advantages of the DNA markers that are currently used to characterize the genetic diversity of several cultivated and wild species of *Passiflora* (Oliveira et al., 2005, 2013; Cerqueira-Silva et al., 2012, 2015; Bernal-Parra et al., 2014; Paiva et al., 2014). In contrast, a recurring problem of the *Passiflora* germplasm conservation is the development of strategies for sampling representative individuals from experimental and natural populations, which is not currently a target of research.

Considering the intrinsic characteristics of the species of the genus *Passiflora*, such as self-incompatibility and allogamy, make the determination of sample size for conservation a research area of fundamental importance. Therefore, the objective of this study was to evaluate the use of microsatellite markers in the characterization of four species *Passiflora* (*P. cincinnata*, *P. edulis* f. *flavicarpa, P. setacea* and *P. alata*) considered self-incompatible (Ocampo et al., 2016) and to verify changes in allele frequencies and reduction in the number of alleles and the effective population size due to the random selection of plants for germplasm conservation.

2. Material and methods

2.1. Plant material

Four species of passionfruit belonging to subgenus *Passiflora*: one commercial specie (*Passiflora edulis* f. *flavicarpa* -BGP311), and three wild species (*Passiflora cincinnata* Mast. -BGP268*; Passiflora alata* Curtis -BGP004, and *Passiflora setacea* DC -BGP237) from the Passionfruit Germplasm Bank (PGB-Passionfruit) at Embrapa Cassava & Fruit, located in Cruz das Almas, Bahia Brazil (12°48′38″S and 39°06′26″O; 220 m), were analyzed. Each species was represented by one accession (Fig. 1).

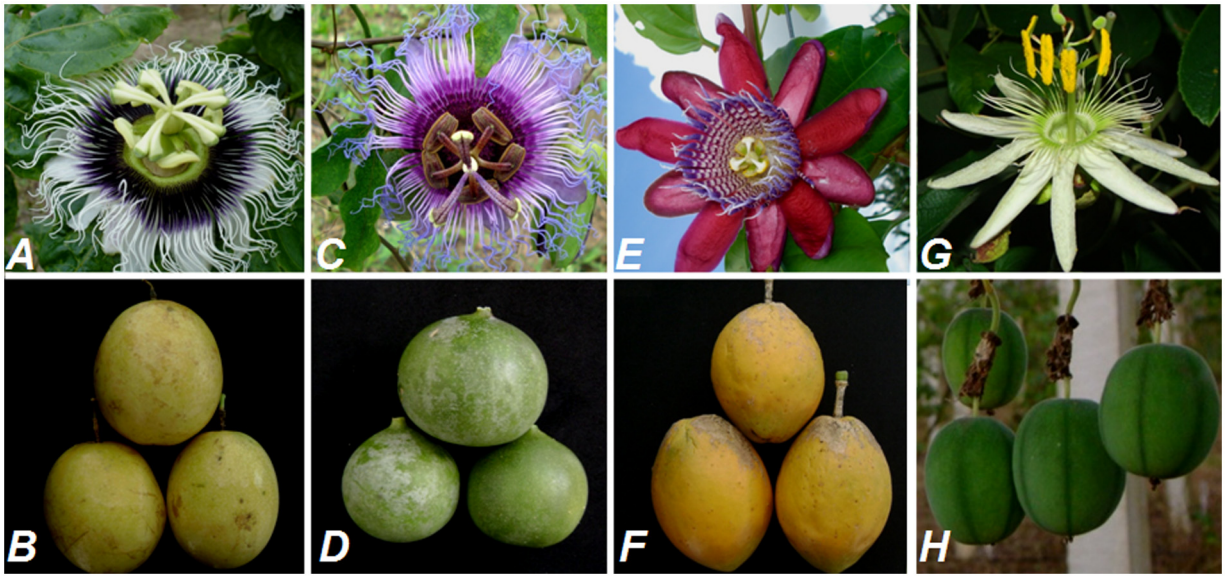

Fig. 1. General view of flowers and fruit of *Passiflora* spp species. A–B) *P. edulis* f. *flavicarpa* (BGP 311); C–D) *P. cincinnata* (BGP268); E–F) *P. alata* (BGP004) and G–H); and *P. setacea* (BGP237).