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Genetic diversity and population structure in *Physalis peruviana* and related taxa based on InDels and SNPs derived from COSII and IRG markers

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A B S T R A C T

The genus *Physalis* is common in the Americas and includes several economically important species, among them is *Physalis peruviana* that produces appetizing edible fruits. We studied the genetic diversity and population structure of *P. peruviana* and characterized 47 accessions of this species along with 13 accessions of related taxa consisting of 222 individuals from the Colombian Corporation of Agricultural Research (CORPOICA) germplasm collection, using Conserved Orthologous Sequences (COSII) and Immunity Related Genes (IRGs). In addition, 642 Single Nucleotide Polymorphism (SNP) markers were identified and used for the genetic diversity analysis. A total of 121 alleles were detected in 24 InDels loci ranging from 2 to 9 alleles per locus, with an average of 5.04 alleles per locus. The average number of alleles in the SNP markers was two. The observed heterozygosity for *P. peruviana* with InDel and SNP markers was higher (0.48 and 0.59) than the expected heterozygosity (0.30 and 0.41). Interestingly, the observed heterozygosity in related taxa (0.4 and 0.12) was lower than the expected heterozygosity (0.59 and 0.25). The coefficient of population differentiation F_{ST} was 0.143 (InDels) and 0.038 (SNPs), showing a relatively low level of genetic differentiation among *P. peruviana* and related taxa. Higher levels of genetic variation were instead observed within populations based on the AMOVA analysis. Population structure analysis supported the presence of two main groups and PCA analysis based on SNP markers revealed two distinct clusters in the *P. peruviana* accessions corresponding to their state of cultivation. In this study, we identified molecular markers useful to detect genetic variation in *Physalis* germplasm for assisting conservation and crossbreeding strategies.

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1. Introduction

The genus *Physalis* consists more than 90 species, native of the Americas, being Mexico the center of diversity of the husk tomato (Vargas-Ponce et al. 2010). The genus includes different species with nutritional, nutraceutical and commercial interests. Among them, *P. peruviana* and other related taxa as *Physalis philadelphica*, *Physalis pruinosa* and *Physalis longifolia* have been characterized for different health related compounds with anti-inflammatory and antioxidant properties (Martínez et al., 2010; Yen et al., 2010; Maldonado et al., 2011; Ramadan, 2011; Jin et al., 2012; Kindscher et al., 2014; Takimoto et al., 2014), as well as others with valuable nutritional properties including vitamins A, B and C, polyunsaturated fatty acids, proteins and minerals (Puente et al., 2011; Ramadan, 2011). Therefore the commercial interest of *P. peruviana*, also known as Cape gooseberry, has increased to the point of being currently the main exported fruit after banana in Colombia (Bonilla et al., 2009).

Accurate knowledge of genetic diversity and relationships among preserved germplasm collections of any crop is essential and important for establishing, managing and ensuring long-term success of appropriate crop improvement programs through breeding (Gwag et al., 2010). Thus, the study on genetic diversity and population structure of germplasm collections has been useful in supporting conservation and genetic improvement strategies (Rao and Hodgkin, 2002; Grandillo, 2014). Furthermore, natural biodiversity found in non-cultivated relatives of crop species are reservoirs representing an important source of genetic variation essential for any crop-breeding program (Grandillo, 2014). Although some *Physalis* species have been widely recognized by

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their nutraceutic and economic importance, little is known about their genetic diversity at the molecular level, mainly because of the lack of available markers in accordance with their current status as orphan species. Dominant markers RAMs (Random Amplified Microsatellites) were the first type used to study the genetic diversity of a Colombian *P. peruviana* collection where high expected heterozigosity (He = 0.2559) was found (Bonilla et al., 2008; Morillo Paz et al., 2011). Later, next-generation sequencing technologies for the rapid identification of SSR loci derived from ESTs were used (Simbaqueba et al., 2011; Garzón-Martínez et al., 2012). Nevertheless, since all SSR loci were located in the UTR regions of the transcriptome, a low polymorphic rate (22%) was found in a panel of 8 accessions from *P. peruviana* and the related species *Physalis floridana*, therefore we decided to develop alternative markers. More recently, 97 tomato markers (COS, SSRs and InDel markers) and 25 *P. peruviana* SSR markers were used for genetic diversity analysis in 38 accessions of *Physalis* (Wei et al., 2012). This study suggested the efficient use of tomato markers in genetic studies of *Physalis* as species from the family Solanaceae, and a high level of polymorphism (92.7% of markers were polymorphic) in accordance with a broad genetic at DNA level in wild and cultivated species. Furthermore, Berdugo et al. (2015), used 328 COSII and 154 Immunity Related Genes (IRGs) to evaluate an F1 population generated between contrasting pathogen response parents. This population showed a total of 127 alleles with an average of 3.18 per locus, a PIC of 0.358 and high values of heterozygosity (Ho: 0.737 and He: 0.449).

Recent advances in sequencing technologies allowed the identification of large sets of Single Nucleotide Polymorphisms (SNPs) (Patel et al., 2015; Yang et al., 2015). SNP genotyping has become the most useful technique in model and non-model species for genetic diversity and population structure analysis, marker-assisted selection and association studies (Frascaroli et al., 2013). Recently, Enciso-Rodríguez et al. (2013) identified 74 IRGs in *P. peruviana*, from which 17 markers were selected and sequenced in a small subset of *P. peruviana* and related taxa allowing the identification of one candidate SNP associated to the resistance response against the fungal pathogen *Fusarium oxysporum*, one of the main constraints to *P. peruviana* production in Colombia.

Since the development and discovery of new markers for non-model species as well as the use of Conserved Ortholog Set of markers (COSII) (Fulton et al., 2002; Wu et al., 2006) are valuable resources for genetic studies of *Physalis*, the aim of the present study was to investigate the genetic diversity and population structure of 60 accessions with a large representation of *P. peruviana*, by the use and comparison of InDels and SNPs derived from COSII and IRG markers, to contribute knowledge on the germplasm genetic base for conservation and use for breeding strategies.

2. Material and methods

2.1. Plant material and DNA isolation

Young leaves of 222 plants belonging to 47 *Physalis peruviana* accessions (an average of 3 plants per accession, each derived from a single seed) and 13 related taxa (an average of 4 plants per accession) were collected from an in vitro germplasm collection maintained at the Colombian Corporation for Agricultural Research (CORPOICA) (Table 1, Supplementary Table 1). The leaves were stored at −70 °C and utilized for genomic DNA isolation using the modified Dellaporta et al. (1983) method, as described by Enciso-Rodríguez et al. (2013).

2.2. Molecular marker selection

A total of 454 molecular markers were tested for genetic diversity analyses on *P. peruviana* and related taxa. We selected 327 COSII markers based on their distribution across the 12 linkage groups of the tomato genome, considering their orthologous nature in a broad range of Solanaceae family species (Fulton et al., 2002; Wu et al., 2006; Bedoya-Reina and Barrero, 2010). Furthermore, 33 COSII genes were selected, based on resistance to biotic factors related ontologies (i.e. against phytopathogenic microorganisms), as a strategy to look for polymorphisms within homologous defense/resistance genes. Each primer pair was obtained from the Solanaceae network database (http://www.solgenomics.net). The remaining 94 markers belonged to IRGs, previously developed from the *P. peruviana* leaf transcriptome (Enciso-Rodríguez et al., 2013).

The 360 COSII markers were screened on five accessions with contrasting responses to *Fusarium oxysporum* which includes *P. peruviana* and related species (Enciso-Rodríguez et al., 2013). In addition, 94 IRGs were tested on six contrasting accessions as described by Enciso-Rodríguez et al. (2013). Polymorphic COSII and IRGs markers were selected and used to genotype 222 samples from 60 accessions. From the original set of markers, we selected monomorphic COSII and IRGs molecular markers for SNP identification on a 64-plant panel (Table 1). Complete marker information is shown in Supplementary Table 2.

Table 1
Summary of plant material used in this study.

Species	Number of accessions	Number of plants used for InDels[a]	Number of plants sequenced
Physalis peruviana	47	171	51
Physalis philadelphica	4	18	4
Physalis floridana	2	9	2
Physalis angulate	2	5	2
Physalis viscosa	1	3	1
Physalis pruinosa	1	3	1
Physalis ixocarpa	1	2	1
Nicandra physalodes	1	3	1
Solanum auriculatum	1	8	1
Total	60	222	64

[a] InDel markers = polymorphic COSII and IRGs on agarose gel.

2.3. PCR amplification and marker visualization

Polymerase chain reactions (PCR) were performed each in 15 μl final reaction volume using 1X PCR Buffer, 2.0 mM $MgCl_2$, 0.2 μM dNTPs, 0.2 μM of each primer, 0.05 U/μl Taq DNA polymerase and 5 ng of genomic DNA. Thermal cycler conditions included a denaturation step at 94 °C for 5 min followed by 35 cycles at 94 °C for 30 s, 56 °C to 60 °C for 1 min (depending on the marker), 72 °C for 2 min, ending with and extension at 72 °C for 10 min. Amplifications were performed in a i-Cycler thermal Cycler (Bio-Rad, Hercules, CA, USA). PCR products were separated on 2% agarose gels stained with ethidium bromide (0.5 μg/ml). Molecular size of the COSII and IRGs bands was estimated using a 1 kb plus ladder as reference.

2.4. Marker sequencing

Each forward and reverse COSII and IRG primer selected for sequencing was modified with a 10-base Multiplex Identifier (MID) adaptor sequence and a 454-sequencing primer (A-primer and B-primer).

Table 2
Summary of markers used for InDels studied and SNPs discovery.

Type of study	Marker type	No. of markers	No. of polymporphic markers	No. of SNPs
InDels analysis	COSII	360	18	–
	IRGs	94	6	–
Sub-Total		454	24	–
SNPs discovery	COSII[a]	15	–	642
	IRGs[a]	18	–	
Sub-Total		33	–	642
Total		487	24	642

[a] Monomorphic markers selected for SNPs discovery.

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