Genetic Diversity Analysis and Phylogenetic Relationship among a Representative Collection of Spanish Pepper (*Capsicum annuum*) Landraces

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Abstract

Spain is one of the most important centers of diversity for *Capsicum annuum* peppers, and a plethora of ecotypes can be found throughout the country. These resources represent a valuable genetic pool which must be characterized in depth both morphologically and molecularly. The aim of the present work was to assess phenotypic and genotypic diversity and relationships within some Spanish pepper landraces. A collection of 12 Spanish pepper landraces and 15 foreign controls was characterized with a set of 46 descriptors from International Plant Genetic Resources Institute (IPGRI, now Bioversity International) and genotyped using Genotyping-by-Sequencing (GBS). The studied collection was genetically diverse. The morphological characterization allowed discrimination of the varieties through fruit size, growth habit and others traits. SNP-based phylogenetics clustered together Spanish accessions. Our results provide useful information of several different ecotypes to boost breeding programs.

Keywords: morphological characterization, single nucleotide polymorphism, paprika landraces

Introduction

Chile peppers and paprika (*Capsicum spp.*) are one of the most important vegetables and spices in the world (FAO, 2014). They are used in a range of dishes and recipes, being *C. annuum* the most common and diverse species (DeWitt and Bosland, 2009). Spain was for a long time the entrance to a considerable genetic flow from the Americas, which explains the great amount of Spanish varieties and landraces nowadays (Rodríguez-Burruezo *et al.*, 2016). These resources represent a valuable genetic pool (Lanteri, 2005; Nicolai, 2012). In order to optimize their use by breeders, these materials must be characterized in depth both morphologically and molecularly. The combination of these two characterization strategies may produce molecular markers correlated to relevant agronomic traits. New technologies like GBS enable the generation of massive raw data that leads to large sets of SNPs

(Single Nucleotide Polymorphism). The aim of the present work was to assess phenotypic and genotypic diversity and relationships within Spanish pepper landraces.

Materials and methods

Plant material consisted on an encompassing collection of 12 *C. annuum* accessions from different origins within Spain, as well as 15 non-Spanish control accessions that represent the variability of the species (Tab. 1). The collection was initially characterized with a set of 46 IPGRI descriptors (IPGRI, 1995) (Tab. 2), ANOVA and Principal Component Analysis was performed to identify significant differences among accessions. DNA was extracted from one plant per genotype by modified Doyle and Doyle method (1990). Afterwards, Genotyping-by-Sequencing (GBS) methodology described by Elshire *et al.* (2011) was followed to genotype the whole collection. Consequently SNP-

Table 1. Plant material. Spanish landraces and foreign controls

Name	Code	Origin	Fruit type (IPGRI)
Spanish landraces			
<i>Arnoia</i>	Arn	Galicia, Spain	Blocky
<i>Bola DOP</i>	Bol	Murcia, Spain	Almost round
<i>Calahorra</i>	Cal	La Rioja, Spain	Blocky
<i>Guernika</i>	Guer	País Vasco, Spain	Elongated
<i>Guindilla Ibarra</i>	Iba	País Vasco, Spain	Elongated
<i>Largo de Reus</i>	Reus	Tarragona, Spain	Blocky
<i>M. Fresno de la Vega</i>	Fres	León, Spain	Blocky
<i>Najerano</i>	Naj	La Rioja, Spain	Triangular
<i>Padrón</i>	Pad	Galicia, Spain	Blocky
<i>Pimiento del Bierzo IGP</i>	Bie	León, Spain	Blocky
<i>Piquillo IGP</i>	Piq	Navarra, Spain	Triangular
<i>Valenciano</i>	VLC	Valencia, Spain	Blocky
Foreign control accessions			
<i>Ací Sivri</i>	Ací	Turkey	Elongated
<i>Ancho 101</i>	101	Mexico/USA	Triangular
<i>Ancho mulato</i>	Mul	Mexico	Triangular
<i>Chile de Árbol</i>	Árb	Mexico	Elongated
<i>Chile Serrano</i>	Serr	Mexico	Triangular
<i>Chimayo</i>	Chim	New Mexico, USA	Blocky
<i>Di Senise IGP</i>	Sen	Potenza, Italy	Elongated
<i>Jalapeño M</i>	Jal	Mexico	Elongated
<i>Numex Big Jim</i>	Bjim	New Mexico, USA	Elongated
<i>Pasilla Bajío</i>	Baj	Mexico	Elongated
<i>Petit Marsellais</i>	Pet	France	Blocky
<i>Poivre Rouge de Bresse</i>	PRB	France	Elongated
<i>Tendre Châteaurenard</i>	Chat	France	Blocky
<i>Torpedo Bangalore</i>	Ban	India	Elongated
<i>Habanero rojo (C. chinense)</i>	Haba	Pensilvania, USA	Campanulated

Table 2. *Capsicum* IPGRI descriptors

Plant			
Stem colour	Stem shape	Stem pubescence	Nodal anthocyanin
Leaf colour	Leaf shape	Leaf pubescence	Lamina margin
Mature leaf length	Mature leaf width		
Inflorescence			
Days to flowering	Number of flowers per axil	Flower position	Corolla colour
Corolla spot colour	Corolla shape	Corolla length	Anther colour
Anther length	Filament colour	Filament length	Stigma exertion
Male sterility	Calyx Pigmentation	Calyx margin	Calyx annular constriction
Fruit			
Days to fruiting	Anthocyanin spots	Fruit color at intermediate stage	Fruit color at mature stage
Shape	Length	Width	Weight
Pedicle length	Fruit wall thickness	Shape at pedicle attachment	Neck at base of fruit
Fruit shape at blossom end	Fruit blossom end appendage	Fruit cross-sectional corrugation	Number of locules
Ripe fruit pedicle with fruit persistence	Ripe fruit pedicle with stem persistence	Fruit surface	Placenta length

Figure 1. Fruit varietal types. Left to right and top to bottom: Almost round: Bola; Campanulated: Habanero rojo; Triangular: Piquillo, Najerano; Elongated: Guernika, Guindilla Ibarra; Blocky: Morrón Fresno de la Vega, Largo de Reus

Figure 2. Varieties distribution based on analysis of principal component 1 (27.4%) and principal component 2 (10.2%). On the left side of the graph are smaller, elongated varieties (Iba, Bjim, Baj), while on the right side there are blocky and bigger landraces (Naj, Reus, VLC).

calling was performed using Glaubitz *et al.* (2014) protocol. Finally, phylogenetic analysis was done relying on SNP-based genetic distances to assess the degree of relatedness between genotypes, using SNPhylo (Lee *et al.*, 2014).

Results and discussion

The morphological characterization allowed discrimination of the varieties through principal component analysis (Fig. 2). Out of the initial 46 IPGRI descriptors, 36 showed significant differences. The first two components explain 27.4% and 10.2% of total variability. Several traits such as fruit shape and size, plant height and growth habit, showed statistical difference for

several landraces (data not shown). On the PCA, varieties grouped mainly based on shape. On the basis of the genetic distance matrix a phylogenetic tree was constructed. Landraces of the same origin clustered together (Fig. 3). Even though there are similarities within certain landraces, the whole collection was found to be phenotypically and genetically diverse, which supports the initial assessment of Spanish pepper landraces as invaluable resources as breeding materials for future European pepper trade.

Conclusion

Our results provide useful information of several different ecotypes to boost breeding

Figure 3. Phylogenetic tree based on genetic distance. Circled: *C. chinense* control Habanero (green); Mexican/U.S. varieties (yellow); Blocky-Spanish/European landraces (red); Elongated-Spanish/European varieties (blue).

programs, germplasm management and conservation of landraces of *C. annuum* in Spain.

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