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Original Article

Use of Molecular Markers to Assist the Development of Inbred Lines under Open Field Conditions: the Case of Criollo Peppers (*Capsicum annuum* L.) from Mexico

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Abstract

Chile peppers are one of the most important crops in Mexico and a plethora of ecotypes can be found there. Most of them are ancient open-pollinated (OP) landraces selected by farmers for uniform phenotype but with an inherent level of genetic diversity, called *criollos*. In this work 15 pepper accessions, encompassing 2 *criollo* lines, their open-pollinated progenies, and 5 controls, were characterized with a set of 36 IPGRI descriptors and 23 SSR markers to assess the effect of open pollination in the inbreeding process. Heterozygosity levels were comprised between 12 and 47% in the progenies, which were similar or lower than those values from parent plants and similar or higher than control cultivars. Also, both progenies and parents showed similar levels of agronomic and morphological uniformity. Our results suggest that this OP program is efficient in terms of reaching enough agronomic uniformity in *criollo* Ancho peppers while preserving certain genetic diversity to confer adaptation to climate change.

Keywords: Chile pepper heirlooms, climate change, genetic diversity, morphological characterization, participatory breeding, simple sequence repeat (SSR)

Introduction

Peppers (*Capsicum* spp.) are one of the most important vegetables in the world, encompassing a worldwide production of thirty-six million t (FAOSTAT, 2014). *Capsicum annuum* is the most diverse and commonly cultivated. Since Mexico is the primary diversity centre of this species, an extraordinary range of varietal types and cultivars can be found there (DeWitt and Bosland, 1996; Kraft, 2009).

Some of the breeding efforts directed to this species in this country have been towards the improvement of valuable landraces and heirlooms (Kraft, 2009). Mexican institutions labour has been improving the *criollo* peppers like the Ancho type, an ancient open-pollinated (OP) landrace that may be a strong candidate as pre-breeding material or as diverse population with higher adaptation/resilience to climate change (Votava *et al*., 2005; Aguilar-Meléndez *et* al., 2009; Madosa *et al*., 2010). The governmental initiative consists in participatory breeding programs geared towards getting farmers involved for identification of the best individuals from their fields. Then open pollination seeds from these individuals are harvested for the next generation. This approach should provide a

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reasonable balance between both inbreeding and preserving the essential level of diversity for the landrace identity itself (Kraft, 2009).

The goal of this experiment was to assess phenotypic and genotypic diversity within *criollo* peppers as well as allele fixation levels as a result of this traditional plant breeding method.

Materials and Methods

Plant material

Two accessions of OP chile Ancho type (A and B), their respective progenies (a1, a2, a3, a4 and b1, b2, b3, b4), and a set of five control varieties – three OP landraces (C1: 'Serrano', C2: 'Puya', C3: 'Pasilla') and two relatively modern cultivars (M1: 'Numex Garnet' and M2: 'Modern Pasilla') were included in this experiment. Plant materials were provided by scientists of the Chile breeding program of Universidad Autónoma de Aguascalientes (UAA, Mexico) (Fig. 1).

Experimental procedures

Eight plants per accession were characterized according to thirty-six IPGRI (1995) plant, inflorescence, fruit, and seed descriptors. ANOVA and Principal Components Analysis (PCA) were calculated to assess the differences among individuals using Statgraphics Centurion XVI (StatPoint Technologies, Inc.).

Subsequently, DNA was extracted using modified CTAB method (Doyle and Doyle, 1990) from a pool of the eight plants of each accession. A 23 Single Sequence Repeat (SSR) marker collection (Minamiyama *et al*., 2006; Portis *et al*., 2006; Nagy *et al*., 2007; Yi *et al*., 2006), enriched with M13 tail and a fluorescent dye (Schuelke, 2000), was used to genotype the collection throughout capillary electrophoresis and ABI PRISM® 3100-Avant (Applied Biosystems, USA). Genetic parameters, such as Heterozygosity (H), Polymorphic Information Content (PIC), Principal Coordinates Analysis (PCoA), and distance matrix were calculated using GenAlex 6.5 (Peakall and Smouse, 2006) and PowerMarker 3.25 (Liu and Muse, 2005).

Results and Discussion

Phenotypic characterization

From the 36 descriptors, only 15 showed significant variation among our collection, which were used to calculate the first two principal components (PC). PC1 and PC2 explained 39.8 and 19.0% of the collection total variability, respectively. For PC1 traits like stem length, seed weight, fruit weight and shape, fruit cross-sectional corrugation (positive values) and anther colour (negative) were the most discriminant ones (Fig. 2a). PC2 showed a similar behaviour where plant and fruit traits accounted for higher discriminant values (fruit surface, nodal anthocyanin (positive values), fruit shape at blossom end, and chroma at red stage (negative value)) (Fig. 2a), as reported by Pereira-Dias *et al.* (2015) in a collection of Spanish peppers. Based on that, both OP lines and their progenies were clustered together on the right side of the graph (triangular, larger fruits), as it was expected, even though there is no clear separation of the two families, indicating that standard phenotype is maintained despite the lack of controlled pollination. In the middle, the elongated, medium size fruits with a wrinkled surface, and to the left the smaller fruits with cayenne forms, clearly separated from each other and the rest of clusters (Fig. 2b).

Molecular analysis

Six SSR markers were not reliably distinguishable and therefore excluded from the analysis. The remaining 17 SSR allowed the identification of 43 different alleles, with an average of 2.5 alleles per SSR marker, ranging between 2 and 5. PIC mean value was 0.30 (ranging from 0.19 to 0.41). Our results are in agreement with those from Lee *et al.* (2003), Portis *et al.* (2006) and Nagy *et al.* (2007), although PIC mean value was slightly lower, perhaps due to individual's relatedness.

Fig. 1. Illustration of the average fruit type for each parental line, progeny, and controls. From top to bottom, left to right: parent A and progenies a1-4 (upper row); intermediate row parent B and progenies b1-4 (middle row); controls C1-3 and M1-2 (lower row)

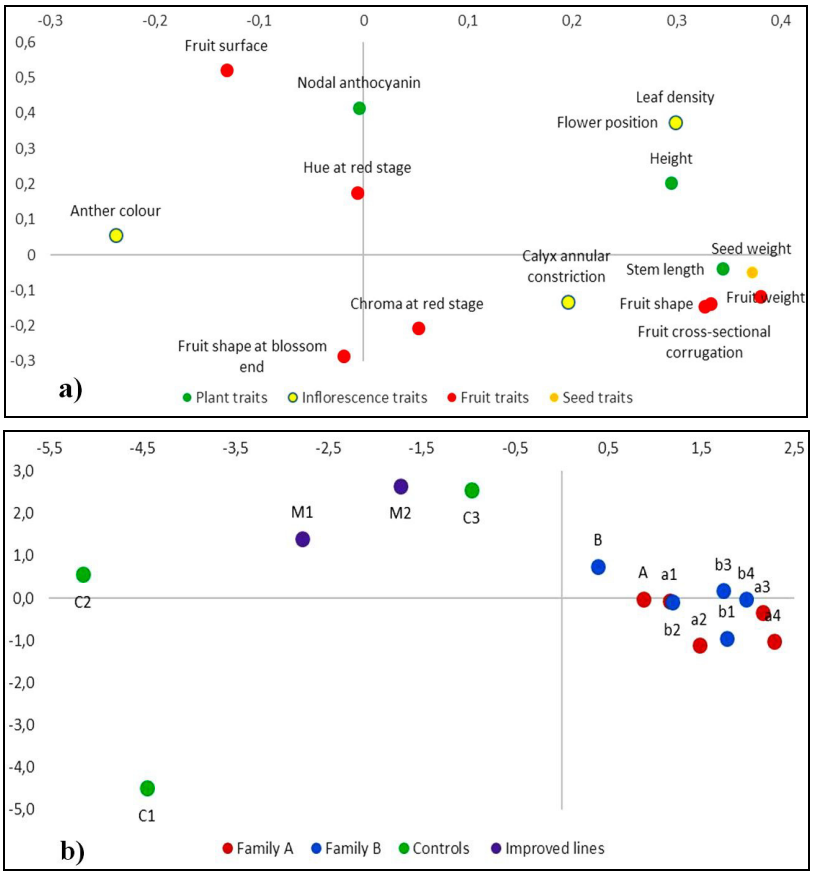

Fig. 2. PC analysis for the first two components corresponding to: i) the distribution of evaluated IPGRI descriptors (upper, Fig. 2a) for plant (green), inflorescence (yellow), fruit (red), and seed (orange) and ii) the distribution of plant accessions based on IPGRI descriptors (lower, Fig. 2b) A, B: parental lines; a1-4, b1-4: progenies; C1-3: OP landraces controls; M1-2: modern cultivars controls

Table 1. Heterozygosity values per accession and mean values for progenies

Family A	Ho	Family B	Ho	Controls	Ho
A	0.29	B	0.41	C1	0.47
a1	0.29	b1	0.29	C2	0.12
a2	0.29	b2	0.47	C3	0.12
a3	0.18	b3	0.12	M1	0.41
a4	0.29	b4	0.35	M2	0.12
μ progenies	0.26		0.31		-

A, B: parent lines; a1-4, b1-4: progenies; C1-3: OP landrace control; M1-2: modern cultivar.

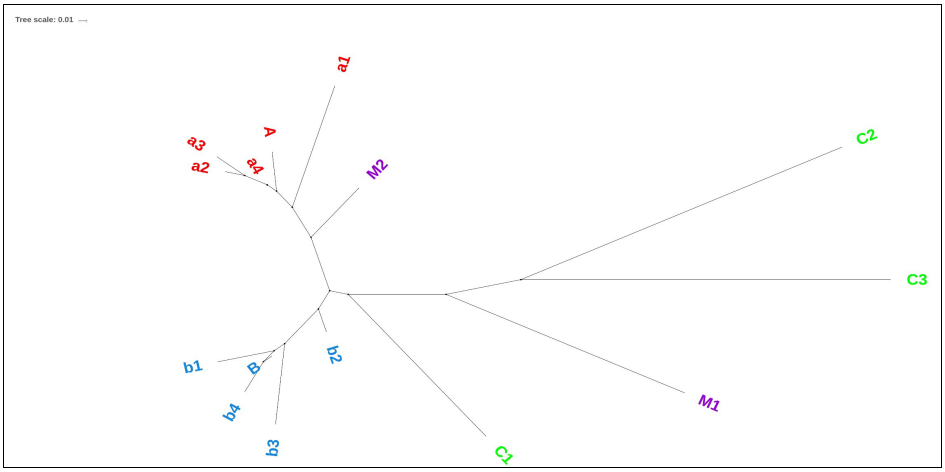

Fig. 3. iTOL's dendrogram (Letunic and Bork, 2016) based on Nei's genetic distances (Nei *et al.*, 1983). A, B: parental lines; a1-4, b1-4: progenies; C1-3: OP landraces controls; M1-2: modern cultivars controls

Observed mean heterozygosity (Ho) was 30% while mean expected heterozygosity (He) was 37%. Ho is higher than expected for an autogamous species. This result might be explained by two factors: i) DNA of eight plants per variety was pooled and ii) open-pollination conditions favoured cross-pollinations. Also, He value may be so high because of the great number of alleles present. Then as a whole, progenies A and B averaged similar or higher homozygosity than their corresponding parental lines (Table 1).

Phylogenetic relationships

Based on the genetic distance matrix a dendrogram was constructed (Fig. 3). Clearly, there are two main groups, one corresponding to family A (red) and the other one to family B (blue). Our results suggest that both families show enough genetic differences to be separated despite being closely related phenotypically. Even within families, different levels of genetic fixation are found among progenies and thus, some of them appear closer to the parental line than others.

Conclusions

For chile Ancho breeding a combination between individual selection with open-pollination conditions proved to be a low-cost method that allows: i) the improvement of lines as well as retaining ii) the expected morphotype inherent to the variety and iii) a certain degree of genetic diversity. The fact that progeny plants had a relatively high level of heterozygosity while being agronomically and morphologically uniform, may provide these materials resilience and adaptation to environmental stress factors and climate change.

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