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Assessing genetic and phenotypic diversity in pepper (*Capsicum annuum* L.) landraces from North-West Spain

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ABSTRACT

Pepper (*Capsicum annuum* L.) is one of the most valuable vegetables in the world. Over the last decades, highly performing cultivars have progressively replaced the diversified and heterogeneous landraces worldwide, causing wide genetic erosion in this crop. The recovery of these ancient landraces, which might preserve alleles of agricultural interest and local adaptations, results of pivotal importance for the development of new varieties and the maintenance of a sustainable agriculture. In the present work, a collection of twenty-six landrace-derived inbred lines and three landraces from North-West Spain were evaluated for their agronomic performance and genetic diversity based on a set of twenty-seven morphological descriptors and twenty microsatellite markers. The collection featured phenotypic variability for all the studied traits, which were influenced by the location, except for the yield. The principal component analysis divided the landraces in well-defined groups, with only Arnoia, Punxin and Blanco Rosal showing some degree of overlapping. The greater part of the variance was accounted for traits such as fruit weight, pericarp thickness and fruit shape and color. The molecular analyses suggested a high level of genetic diversity within the collection and the presence of specific alleles, which were not previously detected in other Spanish pepper landraces. Multivariate and Bayesian clustering showed that landraces were primarily grouped according to their geographical origin and secondarily in agreement with the characteristics of their fruits. Six groups of landraces, with a great genetic differentiation, were clearly identified. Only the landraces Mougan and Arnoia possessed an allele associated to the pungency character.

1. Introduction

The genus *Capsicum* (family *Solanaceae*) originated in the tropical South American region centered in what is now Bolivia (Eshbaugh, 1993). Nowadays, the number of recognized species in the genus is twenty-seven, five of which were domesticated from distinct events at different primary diversification centers (Andrews, 1995; Baral and Bosland, 2002). Among these five, *Capsicum annuum* L. is the most widespread and economically important *Capsicum* species worldwide as well as the most used in commercial cultivar breeding programs (Bosland and Votava, 2000). *C. annuum* was domesticated in Mexico from the wild bird pepper or 'Chiltepin' (*C. annuum* var. *glabriusculum*) and subsequently introduced to Europe by Columbus in the 15th century (Andrews, 1995; Perry et al., 2007; Kraft et al., 2014). Afterwards, it was rapidly distributed to Africa, India and China, where it came into wide cultivation giving way to a current crop of immense cultural and economic importance because of its multiple uses (Bosland and Votava, 2000; Kumar et al., 2006). Thousands of years of human selections in multiple environments and cultural contexts led to the impressive phenotypic diversity of contemporary *C. annuum* fruits (Nuez et al., 1996; Djian-Caporalino et al., 2007; Nicolaï et al., 2013). In general, continued selection was driven to obtain lines with non-deciduous, pendant, larger and non-pungent fruits with greater shape variation and increased fruit mass (Paran and van der Knaap, 2007). The main negative effect of migrations and consequent artificial selections in the secondary diversification centers was the dramatic reduction in the genetic basis of pep-

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per, as occurred for the majority of cultivated species (Tang et al., 2010). Such genetic erosion has become more evident during the past century, when modern breeding promoted the development and worldwide utilization of highly performing commercial cultivars and hybrids, which are genetically uniform and therefore more vulnerable to any biotic or abiotic stress (Votava et al., 2005). At the present time, with the imminent climate change and the worldwide increasing demand of food, to recover the lost genetic variability and made it available to plant breeders become a mandatory task. In this context, pepper landraces, i.e. native varieties empirically selected by farmers over time and well adapted to the agro-environments in which they have been cultivated for long time, represent valuable reservoirs of genetic diversity that has not been fully exploited (Zeven, 1998; Pacheco-Olvera et al., 2012; Liu et al., 2013). Additionally, landraces are commonly associated to better sensory traits, which would serve to explore new niche markets oriented to worldwide consumers willing to pay higher prices for new pepper varieties combining improved taste, healthy benefits and nutritional properties (Casals et al., 2011; Patil et al., 2014).

In Spain, as in other parts of Southern Europe, the versatility of agro-climatic regions and the heterogeneity of the land favoured the survival in cultivation of a large number of specifically adapted pepper landraces very diverse phenotypically (Carravedo et al., 2005; González-Pérez et al., 2014). Part of these landraces was confined to North-West Spain (region of Galicia), where they suffered from selection by farmers, generally directed at fruit attributes, under restricted areas. As a consequence, groups of morphologically recognizable landraces have been defined based on ethnobotanical characteristics in the North (Couto, Couto Grande and Piñeira), Center (Padron and Mougan) and South (Blanco Rosal, Arnoia, Punxin and Oimbra) of Galicia (unpublished data). These landraces are still broadly cultivated in NW Spain, where they sustain the local economy of small horticultural cooperatives. Seeds from different populations of each local variety were collected by the Centro de Investigaciones Agrarias de Mabegondo (CIAM) (A Coruña, Spain), where landrace-derived inbred lines have been obtained during the last decade. This germplasm collection has been partially investigated and data revealed that some of these landraces contain remarkable amounts of vitamin C and antioxidant compounds (unpublished data) and might result exceptionally valuable for the processing industries (Rodriguez-Bao et al., 2004). Such peculiarities make this collection an interesting resource that might be promptly employed for the breeding and scientific communities, as long as a meticulous characterization is being performed. For that purpose, the combination of a morphological approach together with a molecular evaluation will invariably result in more reliable and accurate conclusions (Geleta et al., 2005; Zeinalabedini et al., 2012; Mercati et al., 2014). Among many types of molecular markers that have been developed during the past decades, including the lately released Affymetrix GeneChip array (Ashrafi et al., 2012; Hill et al., 2013), microsatellites or Simple Sequence Repeats (SSRs) are still the most attractive ones for genetic diversity analysis and breeding in plants (Varshney et al., 2005). SSRs are co-dominant, multi-allelic, highly variable and they can be assayed in any laboratory with minimum facilities as well as automated with capillary sequencers for moderate throughput (Schuelke, 2000). In addition, microsatellites performed superiorly than SNPs in resolving population structure (Hamblin et al., 2007; Singh et al., 2013).

The main goal of the present work was to evaluate a collection of twenty-six inbred lines derived from nine different pepper landraces and three landraces still cultivated in NW Spain. The genetic relationships and the level of genetic diversity within and among landraces were investigated in a set of twenty publicly available SSR markers. Likewise, the agronomic performance of lines was assessed with twenty-seven morphological traits (IPGR descriptors) in two different locations.

Table 1
Common name and origin of the landraces from NW Spain used in the present work.

Landrace	Lines[a]	Origin	
		Region	Province
Arnoia	AR330-05, AR55, AR65, AR45, AR274-05	Arnoia	Orense
Blanco Rosal	BR97-04, BR113-05	O Rosal	Pontevedra
Couto	Co5A, Co10A, Co12	Narón	A Coruña
Couto Grande	CG-10-117	Narón	A Coruña
Mougan	MG279-05, MG77-04, MG101-04, MG335-05, MG14-04, MG318-05	Guntin	Lugo
Oimbra	OI27-05, OI30-04	Oimbra	Orense
Padron	ETH80-05, ETH134-05, ETH209-05, PA145, ETH211-05, ETH166-05	Herbón	A Coruña
Piñeira	PI309-05, PI275-05	Ribadeo	Lugo
Punxin	PX74-05, PX118-04	Punxin	Orense

[a] All lines, except AR45, AR55 and AR65, are landraces-derived inbred lines.

2. Materials and methods

2.1. Plant material

Twenty-six pepper (*C. annuum*) inbred lines derived from nine different landraces, collected as seeds from farmers in the main growing regions of Galicia (NW Spain) and three landraces (AR45, AR55 and AR65), were used in this work (Table 1, Fig. S1).

2.2. Agro-morphological characterization

The twenty-nine lines were evaluated over three years (2005–2007) in two experimental fields located in Northern and Southern Galicia. The first one was at the Centro de Investigaciones Agrarias de Mabegondo (Mabegondo, A Coruña, Spain) (43° 15′N, 8° 18′W). The second field was located at Estación Experimental Agrícola de Baixo Miño (Salceda de Caselas, Pontevedra, Spain) (42° 6′N, 8° 33′W), about 10 Km far from the border with Portugal. Both areas, approximately 200 Km distant, belong to the same climate region (Mediterranean maritime) according to the classification of Papadakis (1975) and they exhibit little differences in their agro-climatic conditions. During the time of experiments (2005–2007) slightly higher mean temperatures were observed in Salceda (13.5 °C) with respect to Mabegondo (11 °C), with averages per year of 14 °C, 13 °C and 13.5 °C for the first and 11 °C, 12 °C and 11.5 °C for the second site. On the contrary, the rainfall accomplished better at the latter location (mean values of 85.4 L/m^2 against 71.2 L/m^2). Thus, average values per year (2005, 2006 and 2007) for Mabegondo were 70.1, 105.8 and 80.2 L/m^2, while 62.8, 82.4 and 68.5 L/m^2 for Salceda. Seeds were sown under greenhouse conditions and the seedlings were transplanted to the field in the month of April. The experimental design was a complete randomized block with three replications for a total of 84 plants per plot. Agro-morphological data were collected from 10 plants and 25 fruits per replicate. Twenty-seven traits associated to both