



Screening old peppers (*Capsicum* spp.) for disease resistance and pungency-related traits



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ABSTRACT

A broad panel of *Capsicum* spp. landraces, mostly originating in the Andean region, was assessed with thirteen molecular markers linked to disease resistance and pungency-related traits. *Tsw* and *L⁴* were the most common loci observed within the collection, although resistant alleles associated to *Pvr4*, *Phyto.5.2*, and *Cmr1* were also recorded in approximately 30% of pepper genotypes. South America and, namely the Andean region, revealed as a promising source of disease resistances, particularly against *potyvirus*s. Clustering analysis split the collection into forty groups, each one carrying a distinctive combination of resistant alleles. *Capsicum annuum* and *C. chinense* showed the greatest variability, while the other *Capsicum* species generated homogeneous clusters with well-defined haplotypes. *Capsicum chinense* appeared as the most promising species for breeding purposes, with various accessions showing potential resistance to more than 80% of assessed diseases. PCoA analysis did not show a connection between those resistant genotypes and particular geographical regions. The pungency trait was predicted for 85.5% of pepper accessions. Markers linked to capsinoids content did not amplify or did not displayed polymorphism.

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1. Introduction

Pepper (*Capsicum* spp) is one of the most important vegetables and spices worldwide due to its versatility for culinary usages, medical practices, and use as a highly pungent extract in animal repellents and insecticides (FAOSTAT, 2015). This genus belongs to the Solanaceae family and currently harbours 38 species, of which five are domesticated: *C. annuum* L., *C. chinense* Jacq., *C. frutescens* L., *C. baccatum* L., and *C. pubescens* Ruiz et Pav. (USDA-ARS, 2015). Conventionally, the *Capsicum* species have been organized into three complexes based on cytogenetics and cross fertility. The *C. annuum* complex contains *C. annuum*, *C. chinense*, *C. frutescens*, and *C. galapagoense* Hunziker. The first three species are integrated into a morphological continuum and they are potentially crossable with ease (Onus and Pickersgill, 2004). The *C. baccatum* complex is composed of *C. baccatum*, *C. praetermissum* Heiser et Smith and *C. tovarii* Eshbaugh, Smith et Nickrent. Finally, the *C. pubescens* complex is constituted by *C. pubescens*, *C. cardenasii* Heiser et Smith, and *C. eximium* Hunziker (Walsh and Hoot, 2001). The genus has its ori-

gins in the tropical South American region centered on what is now Bolivia. The five cultivated species were independently domesticated as far back as 6000 B.C. in either Mesoamerica or South America, and the Andean region constituted one of the primary and most significant centers for their diversification (Perry et al., 2007). After the first trip of Columbus, peppers moved to Europe and from there to Africa, India, and China, being *C. annuum* the most successful in this conquest, while *C. baccatum* and *C. pubescens* remained mostly relegated to the Andean region (Bosland and Votava, 2012).

Despite the enormous significance of this crop, diseases still represent one of the major limiting factors in pepper cultivation. Among soil-borne diseases, stand out those caused by the Oomycete *Phytophthora capsici* (phytophthora root rot, stem rot, foliar rot, and fruit rot), the nematode *Meloidogyne incognita* (root-knot), and the bacteria *Xanthomonas campestris* pv. *vesicatoria* (bacterial leaf spot) (Pernezny et al., 2003). Pepper may also suffer from various viral diseases, which involved different genus of viruses; *Tobamovirus*, including Tobacco mosaic virus (TMV) and Tomato mosaic virus (ToMV), *Tospovirus*, such as Tomato spotted wilt virus (TSWV), *Cucumovirus*, like *Cucumber mosaic virus* (CMV), and *Potyvirus*. Regarding the last genus, pepper may be infected by different pathotypes (-0, -1, -2) of potato virus Y (PVY), tobacco etch virus (TEV), and pepper mottle virus (PepMoV) (Kenyon et al.,

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Table 1
Molecular markers used in this study.

Marker name	Marker type	Trait	Loci	Source
OpD04.717	SCAR	<i>P. capsici</i>	<i>Phyto.5.2</i>	Quirin et al. (2005)
060I2END	SCAR	Tobamovirus	<i>L4</i>	Yang et al. (2009)
SCAR_CD	SCAR	Nematode	<i>Me1, Me7</i>	Djian-Caporalino et al. (2007)
PR-Bs3	SCAR	<i>X. campestris</i>	<i>Bs3</i>	Römer et al. (2010)
CaTm-int1	CAPS	CMV	<i>Cmr1</i>	Kang et al. (2010)
SCAC568	CAPS	TSWV	<i>Tsw</i>	Moury et al. (2000)
<i>Pvr1-S/pvr1-R1</i>	CAPS	Potyvirus	<i>Pvr1</i>	Yeam et al. (2005)
<i>pvr1-R2</i>	CAPS	Potyvirus	<i>Pvr1</i>	Yeam et al. (2005)
CSO	CAPS	Potyvirus	<i>Pvr4</i>	Caranta et al. (1999)
T200A	Tetra-ARMS	Potyvirus	<i>pvr2</i>	Rubio et al. (2008)
MAP1	CAPS	Pungency	<i>Pun1</i>	Rodríguez-Maza et al. (2012)
CAPS_ <i>p-amt</i> ²	CAPS	Capsinoids	<i>pAMT</i>	Tanaka et al. (2010a)
SCAR_ <i>p-amt</i> ⁶	SCAR	Capsinoids	<i>pAMT</i>	Jeong et al. (2015)

2014). Some of those diseases can be managed with chemical treatments. However, environmental and consumer concerns, as well as the risk of insensitivity built up in plant pathogens, recommend the development of resistant cultivars as a more effective way to reduce the impact of diseases (Gullino and Kuijpers, 1994). Many resistance genes and quantitative trait loci (QTL) against the major pepper pathogens have been identified and various have been successfully introgressed into commercial varieties (Wang and Bosland, 2006). Pepper resistances are reported to be under both polygenic and monogenic control, the latter being based on either dominant or recessive genes (Sarath Babu et al., 2011). However, very few resistances combine all the characteristics required for a complete solution and the majority is hampered by the appearance of new pathogen races or the instability of genes in changeable environments (Stall et al., 2009; Tomita et al., 2011). Therefore, a continuous supply of disease resistance sources is needed to keep up with the pace imposed by pathogens and the demands of growers. *Capsicum* germplasm collections represent a great genetic wealth for that purpose. However, screening genebanks for disease resistance is often hindered by several drawbacks. Phenotypic evaluations are lengthy, laborious, expensive and sometimes very complex, since several resistance tests cannot be always performed on the same plants. The use of DNA markers closely linked to genes of interesting agronomic traits represents a more efficient and less costly alternative approach, enabling the exploration of vast collections and the accurate prediction of desired plant phenotypes (Yang et al., 2008). Those molecular markers have also proved helpful for a rapid and efficient transfer of useful traits into commercial varieties through marker-assisted selection (MAS) programs. In addition, the molecular identification of accessions potentially carrying multiple resistances, as it was the case of the Mexican *C. annum* landrace ‘Criollo de Morelos 334’ (‘CM334’), would facilitate the accumulation (“pyramiding”) of various resistance genes into the same genotype (Yang et al., 2015). To date, several molecular markers associated to genes or QTL that confer resistance to the major pepper pathogens have been reported (Caranta et al., 1999; Römer et al., 2010; Rubio et al., 2008).

Apart from being a promising source of disease resistances, pepper genebank collections have also revealed as valuable reservoirs of organoleptic traits related to nutraceutical compounds, such as capsaicinoids and capsinoids (Singh et al., 2009; van Zonneveld et al., 2015). DNA markers for the principal genes governing the presence of those compounds were developed as well (Tanaka et al., 2010a; Rodríguez-Maza et al., 2012). The main goal of the current work was to screen a *Capsicum* germplasm panel with a set of markers linked to disease resistance and major organoleptic traits, such as pungency. For that purpose, we selected a set of *Capsicum* spp. accessions mostly originating from the Andean region (Ecuador, Peru, and Bolivia), the primary diversification center of the genus *Capsicum*, but also from secondary centers such as Spain, which

might constitute the entrance of pepper to Europe (González-Pérez et al., 2014). It is expected that these regions harbour larger genetic diversity and preserve more promising variability and valuable traits than other places across the world.

2. Materials and methods

2.1. Plant material

This work was performed in a collection of one hundred eighty *Capsicum* spp. accessions including domesticated and wild species: *C. annum* (58), *C. chinense* (41), *C. frutescens* (17), *C. pubescens* (21), *C. baccatum* (4) *C. baccatum* var. *pendulum* (27), *C. baccatum* var. *baccatum* (3), *C. chacoense* (5), *C. eximium* (2), *C. cardenasii* (1) and *C. tovarii* (1) (Table S1). The material was kindly provided by the USDA, ARS, Plant Genetic Resources Conservation Unit (USA), the Center for Genetic Resources (CGN, The Netherlands), genebanks at the Leibniz Institute of Plant Genetics and Crop Plant Research (Germany), the Institute for Conservation and Improvement of Valencian Agrodiversity (COMAV, Spain), and the Vegetable Germplasm Bank of Zaragoza (BGHZ, Spain). The taxonomical classification used in this work was obtained from the different genebanks, except for seventeen accessions (marked with an asterisk in Table S1), which were detected as taxonomically misclassified in a recent work (Silvar and García-González, 2016). The panel of *Capsicum* spp. was selected trying to cover a wide range of geographical areas, especially those from the Andean region (Ecuador, Peru and Bolivia). When possible, data on the improvement status and population type of each accession was extracted from genebanks databases. No clear information was found for the majority of them. However, detailed investigation on plant inventories holding collection sites and donors, suggest that most of the accessions might be considered local varieties or landraces. A few pepper cultivars were also included. Available data on disease phenotypes were retrieved from genebanks as well (Table S1).

2.2. Molecular marker analysis

DNA was isolated from young leaves of one plant per accession using the CTAB method (Doyle and Doyle, 1987). DNA qualities were evaluated on agarose gels, concentrations were determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington DE) and adjusted to 25 ng μL^{-1} .

Molecular markers linked to resistance and organoleptic traits are indicated in Table 1. The PCR conditions were standardized for OpD04.717, SCAR_CD, CaTm-int1, SCAC568, 060I2END, *Pvr1-R1*, and *pvr1-R2*, according to Di Dato et al. (2015). The amplification of CSO, PR-Bs3, T200A, CAPS-*p-amt*², SCAR-*p-amt*⁶ was performed as described in their original reports (Caranta et al., 1999; Römer et al., 2010; Rubio et al., 2008; Tanaka et al., 2010a; Jeong et al.,

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