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

3
4 Cristina Silvar^{1*} and Carlos A. García-González^{1,2}

5 ¹ Grupo de Investigación en Biología Evolutiva, Departamento de Biología Animal, Biología
6 Vexetal e Ecoloxía. Universidade da Coruña, Campus da Zapateira s/n, 15071 A Coruña, España

7
8 ² Unidad Académica de Ciencias Químicas y de la Salud, Universidad Técnica de Machala, Vía
9 Machala Pasaje Km. 5.5, 070150 El Oro, Ecuador

10
11 *Corresponding author (e-mail): Cristina Silvar (c.silvar@udc.es)

18 **Abstract**

19 A broad panel of *Capsicum* spp. landraces, mostly originating in the Andean region, was assessed
20 with thirteen molecular markers linked to disease resistance and pungency-related traits. *Tsw* and *L*⁴
21 were the most common loci observed within the collection, although resistant alleles associated to
22 *Pvr4*, *Phyto.5.2*, and *Cmr1* were also recorded in approximately 30 % of pepper genotypes. South
23 America and, namely the Andean region, revealed as a promising source of disease resistances,
24 particularly against *potyviruses*. Clustering analysis split the collection into forty groups, each one
25 carrying a distinctive combination of resistant alleles. *Capsicum annuum* and *C. chinense* showed
26 the greatest variability, while the other *Capsicum* species generated homogeneous clusters with
27 well-defined haplotypes. *Capsicum chinense* appeared as the most promising species for breeding
28 purposes, with various accessions showing potential resistance to more than 80 % of assessed
29 diseases. PCoA analysis did not show a connection between those resistant genotypes and particular
30 geographical regions. The pungency trait was predicted for 85.5 % of pepper accessions. Markers
31 linked to capsinoids content did not amplify or did not displayed polymorphism.

32

33

34 **Keywords:** molecular markers ; resistant loci ; capsaicin ; capsinoids ; Andean region

35

36 **1. Introduction**

37 Pepper (*Capsicum* spp) is one of the most important vegetables and spices worldwide due to its
38 versatility for culinary usages, medical practices, and use as a highly pungent extract in animal
39 repellents and insecticides (FAOSTAT, 2015). This genus belongs to the Solanaceae family and
40 currently harbours 38 species, of which five are domesticated: *C. annuum* L., *C. chinense* Jacq., *C.*
41 *frutescens* L., *C. baccatum* L., and *C. pubescens* Ruiz et Pav. (USDA-ARS, 2015). Conventionally,
42 the *Capsicum* species have been organized into three complexes based on cytogenetics and cross
43 fertility. The *C. annuum* complex contains *C. annuum*, *C. chinense*, *C. frutescens*, and *C.*
44 *galapagoense* Hunziker. The first three species are integrated into a morphological continuum and
45 they are potentially crossable with ease (Onus and Pickersgill 2004). The *C. baccatum* complex is
46 composed of *C. baccatum*, *C. praetermissum* Heiser et Smith and *C. tovarii* Eshbaugh, Smith et
47 Nickrent. Finally, the *C. pubescens* complex is constituted by *C. pubescens*, *C. cardenasii* Heiser et
48 Smith, and *C. eximium* Hunziker (Walsh and Hoot 2001). The genus has its origins in the tropical
49 South American region centered on what is now Bolivia. The five cultivated species were
50 independently domesticated as far back as 6000 B.C. in either Mesoamerica or South America, and
51 the Andean region constituted one of the primary and most significant centers for their
52 diversification (Perry et al., 2007). After the first trip of Columbus, peppers moved to Europe and
53 from there to Africa, India, and China, being *C. annuum* the most successful in this conquest, while
54 *C. baccatum* and *C. pubescens* remained mostly relegated to the Andean region (Bosland and
55 Votava 2012).

56 Despite the enormous significance of this crop, diseases still represent one of the major limiting
57 factors in pepper cultivation. Among soil-borne diseases, stand out those caused by the Oomycete
58 *Phyphthora capsici* (phytophthora root rot, stem rot, foliar rot, and fruit rot), the nematode
59 *Meloidogyne incognita* (root-knot), and the bacteria *Xanthomonas campestris* pv. *vesicatoria*
60 (bacterial leaf spot) (Pernezny et al., 2003). Pepper may also suffer from various viral diseases,

61 which involved different genus of viruses; *Tobamovirus*, including Tobacco mosaic virus (TMV)
62 and Tomato mosaic virus (ToMV), *Tospovirus*, such as Tomato spotted wilt virus (TSWV),
63 *Cucumovirus*, like *Cucumber mosaic virus* (CMV), and *Potyvirus*. Regarding the last genus, pepper
64 may be infected by different pathotypes (-0, -1, -2) of potato virus Y (PVY), tobacco etch virus
65 (TEV), and pepper mottle virus (PepMoV) (Kenyon et al., 2014). Some of those diseases can be
66 managed with chemical treatments. However, environmental and consumer concerns, as well as the
67 risk of insensitivity built up in plant pathogens, recommend the development of resistant cultivars
68 as a more effective way to reduce the impact of diseases (Gullino and Kuijpers 1994). Many
69 resistance genes and quantitative trait loci (QTL) against the major pepper pathogens have been
70 identified and various have been successfully introgressed into commercial varieties (Wang and
71 Bosland 2006). Pepper resistances are reported to be under both polygenic and monogenic control,
72 the latter being based on either dominant or recessive genes (Sarath Babu et al., 2011). However,
73 very few resistances combine all the characteristics required for a complete solution and the
74 majority is hampered by the appearance of new pathogen races or the instability of genes in
75 changeable environments (Stall et al., 2009, Tomita et al., 2011). Therefore, a continuous supply of
76 disease resistance sources is needed to keep up with the pace imposed by pathogens and the
77 demands of growers. *Capsicum* germplasm collections represent a great genetic wealth for that
78 purpose. However, screening genebanks for disease resistance is often hindered by several
79 drawbacks. Phenotypic evaluations are lengthy, laborious, expensive and sometimes very complex,
80 since several resistance tests cannot be always performed on the same plants. The use of DNA
81 markers closely linked to genes of interesting agronomic traits represents a more efficient and less
82 costly alternative approach, enabling the exploration of vast collections and the accurate prediction
83 of desired plant phenotypes (Yang et al., 2008). Those molecular markers have also proved helpful
84 for a rapid and efficient transfer of useful traits into commercial varieties through marker-assisted
85 selection (MAS) programs. In addition, the molecular identification of accessions potentially

86 carrying multiple resistances, as it was the case of the Mexican *C. annuum* landrace 'Criollo de
87 Morelos334' ('CM334'), would facilitate the accumulation ("pyramiding") of various resistance
88 genes into the same genotype (Yang et al., 2015). To date, several molecular markers associated to
89 genes or QTL that confer resistance to the major pepper pathogens have been reported (Caranta et
90 al., 1999, Römer et al., 2010, Rubio et al., 2008).

91 Apart from being a promising source of disease resistances, pepper genebank collections have also
92 revealed as valuable reservoirs of organoleptic traits related to nutraceutical compounds, such as
93 capsaicinoids and capsinoids (Singh et al., 2009, van Zonneveld et al., 2015). DNA markers for the
94 principal genes governing the presence of those compounds were developed as well (Tanaka et al.,
95 2010a, Rodríguez-Maza et al., 2012). The main goal of the current work was to screen a *Capsicum*
96 germplasm panel with a set of markers linked to disease resistance and major organoleptic traits,
97 such as pungency. For that purpose, we selected a set of *Capsicum* spp. accessions mostly
98 originating from the Andean region (Ecuador, Peru, and Bolivia), the primary diversification center
99 of the genus *Capsicum*, but also from secondary centers such as Spain, which might constitute the
100 entrance of pepper to Europe (González-Pérez et al., 2014). It is expected that these regions harbour
101 larger genetic diversity and preserve more promising variability and valuable traits than other places
102 across the world.

103

104 **2. Materials and methods**

105 *2.1. Plant material*

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

124 *2.2. Molecular marker analysis*

125 DNA was isolated from young leaves of one plant per accession using the CTAB method (Doyle
126 and Doyle, 1987). DNA qualities were evaluated on agarose gels, concentrations were determined

127 with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington DE) and
128 adjusted to 25 ng μL^{-1} .
129 Molecular markers linked to resistance and organoleptic traits are indicated in Table 1. The PCR
130 conditions were standardized for OpD04.717, SCAR_CD, CaTm-int1, SCAC568, 060I2END,
131 *PvrI*-R1, and *pvrI*-R2, according to Di Dato et al. (2015). The amplification of CSO, PR-*Bs3*,
132 T200A, CAPS-*p-amt*², SCAR-*p-amt*⁶ was performed as described in their original reports (Caranta
133 et al., 1999, Römer et al., 2010, Rubio et al., 2008, Tanaka et al., 2010a, Jeong et al., 2015). SCAR
134 markers were directly visualized under UV light on 2-3 % agarose gels stained with GreenSafe
135 Premium (NZYTech). PCR products derived from amplifications with CAPS markers were
136 digested with the corresponding restriction enzymes. Digestions were carried out in a 15 μL volume
137 using 1U of the corresponding endonuclease (Thermo Fisher Scientific) with an incubation time of
138 3 h. The restricted fragments were separated in 2.5 % agarose gels and visualized as described
139 above. The marker MAP1 was detected in an ABI PRISM 3130 genetic analyzer (Applied
140 Biosystems) after PCR amplification and 6-FAM labelling with a forward tailed primer, as
141 described by Rodriguez-Maza et al. (2012).

142 2.3. Data analysis

143 All marker data were used to generate a 0/1 matrix (presence/absence of each allele at the marker
144 locus). Pairwise similarities were calculated using the Jaccard coefficient (Jaccard, 1908). A tree
145 depicting the patterns of clustering for the different pepper genotypes was built from the similarity
146 matrix using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Graphical
147 representation of the tree was performed with MEGA 6.0 software (Tamura et al., 2013). Genetic
148 discrimination among different geographical regions was additionally performed through a principal
149 coordinate analysis (PCoA). All analyses were carried out using R 3.0.2 software (R Development
150 Core Team, 2014).

151

152 3. Results

153 3.1. Screening for disease resistance and pungency-related traits

154 A summary for the screening of the 180 pepper genotypes evaluated with 10 markers linked to
155 QTLs or alleles for disease resistance and 3 markers associated to pungency-related traits is shown
156 in Table S2. For the sake of clarity, accessions from *C. baccatum* var. *pendulum* and var. *baccatum*
157 were considered as a whole group. Fifty-six pepper genotypes showed the resistant allele for the
158 OpD04.717 marker associated to the *Phyto.5.2* locus (Fig. 1, Table 2). The majority of those belong
159 to *C. chinense* and *C. annuum* species. All five *C. chacoense* individuals amplified the fragment of
160 almost 700 bp, while none of the evaluated *C. pubescens* accessions displayed the resistant allele
161 (Table 2). The allele for resistance in the dominant marker 060I2END linked to the L^4 locus
162 appeared in the majority (range between 81 % and 92.7 %) of domesticated species, except for *C.*
163 *annuum*, for which only the 5.2 % of genotypes did not show amplification of the ca. 750 bp
164 amplicon (Fig. 1). A 100 % of non-domesticated *C. chacoense*, *C. eximium*, *C. cardenasii*, and *C.*
165 *tovarii* exhibited the 060I2END resistant allele (Table 2). The 107 bp PCR product associated to
166 resistant genotypes in marker SCAR_CD (Fig. 1) came out in the majority (92.7 %) of *C. chinense*
167 accessions and various (17.2 %) *C. annuum* genotypes. Interestingly, all the other species displayed
168 lack of resistance (Table 2). The marker CaTm-int1, linked to the major resistant gene *Cmr1* against
169 CMV, resulted in two fragments (150 bp and 300 bp) in the resistant peppers after the digestion
170 with *HinfI* (Fig. 1). Resistance to CMV was only detected in 94.1 % and 90.2 % of *C. frutescens*
171 and *C. chinense* accessions, respectively (Table 2). The CAPS marker SCAC568, linked to the
172 *Tswv* gene, which confer resistance to TSWV, was digested with *TaqI*, providing two fragments
173 (220 bp and 320 bp) in the susceptible genotypes (Fig. 1). Broad resistance to TSWV was found in
174 *C. baccatum*, *C. frutescens*, and *C. chinense* for which 100 %, 100 % and 97.6 % respectively, of
175 tested individuals were recorded as resistant (Table 2). The SCAC568 undigested fragment
176 associated to resistance was also observed for all *C. chacoense*, *C. eximium*, and *C. cardenasii*

177 accessions, while lack of amplification was found for the single *C. tovarii* genotype (Table 2).
 178 Curiously, all *C. pubescens* accessions from Ecuador and the majority of Peru did not amplify any
 179 susceptible or resistant allele (Table S2). The SCAR marker PR-*Bs3*, diagnostic for the R gene *Bs3*,
 180 which provides resistance against various races of *Xanthomonas campestris* pv. *vesicatoria* was
 181 detected as an InDel polymorphism located in the *Bs3* promoter. Amplicons derived from resistant
 182 accessions were 13 bp shorter than those obtained in susceptible genotypes (Fig. 1). The PR-*Bs3*
 183 resistant allele was only recorded in two *C. annuum* individuals; C66 from India and PI585238
 184 from Ecuador (Table S2). Interestingly, few *C. pubescens* accessions (PI585262, CGN23768,
 185 CAP357, CAP1486) from Ecuador and Peru, together with the two *C. eximium*, and the single *C.*
 186 *cardenasii* accessions displayed an unexpected fragment of ca. 150 bp. The codominant marker
 187 CSO, linked to the *Pvr4* locus for resistance to PVY, was recorded as a 444 bp fragment in resistant
 188 genotypes after restriction with *AlwNI* (Fig. 1). Potential resistance to PVY was mostly observed in
 189 *C. baccatum* (85.3 %) and *C. chinense* (56.1 %). Remarkably, no individual of *C. frutescens* (except
 190 for PI439488 from Bolivia) and *C. pubescens* (except for CAP217, CAP357, CAP1486, and
 191 BGV013702 from Peru) showed amplification of the CSO marker. Similarly, the wild species did
 192 not amplify either (Table S2). The combination of allele-specific CAPS markers *PvrI*-S, *pvrI*-R1,
 193 and *pvrI*-R2 allowed detecting the dominant *PvrI*⁺ susceptible allele and the resistant alleles *pvrI*,
 194 *pvrI*¹, and *pvrI*² at the *pvrI* locus. Thus, *PvrI*-S PCR products, digested with *BsrI*, generated the
 195 expected fragments of 133 and 578 bp in susceptible genotypes *PvrI*⁺/*PvrI*⁺ (Fig. 1). In contrast,
 196 the *pvrI*-R1 primer set distinguished the *pvrI* recessive allele after restriction of amplicons with
 197 *Fnu4HI*, which results in three fragments of 556, 86, and 69 bp, although the discrimination of the
 198 last two was impossible on 2.5 % agarose gels (Fig. 1). The *pvrI*² genotypes was recorded by using
 199 the *pvrI*-R2 marker and subsequent digestion with *HindIII*, which produces a fragment of 380 bp
 200 and an agarose unresolved product of 32 bp (Fig. 1). Finally, the combination of genotyping results
 201 from all three markers permit to elucidate the presence of the *pvrI*¹. The *pvrI* allele, which confers

202 resistance against TEV, PVY (0), and PepMoV was detected in 15 accessions of *C. chinense* (Table
 203 S2). Only two *C. annuum* genotypes (BGV005857 and C51) possess the *pvrI*² allele, which avoids
 204 the replication of the *potyviruses* TEV and PVY (0, 1). The *pvrI*¹ allele, which protects pepper only
 205 against PVY (0), was observed in thirteen *C. annuum* and three *C. chinense* (Table S2). Curiously,
 206 four *C. chinense* accessions from Ecuador and Peru showed two fragments (133 bp and 578 bp) for
 207 the *PvrI*-S marker, which suggest the existence of the *PvrI*⁺ allele and therefore the potential
 208 susceptibility of those plants. However, the *pvrI*-R1 marker tested on these accessions produced the
 209 expected fragments (69 bp+89 bp+556 bp) for the presence of the *pvrI* allele (Table S2). Potential
 210 resistance against PVY and TEV was further investigated with the tetra-ARMS marker T200A,
 211 which is able to discriminate the dominant allele conferring susceptibility *Pvr2*⁺ from the recessive
 212 resistant alleles *pvr2*¹, *pvr2*², and *pvr2*³. The expected amplicon of 258 bp was detected in all fifteen
 213 *C. annuum* accessions that displayed a *pvrI*¹ or *pvrI*² allele at the *pvrI* locus. The other accessions
 214 belonging to species other than *C. annuum* exhibited the 199 bp amplicon and they were considered
 215 as potentially susceptible (Fig. 1, Table S2).

216 The putative presence of the pungency trait in the *Capsicum* accessions was investigated by using
 217 the molecular marker MAP1, which was developed based on a DNA sequence with high similarity
 218 to *Pun1* locus (Rodriguez-Maza et al., 2012). Polymorphism was detected with allele sizes of 514
 219 bp for potentially pungent accessions and 497 bp for non-pungent accessions (data not shown).
 220 Thirty-three out of 58 (56.9 %) *C. annuum* accessions showed the pungent allele. Similarly, the 514
 221 bp fragment was observed for all non *annuum* pepper, except for *C. frutescens* C452, who displayed
 222 a heterozygous genotype (Table S2). Interestingly, all tested *C. pubescens* accessions exhibited a
 223 fragment of 519 bp and they were considered as potentially pungent (Table 2). The CAPS marker
 224 developed for the Himo-type allele of the *pAMT* gene (*p-amt*²) (Tanaka et al., 2010a) was tested in
 225 the whole collection. Polymorphism was not found in any out of 180 genotypes after restriction
 226 with *RsaI* (Fig.1, Table S2). The SCAR marker recently reported by Jeong et al. (2015) in the

dysfunctional *p-amt*⁶ allele of *C. chinense* SNU11-001 did not amplify any *Capsicum* accession (data not shown).

The 180 pepper accessions were grouped according to their place of origin in order to investigate the existence of geographical regions harboring higher number of alleles for promising traits (Table 3). Pepper accessions from the Andean region (Ecuador, Peru, and Bolivia) showed resistant alleles for the majority of tested markers. The highest number of those alleles was recorded for 060I2END and SCAC568, linked to loci for TSWV and *Tobamovirus* resistance (Table 3). Moderate presence of potential resistance to *P. capsici*, *Meloidogyne* spp., and CMV was also found in the countries from the Andean region (Table 3). The *pvr1*, *prv1*¹, and *pvr1*² alleles were detected in Ecuador, *prv1*¹ and *pvr1* in Peru and only *prv1*¹ in Bolivia. Resistance to *potyviruses* PVY and PepMoV seems to be due also to the presence of the *Pvr4* resistance gene, which appeared in almost half of individuals from Peru and Bolivia. The larger part of Andean peppers (87.5 % from Ecuador, 95.1 % from Bolivia, and 90.5 % from Peru) was considered as potentially pungent (Table 3). Half of Mexican peppers were catalogued as resistant to *P. capsici* and TSWV, while Brazilian accessions exhibited a high number of resistant alleles for TSWV and CMV. In general, low levels of resistance were recorded in pepper landraces from Spain (Table 3), which are mostly *C. annuum* (Table S1).

3.2. Genotypes clustering and distribution of traits

A clustering analysis, based on the presence/absence of resistance/pungency alleles, was performed on the collection of pepper landraces in order to identify homogeneous groups of accessions carrying multiple alleles for traits of interest. Firstly, fifteen genotypes that did not show any potential resistance/pungency and five individuals which exhibited more than 20 % of missing data were removed to avoid distortion of group numbers. The remaining 160 *Capsicum* accessions were clustered in 40 groups resulting in various expected phenotypes for the diseases under study (Fig. 2, Table S3). Sixteen accessions, eleven of them being *C. annuum*, displayed a distinctive combination

252 of alleles and they were clustered into single groups. Among those the *C. annuum* accessions C96,
253 CAP524, C141, C1, PI260436, and C106 accumulated the highest number of resistance genes,
254 mainly against PVY and *P. capsici*, but also against TSWV or nematodes (Table S3). The other *C.*
255 *annuum* landraces were primarily clustered in groups 5, 9-19, and the majority displayed resistance
256 to only one disease, mostly to those caused by *P. capsici* or *potyviruses* (Fig. 2). Group 12, which
257 comprised the highest number of *C. annuum* genotypes, did not show any resistance genes, but they
258 carried the allele for pungency (Table S3). The other *Capsicum* species made compact clusters with
259 well-defined haplotypes of specific alleles for disease resistance, all of them carrying an allele for
260 pungency. *Capsicum pubescens* and *C. baccatum* were represented in groups 38 and 39 and they
261 show resistance to *Tobamovirus*, TSWV, and PVY (0, 1, 2) (Fig. 2, Table S3). Remarkably, group
262 38 contained those *C. pubescens* from Ecuador and Peru that did not amplify for markers SAC568
263 and/or CSO, and they were catalogued as potentially susceptible to TSWV and/or *potyvirus*,
264 although they grouped together with the resistant genotypes (Table S3). The group 40 comprised
265 the majority of *C. frutescens* accessions, which possessed likely resistance to *Tobamovirus*, CMV,
266 and TSWV (Fig. 2, Table S3). *Capsicum chinense* accessions were distributed among groups from
267 20 to 29 and they exhibited the highest number of resistant-gene linked markers. Thus, groups 21,
268 23, 25, and 29 arose as exceptional sources of potential resistance to major pathogens of pepper,
269 carrying up to seven resistant alleles (Fig. 2, Table S3). All *C. chacoense* genotypes clustered
270 together under group 37 and they possessed a singular combination of resistant genes against *P.*
271 *capsici*, *Tobamovirus*, and TSWV (Fig. 2, Table S3). Interestingly, the seventeen accessions, which
272 were previously detected as taxonomically displaced (Silvar and García-González 2016) showed a
273 pattern of resistances that accurately coincide with the taxonomical group of their current
274 classification.

275 PCoA was performed independently for those species (*C. annuum* and *C. chinense*) showing the
276 greatest variability, with the aim of assessing hypothetical geographic patterns of genetic

277 differentiation associated to disease resistance (Fig. S1). The first coordinate explained a variation
278 between 23.15 % and 17.04 %, for *C. annuum* and *C. chinense*, respectively. Clustering of
279 genotypes based on their geographical origin was not observed for any of the *Capsicum* spp. In
280 contrast, patterns of grouping responded to the predicted phenotypes for disease resistance and
281 pungency and they served to identify those more promising genotypes for disease resistance within
282 each species (Fig. S1).

283 4. Discussion

284 Pathogens are a threat for the adequate growth and yield of most *Capsicum* species all around the
285 world. In this context, search for new sources of resistance still represents a challenge nowadays,
286 and pepper germplasm collections are a promising target for that purpose. All the same, the
287 exploration of valuable organoleptic traits on genetic resources would benefit growers and
288 consumers, favoring the emergence of new niche markets. In the present work, a collection of
289 pepper accessions, mostly originating from the Andean region, the primary diversification center of
290 *Capsicum* spp., was screened with a set of molecular markers linked to genes or QTLs for disease
291 resistance and pungency-related traits.

292 *Phyto.5.2* locus was reported to have a large effect on *P. capsici* resistance and to condition a broad
293 resistance across multiple isolates (Thabuis et al., 2003). The dominant OpD04.717 allele, linked to
294 that locus, was recorded for all *C. chinense* accessions under study and for few *C. annuum*
295 individuals. Remarkably, resistance to this pathogen was also found in *C. chacoense* accessions,
296 which contradicts results of Di Dato et al. (2015), who did not identify any resistant genotype
297 within that species. *Capsicum annuum* and *C. chinense* resistant accessions came mainly from
298 Central America and the Caribbean region, where the most durable source of resistance to *P.*
299 *capsici*, 'CM334', was identified (Bosland and Lindsey, 1991). *Capsicum annuum*, *C. chinense* and
300 to a lesser extent *C. baccatum* have been described as source of resistance against several or
301 specific *P. capsici* races (Sarath Babu et al., 2011). Our *C. annuum* resistant panel includes well-
302 known accessions from Mexico such as 'PI201234', 'Jalapeño' or 'Chile de Arbol' but also some
303 local varieties from Peru, Bolivia, and Spain. Previous clustering analysis on those resistant *C.*
304 *annuum* revealed that Spanish accessions belonged to different clades than Mexican ones
305 (González-Pérez et al., 2014). Such evidence suggests that Spanish and Mexican *C. annuum*
306 peppers are not genetically closely related, and they might possess different alleles for resistance to
307 *P. capsici*.

308 Screening for resistance to nematodes was performed with the SCAR_CD marker, which is
309 associated to *Me1-Mech2*, *Me7-Mech1* resistance genes of the *Me*-cluster anchored on the P9
310 chromosome. Resistant alleles to this root-knot disease were recorded in few *C. annuum* and the
311 majority of *C. chinense*, but they were totally absent in the other species. Several sources of
312 resistance to *M. incognita* were reported in *C. annuum*, *C. chinense*, *C. chacoense*, and *C.*
313 *frutescens* (Fery et al., 1998, Oka et al., 2004). However, in our work, nematode resistance was not
314 observed in any *C. frutescens* or *C. chacoense* genotypes. This might be attributed to the presence
315 in those species of distinctive genes or alleles controlling resistance. Indeed, Gisbert et al. (2013)
316 reported resistant amplicons in *C. frutescens* for the CAPS_F4R4 marker, linked to *Me7-Mech1*
317 alleles, but not for the SCAR_CD marker. Such results suggest that the accurate assessment for
318 nematode resistance, especially on non-*annuum* species, should be performed with a broader set of
319 markers covering various alleles at specific loci.

320 The *R* gene *Bs3* provides resistance against *Xanthomonas* races 0, 1, 4, 7, and 9 and it is currently
321 the gene of choice for deployment in commercial varieties, on which strains of the pathogen have
322 overcome other bacterial spot resistance genes (Römer et al., 2007). In the present work, the
323 resistant allele linked to that gene was detected in two *C. annuum* individuals from Ecuador and
324 India. Those accessions could be useful donors for new pepper varieties now that KASP markers
325 have been developed, which leaves behind the potential ambiguity of scoring a 13-bp deletion in
326 agarose gels (Holdsworth and Mazourek 2015).

327 A likely resistance source to *cucumber mosaic virus* (CMV) was found in 94.1 % of *C. frutescens*
328 and 90.2 % of *C. chinense* accessions, but not in any genotype from other domesticated or wild
329 species. Our results are based on marker CaTm-int1, linked to *Cmr1*, the only single dominant
330 resistance gene against CMV identified in *C. annuum* cv. 'Bukang' (Kang et al., 2010). *Capsicum*
331 *chinense* and *C. frutescens* were also reported as good source of resistance against CMV by Di Dato
332 et al. (2015) by using the same CAPS marker, but also by means of phenotypic assays (Grube et al.,

2000). Nevertheless, *Tospovirus* resistance in our *C. annuum* panel should not be discarded and might be explained by the presence of any other of the multiple mechanisms for CMV resistance described in that species, from qualitative to quantitative genetic loci (Suzuki et al., 2003, Yao et al., 2013).

Potential resistance to TSWV, based on the *Tsw* dominant resistant allele, was recorded in the whole panels of *C. chinense*, *C. frutescens*, *C. baccatum*, *C. chacoense*, *C. eximium*, and *C. cardenasii*, together with few accessions of *C. annuum*. These results are in agreement with those from Cebolla-Cornejo et al. (2003) and Di Dato et al. (2015), with the additional alleles found in *C. annuum* individuals from Mexico, Peru, and Spain, which might constitute straightforward candidates for resistance exploitation.

The absence of SCAC568 amplification on *C. pubescens* accessions from Ecuador and Peru was surprising and, to the best of our knowledge, not reported earlier. This might be the result of parallel recombination affecting the genetic linkage between the marker and the resistant gene, as a consequence of human selection for other traits of interest in Ecuadorian *C. pubescens* landraces.

Among the known genetic factors conferring resistance to *tobamovirus*, the L^4 allele at the *L* locus is considered to have the broadest resistance spectrum, mediating resistance against P₀, P₁, P_{1,2}, and P_{1,2,3} pathotypes (Kim et al., 2008). The resistant dominant allele for the marker 060I2END, associated to L^4 , was found in almost all accessions from domesticated and wild species, with the exception of *C. annuum*, on which potential resistance to *tobamovirus* was only recorded in three landraces. Similar results were reported recently by Di Dato et al. (2015). Moreover, the current work revealed further sources of *C. chacoense* genotypes carrying the L^4 allele, apart from those previously described (Yang et al., 2012).

Resistance to *potyviruses* was investigated by using different markers linked to *pvr1*, *pvr2*, and *pvr4* resistant genes (Caranta et al., 1999, Yeaman et al., 2005, Rubio et al., 2008). To date, at least 11 alleles are known to naturally occur at the *pvr1* locus conferring a characteristic spectrum of

358 resistance against distinctive sets of pathotypes or strains of TEV, PVY, and PepMoV (Ibiza et al.,
359 2010). Among those, three major recessive resistant alleles (*pvr1*, *pvr1*¹, and *pvr1*²) have been the
360 most widely used in breeding strategies (Mazourek and Wyatt, 2013). In the present work, *C.*
361 *chinense* lines arise as the most promising resource against *potyviruses*, since the predominant *pvr1*
362 allele protects pepper plants against TEV, PVY (0), and PepMoV (Yeam et al., 2005). Moreover,
363 *pvr1* alleles were primarily recorded across the Andean countries, likely connected with the
364 presence of more virulent PVY pathotypes than in Europe (Caranta et al., 1999). Curiously, four *C.*
365 *chinense* accessions from Ecuador showed a positive scoring for both *Pvr1*⁺ and *pvr1* alleles. Such
366 output suggests the heterozygosity of those lines at the *pvr1* locus, and a plausible susceptibility due
367 to the recessive inheritance of the *pvr1* resistant allele, as being demonstrated in *C. annuum* (Lee et
368 al., 2013). Potential resistance to *potyviruses* in the *Capsicum* spp. panel was further assessed with
369 the T200A marker, linked to the formerly known as *pvr2* locus (Rubio et al., 2008). The resistant
370 amplicon was recorded exactly in the same *C. annuum* genotypes that were predicted to carry a
371 *pvr1* allele for resistance, bringing to light the great potentiality of those lines as a source for
372 resistance against PVY. The dominant locus *Pvr4*, which controls a complete inhibition of viral
373 replication and accumulation, was investigated as well. The resistant allele was observed in all but
374 three *C. baccatum*, 56.1 % of *C. chinense*, and 12.1 % of *C. annuum*, suggesting that those lines
375 carry potential resistance to PVY (0, 1, 2) and PepMoV. Remarkably, the CSO marker linked to
376 *Pvr4* locus did not amplify in the wild species, either in the majority of genotypes belonging to *C.*
377 *frutescens* and *C. pubescens*. As far as we know, no previous report has emphasized the lack of
378 amplification of this CAPS marker in particular *Capsicum* spp. This might be explained by the
379 origin of *Pvr4* locus, which was identified in the Mexican *C. annuum* line 'Criollo de Morelos 334'.
380 Thus, the absence of transferability might be connected to DNA sequence modifications at specific
381 genomic regions in genetically distant *Capsicum* spp.

382 Pungency is one of the major quality traits of peppers, and is due to the presence of secondary
 383 metabolites called capsaicinoids. The genetic control of this trait resides on the allelic variants at
 384 *Pun1* locus for *C. annuum* (*pun1*), *C. chinense* (*pun1*²), and *C. frutescens* (*pun1*³), and the *pun2*
 385 locus for *C. chacoense* (Stewart et al., 2007, Stellari et al., 2010). A codominant molecular marker,
 386 MAP1, was developed on a DNA sequence highly similar to the *Pun1* locus, based on a 15 bp
 387 deletion observed in non-pungent peppers (Rodriguez-Maza et al., 2012). In our *Capsicum*
 388 collection, the MAP1 allele for pungency was found in 33 (56.9 %) *C. annuum* accessions and in all
 389 individuals from the non-*annuum* species, which agree with results by Rodriguez-Maza et al. (2012)
 390 and Di Dato et al. (2015). However, the fact that this marker has not been tested in non-pungent
 391 *Capsicum* species other than *C. annuum*, somewhat mistrust its reliability, since the loss of
 392 pungency on those materials might be controlled by other different allelic mutations or loci (Stellari
 393 et al., 2010).

394 Apart from capsaicinoids, various pepper varieties possess structurally analogues compounds,
 395 named capsinoids, which are less pungent but show similar bioactive effects (Sasahara et al., 2010).
 396 Genetic factors controlling high levels of capsinoids in *C. annuum*, *C. chinense*, and *C. frutescens*
 397 are mainly related to mutations disrupting the function of the putative aminotransferase (*pAMT*)
 398 gene, which regulates the conversion of vanillin into vanillyl alcohol, promoting the capsinoids
 399 biosynthetic pathway (Tanaka et al., 2010b, Park et al., 2015, Tanaka et al., 2015). The CAPS
 400 marker used in this work is associated to the *p-amt*² allele that was described as a single nucleotide
 401 modification in the Japanese *C. annuum* cv. 'Himo' (Tanaka et al., 2010a, Tanaka et al., 2015). In
 402 contrast, the SCAR marker developed by Jeong et al. (2015), arise from the *pamt* mutation
 403 identified in *C. chinense* 'SNU11-001' line, and is highly similar to the *p-amt*⁶ allele (Jang et al.,
 404 2015, Tanaka et al., 2015). The CAPS-*p-amt*² marker amplified all accessions within our *Capsicum*
 405 panel, but no polymorphism was detected, even not in the *C. annuum* genotypes. This was
 406 somehow expected considering the high specificity of that CAPS marker, developed on the basis of

407 a single-nucleotide substitution on the *pAMT* coding region from cultivar 'Himo'. Surprisingly, the
408 SCAR- *p-amt*⁶ marker did not generate any of the expected amplicons, albeit various adjustments
409 on the PCR conditions were performed (data not shown). Such results connote a certain lack of
410 marker robustness when using with pepper materials other than those on which primer sets were
411 designed and suggest that further optimization should be performed. Essential requisites for the
412 usefulness of a diagnostic marker are not only its functionality but also its universality for the
413 exploration of complex and large genetic pools. In addition, the novelty of this SCAR marker
414 (Jeong et al., 2015) made it difficult to find comparable data in the literature that support our
415 conclusions. Therefore, molecular analysis did not clearly establish the potential presence of
416 capsinoids in our pepper accessions. HPLC analysis carried out in mature fruits of some of our
417 Spanish *C. annuum* genotypes did not reveal any capsinoids content either (unpublished data).
418 However, further work is still needed, especially on *Capsicum* spp. from the Andean region, on
419 which high levels of capsinoids were reported previously (Singh et al., 2009).

420 Clustering analysis provided a representation of groups of accessions with similar predicted
421 phenotypes, which would facilitate the selection of germplasm for further evaluation. The majority
422 of *C. frutescens*, *C. baccatum*, *C. pubescens*, and *C. chacoense* accessions formed homogeneous
423 groups with low variability, while *C. annuum* and *C. chinense* were distributed among various
424 clusters, showing accessions with singular combinations of genes as well as genotypes genetically
425 distant from others of the same species. Although most of the accessions analyzed were able to
426 transfer at least one resistance gene, the most promising species for breeding purposes was *C.*
427 *chinense*, from which up to six accessions were recorded as likely resistant to *P. capsici*,
428 *Tobamovirus*, nematodes, CMV, TSWV, TEV, PepMoV, and PVY (0, 1, 2). Those resistances
429 could be readily introgressed into *C. annuum* through hybridizations, since both species belong to
430 the *C. annuum* complex and they are sexually compatible (Yoon et al., 2004). *Capsicum frutescens*
431 represents another crossable species within the same gene pool that has been previously described

432 as a notable source of disease resistance (Grube et al., 2000, Oka et al., 2004). In the present work,
433 *C. frutescens* accessions displayed potential resistant genes against *Tobamovirus*, CMV, and
434 TSWV. Unfortunately, resistance to *potyviruses* could not be plainly established due to the lack of
435 amplification in marker CSO, linked to the *Pvr4* locus, which mediates inhibition of PepMoV and
436 PVY (0, 1, 2). However, we cannot discard that those lines contain other genes, such as *pvr3*, *pvr5*
437 or *pvr7*, which are unlinked to the *pvr1* locus, and confer resistance to several *potyviruses* (Wang
438 and Bosland 2006). The lowest number of alleles for resistance was mainly recorded in *C. annuum*
439 accessions, many of them displaying hypothetical susceptibility for all the pathogens targeted in this
440 study. *Capsicum annuum* is the most widely cultivated species all over the world, and the one
441 subjected to the highest pressure of human selections (Bosland and Votava 2012). The
442 domestication and subsequent artificial selections might be likely accompanied by undesirable loss
443 of resistant alleles in resulting *C. annuum* cultivated germplasm (Doebley et al., 2006). Indeed, the
444 broadest resistant *C. annuum* accessions in our collection were those from Mexico, the centre of
445 domestication for this *Capsicum* species (Kraft et al., 2014). Analysis of cluster for the pungency
446 trait disclosed interesting groups within the *C. annuum* species, which might be exploited either for
447 the combined introgression of various disease resistances and pungency (e.g. groups 1-5), or for the
448 introgression of disease resistances in non-pungent genotypes (e.g. groups 6, 9, 10, and 17).
449 Forty-five phenotypic data points for 31 pepper accessions were retrieved from genebank databases
450 and compared to the molecular data obtained in the present work. In 40 (88.8 %) cases, a precise
451 correspondence was observed between the disease phenotype and the presence/absence of the
452 relative molecular marker. Such results suggest a high reliability for the set of markers employed
453 and guarantee their usefulness for accurate prediction of disease resistance in the absence of
454 phenotypic evaluations.

455 5. Conclusions

456 Our results bring to light the significance of peppers coming from the primary centres of *Capsicum*
457 diversification, which revealed as a promising source of resistance to particular or multiple
458 pathogens, including oomycetes, nematodes, viruses and bacteria. Those peppers might also have
459 retained other valuable traits with commercial, healthy and nutritional attributes, as it has been
460 demonstrated recently for comparable genetic resources (van Zonneveld et al., 2015).
461

462 **Acknowledgements**

463 This work was partially supported by Xunta de Galicia (Project EM2014/048) and by the Spanish
464 Institute for Agricultural and Food Research and Technology (INIA), co-financed by the European
465 Regional Development Fund (FEDER) (Project RTA2011-00118-C02-02). We gratefully
466 acknowledge provision of germplasm by USDA, ARS, Plant Genetic Resources Conservation Unit,
467 Gene Bank at the Leibniz Institute of Plant Genetics and Crop Plant Research, Center for Genetic
468 Resources, Institute for Conservation and Improvement of Valencian Agrobiodiversity, and Vegetable
469 Germplasm Bank of Zaragoza. The authors declare no conflicts of interest.

470

471

472 **References**

- 473 Bosland, P.W., Lindsey, D.L., 1991. A seedling screen for phytophthora root rot of pepper,
474 *Capsicum annuum*. Plant Dis. 75, 1048–1050.
- 475 Bosland, P.W., Votava, E.J., 2012. Peppers: vegetable and spice *Capsicum*. CABI Publishing,
476 Oxon. 204 p.
- 477 Caranta, C., Thabuis, A., Palloix, A., 1999. Development of a CAPS marker for the *Pvr4* locus: A
478 tool for pyramiding potyvirus resistance genes in pepper. Genome 4, 1111–1116.
- 479 Cebolla-Cornejo, J., Soler, S., Gomar, G., Soria, M.D., Nuez, F., 2003. Screening *Capsicum*
480 germplasm for resistance to tomato spotted wilt virus (TSWV). Ann. Appl. Biol. 143, 143-152.
- 481 Di Dato, F., Parisi, M., Cardi, T., Tripodi, P., 2015. Genetic diversity and assessment of markers
482 linked to resistance and pungency genes in *Capsicum* germplasm. Euphytica 204, 103–119.
- 483 Djian-Caporalino, C., Fazari, A., Arguel, M.J., Vernie, T., VandeCastele, C., Faure, I., Brunoud,
484 G., Pijarowski, L., Palloix, A., Lefebvre, V., Abad, P., 2007. Root-knot nematode (*Meloidogyne*
485 spp.) *Me* resistance genes in pepper (*Capsicum annuum* L.) are clustered on the P9 chromosome.
486 Theor. Appl. Genet. 114, 473–486.
- 487 Doebley, J.F., Gaut, B.S., Smith, B.D., 2006. The molecular genetics of crop domestication. Cell
488 127, 1309–1321.
- 489 Doyle, J.J., Doyle, J.J., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf
490 tissue. Phytochem. Bull. 19, 11–19.
- 491 FAOSTAT, 2015. [<http://faostat.fao.org/site/535/DesktopDefault.aspx?PageID=535#ancor>].
492 Accessed 20 April 2016.
- 493 Fery, R.L., Thies, J.A., 1998. PA-353, PA-398, and PA-426, southern root-knot nematode resistant
494 *Capsicum chinense* Jacq. germplasm lines. Hort. Sci. 33, 760e761.
- 495 Gisbert, C., Trujillo-Moya, C., Sánchez-Torres, P., Sifres, A., Sánchez-Castro, E., Nuez, F., 2013.
496 Resistance of pepper germplasm to *Meloidogyne incognita*. Ann. Appl. Biol. 162, 110–118.
- 497 González-Pérez, S., Garces-Claver, A., Mallor, C., Saenz de Miera, L.E., Fayos, O., Pomar, F.,
498 Merino, F., Silvar, C., 2014. New insights into *Capsicum* spp relatedness and the diversification
499 process of *Capsicum annuum* in Spain. PloS One 9, e116276.

500 Grube, R.C., Zhang, Y., Murphy, J.F., Loaiza-Figueroa, F., Lackney, V.K., Provvidenti, R., Jahn,
501 M.K., 2000. New source of resistance to Cucumber mosaic virus in *Capsicum frutescens*. Plant Dis.
502 84, 885-891.

503 Gullino, M.L., Kuijpers, L.A.M., 1994. Social and political implications of managing plant diseases
504 with restricted fungicides in Europe. Annu. Rev. Phytopathol. 32, 559–579.

505 Holdsworth, W.L., Mazourek, M., 2015. Development of user-friendly markers for the *pvr1* and
506 *Bs3* disease resistance genes in pepper. Mol. Breed. 35, 28-33.

507 Ibiza, V.P., Canizares, J., Nuez, F., 2010. EcoTILLING in *Capsicum* species: searching for new
508 virus resistances. BMC Genomics 11, 631.

509 Jaccard, P., 1908. Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44,
510 223–270.

511 Jang, S., Han, K., Jo, Y.D., Jeong, H.J., Siddique, M.I., Kang, B.C., 2015. Substitution of a
512 dysfunctional *pAMT* allele results in low pungency but high levels of capsinoid in *Capsicum*
513 *chinense* ‘Habanero’. Plant Breed. Biotech. 3, 119–128.

514 Jeong, H.S., Jang, S., Han, H., Kwon, J.K., Kang, B.C., 2015. Marker-assisted backcross breeding
515 for development of pepper varieties (*Capsicum annuum*) containing capsinoids. Mol. Breed. 35,
516 226-236.

517 Kang, W.H., Hoang, N.H., Yang, H.B., Kwon, J.K., Jo S.H., et al., 2010. Molecular mapping and
518 characterization of a single dominant gene controlling CMV resistance in peppers (*Capsicum*
519 *annuum* L.). Theor. Appl. Genet. 120, 1587–1596.

520 Kenyon, L., Kumar, S., Tsai, W.S., Hughes, J., 2014. Virus diseases of peppers (*Capsicum* spp.)
521 and their control. Adv. Virus Res. 90, 297–354.

522 Kim, H.J., Han, J.H., Yoo, J.H., Cho, H.J., Kim, B.D., 2008. Development of a sequence
523 characteristic amplified region marker linked to the *L⁴* locus conferring broad spectrum resistance to
524 tobamoviruses in pepper plants. Mol. Cells 25, 205–210.

525 Kraft, K.H., Brown, C.H., Nabhan, G.P., Luedeling, E., Luna Ruiz, J.J., d’Eeckenbrugge, G.C.,
526 Hijmans, R.J., Gepts, P., 2014. Multiple lines of evidence for the origin of domesticated chili
527 pepper, *Capsicum annuum*, in Mexico. Proc. Nat. Acad. Sci. USA 111, 6165–6170.

528 Lee, J.M., Jahn, M.M., Yeam, I., 2013. Allelic relationships at the *pvr1* locus in *Capsicum annuum*.
529 Euphytica 194, 417–424.

530 Mazourek, M., Wyatt, L.E., 2013. Candidate gene approaches in *Capsicum*. In, Kang B.C., Kole C.
531 (eds) Genetics, genomics and breeding of peppers and eggplants. CRC Press, London, pp 56–76.

532 Moury, B., Pflieger, S., Blattes, A., Lefebvre, V., Palloix, A., 2000. A CAPS marker to assist
533 selection of Tomato spotted wilt virus (TSWV) resistance in pepper. Genome 43, 137–142.

534 Onus, A.N., Pickersgill, B., 2004. Unilateral incompatibility in *Capsicum* (*Solanaceae*): occurrence
535 and taxonomic distribution. Ann. Bot. 94, 289–295.

536 Oka, Y., Offenbach, R., Pivonia, S., 2004. Pepper rootstock graft compatibility and response to
537 *Meloidogyne javanica* and *M. incognita*. J. Nematol. 36, 137–141.

538 Park, Y.J., Nishikawa, T., Minami, M., Nemoto, K., Iwasaki, T., Matsushima, K., 2015. A low
539 pungency S3212 genotype of *Capsicum frutescens* caused by a mutation in the putative
540 aminotransferase (*p-AMT*) gene. Mol. Genet. Genom. 290, 2217–2224.

541 Pernezny, K., Roberts, P.D., Murphy, J.F., Goldberg, N.P., 2003. Compendium of pepper diseases.
542 63 pp.

543 Perry, L., Dickau, R., Zarrillo, S., Holst, I., Pearsall, D.M., Piperno, D.R., et al., 2007. Starch fossils
544 and the domestication and dispersal of chili peppers (*Capsicum* spp. L.) in the Americas. Science
545 315, 986–988.

546 Quirin, E.A., Ogundiwin, E.A., Prince, J.P., Mazourek, M., Briggs, M.O., Chlanda, T.S., Kim, K.T.,
547 Falise, M., Kang, B.C., Jahn, M.M., 2005. Development of sequence characterized amplified region
548 (SCAR) primers for the detection of *Phyto.5.2*, a major QTL for resistance to *Phytophthora capsici*
549 Leon. in pepper. Theor. Appl. Genet. 110, 605–612.

550 R Development Core Team, 2014. R, a language and environment for statistical computing. R
551 Foundation for Statistical Computing, Vienna, Austria. [<http://www.r-project.org/>].

552 Rodríguez-Maza, M., Garcés-Claver, A., Park, S.W.B., Kang, C., Arnedo-Andrés, M.S., 2012. A
553 versatile PCR marker for pungency in *Capsicum* spp. Mol. Breed. 30, 889–898.

554 Römer, P., Hahn, S., Jordan, T., Strauß, T., Bonas, U., Lahaye, T., 2007. Plant-pathogen recognition
555 mediated by promoter activation of the pepper *Bs3* resistance gene. Science 318, 645–648.

556 Römer, P., Jordan, T., Lahaye, T., 2010. Identification and application of a DNA-based marker that
557 is diagnostic for the pepper (*Capsicum annuum*) bacterial spot resistance gene *Bs3*. Plant Breed.
558 129, 737–740.

559 Rubio, M., Caranta, C., Palloix, A., 2008. Functional markers for selection of potyvirus resistance
560 alleles at the *pvr2-elF4E* locus in pepper using tetra-primer ARMS-PCR. Genome 51, 767–771.

561 Sarath Babu, B., Pandravada, S.R., Prasada Rao, R.D.V.J., Anitha, K., Chakrabarty, S.K.,
562 Varaprasad, K.S., 2011. Global sources of pepper genetic resources against arthropods, nematodes
563 and pathogens. Crop Protec. 30, 389–400.

564 Sasahara, I., Furuhashi, Y., Iwasaki, Y., Inoue, N., Sato, H., Watanabe, T., Takahashi, M., 2010.
565 Assessment of the biological similarity of three capsaicin analogs (capsinoids) found in non-
566 pungent chili pepper (CH-19 Sweet) fruits. Biosci. Biotech. Biochem. 74, 274–278.

567 Silvar, C., García-González, C.A., 2016. Deciphering genetic diversity in the origins of pepper
568 (*Capsicum* spp.) and comparison with worldwide variability. Crop Sci. 56, 3100–3111.

569 Singh, S., Jarret, R., Russo, V., Majetich, G., Shimkus, J., Bushway, R., Perkins, R., 2009.
570 Determination of capsinoids by HPLC-DAD in *Capsicum* species. J. Agri. Food Chem. 57, 3452–
571 3457.

572 Stall, R.E., Jones, J.B., Minsavage, G.V., 2009. Durability of resistance in tomato and pepper to
573 *Xanthomonas* causing bacterial spot. Annu. Rev. Phytopathol. 47, 265–284.

574 Stellari, G.M., Mazourek, M., Jahn, M.M., 2010. Contrasting modes for loss of pungency between
575 cultivated and wild species of *Capsicum*. Heredity 104, 460–471.

576 Stewart, C., Mazourek, M., Stellari, G.M., O’Connell, M., Jahn, M., 2007. Genetic control of
577 pungency in *C. chinense* via the *Pun1* locus. *Journal of Experimental Botany*, 58, 979–991.

578 Suzuki, K., Kuroda, T., Miura, Y., Murai, J., 2003. Screening and field traits of virus resistant
579 source in *Capsicum* spp. Plant Dis. 87, 779–783.

580 Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6, Molecular
581 Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 30, 2725–2729.

582 Tanaka, Y., Hosokawa, M., Miwa, T., Watanabe, T., Yazawa, S., 2010a. Newly mutated putative-
 583 aminotransferase in non-pungent pepper (*Capsicum annuum*) results in biosynthesis of capsinoids,
 584 capsaicinoid analogues. J. Agri. Food Chem. 58, 1761–1767.

585 Tanaka, Y., Hosokawa, M., Miwa, T., Watanabe, T., Yazawa, S., 2010b. Novel loss-of-function
 586 putative aminotransferase alleles cause biosynthesis of capsinoids, non-pungent capsaicinoid
 587 analogues, in mildly pungent chili peppers (*Capsicum chinense*). J. Agri. Food Chem. 58, 11762–
 588 11767.

589 Tanaka, Y., Sonoyama, T., Muraga, Y., Koeda, S., Goto, T., Yoshida, Y., Yasuba, K., 2015.
 590 Multiple loss-of-function putative aminotransferase alleles contribute to low pungency and
 591 capsinoid biosynthesis in *Capsicum chinense*. Mol. Breed. 35, 142–155.

592 Thabuis, A., Palloix, A., Pflieger, S., Daubeze, A.M., Caranta, C., Lefebvre, V., 2003. Comparative
 593 mapping of *Phytophthora* resistance loci in pepper germplasm, evidence for conserved resistance
 594 loci across Solanaceae and for a large genetic diversity. Theor. Appl. Genet. 106, 1473–1485.

595 Tomita, R., Sekine, K.T., Mizumoto, H., Sakamoto, M., Murai, J., Kiba, A., Hikichi, Y., Suzuki, K.,
 596 Kobayashi, K., 2011. Genetic basis for the hierarchical interaction between Tobamoviruses and *L*
 597 resistance gene alleles from different pepper species. Mol. Plant Microbe Interact. 24, 108–117.

598 USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network
 599 (GRIN), 2015: National Germplasm Resources Laboratory, Beltsville, Maryland.
 600 [<https://npgsweb.ars-grin.gov/gringlobal/search.aspx> (accessed 23 November 2015)]. Accessed 15
 601 December 2015.

602 van Zonneveld, M., Ramirez, M., Williams, D., Petz, M., Meckelmann, S., et al., 2015. Screening
 603 genetic resources of *Capsicum* peppers in their primary center of diversity in Bolivia and Peru.
 604 PLoS One 10, e0134663.

605 Walsh, B.M., Hoot, S.B., 2001. Phylogenetic relationships of *Capsicum* (Solanaceae) using DNA
 606 sequences from two noncoding regions, the chloroplast atpB-rbcL spacer region and nuclear waxy
 607 introns. Int. J. Plant Sci. 162, 1409–1418.

608 Wang, D., Bosland, P.W., 2006. The genes of *Capsicum*. HortSci. 41, 1169–1187.

- 609 Wang, Y., Diehl, A., Wu, F., Vrebalov, J., Giovannoni, J., Siepel, A., Tanksley, S.D., 2008.
610 Sequencing and comparative analysis of a conserved syntenic segment in the *Solanaceae*. *Genetics*
611 180, 391–408.
- 612 Yao, M., Li, N., Wang, F., Zhibiao, Y.E., 2013. Genetic analysis and identification of QTLs for
613 resistance to cucumber mosaic virus in chilli pepper (*Capsicum annuum* L.). *Euphytica* 193, 135–
614 145.
- 615 Yang, H., Renshaw, D., Thomas, G., Buirchell, B., Sweetingham, M.S., 2008. A strategy to develop
616 molecular markers applicable to a wide range of crosses for marker assisted selection in plant
617 breeding, a case study on anthracnose disease resistance in lupin (*Lupinus angustifolius* L.). *Mol.*
618 *Breed.* 21, 473–483.
- 619 Yang, H.B., Liu, W.Y., Kang, W.H., Jahn, M., Kang, B.C., 2009. Development of SNP markers
620 linked to the *L* locus in *Capsicum* spp. by a comparative genetic analysis. *Mol. Breed.* 24, 433–446.
- 621 Yang, H.B., Liu, W.Y., Kang, W.H., Kim, J.H., Cho, H.J., Yoo, J.H., Kang, B.C., 2012.
622 Development and validation of *L* allele-specific markers in *Capsicum*. *Mol. Breed.* 30, 819–829.
- 623 Yang, H., Jian, J., Li, X., Renshaw, D., Clements, J., Sweetingham, M.W., Tan, C., Li, C., 2015.
624 Application of whole genome resequencing data in the development of diagnostic DNA markers
625 tightly linked to a disease-resistance locus for marker-assisted selection in lupin (*Lupinus*
626 *angustifolius*). *BMC Genomics* 16, 660.
- 627 Yeam, I., Kang, B.C., Lindeman, W., Frantz, J.D., Faber, N., Jahn, M.M., 2005. Allele-specific
628 CAPS markers based on point mutations in resistance alleles at the *pvr1* locus encoding eIF4E in
629 *Capsicum*. *Theor. Appl. Genet.* 112, 178–186.
- 630 Yoon, J.B., Do, J.W., Yang, D.C., Park, H.G., 2004. Interspecific cross compatibility among five
631 domesticated species of *Capsicum* genus. *J. Korean Soc. Hort. Sci.* 45, 324–329.

632

633 **Supporting information**

634

635 **Table S1** List of *Capsicum* spp. used in this work

636

637 **Table S2** Output for each accession after analysis with molecular markers linked to resistance and
638 organoleptic traits. +: genotypes carrying the allele identified by the marker; -: genotypes not
639 carrying the allele identified by the marker. "na" stands for the absence of amplification

640 **Table S3** Groups of accessions after cluster analysis and predicted phenotypes based on the tested
641 markers. R/S indicates putative resistance/susceptibility, and P/NP pungency/non pungency. "na"
642 stands for the absence of amplification

643 **Fig. S1.** Principal Coordinate Analysis of *C. annuum* (a) and *C. chinense* (b) according to their
644 geographical origin

645

646

647

648

649

650

651

652

653 **Tables**

654

655 **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- <i>Bs3</i>	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</i> -S/ <i>pvr1</i> -R1	CAPS	Potyvirus	<i>Pvr1</i>	Yeam et al. (2005)
<i>pvr1</i> -R2	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)

656

657

658 **Table 2** Number (percentage) of genotypes within each *Capsicum* species with a positive allele (see Table S2) for each marker. “na” indicates
659 absence of amplification.

Species	OpD04. 717	060I2END	SCAR_ CD	PR- Bs3	CaTm- int1	SCAC568	Pvr1-S	pvr1-R1	pvr1- R2	CSO	T200A	MAP1
<i>C. annuum</i> (58)	10 (17.2)	3 (5.2)	10 (17.2)	2 (3.4)	0	4 (6.9)	43 (74.1)	0	2 (3.4)	7 (12.1)	15 (25.9)	33 (56.9)
<i>C. chinense</i> (41)	39 (95.1)	38 (92.7)	38 (92.7)	0	37 (90.2)	40 (97.6)	26 (63.4)	15 (36.6)	0	23 (56.1)	0	41 (100)
<i>C. frutescens</i> (17)	1 (5.9)	15 (88.2)	0	0	16 (94.1)	17 (100)	17 (100)	0	0	1 (5.9)	0	17 (100)
<i>C. baccatum</i> (34)	1 (2.9)	30 (88.2)	0	0	0	34 (100)	34 (100)	0	0	29 (85.3)	0	33 (97.1)
<i>C. pubescens</i> (21)	0	17 (81.0)	0	0	0	4 (19.0)	21 (100)	0	0	2 (9.5)	0	21 (100)
<i>C. chacoense</i> (5)	5 (100)	5 (100)	0	0	0	5 (100)	5 (100)	0	0	na	0	5 (100)
<i>C. eximium</i> (2)	0	2	0	0	0	2	0	0	0	na	0	2
<i>C. cardenasii</i> (1)	0	1	0	0	0	1	0	0	0	na	0	1
<i>C. tovarii</i> (1)	0	1	1	0	0	na	1	0	0	na	0	1
Total (180)	56 (31.1)	112 (62.2)	49 (27.2)	2 (1.1)	53 (29.4)	107 (59.4)	147 (81.6)	15 (8.3)	2 (1.1)	62 (34.4)	15 (8.3)	154 (85.5)

660

661

662

663 **Table 3** Number (percentage) of genotypes within each geographical region with a positive allele (see Table S2) for each marker

664

Region	OpD04.717	060I2END	SCAR_CD	PR-Bs3	CaTm-int1	SCAC568	<i>Pvr1</i> -S	<i>pvr1</i> -R1	<i>pvr1</i> -R2	CSO	T200A	MAP1
Ecuador	11 (22.9)	41 (85.4)	14 (29.2)	1 (2.1)	18 (37.5)	31 (64.6)	40 (83.3)	5 (10.4)	1 (2.1)	18 (37.5)	4 (8.3)	42 (87.5)
Peru	13 (31.7)	32 (78.0)	12 (29.3)	0	13 (31.7)	30 (73.2)	33 (80.5)	6 (14.6)	0	20 (48.8)	2 (4.9)	39 (95.1)
Bolivia	6 (28.6)	15 (71.4)	8 (38.1)	0	6 (28.6)	14 (66.7)	16 (76.2)	0	0	10 (47.6)	2 (9.5)	19 (90.5)
Mexico	3 (50.0)	1 (16.7)	1 (16.7)	0	0	3 (50.0)	3 (50.0)	0	0	2 (33.3)	3 (50.0)	4 (66.7)
Brazil	2 (33.3)	3 (50.0)	2 (33.3)	0	5 (83.3)	6 (100)	6 (100)	0	0	2 (33.3)	0	6 (100)
America (others)	9 (75.0)	8 (66.7)	6 (50.0)	0	5 (41.7)	9 (75.0)	8 (66.7)	2 (16.6)	1 (8.3)	5 (41.7)	2 (16.7)	11 (91.6)
Spain	4 (12.9)	1 (3.2)	3 (9.7)	0	1 (3.2)	2 (6.5)	28 (90.3)	1 (3.2)	0	2 (6.5)	2 (6.5)	19 (61.3)
Europe-Asia (others)	3 (37.5)	4 (50.0)	2 (25.0)	1 (12.5)	4 (50.0)	5 (62.5)	8 (100)	0	0	3 (37.5)	0	7 (87.5)
Unknown	5 (71.4)	7 (100)	1 (14.3)	0	1 (14.3)	7 (100)	5 (71.4)	1 (14.3)	0	0	0	6 (85.7)

665

666

667

668 **Figure legends**

669

670 **Fig. 1.** Patterns of DNA fragments after analysis with eleven markers. For the interpretation of +
671 and – symbols see Table S2. Ladder (50 bp - 1000 bp) corresponds with NZYDNA Ladder VI
672 (NZYTech, Portugal).

673

674 **Fig. 2.** UPGMA cluster analysis of 180 *Capsicum* accessions based on data obtained from markers
675 linked to disease resistance and pungency traits. Numbers on each branch indicate a different group
676 with a particular combination of predicted resistances (see Table S3). Wild species are not coloured.

677

678

679

680

Figure 1

Figure 2