

Tracing back the history of pepper (*Capsicum annuum* L.) in the Iberian Peninsula

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Abstract

Capsicum annuum was the first *Capsicum* introduced into Europe, likely through Spain and Portugal, at the end of the XVth century. Therefore, the Iberian Peninsula constitutes a significant secondary diversification centre, where part of the original pepper gene pool brought back from America might be preserved in the form of landraces. A previous work suggested that the diversification process of *C. annuum* in Spain may occur from an ancient population, still represented by some landraces with ancestral traits. The current work is focused on clarifying the origins and genetic relationships among the pepper landraces from the Iberian Peninsula. For that purpose, a larger number of Spanish peppers, a collection of Portuguese landraces and a panel of *C. annuum* resources from Mexico, including the wild relative *C. annuum* var. *glabriusculum* were genotyped with the DArTseq technology. Sequencing output consisted of 27,159 tags, of which 5,007 SNPs were selected for further analyses. Clustering and *STRUCTURE* approaches clearly differentiated wild and Mexican peppers from those originating in the Iberian Peninsula. Various Spanish and Portuguese accessions clustered within the Mexican group, while the others were primarily organized following a geographical pattern, although their genomic composition was not extremely different.

1. Introduction

Pepper (*Capsicum* spp) is one of the most important vegetables and spices in the worldwide trade. The genus has its origins in the tropical South American region centered in what is now Bolivia. The five most common cultivated species (*C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* and *C. pubescens*) were independently domesticated as far back as 6000 B.C. in either Mesoamerica or South America [1]. After the first trip of Columbus and subsequent voyages of exploration to the New World, peppers moved worldwide, suffering from additional diversification at the secondary centers, which resulted in the awesome phenotypic variability that can be observed nowadays. *C. annuum* was domesticated in Mexico from the wild bird pepper (*C. annuum* var. *glabriusculum*) and it was likely the first pepper that entered Europe, being Spain the place of arrival [2]. Afterwards, the extensive trading routes of Spanish and Portuguese helped to disperse peppers around the globe. Therefore, the Iberian Peninsula constitutes a significant secondary diversification centre, where part of the original pepper gene pool brought back from America might be preserved in the form of landraces. Landraces

represent native varieties empirically selected by farmers over time and well adapted to specific agro-climatic conditions. In the Iberian Peninsula, hundreds of phenotypically very diverse pepper landraces can still be found all over the territory due the heterogeneity of the land and the versatility of agro-climatic regions. However, the origins and relationships among these landraces have remained under-researched. In a previous work, thirty-nine Spanish landraces from the Vegetable Germplasm Bank of Zaragoza were investigated with a broad set of microsatellite (SSRs) markers. Results suggested that the diversification process of *C. annuum* in Spain may occur from an ancient population, still represented by some landraces with ancestral traits, such as the erect fruits [3]. The main goal of the present work was to investigate the genetic diversity, structure and population dynamics of pepper in the Iberian Peninsula. For that purpose, a larger number of Spanish peppers and a collection of Portuguese landraces were analysed and compared to a set of *C. annuum* resources from Mexico, including the wild relative *C. annuum* var. *glabriusculum*.

2. Materials and Methods

2.1. Plant material

A total of ninety-four pepper landraces from Spain (62), Portugal (19) and Mexico (10) were selected from the Vegetable Germplasm Bank of Zaragoza (BGHZ) (Spain), the Portuguese Plant Germplasm Bank (BPGV) (Portugal), the Center for Genetic Resources (CGN) (Netherlands) and the Gene Bank at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) (Germany). All accessions were chosen based on the passport data stored at each gene bank, trying to cover a wide range of geographical areas and the maximum phenotypic variability. Three *C. annuum* var. *glabriusculum* from the germplasm collection at INRA were kindly provided by Dr. Alain Palloix.

2.2. Genotyping

Total genomic DNA was isolated from young leaves of each accession using the NucleoSpin Plant XL kit (Macherey–Nagel), following the manufacturer’s instructions. Genotyping was performed with a genotyping-by-sequencing approach (DArTseq) provided by the company Diversity Arrays Technology (Canberra, Australia, <http://www.diversityarrays.com/>). This system combines complexity reduction methods with next-generation sequencing platforms, targeting primarily genic regions.

2.3. Data analysis

DArTseq output was received as a file with tags sequences with SNP polymorphisms aligned through BlastN to the pepper reference genome (release v.1.55, <http://peppergenome.snu.ac.kr>).

Genetic relationships among accessions were depicted by using the Neighbour-Joining (NJ) algorithm. The software *STRUCTURE* v2.3.4 was employed to assign individuals to populations based on their genomic composition. The tests were performed using an admixture model with correlated allele frequencies. Each run consisted of a burning period of 50,000 steps and 50,000 MCMC repetitions. The most likely number of populations (*K*) was inferred with the Evanno correction method [4]. The accessions were sorted into sub-populations based on their maximum membership probabilities (threshold level of 70%). The ones not showing a clear membership were classified as admixture.

3. Results and Discussion

3.1. Genotypic analysis

In total, 27,159 DArTseq tag sequences presented SNP polymorphisms. Of these, 22,531 (82.9%) were successfully aligned to any of the twelve pepper chromosomes (Table 1). The number of assigned tags ranged from 1,337 (chromosome P8) to 2,699 (P3). SNPs with over 10% of missing data or heterozygous alleles were removed, leaving 19,988 tags. Those SNPs with a major allele frequency above 0.95 were recorded as monomorphic. In total, 5,007 curated polymorphisms were used for further analyses.

Table 1
Statistics of the DArTseq output

| Chr. | # tags | # P | %P | PIC |
|------|--------|-----|-------|-------|
| P1 | 2,340 | 430 | 23.28 | 0.294 |
| P2 | 2,062 | 360 | 20.93 | 0.288 |
| P3 | 2,699 | 561 | 26.70 | 0.304 |
| P4 | 1,653 | 263 | 20.38 | 0.327 |
| P5 | 1,636 | 283 | 24.39 | 0.297 |
| P6 | 2,147 | 354 | 21.44 | 0.297 |
| P7 | 1,740 | 349 | 28.26 | 0.302 |
| P8 | 1,337 | 255 | 23.69 | 0.296 |
| P9 | 1,721 | 363 | 32.55 | 0.328 |
| P10 | 1,775 | 337 | 27.80 | 0.317 |
| P11 | 1,814 | 348 | 28.24 | 0.324 |
| P12 | 1,607 | 300 | 25.32 | 0.313 |
| NA | 4,628 | 804 | 25.47 | 0.317 |

Chr: pepper chromosome; # P: number of polymorphic tags; % P: percentage of polymorphism; PIC: Polymorphism Information Content; NA: not assigned

The chromosome P8 showed the lowest number of polymorphic loci (255), whereas chromosome P3 displayed the highest (561) (Table 1). The percentage of polymorphism slightly varied among chromosomes, ranging from 20.38% (P4) to 32.55% (P9). The lowest average Polymorphism Information Content (PIC) value was found in chromosome P2, while the highest was observed in chromosomes P4 and P9 (Table 1). The expected heterozygosity (H_e) for the whole set of *C. annuum* accessions was 0.29. Similar parameters of genetic diversity (polymorphism around 25% and H_e values close to 0.29) were reported in a diverse panel of *C. annuum* lines genotyped with the Affymetrix® GeneChip® Pepper Array [5].

3.2. Cluster and structure analyses

The NJ tree clearly separated the wild *C. annuum* and the Mexican accessions in a well-defined branch (group I), differentiated from peppers originating in the Iberian Peninsula (Fig. 1). Interestingly, four Spanish and two Portuguese peppers were also clustered within this group.

As previously reported by Nicolai *et al.* [6], the *C. annuum* var. *glabriusculum* PM669 and PM670 from Panama and Colombia, respectively, appeared closely related and slightly distant from the Mexican one (PM647). The majority of accessions from Portugal were comprised in groups III, VII and VIII, while the others are mostly represented by Spanish landraces (Fig. 1).

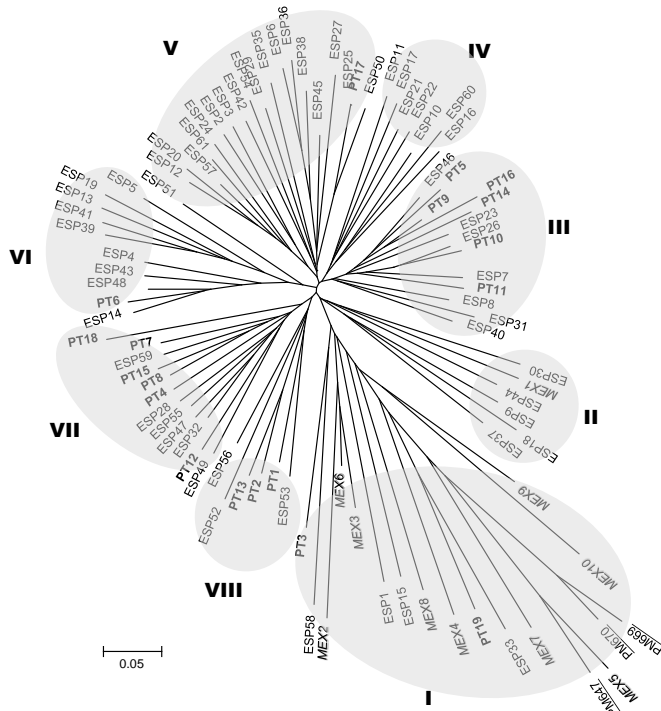


Figure 1

NJ tree of 94 *C. annuum* accessions based on 5,007 SNPs. ESP: Spanish accessions, PT: Portuguese accessions (in **bold**), MEX: Mexican accessions (in *italics*). PM647, PM669 and PM670 (underlined) are wild *C. annuum*.

The analysis with the software *STRUCTURE* pointed to the presence of three populations ($\Delta K=3$), after the Evanno correction [4]. The accessions were sorted according their membership coefficients in three groups: A, comprising 7 genotypes, B, including 8 and C, consisting of 42 accessions. The remaining 36 *C. annuum* did not show clear memberships and they were considered to possess a mixed genomic composition, either between clusters A and B or between clusters B and C (Fig. 2). Accessions in population A primarily corresponded to those in the group I defined by the NJ tree, population B comprised accessions from groups II and VI and finally population C included mostly accessions from clusters III, IV and VII (Fig. 2). Clustering patterns derived from both analyses partially responded to geographical factors. Thus, accessions from the border regions in Spain and Portugal tended to group together. Similarly, Northern Spanish accessions primarily separated from those originating in the South. The genetic organization of peppers attending to morphological fruit traits is also expected [3]. However, owing to the phenotypic characterization of several accessions, mainly from Portugal, is on the way, clear morphological groups could not be established yet. Further work will also cover the exploration, based on the DArTseq tag sequences of those genomic regions controlling

traits of interest, such as pungency.

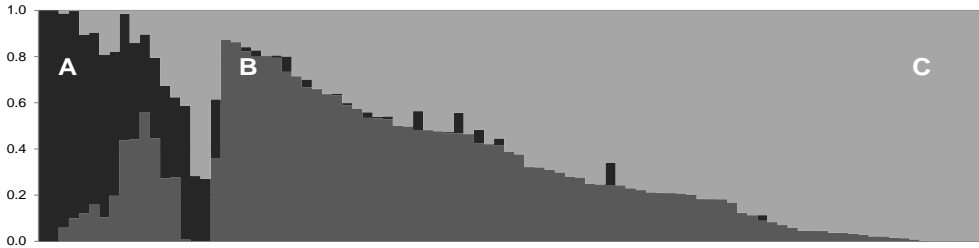


Figure 2

STRUCTURE analysis on 94 *C. annuum* accessions based on 5,007 SNPs. Accessions are ordered according to their membership coefficients. Cluster A is shown in black, cluster B in dark grey and cluster C in light grey.

4. Acknowledgements

This work was supported by the Spanish Institute for Agricultural and Food Research and Technology (INIA), co-financed by the European Regional Development Fund (FEDER) (Project RTA2011–00118–C02–02) and by Xunta de Galicia (Project EM2014/048). We gratefully acknowledge provision of germplasm by BGHZ, BPGV, CGN and IPK. We are highly grateful to Dr. Alain Palloix for providing the *C. annuum* var. *glabriusculum* accessions.

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Proceedings

of

**XVIth EUCARPIA Capsicum
and Eggplant Working Group Meeting**
in memoriam Dr. Alain Palloix

12 -14 September 2016
Kecskemét, Hungary



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The papers in these proceedings were double refereed by members of the International Scientific Committee in a process that involved, detailed reading of the papers, reporting of comments to authors, modifications of papers by authors and re-evaluation of resubmitted papers to ensure quality of content.

Published by

Diamond Congress Ltd., the secretariat of the Conference
H-1012 Budapest, Vérmező út 8.
Technical editor & responsible publisher: Róbert Hohol
www.diamond-congress.hu

ISBN 978-615-5270-27-7



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