# Unlocking genomic diversity of pepper (Capsicum spp.) collections held in genebanks: perspectives for breeding and germplasm management

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## Title

Unlocking genomic diversity of pepper (Capsicum spp.) collections held in genebanks: perspectives for breeding and germplasm management

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1 CREA Research Centre for Vegetable and Ornamental Crops, Pontecagnano Faiano (SA), Italy. 2 DISAFA, University of Torino, Torino, Italy. 3 AVRDC The World Vegetable Centre, Taiwan. 4 Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universitat Politècnica de València, Valencia, Spain. 5 Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany. 6 Wageningen University & Research WUR, Wageningen, The Netherlands. 7 INRA, UR1052 GAFL, Avignon, France. 8 Bati Akdeniz Agricultural Research Institute BATEM, Antalya, Turkey. 9 Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria 10The Volcani Centre, Bet Dagan, Israel. 11Agenzia Nazionale per le Nuove Tecnologie, l'Energia e lo Sviluppo Economico Sostenibile (ENEA), Roma, Italy

## Abstract

BACKGROUND The genetic diversity present in cultivars, landraces and wild relatives of modern crops, represents the source of agriculturally important genes to be discovered in our attempts of making crops more resilient and better adapted to the challenges of modern agriculture. In this context, genebanks are pivotal for the collection, preservation and distribution of germplasm; however, better knowledge of the available genetic variability would help improve the management of plant genetic resources. Here we report the analysis of genome-wide genotyping-by-sequencing (GBS) data on 9,659 pepper accessions retrieved from the major European (CGN, INRA, IPK, UPV) and Asian (AVRDC) genebanks, universities and research centres. The collection analyzed is so far the largest and the most diverse ever studied in pepper. The main objectives of this study are to: a) evaluate the genetic diversity using a large set of single-nucleotide polymorphism (SNP) data; b) detect the overlaps within collections and between genebanks; c) detect misclassification of accessions; d) develop core collections for association mapping studies. MATERIALS & METHODS Germplasm studied comprised 7,253 C. annuum accessions, 1,795 accessions belonging to the other four domesticated species (C. frutescens, C. chinense, C. baccatum, C. pubescens) and 43 accessions belonging to the wild species (Table 1). About 3% of the collection was

represented by unknown species. Passport information and 42 phenotypic descriptors for plant, flower and fruit traits have been provided by genebank curators in order to enhance the description of plant material. A two restriction enzyme GBS protocol was used [1]; PstI-MspI have been selected based on in silico digestion of the reference genome accession (CM334) [2]. RESULTS GeGBS data provided more than 10,000 SNPs evenly distributed on the genome and used to characterize genetic diversity, population structure and phylogenetic relationships. The analysis allowed identifying five main clusters (K) corresponding to the most represented Capsicum species in the whole collection (Figure 1). Capsicum annuum was subdivided into two sub clusters and include admixed samples. Three other clusters were observed for C. baccatum, C. frutescens and C. chinense, respectively. The remaining species, including those corresponding to the group of purple flowers clustered in an admixed group. The principle component analysis (PCA) plot for the first two principal components suggests a geographical differentiation of the Caribbean, Central Asia and European gene-pools, and a distinction of the three main clades according to the most updated classification [3]. Moreover, the observed ~1,000 admixed accessions, and the incomplete separations between C. frutescens and C. chinense, revealed possible taxonomic misclassifications in the genebanks or gene flow between species resulting in introgressions. The genetic and phenotypic variation existing in the collection studied will be exploited for genome-wide association studies (GWAS). 178 Capsicum and Eggplant EUCARPIA Meeting 2019 | BG-O/03 DISCUSSION & CONCLUSION The genetic information provided in this study is appropriate for a deep investigation of the diversity present across the different Capsicum collections maintained by genebanks. Appropriate strategies including "germplasm genomics" can optimize the management of resources, the exchange of material between genebanks and their use for breeding purposes. Beyond genetic diversity, data can be used for the development of core collections to be exploited in GWAS, facilitating the identification of genomic regions associated with valuable traits and enhancing the efforts made by genebanks in the extensive collection and characterization of Capsicum germplasm resources.

REFERENCES [1] Elshire et al., 2011, Plos One 6: e19379. doi.org/10.1371/journal.pone.0019379 [2] Kim et al., 2014, Nature Genetics 46:270–278 [3] Carrizo-Garcia et al., 2016, Ann. Bot. 118:35-51. doi: 10.1093/aob/mcw079 Figure 1. Population structure of the Capsicum germplasm collection. Five subpopulation clusters inferred by STRUCTURE are represented by different colors : 1) C. baccatum ; 2) C. annuum ; 3) C. chinense and C. frutescens ; 4) C. annuum ; 5) C. chinense, C. frutescens and C. annuum ; adm = admixed 179 BG-O/03 | Capsicum and Eggplant EUCARPIA Meeting 2019 Table 1. Summary of plant material provided by genebanks and used in this study
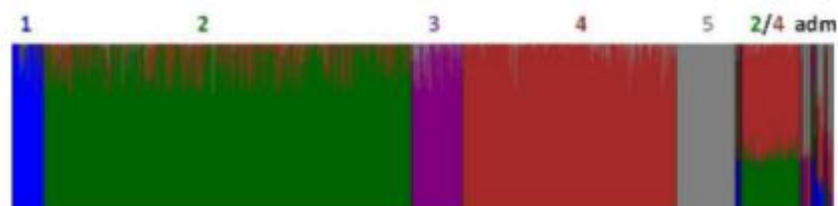
Figure 1. Population structure of the Capsicum germplasm collection. Five subpopulation clusters inferred by STRUCTURE are represented by different colors : 1) C. baccatum ; 2) C. annuum ; 3) C. chinense and C. frutescens ; 4) C. annuum ; 5) C. chinense, C. frutescens and C. annuum ; adm = admixed

| Species | N° of accessions sequenced | AVRDC | IPK | UPV | INRA | WUR | ARO | CREA | Units | BATEM | HVCRI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C. annuum | | 4166 | 908 | 935 | 629 | 264 | 215 | 209 | 94 | 45 | 38 |
| C. annuum var. glabriusculum | 7523 | | 4 | | | | | 3 | | | |
| C. frutescens | 728 | 474 | 160 | 10 | 39 | 15 | 21 | 8 | 1 | | |
| C. chinense | 627 | 325 | 50 | 7 | 104 | 71 | 31 | 37 | 2 | | |
| C. baccatum | | 101 | 3 | 24 | 86 | | 8 | | 1 | | |
| C. baccatum var. baccatum | 395 | 3 | 3 | | | 7 | | 2 | | | |
| C. baccatum var. pendulum | | 115 | 23 | | | 17 | | 2 | | | |
| C. pubescens | 45 | 8 | 21 | | 7 | 2 | 2 | 2 | 3 | | |
| C. chacoense | 26 | 10 | 5 | | | 4 | 4 | 1 | | | |
| C. eximium | 8 | 2 | 4 | | 2 | | | | | | |
| C. praetermissum | 7 | | 5* | | | | | 1 | 1 | | |
| C. cardenasii | 1 | | 1 | | | | | | | | |
| C. tovari | 1 | | | | | 1 | | | | | |
| Undefined | 298 | 286 | 7 | 3 | 1 | | | | 1 | | |
| TOTAL | 9659 | 5490 | 1194 | 979 | 870 | 383 | 281 | 265 | 103 | 45 | 38 |

* Listed as C. baccatum subsp. praetermissum

Table 1. Summary of plant material provided by genebanks and used in this study