



THE EUROPEPLAND PROJECT: IMPLEMENTING A TRANS-EUROPEAN PEPPER LANDRACE COLLECTION FOR RESILIENT AGRICULTURE



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Background

Global agriculture faces unprecedented challenges: rising food demand, resource scarcity, and severe climate change. Developing resilient, high-yielding cultivars that minimize natural resource exploitation is crucial for sustainable food systems. Landraces (LRs)—locally adapted traditional varieties—constitute an invaluable source of germplasm to meet these future agricultural needs. Beyond intensive farming and large-scale retail, LRs are vital for sustaining smallholder rural economies. The European Mediterranean basin is a major center of secondary diversification for pepper (*Capsicum annuum* L.), harboring immense phenotypic diversity in plant and fruit traits. Due to generations of on-farm selection, Mediterranean LRs exhibit broad adaptation to contrasting environments, serving as a genetic reservoir for resilience traits. While these traditional varieties possess critical alleles for climate-smart breeding, their exploitation is frequently hindered by knowledge gaps and incomplete characterization. Advanced high-throughput genotyping technologies offer a powerful, comprehensive approach to resolve population structures, bridge existing characterization gaps, and optimize LR collection management for precision breeding. In this context, the EuroPepLand project aims to establish a trans-European pepper LR core collection to support breeders in developing climate-resilient varieties. By integrating these advanced genomic tools with comprehensive phenotypic descriptors, the project characterizes *Capsicum* landraces stored across major European genebanks and research institutions.

Collection establishment

Thirteen European partners provided the seeds for the accessions under the Standard Material Transfer Agreement system. A total of 441 landraces were assembled to capture broad phenotypic diversity in fruit size, shape, color, pungency, and adaptation (**Figure 1**). This collection reflects the diversity of European production areas across the Mediterranean basin and the Balkans (**Figure 2**), spanning both coastal and inland regions.

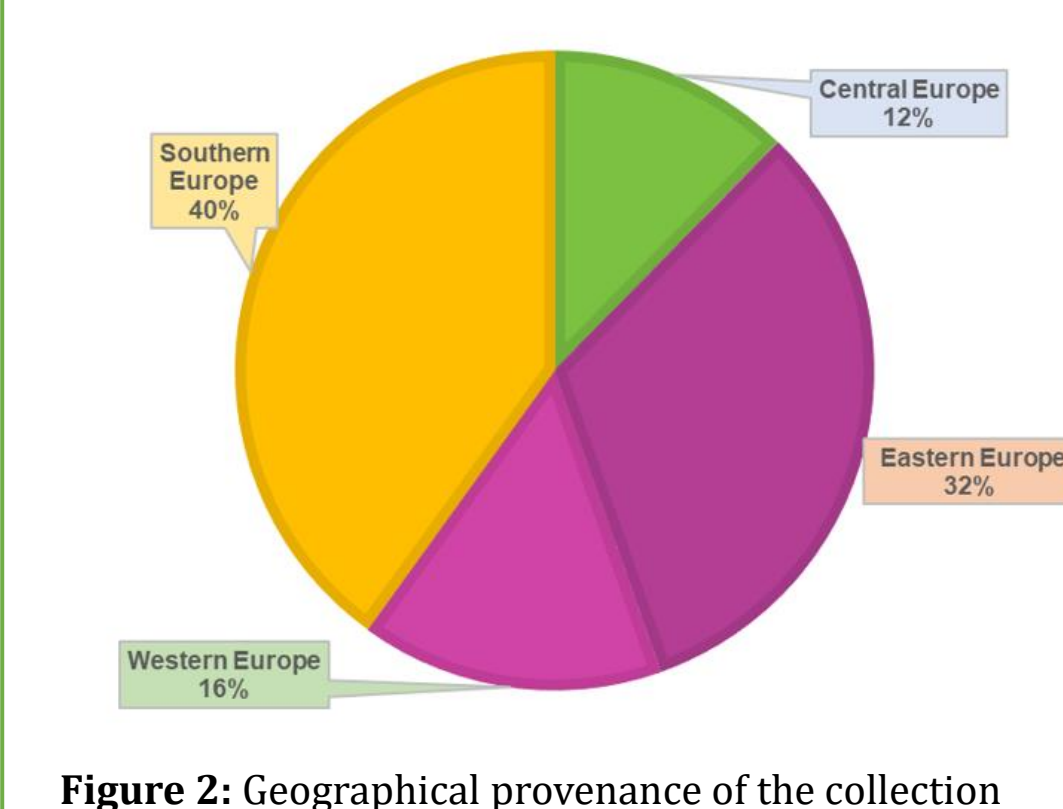


Figure 2: Geographical provenance of the collection

Seeds were sown in pots under climate-grown chamber conditions. Nucleic acid isolation was performed using the DNeasy plant mini kit (Qiagen, Hilden, Germany). The DNA purity was estimated by absorbance at 280 and 260 nm, respectively, using a UV-Vis Nanodrop (Thermo Scientific, Wilmington, DE, USA), and the integrity by electrophoresis. Concentration was measured using the Qubit 3 Fluorometer (ThermoFisher, Waltham, MA, USA). Genotyping was performed using the 10K pepper Illumina Infinium Arrays provided by SGS Trait Genetics.



Figure 1: Phenotypic diversity of the EuroPepLand collection

Results

The final array matrix consisted of 9,928 SNPs distributed across the twelve pepper chromosomes, with chromosome 8 harbouring the lowest number of probes (376) and chromosome 3 the highest (872). In total, 149,272 polymorphic sites were identified. Population structure analysis revealed diversity based on geographical provenance, with germplasm retrieved from western and southern Europe clustering separately from that of eastern Europe (**Figure 3**).

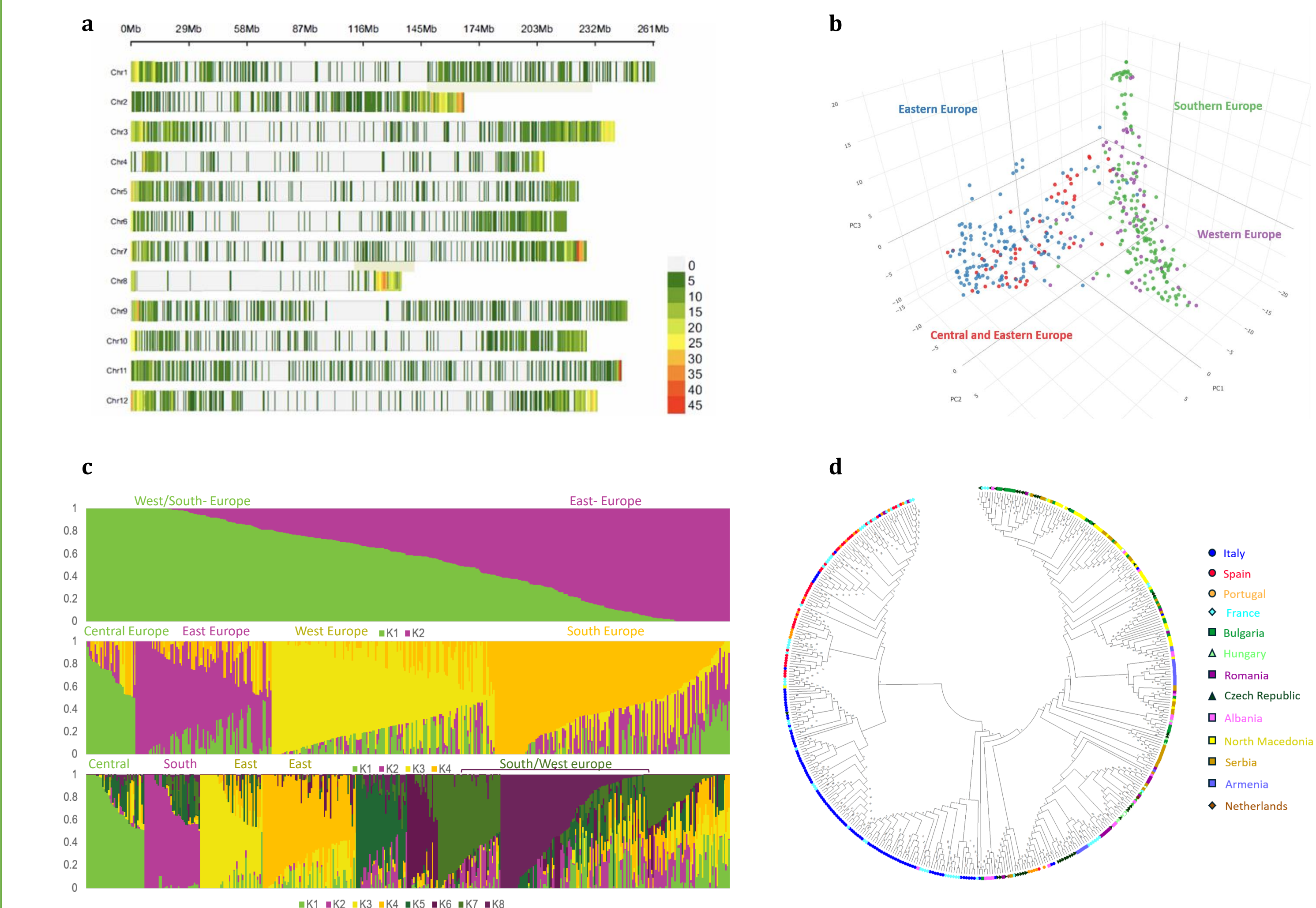


Figure 3: Genomic diversity of the EuroPepLand collection. a) SNP density plot chromosome wise representing number of SNPs within 1 Mb window size. The horizontal axis shows the chromosome length (Mb); the different color depicts SNP density; b) Three-dimensional (3D) PCA and c) genetic structure based on K=2, K=4 and K=8 subpopulations highlighting diversity across the main European regions of origin; d) Phylogenetic tree based on the country of origin of the accessions.

In order to test the potential of the collection for candidate gene discovery, a genome-wide association study has been performed for pungency. Significant associations were found within the 135-136 Mbp region on chromosome 2 corresponding to the *PUN1* gene, the main gene responsible for spiciness in *C. annuum* encoding for an acyltransferase with capsaicin synthase activity (**Figure 4**).

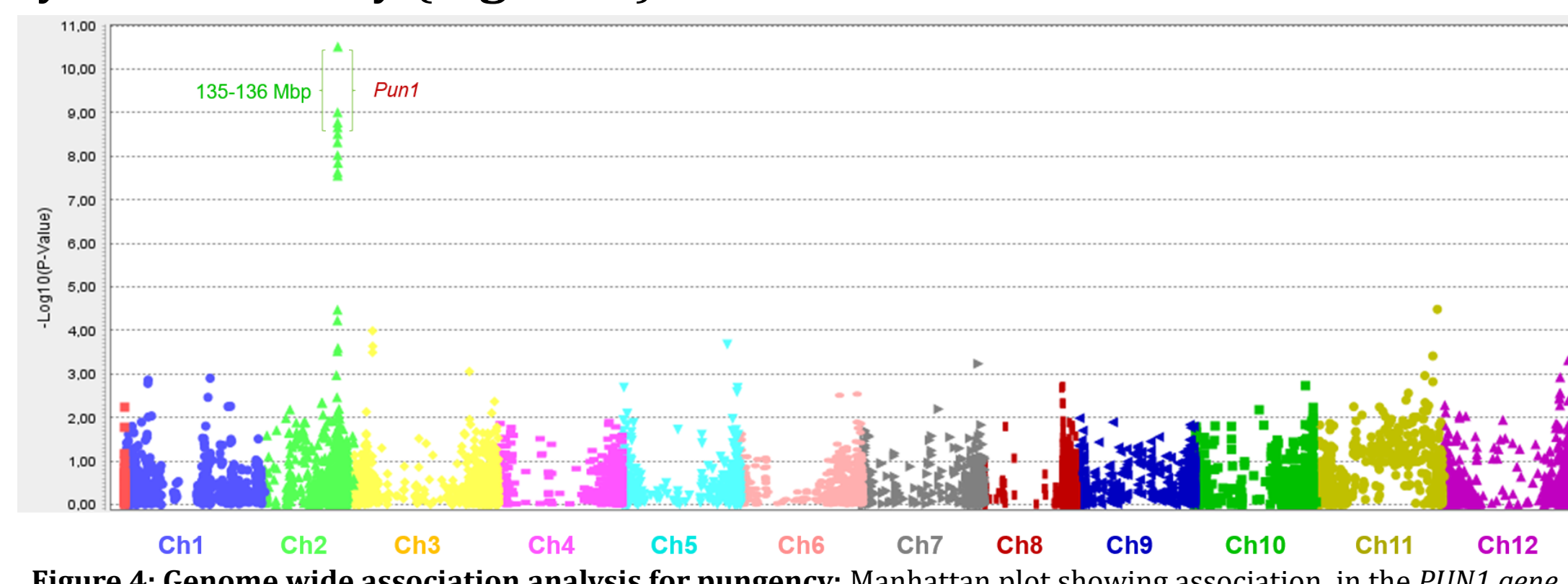


Figure 4: Genome wide association analysis for pungency: Manhattan plot showing association in the *PUN1* gene

A phenotyping experiment in agreement with the CNR and CREA IT partners has been also performed in the PhenoHort facility built in the glasshouse of CREA—Research Centre for Vegetable and Ornamental Crops, Pontecagnano Faiano, Italy. Eleven selected accessions have been tested for their tolerance level to salt stress (**Figure 5**). The PlantEye F500 scanner (Phenospec, Heerlen, The Netherlands), consisting of a dual digital sensor combining multispectral imaging with 3D vision was used for the analysis.

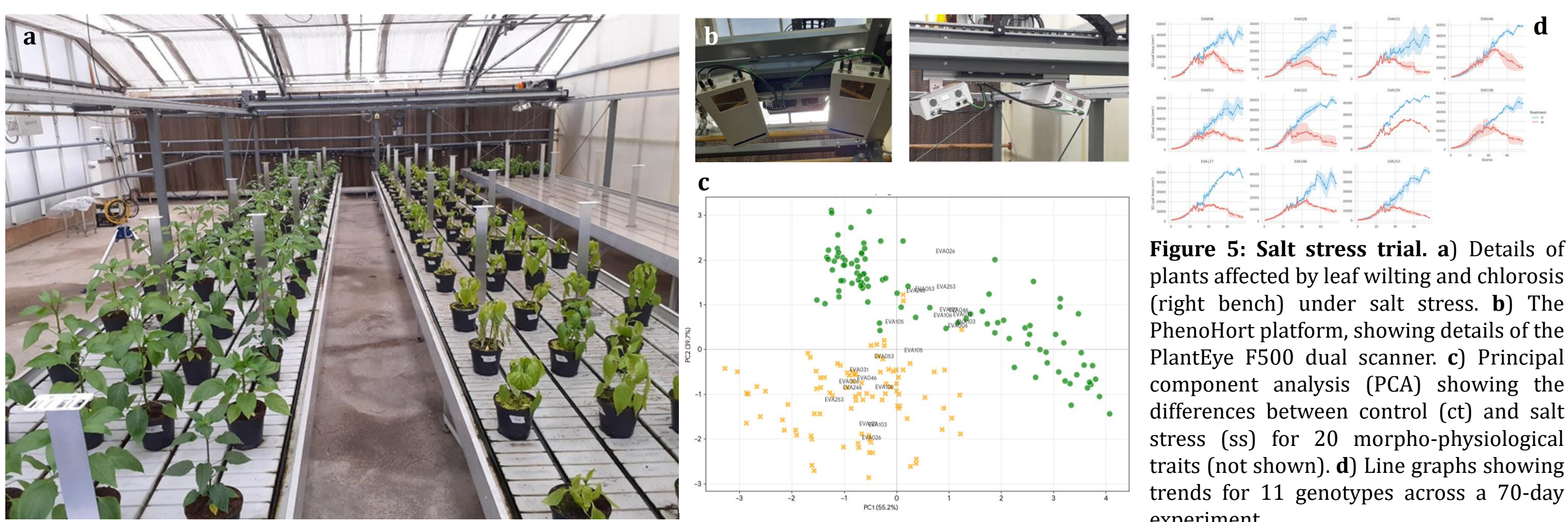


Figure 5: Salt stress trial. a) Details of plants affected by leaf wilting and chlorosis (right bench) under salt stress. b) The PhenoHort platform, showing details of the PlantEye F500 dual scanner. c) Principal component analysis (PCA) showing the differences between control (ct) and salt stress (ss) for 20 morpho-physiological traits (not shown). d) Line graphs showing trends for 11 genotypes across a 70-day experiment.

Summary and prospects

EuroPepLand encourages the creation of a network of researchers and genebank managers aiming to enhance the catalogue of information on European local varieties by creating a comprehensive collection, providing phenotyping information, and deeply assessing genetic diversity. The molecular characterization of the collection lays the foundation for the rational management and conservation of accessions through the identification of specific subpopulations and accessions with high levels of redundancy or similarity. The construction of a collection containing a large part of the genetic and phenotypic diversity will allow users to screen this diversity and perform candidate gene identification via association mapping. Therefore, the collection will be a powerful tool for research and breeding purposes. The generated phenotypic and genomic data will be made open-access, with all new information fully integrated into the EURISCO database. Through engaging with holding institutions and the EVA Pepper network, this collection will be integrated into the European genebank integrated system to ensure the long-term conservation and its availability for applied research and breeding purposes. Within this broad framework, the project will improve the value of the food chain for high-quality local products, with concrete and positive impacts on end-users.

