

Unveiling root system variability in lentil core collection through automated high-throughput phenotyping

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Introduction

Lentil (*Lens culinaris* subsp. *culinaris*) is a key legume crop for sustainable agriculture. However, climate change, especially drought, is reducing yield stability. Root system architecture (RSA) plays a crucial role in water uptake and stress adaptation. This study evaluates RSA traits in a subset of 64 lentil genotypes selected from the INCREASE H2020 collection. The goal is to identify genotypes with root traits favorable to drought resilience and to potential breeding improvement.

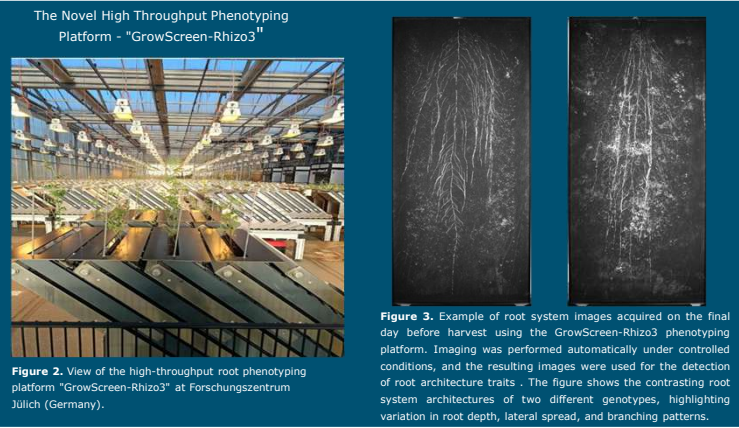
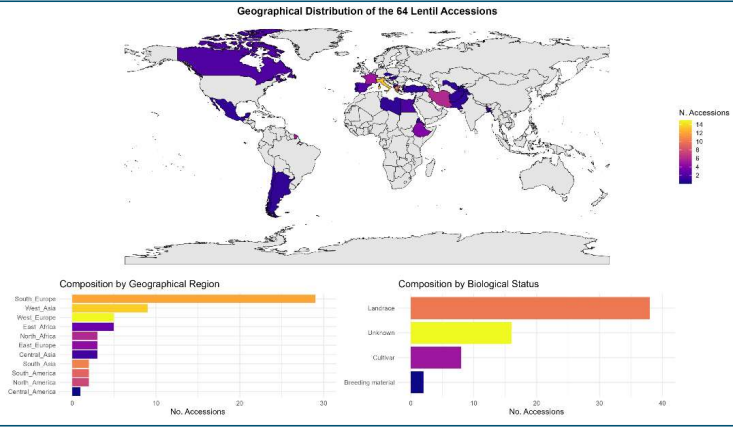


Figure 1. The world map and bar plots show the geographical and biological distribution of the 64 lentil accessions. Accessions are broadly distributed across ten regions, with the highest representation from South Europe and West Asia. The majority are landraces, underlining their value in pre-breeding and adaptation studies.

Material and Method

A core set of 64 SSD lentil genotypes was selected based on genetic diversity, phenotypic variation, and passport data (Figure 1). Plants were grown for 26 days in rhizotrons under controlled conditions using the GrowScreen-Rhizo3 platform (Forschungszentrum Jülich, Germany), following a randomized block design with three replicates per genotype. Root and shoot traits were acquired through daily high-resolution imaging (Figure 2-3). The dataset included 14 quantitative traits describing root system architecture and biomass allocation (e.g., Total Root Length, Root System Depth, Convex Hull Area, Root Fresh and Dry Weight). Trait distributions were first evaluated using the Shapiro-Wilk test. One-way ANOVA was then performed, revealing significant genotype effects for most traits. Trait interdependence was assessed via Pearson's correlation (Figure 4). To reduce dimensionality and explore multivariate patterns, a Principal Component Analysis (PCA) was conducted (Figure 5).

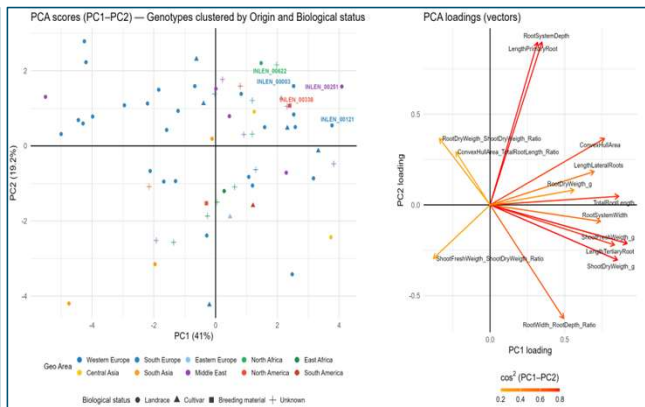


Figure 4. Pearson correlation matrix among 14 root and biomass traits measured across all lines. The upper panels report Pearson correlation coefficients, with color intensity reflecting the strength and direction of the associations. The diagonal panels display the distribution of each trait (density plots), providing information on their variability and deviation from normality. The lower panels show scatter plots of pairwise relationships, allowing visualization of the dispersion of data points, potential clustering, and the linearity of associations.

Figure 5. Principal Component Analysis (PCA) based on 14 root architecture traits measured on 64 lentil genotypes. Left: genotypes are plotted in the space defined by the first two principal components (Dim1 = 41.0%, Dim2 = 19.2%), colored according to their geographical origin (Geo Area) and distinguished by point shape according to their biological status (Landrace, Cultivar, Breeding material, Unknown). Labels indicate the top five genotypes based on the Figure 6 Plot, with text color matching their geographical origin. Right: loading plot showing the contribution and orientation of each trait to the first two principal components. Vector length and proximity to the circle indicate the relative contribution of each trait to the PCA axes, with color intensity reflecting the quality of representation (cos² values, 0.2–0.8).

The adequacy of the data for PCA was confirmed by a significant Bartlett's test ($\chi^2 = 3268.02$, $df = 91$, $p < 0.001$) and a marginal Kaiser-Meyer-Olkin measure (KMO = 0.56). A weighted percentile-based scoring system was applied to evaluate genotypic performance across six root traits (Convex Hull Area, Lateral Root Length, Root Dry Weight, Root System Depth, Total Root Length, and Root/Shoot Ratio). Trait values were converted to percentiles (0–1 scale) and combined using trait-specific weights, prioritizing soil exploration capacity. The resulting composite index was used to identify the top-performing genotypes, which were subsequently visualized using parallel coordinate plots (Figure 6).

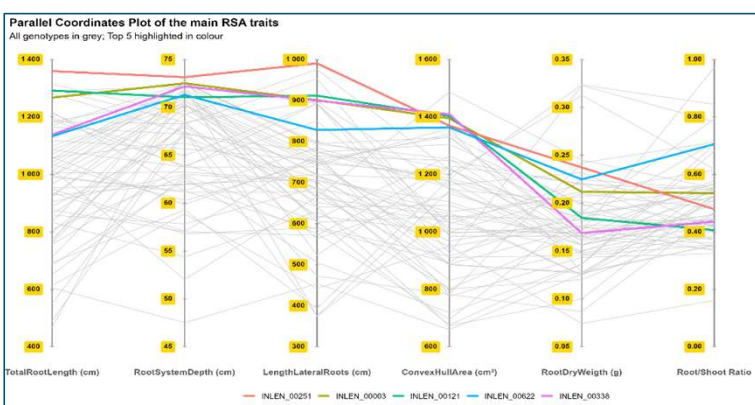


Figure 6. Parallel Coordinates Plot showing the Top 5 genotypes based on a weighted percentile-based scoring system was applied to evaluate the performance across six root traits (Convex Hull Area, Lateral Root Length, Root Dry Weight, Root System Depth, Total Root Length, and Root/Shoot Ratio) of the lentil genotypes.

Results

Significant phenotypic variation was detected across genotypes for all measured traits. Root system depth, lateral root length, and root dry biomass exhibited the greatest variability. The PCA mainly driven by traits associated with elongation and biomass accumulation. The parallel coordinate plot (Figure 6), revealed genotypes such as INLEN_00251, INLEN_00003, and INLEN_00121 as consistent top performers. These accessions combined high scores in depth, in width and in root area expansion, suggesting their potential for water foraging.

Conclusion

The wide phenotypic variability observed is suitable for genome-wide association studies (GWAS), which could be performed on the collection to identify loci controlling key RSA traits. By combining phenotypic and genotypic data (already available), our project aim to enable the selection of drought resistant lines suitable for cultivation in the Mediterranean area.