

# High throughput phenotyping of root system architecture traits in lentil core collection

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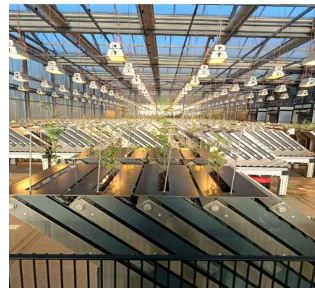


## Introduction

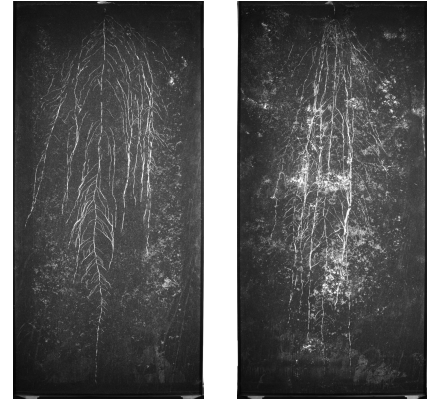
Lentil (*Lens culinaris* subsp. *culinaris*) is a key legume crop for sustainable agriculture. Climate changes, especially drought, is reducing yield stability. Root system architecture (RSA) plays a crucial role in water uptake and stress adaptation. This study evaluates RSA traits in 64 SSD (Single Seed Descent) lentil genotypes belonging to a larger collection used in the INCREASE H2020 project. The goal is to identify genotypes with root traits favorable to drought resilience and breeding improvement.

## Materials and Method

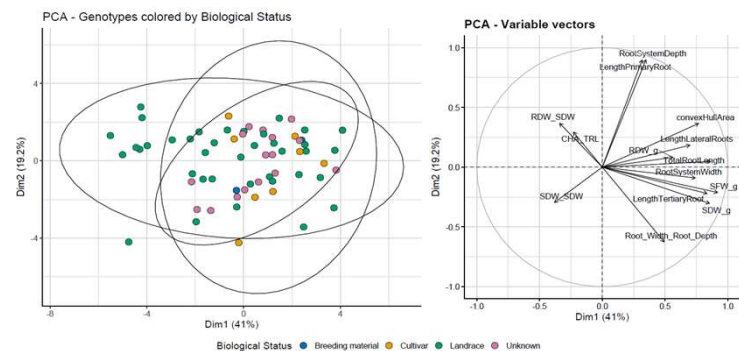
A core set of 64 lentil genotypes was selected based on genetic diversity, phenotypic variation and passport data. Plants were grown for 26 days in rhizotrons under controlled conditions using the GrowScreen-Rhizo3 platform (Forschungszentrum Jülich, Germany; Figure 1), following a randomized block design with three replicates per genotype. Root and shoot traits were acquired through daily high-resolution imaging (Figure 2). 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 3). To reduce dimensionality and explore



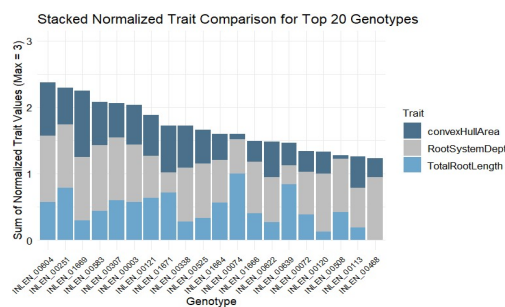
**Figure 1.** View of the high-throughput root phenotyping platform "GrowScreen-Rhizo3" at Forschungszentrum Jülich (Germany).



**Figure 2.** Example of root system images, acquired on the final day before harvest, using the GrowScreen-Rhizo3 phenotyping platform. Imaging was performed automatically under controlled conditions, and the resulting images were used for quantitative analysis of root architecture. The figure shows the contrasting root system architectures of two different lentil genotypes, highlighting variation in root depth, lateral spread, and branching patterns.

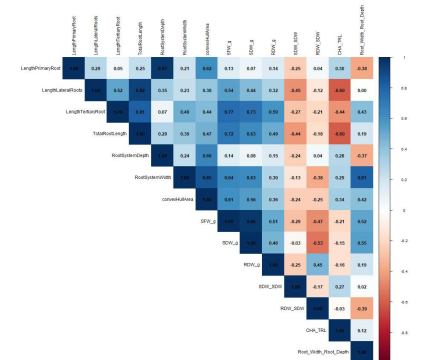


**Figure 4.** Principal Component Analysis (PCA) based on 14 root architecture traits measured on 64 lentil genotypes. Left: genotypes are plotted in the space defined by the first two principal components (Dim1 = 41%, Dim2 = 19.2%) and colored according to their Biological Status (Breeding material, Cultivar, Landrace, Unknown). Ellipses represent 95% confidence regions for each group. Right: loading plot showing the contribution and direction of each trait to the two principal components. Vectors closer to the circle and longer in length indicate traits with higher contribution to the PCA axes.



**Figure 5.** Stacked bar plot of normalized root traits (Total Root Length, Root System Depth, Convex Hull Area) for the 20 top-performing lentil genotypes. Bars represent the sum of min-max normalized values (0–1) across traits; the maximum cumulative value is 3. Genotypes were selected based on a scoring system that awarded one point for each trait above the population mean. This visualization highlights both balanced performers and trait-specific contributors.

multivariate patterns, a Principal Component Analysis (PCA) was conducted (Figure 4). 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 normalized scoring system was used to rank genotypes based on key traits, and top performers were visualized using a stacked bar chart (Figure 5).



**Figure 3.** Pearson correlation matrix among 14 root and biomass traits measured across all the 64 lines. Positive and negative correlations are indicated by blue and red gradients, respectively. Strong associations were observed among root length components, biomass fractions, and architectural descriptors.

## Results

Significant phenotypic variation was detected across the 64 genotypes for all measured traits. Root system depth, lateral growth, and shoot-to-root biomass allocation exhibited the highest variability. The first two principal components of the PCA accounted for 60.2% of the total variation and were mainly driven by traits related to root elongation and biomass accumulation. Several genotypes displayed both deeper root systems and broader lateral expansion, key traits associated with enhanced water uptake under drought conditions.

## Discussion

The observed diversity in root system architecture highlights a strong potential for selection in drought prone environments. The genotypes showing deeper roots, greater lateral spread and higher root biomass represent valuable candidates for improving water uptake efficiency and yield stability in this crop.

## Conclusion

The wide phenotypic variability observed is suitable for genome-wide association studies (GWAS), which could be performed to identify loci controlling key RSA traits. Combining phenotypic and genotypic data will accelerate the identification of genetic determinants underlying drought resilience lines for the Mediterranean cultivation area.