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SHOVELOMICS AND CLEAR POT ANALYSES HIGHLIGHT EXTENSIVE NATURAL GENETIC VARIATION FOR ROOT SYSTEM ARCHITECTURE IN BARLEY

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In plants, roots and root system architecture (RSA) are involved to adaptation, response to abiotic stresses, water and nutrient uptake and in anchoring the aboveground part of plants in the soil. Recent studies carried out in cereal crops and wild relatives under both controlled and field conditions have highlighted the existence of untapped diversity for belowground traits. Unlike maize and rice, the analysis of natural genetic variation for RSA in barley has lagged behind, hampering our ability to dissect its genetic bases and exploit the existing variability for breeding.

In the present study, we examined the phenotypic diversity and distribution of different belowground traits in a diverse panel of 415 barley accessions, including modern and old cultivars and landraces, characterized at molecular level using more than 300 k genome wide markers. This diverse barley panel was sown during the fall of two consecutive growing seasons (2021–2022 and 2022–2023) in Fiorenzuola d'Arda (Italy), following an alpha-lattice experimental design with two replicates. RSA of these plants was dissected and examined phenotyping 80 belowground traits (e.g., root angle, root number, root density, root area, etc.) at late developmental stages (Zadok growth scale 69) in field conditions using shovelomics methodology and image analysis software. Moreover, several seminal root traits (e.g., seminal root number, seminal root angle) of the same genotypes were phenotyped in barley plantlets grown in controlled conditions using the clear pot method.

Data analyses showed that for belowground traits, broad sense heritability

ranges from 0.3 (root area) to 0.9 (width skeleton), highlighting that RSA might be a target of genetic improvement. The adjusted means of belowground traits measured in seedlings were correlated with those computed in field conditions at late developmental stages. Overall, our results point out several positive correlations.

For dissecting the genetic bases of RSA of seedlings and adult barley plants, phenotypic data of 372 out 415 barley accessions were combined with genotypic information to carry out genome-wide association analyses (GWAS) using single marker regression (GLM, MLM, SUPER) and multi-locus (FarmCPU, MLMM and BLINK) models for continuous and discrete traits. GWAS analyses detected several peaks of potential interest across all seven barley chromosomes, particularly using Bonferroni's threshold GWAS based on SUPER model outputted the highest number of significant markers. Moreover, using the same genotypic and phenotypic information, we fitted and cross-validated several genomic regression models for continuous and discrete belowground traits using GBLUP and threshold-GBLUP, respectively.

Overall, the extensive natural variation for belowground traits detected in our barley panel along with dense genotyping revealed several QTLs underlying RSA, paving the way to exploit these data for barley improvement and varietal innovation.