

Genome wide association mapping identifies major QTLs affecting seminal root traits in barley

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BACKGROUND

Barley (*Hordeum vulgare* L.) is the fourth most important cereal in the world after wheat, rice and corn. Barley is also a popular model for genetic studies regarding adaptation to different environmental conditions. The root system plays a fundamental role for absorption of water and nutrients and a better knowledge about the genetic basis of root trait variation is crucial to successfully target root traits in breeding programs (De Dorlodot *et al.* 2007).

OBJECTIVE. In this study we investigated the genetic control of seminal root traits variation in a barley germplasm collection (WHEALBI collection) (<https://www.whealbi.eu/>) representative of the genetic variation in this species, using GWA mapping and leveraging on the available high density genotype information on this collection.

MATERIALS AND METHODS

- Plant materials and phenotyping.** The almost complete barley WHEALBI collection (<https://www.whealbi.eu/>) encompassing 485 accessions, was utilized. Phenotypic data concerning root traits, seminal root number (SRN), root growth angle (RGA), total root length (RTL), average root length (ARL) and root dry weight (RDW) were collected using a semi-hydroponic system at the seedling level (13 days from germination) (Kirschner *et al.* 2021). Roots images have been analyzed using two software: ImageJ (<http://imagej.nih.gov/ij/>) and GIMP ([GIMP - GNU Image Manipulation Program](http://www.gimp.org/))
- GWA.** SNP Genotypic data were initially provided by WHEALBI (Bustos-Korts *et al.* 2019), and further improved in this study. Genome Wide Association Study (GWAS) was performed using BLINK model (Huang *et al.*, 2019) implemented in the GAPIT R package (Lipka *et al.*, 2012).

REFERENCES

- De Dorlodot S., *et al.* (2007). Root system architecture: opportunities and constraints for genetic improvement of crops. *Trends in Plant Science* 12, 474–481.
- Kirschner K. G. *et al.* (2021). *ENHANCED GRAVITROPISM 2* encodes a STERILE ALPHA MOTIVE containing protein that controls root growth angle in barley and wheat. *PNAS* 118 No. 35
- Bustos Korts D, *et al.* (2019). Exome sequences and multi-environment field trials elucidate the genetic basis of adaptation in barley. *TPI* 99:1172-91
- Huang, M. *et al.* (2019). BLINK: A package for the next level of genome-wide association studies with both individuals and markers in the millions. *Gigascience* *giy154*
- Lipka, A. E. *et al.* (2012). GAPIT: genome association and prediction integrated tool. *Bioinformatics* 28, 2397–2399

RESULTS

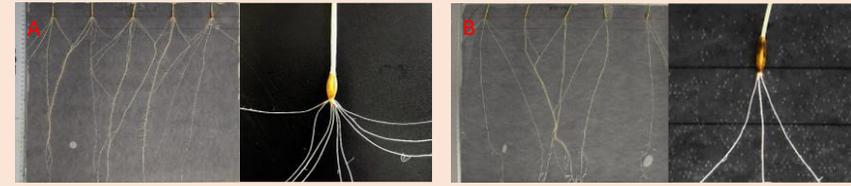


Figure 1. Example of variation for root architecture within WHEALBI. A) Panel of WB_254 and magnification of a single seedling with nine seminal roots. B) Panel of WB_494, wild accession, and seedling with three seminal roots.

Table 1. Variation of root system architecture traits within WHEALBI.

ID (unit)	Minimum	Maximum	Average	h^2
SRN (no.)	3.00 ± 0.00	8.20 ± 0.57	5.88	0.87
RGA (angle)	17.23 ± 5.96	118.51 ± 8.86	55.66	0.81
RTL (cm)	60.3 ± 0.42	230.46 ± 8.22	154.79	0.55
ARL (cm)	18.44 ± 2.31	36.27 ± 4.94	26.36	0.78
RDW (mg)	3.43 ± 0.61	28.01 ± 0.58	17.67	0.55

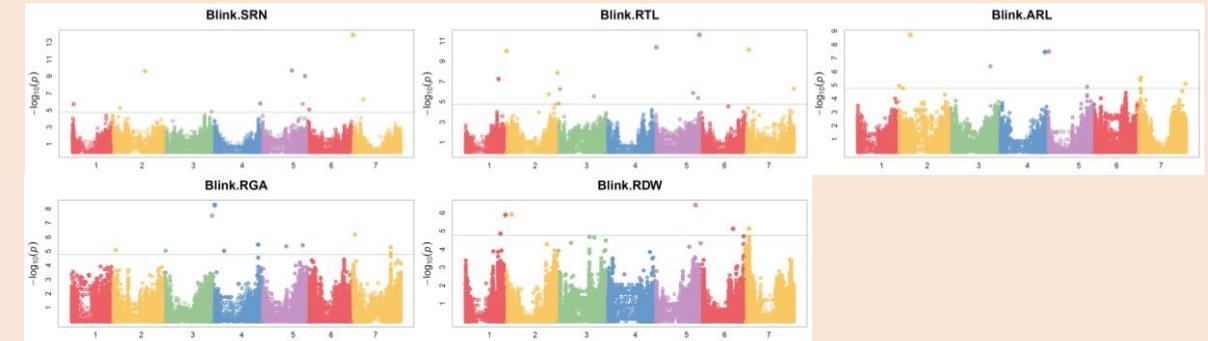


Figure 2. Manhattan plots for GWA mapping results for root traits within the WHEALBI barley collection. Horizontal axis presents seven chromosomes (1H–7H). Vertical axis shows $-\log_{10}(P)$ values of marker-trait associations. Horizontal green line shows probability threshold value ($-\log_{10} P = 4.75$) for association.

SUMMARY AND CONCLUSIONS

- Phenotypic and genetic variability was observed for all traits (Figure 1, table 1)
- Variation for seminal root trait was extensive and h^2 ranged between 0.55 and 0.87, for RDW and SRN, respectively
- 51 QTL were identified for seminal root traits (figure 2) and specifically 11 for SRN, 14 for RTL, 10 for ARL, 10 for RGA and 6 for RDW.

For SRN we identified two major QTLs on chromosome 5H and 7H that showed high R^2 value (respectively 0.16 and 0.13). They have showed also the major absolute effect, respectively 0.91 and 1.15

These results will be the starting point toward the identification of genes involved in root development and will enable to test hypothesis about how breeding shaped root system architecture (eg. increase of number of seminal roots linked with domestication) in barley.