

# VERNALIZATION1 Modulates Root System Architecture in Wheat and Barley

Dear Editor,

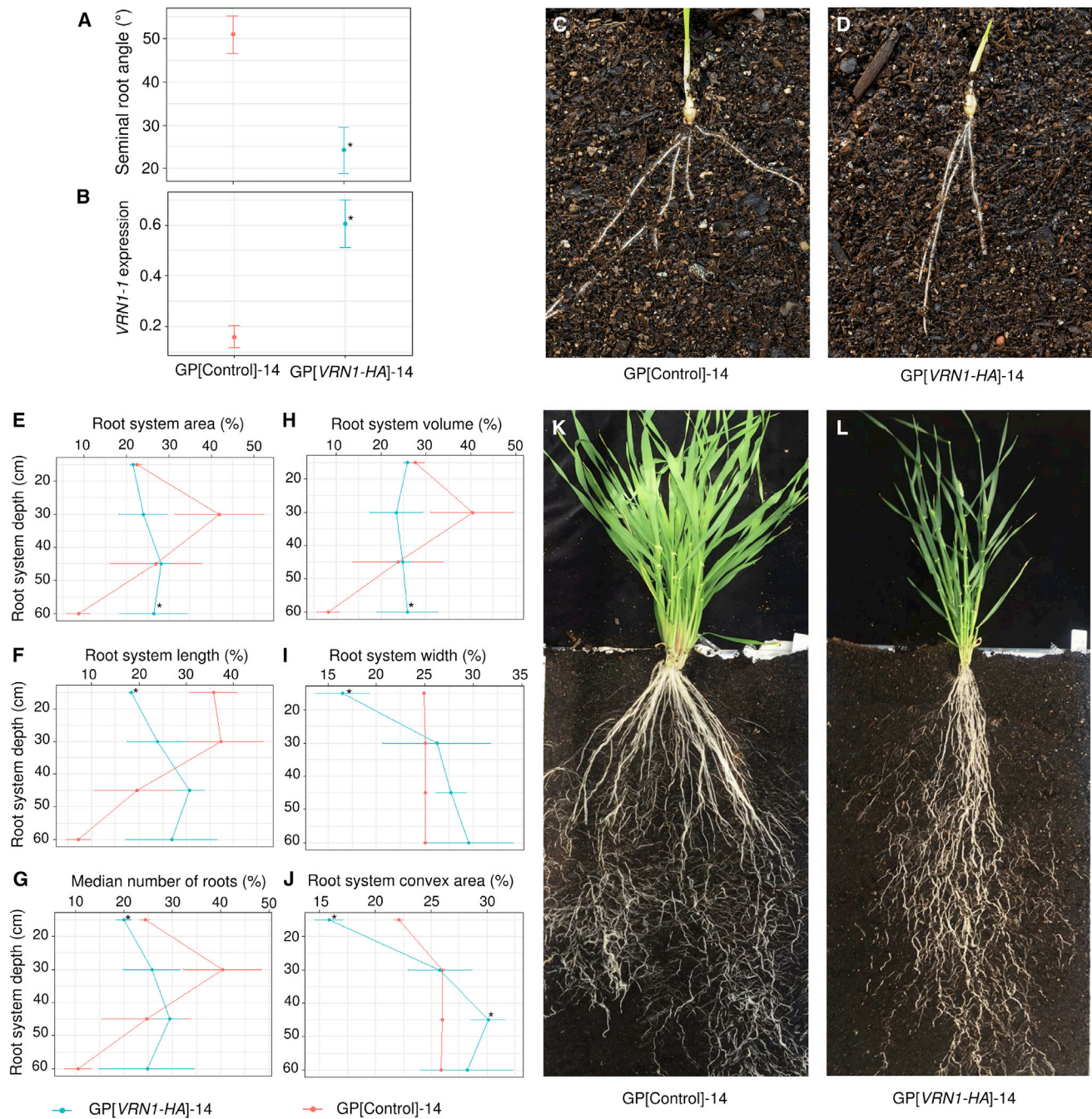
Roots play a key role in plant growth regulation. It is well described that the below-ground plant architecture has a significant impact on plant performance under abiotic constraints and maintains stability under increased grain load (Lynch, 2013). Although loci influencing root traits have been shown to affect grain yield and agronomic performance (e.g., Canè et al., 2014), knowledge about the genetic control of root growth in major grain crops is limited. Here, we demonstrate that *VERNALIZATION1* (*VRN1*), a key regulator of flowering behavior in cereals (Deng et al., 2015), also modulates root architecture in wheat and barley. Our discoveries provide unexpected insight into underground functions of a major player in the flowering pathway.

We initially mapped a highly significant quantitative trait locus (QTL) for nodal root angle index (NRI; Supplemental Figure 1, Supplemental Materials and Methods) on *Triticum aestivum* chromosome 5B using 219 hexaploid winter wheat accessions (Supplemental Figure 2, Supplemental Tables 1–3). This QTL consists of six single-nucleotide polymorphism (SNP) markers, which are in strong linkage disequilibrium (LD;  $r^2 \geq 0.7$ ) and span the B-subgenome homoeolog of *VERNALIZATION1* (*VRN1*) (223 185 538–223 597 558 bp), one of the most important and well-characterized developmental genes in the temperate cereals wheat and barley. The MADS-box transcription factor encoded by *VRN1* is well known for its regulation of genes influencing reproductive plant organs and flowering (Deng et al., 2015). The *VRN1* wild-type “winter” allele, *v*, confers a requirement for prolonged exposure to cold (vernalization) as a prerequisite for flowering in most winter-type wheat and barley, however a deletion in the first intron enables spring-sown plants to flower without prior vernalization, thus referred to as the “spring” allele (*a*) (Trevaskis et al., 2006). To investigate the connection between the major *VRN1* polymorphism and variation in wheat root growth, we evaluated three hexaploid wheat near-isogenic lines (NILs) carrying different combinations of winter and spring alleles at the A, B, and D subgenome homeologs of *VRN1* in a common genetic background (Supplemental Materials and Methods). The presence of the winter alleles consistently reduced root angle at all growth stages under greenhouse and field conditions (Supplemental Figures 3–5). Root length varied between the different NILs during the lifecycle (Supplemental Figures 6 and 7); winter alleles (*vvv*) were associated with reduced root length at seedling stage but increased length at anthesis, most likely due to delayed anthesis and an extended vegetative period (Supplemental Figure 8). Root biomass in the bottom half of the soil profile was similar for all NILs at anthesis (Supplemental Figure 9) but post-anthesis, NILs carrying the spring allele (*vav* and *vaa*) produced significantly more roots (5.3%) at depth (60–80 cm) compared with winter types (*vvv*). Interestingly, these lines displayed a

significantly reduced root-to-shoot ratio (R/S) in comparison with winter (*vvv*) NILs at anthesis (Supplemental Figures 10 and 11). Our results suggest that *VRN1* influences temporal and spatial root growth in wheat throughout the whole plant lifecycle, with the B-subgenome homoeolog *VRN-B1* imparting the strongest phenotypic effect in our study.

For cross-species comparison, we assayed similar root architectural traits in barley NILs that differ for prevalent *VRN1* alleles (designated *VRN-H1*) in a common genetic background (Supplemental Materials and Methods). Intriguingly, each *VRN-H1* spring allele was associated with a unique root phenotype under greenhouse and field conditions, suggesting divergent selection of functional allelic variants in barley (Supplemental Figures 12 and 13). As expected, *VRN-H1* spring alleles significantly influenced above-ground plant development, including days to anthesis and tiller number (Supplemental Figure 14). However, in contrast to wheat, no significant differences in root length at seedling stage were evident among the barley NILs. As observed previously in wheat, spring alleles in barley were associated with reduced root elongation and maximum root length between anthesis and maturity (Supplemental Figure 15). However, each spring *VRN1-HA* allele was associated with unique root system architecture at the mature stage. Compared with the wild-type, all barley NILs carrying spring alleles produced a higher proportion of roots at moderate soil depths (20–60 cm), particularly during the grain-filling stage (Supplemental Figure 16), although their root biomass and R/S were lower during the seedling stage (Supplemental Figure 17). Notably, the *VRN1-1* allele, which is common in Australian barley varieties grown in regions with highly variable rainfall and severe seasonal drought, was associated with narrow root growth behavior in early plant development and prolonged root growth at the deepest soil level (60–80 cm) during grain filling. This suggests that selection for *VRN-H1* variants that simultaneously induce early flowering and maintain “steep, cheap, and deep” root systems (Lynch, 2013) provides a dual mechanism imparting flowering-mediated drought escape coupled with improved water or nutrient acquisition. Interestingly, the NIL carrying a deletion of *VRN-H2*, an important plant developmental gene and the known signaling target of *VRN-H1* in the vernalization response, did not show narrow root growth angles in barley (Supplemental Figures 12 and 13) but exhibited a shorter root length at flag-leaf emergence and a unique root system distribution (Supplemental Figures 15 and 16).

To confirm that the influence of *VRN1* on below-ground plant development was caused by pleiotropy rather than linkage, we



**Figure 1. Comparisons of Root Phenotypes and Gene Expression between the *VRN-1H* Transformed Line GP[*VRN1-HA*]-14 and Barley Cultivar Golden Promise Control Line GP[Control]-14.**

(A) Seminal root angle; (B) *VRN1-1* expression in bulked seedling tissue; (C) GP[Control]-14 root system at 5 days post sowing; (D) GP[*VRN1-HA*]-14 root system at 5 days post sowing. (E–J) Variation in root system parameters at varying soil depths: (E) total root system area; (F) total root system length, (G) median number of roots, (H) total root system volume, (I) root system width; (J) root system convex area. (K) GP[Control]-14 root system at 5 weeks post sowing, and (L) GP[*VRN1-HA*]-14 root system at 5 weeks post sowing. BLUEs and standard errors are displayed for each line (GP[Control]-14: pink, GP[*VRN1-HA*]-14: blue), except in (A) where means and standard errors are displayed. *t* tests were used to determine statistical significance in (A and B),  $p \leq 0.05$ . Fisher's LSD 0.05 test was used to determine significant differences in (D–J). \* indicates statistical significance.

assessed root phenotypes in three transgenic lines of the spring barley cultivar Golden Promise that carry an additional copy of *VRN1-HA* (Supplemental Materials and Methods). Coordination of above-ground and below-ground architecture in barley by *VRN-1H* was highlighted by the striking reflection of shoot and

root architecture in transformed line GP[*VRN1-HA*]-14, in which a significant increase in overall *VRN-1H* expression was detected as compared with the non-transformed control (Figure 1). The additional *VRN1-HA* allele drastically altered all root parameters measured at seedling and adult plant stages (Figure 1E–J,

Supplemental Figure 18A) and significantly reduced spike length and DTA, consistent with recent findings of additional *VRN1* functions (Deng et al., 2015; Supplemental Figure 18B and 18C), while a significant reduction in R/S was only observed in transformed lines GP[*VRN1-HA*]-6 and GP[*VRN1-HA*]-22 (Supplemental Figure 19).

To gain first insights into the biological mechanism with which *VRN1* influences root architecture, we investigated gene expression and used time-lapse imaging to compare root gravitropic responses between barley NILs carrying different alleles (Supplemental Figure 20). Strong differences in gravitropic response were observed depending on the *VRN1* allelic state (Supplemental Movie 1) and high *VRN-H1* expression levels were observed in mature root system tissues of barley NILs carrying *VRN1-1* and the winter wild-type allele. No expression differences could be found between the *VRN-H1* and winter wild-type barley NILs for the barley homolog of *DEEPER ROOTING 1 (DRO1)* (Uga et al., 2013), the first cloned root architectural gene in rice, or the barley homolog of the auxin-induced *GH3-2* gene, confirming that the observed differences in root angle are independent of *DRO1* and auxin sensitivity at the whole-root system level. However, this does not exclude the possibility of significant differences in root tips, as observed for *DRO1* expression in rice (Uga et al., 2013).

We then examined the *VRN1* allelic composition of different wheat populations. By analyzing 132 commercial Australian wheat cultivars (Supplemental Table 4), we confirmed associations of the *VRN-B1* winter-spring polymorphism to variation in root growth using data from high-throughput root phenotyping (Supplemental Materials and Methods). This revealed that seminal root angle was significantly narrower in cultivars carrying the winter allele compared with cultivars with the spring allele ( $p = 0.01$ ) and that *VRN-B1* accounted for 8% of the total variation in root angle, independent of spatial variation ( $p = 0.01$ ) and consistent across various imputation models (Supplemental Figure 21). Although the *VRN-B1* winter allele was almost completely fixed in the EU wheat panel used for the genome-wide association study (Supplemental Table 1), investigation of haplotype variation using the six SNP markers around this locus revealed further diversity. Interestingly, individuals displaying alleles G-G at the two SNPs *Kukri\_c12910\_908* and *Excalibur\_c38433\_291*, which are in strong LD ( $r^2 = 0.84$ ) and directly flank *VRN-B1*, had significantly increased NRI compared with lines with A-A at this position ( $p = 0.00002$ ) (Supplemental Table 5, Supplemental Table 6, Supplemental Figure 22). The phenotypic difference between A-A and G-G genotypes was also detectable at flowering under temperate field conditions (Supplemental Figure 23) and was independent of vernalization or *VRN1* expression in root and shoot tissues (Supplemental Figure 24). This may suggest that novel molecular variants of *VRN1*, distinct from the major winter-spring polymorphism, are responsible for modulation of root development in winter wheat germplasm.

In summary, our results indicate that *VRN1* pleiotropically shapes overall plant morphology in wheat and barley, thereby regulating the balance between above- and below-ground plant architecture. In barley, QTLs for root traits were detected previously in the vicinity of *VRN1* (Arifuzzaman et al., 2014, 2016) and a potential involvement of *VRN1* in overall plant growth was also

proposed in wheat (Eagles et al., 2011). Nevertheless, its direct involvement in cereal root system architecture was unknown to date. However, many related genes from the MADS-box transcription factor family are highly expressed in roots of *Arabidopsis* (Yu et al., 2014), rice (Guo et al., 2013), and soybean (Liu et al., 2015), where they affect underground plant development. Investigations in *Arabidopsis* have demonstrated important roles of MADS-box genes in local auxin accumulation in root primordia or root cap tissue (Yu et al., 2014). Further work to identify additional downstream targets of *VRN1*, connecting well-characterized above-ground and unexplored below-ground expression networks, would help elucidate the molecular functions of this major developmental gene in cereals.

## SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

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## AUTHOR CONTRIBUTIONS

L.T.H., K.P.V.-F., H.R., B.T., B.W., W.F., M.F., R.J.S., A.K.B., G.F., J.C., M.C., K.C., J.F., A.S., S.R.M., E.S.M., D.R.J., J.R.B., G.H., H.E., and I.D.G. conceived the various experimental components and guided the direction of the study. K.P.V.-F., B.W., A.S., I.G., W.F., and M.F. analyzed the winter wheat population. H.R., K.P.V.-F., A.M.-C., and C.R. analyzed the spring wheat population. H.R., A.K., and B.T. generated and analyzed the spring wheat NILs. S.N., L.T.H., and H.E. performed the wheat pedigree network analysis. H.R., B.T., S.R.M., B.C.C., J.R.B., and A.K. generated and analyzed the barley NILs. H.R., B.T., S.R.M., I.D.G., and A.K. generated and analyzed the transgenic barley lines. K.P.V.-F., H.R., I.D.G., R.J.S., and L.T.H. wrote the manuscript.

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## REFERENCES

- Arifuzzaman, M., Sayed, M.A., Muzammil, S., Pillen, K., Schumann, H., Naz, A.A., and Léon, J. (2014). Detection and validation of novel QTL for shoot and root traits in barley (*Hordeum vulgare* L.). *Mol. Breed.* **34**:1373–1387.
- Arifuzzaman, M., Günal, S., Bungartz, A., Muzammil, S., P Afsharyan, N., Léon, J., and Naz, A.A. (2016). Genetic mapping reveals broader role of Vrn-H3 gene in root and shoot development beyond heading in barley. *PLoS One* **11**:e0158718.
- Canè, M.A., Maccaferri, M., Nazemi, G., Salvi, S., Francia, R., Colalongo, C., and Tuberosa, R. (2014). Association mapping for root architectural traits in durum wheat seedlings as related to agronomic performance. *Mol. Breed.* **34**:1629–1645.
- Deng, W., Casao, M.C., Wang, P., Sato, K., Hayes, P.M., Finnegan, E.J., and Trevaskis, B. (2015). Direct links between the vernalization response and other key traits of cereal crops. *Nat. Commun.* **6**:5882.
- Eagles, H.A., Cane, K., and Trevaskis, B. (2011). Veery wheats carry an allele of Vrn-A1 that has implications for freezing tolerance in winter wheats. *Plant Breed.* **130**:413–418.
- Guo, S., Xu, Y., Liu, H., Mao, Z., Zhang, C., Ma, Y., Zhang, Q., Meng, Z., and Chong, K. (2013). The interaction between OsMADS57 and OsTB1 modulates rice tillering via DWARF14. *Nat. Commun.* **4**:1566.
- Liu, W., Han, X., Zhan, G., Zhao, Z., Feng, Y., and Wu, C. (2015). A novel sucrose-regulatory MADS-box transcription factor GmNMHC5 promotes root development and nodulation in soybean (*Glycine max* L. Merr.). *Int. J. Mol. Sci.* **16**:20657–20673.
- Lynch, J.P. (2013). Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Ann. Bot.* **112**:347–357.
- Trevaskis, B., Hemming, M.N., Peacock, W.J., and Dennis, E.S. (2006). HvVRN2 responds to daylength, whereas HvVRN1 is regulated by vernalization and developmental status. *Plant Physiol.* **140**:1397–1405.
- Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai, Y., Ono, K., Kanno, N., et al. (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nat. Genet.* **45**:1097–1102.
- Yu, L.-H., Miao, Z.-Q., Qi, G.-F., Wu, J., Cai, X.-T., Mao, J.-L., and Xiang, C.-B. (2014). MADS-box transcription factor AGL21 regulates lateral root development and responds to multiple external and physiological signals. *Mol. Plant* **7**:1653–1669.