The Plant Genome OPEN ACCESS

ORIGINAL ARTICLE

Genetic control of root architectural traits under drought stress in spring barley (*Hordeum vulgare* L.)

Md. Nurealam Siddiqui[1,2] ⓘ | Melisa Jahiu[1] ⓘ | Mohammad Kamruzzaman[1] |
Miguel Sanchez-Garcia[3] | Annaliese S. Mason[1] | Jens Léon[1,4] | Agim Ballvora[1]

[1]Plant Breeding Department, Institute of Crop Science and Resource Conservation (INRES), University of Bonn, Bonn, Germany

[2]Department of Biochemistry and Molecular Biology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

[3]Department of Biodiversity and Crop Improvement Program, International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco

[4]Field Lab Campus Klein-Altendorf, University of Bonn, Rheinbach, Germany

Correspondence
Agim Ballvora, Plant Breeding Department, Institute of Crop Science and Resource Conservation (INRES), University of Bonn, Bonn, Germany.
Email: ballvora@uni-bonn.de

Assigned to Associate Editor Lee Hickey.

Abstract

Root architectural traits play pivotal roles in plant adaptation to drought stress, and hence they are considered promising targets in breeding programs. Here, we phenotyped eight root architecture traits in response to well-watered and drought stress conditions in 200 spring barley (*Hordeum vulgare* L.) inbred lines over two consecutive field seasons. Root architecture traits were less developed under drought in both seasons when compared with control treatments. Genetic variation in root architectural traits was dissected employing a genome-wide association study (GWAS) coupled with linkage disequilibrium mapping. GWAS uncovered a total of 186 significant single nucleotide polymorphism-trait associations for eight root traits under control, drought, and drought-related indices. Of these, a few loci for root traits were detected on chromosomes 3 and 5, which co-located with QTL identified in previous studies. Interestingly, 13 loci showed simultaneou associations with multiple root traits under drought and drought-related indices. These loci harbored candidate genes, which included a wide range of drought-responsive components such as transcription factors, binding proteins, protein kinases, nutrient and ion transporters, and stress signaling factors. For instance, two candidate genes, *HORVU7Hr3G0713160* and *HORVU6H r3G0626550*, are orthologous to *AtACX3* and *AtVAMPs*, which have reported functions in root length-mediated drought tolerance and as a key protein in abiotic stress tolerance, respectively. Interestingly, one of these loci underlying a high-confidence candidate gene *NEW ENHANCER OF ROOT DWARFISM1* (*NERD1*) showed involvement with root development. An allelic variation of this locus in non-coding region was significantly associated with increased root length under drought. Collectively, these results offer promising multi-trait affecting loci and candidate genes underlying root phenotypic responses to drought stress, which

---

- -

may provide valuable resources for genetic improvement of drought tolerance in barley.

Plain Language Summary

Genetic analysis of multiple root traits in two consecutive years field trials revealed quantitative trait loci, which are simultaneously responsible for several traits of root architecture in spring barley. The multi-trait affecting loci and putative candidate genes modulate root architecture under drought stress.

1 | INTRODUCTION

Among cereals, barley is recognized as one of the seven cultivated major cereal grains, recently ranked fourth in global production after wheat, rice, and maize (Zhou, 2009). Barley (*Hordeum vulgare* L.) is predominantly used as a food crop in many parts of the world (Kent & Evers, 1994). Barley is a versatile crop; it has been utilized for human food and beverage manufacturing since ancient times. It has also been primarily used to feed cattle (Newton et al., 2011). Drought stress is a major yield-limiting factor in barley, causing dramatic grain yield reductions of up to 49%–87% (Samarah, 2005; Samarah et al., 2009). As the most sensitive developmental event of barley growth during drought is the spike initiation phase (Sehgal et al., 2018), dissecting genetic control of drought tolerance during the flowering stage is critical for improving drought stress tolerance.

One of the most promising strategies to enhance drought tolerance in barley is thought to be the design or implementation of a beneficial root system architecture (Ramireddy et al., 2018). The root system is an essential multifunctional organ that plays critical roles in extracting soil-based resources, facilitating absorption and transport of water and nutrients, and balancing root-to-shoot signaling in plants, especially under water-deprivation conditions (Siddiqui, Léon, et al., 2021). When plants experience water deficiency, roots undergo persistent and continuous downwards growth in order to enter deep soil layers (Fan et al., 2017; Koevoets et al., 2016; Maeght et al., 2013; Vyver & Peters, 2017). Under certain conditions, root architecture determines access to water, and a significant association between RSA traits and drought tolerance has been observed in many plant species, including barley (Comas et al., 2013; Naz et al., 2014; Tuberosa et al., 2002). Several reports established a positive correlation between root traits and shoot performance (including grain yield) under drought stress (Hufnagel et al., 2014; Meister et al., 2014; Uga et al., 2013). For instance, deeper roots with greater branching have been recorded in drought-resistant rice varieties when compared with drought-sensitive rice (*Oryza sativa*) varieties (Price & Tomos, 1997). Deeper and thinner root systems are directly associated with crop adaption to soils with water-limited conditions, in contrast to crops with shallow and thick rooting systems (Ram, 2014). Root architectural traits are also characterized by proliferative roots developed through lateral root initiation and elongation, and these characteristics include root number/volume and root surface area (RSA), which aid in water uptake from water-limited soils (Ye et al., 2018). Therefore, RSA traits conferring soil exploration in time and space are the traits that are regarded as the most relevant in crop breeding programs (Comas et al., 2013; Koevoets et al., 2016; Lynch & Brown, 2012; Oyiga et al., 2020; Rogers & Benfey, 2015).

Customizing RSA by classical breeding approaches necessitates thorough investigation and understanding of roots, which are highly complex plant components and usually hidden below ground (Siddiqui et al., 2022). More importantly, RSA is a complex phenomenon, influenced by multiple intrinsic and extrinsic factors and regulated by multiple genetic components (Lynch & Brown, 2012). In addition, precise field-based root phenotyping of diversity panels is highly challenging due to the labor-intensive and complex root harvesting required. Thus, specifically targeting RSA in crop breeding is challenging. Moreover, a systematic approach to develop adaptive RSA in crops is desirable, not only to uncover root-mediated genetic factors for breeding purposes but also for functional studies on the shape and size of root system. However, to date, the genetic mechanisms, components, and variation underlying RSA traits have not been comprehensively investigated in most crops (including barley), particularly in response to drought stress under field conditions.

Previous mapping studies in barley have identified quantitative trait loci (QTL) for seminal root traits such as root angle and root number (Robinson et al., 2016, 2018); however, the genetic control of additional root traits should be explored to provide a deeper understanding of root development in barley and the potential to improve resilience to drought. A high-resolution, haplotype-based genome-wide association study (GWAS) approach to dissect complex RSA traits and identify robust candidate genes in barley under drought conditions is imperative. Therefore, the objective of our study was to identify genomic loci showing associations with simultaneous

effects on several RSA trait changes under drought conditions. To achieve this, we used "shovelomics" approaches for digging out the upper part of the root systems (Klein et al., 2020; Siddiqui, Kailash et al., 2023; Siddiqui, Gabi, et al., 2023; York et al., 2018) to phenotype eight root traits using 200 diverse spring barley genotypes at the complete flowering stage under well-watered and drought stress conditions in two barley growing seasons (2020 and 2021). Phenotypic data were analyzed using mixed linear model (MLM) and GWAS approaches based on 23.8K markers obtained from a 50K iSelect barley single nucleotide polymorphism (SNP) array. We identified a total of 186 genetic loci involved in marker-trait associations, of which 13 loci showed multi-trait associations with eight root traits in response to drought stress. Several candidate genes (and allelic variants) with putative functionality in root development and drought stress tolerance were identified.

2 | MATERIALS AND METHODS

2.1 | Plant materials and field experiments

We used a total of 165 and 200 diverse spring barley (*Hordeum vulgare* L. ssp. *vulgare*) inbred lines comprising advanced breeding lines, landraces, and cultivars to assess the genetic diversity of root architecture traits in 2020 and 2021, respectively. This diversity panel was selected from the Global Barley Breeding Program of the International Center for Agricultural Research in the Dry Areas (ICARDA). The full list with a description of the mapping population is provided in Table S1 (Bouhlal et al., 2022).

The diversity panel was planted during the 2020 and 2021 cropping seasons at the Campus Klein–Altendorf Research Facility (50°37′N, 6°59′E) of the University of Bonn, Germany. Field experiments were conducted under rain-fed (control) and drought stress (rain-out shelter) conditions. The rain-out shelter was built with a roof and two sides, which were designed with the ability to be controlled electronically by a motorized system, and plants were irrigated through an automated sprinkler in order to deliver ~5.00 L/m² of water per day. For the plants to feel the ambient conditions, the shelter was left open, and only closed during rainfall to exclude rainwater. A randomized complete block design was used as a mapping panel for barley genotypes grown under both conditions, with 0.5-m-long rows and 0.21-m between rows plot size. All plots were fertilized using standard agronomic practices. Drought stress was imposed by stopping watering the plots at the tiller initiation stage (BBCH20) and continued until root harvesting at complete flowering stage (BBCH51), as followed by Oyiga et al. (2020). The moisture content of the soil was recorded via a soil moisture sensor EM50 data logger buried in the soil (0- to 30-cm deep). The

Core Ideas

- Root architecture play an important role for acquisition of soil resources under drought conditions.
- Substantial natural genetic variation was observed in root architecture traits in barley germplasm under drought stress.
- Genetic analysis of multiple root traits in 2 years field trials revealed promising loci, which are simultaneously responsible for several traits of root architecture in spring barley.
- The multi-trait affecting loci and putative candidate genes modulate root architecture under drought stress.
- Allelic variation in *NEW ENHANCER OF ROOT DWARFISM1* (*NERD1*) was associated with increased root length under drought stress.

root architectural and anatomical traits were collected from three barley plants of the same genotype, planted in a single row.

2.2 | "Shovelomics" approach for root phenotyping

To characterize root architectural traits under controlled drought stress conditions, the barley roots were harvested at the heading (BBCH51) stage following the "shovelomics" protocol (York et al., 2018). After the collection of the plants, they were carefully washed to remove the soil and unwanted debris and stored in 50% alcohol solution. The phenotypic traits of the root were analyzed using the EPSON scanner (Perfection LA24000) with a resolution of 2400 dpi (Kadam et al., 2017), and images were analyzed using the software WinRHIZO (Regent Instruments Inc.). Root traits assessed included total root length (TRL), projected root area (PA), RSA, root average diameter (RAD), root volume (RV), number of root tips (NRT), number of root forks (NRF), and number of root crossings (NRC).

2.3 | Statistical analysis

The phenotypic data were analyzed and visualized using R Studio version 4.2.3. To evaluate the effect of genotype and treatment interaction on the root architectural characteristics, a two-way analysis of variance (ANOVA) was conducted to analyze the quantitative root traits where the treatment and the genotype were treated as fixed effects. Analyses were

done in R (R Core Team, 2018) using packages "nlme" and "emmeans." Descriptive statistics were used to estimate means, minimum, maximum, standard error, and coefficient of variation (CV) for each trait.

Broad-sense heritability (H^2) was calculated to estimate heritability based on the following formula: $H^2 = V_G/(V_G + V_E/r)$, where r is the number of cultivar replications, V_E is the estimate of error variance for each treatment, and V_G is the estimate of genetic variance as described by Gitonga et al. (2014). The stress plasticity index (SPI) of the root system architectural traits was calculated by comparing the genotypes under drought stress conditions with the genotypes under controlled conditions using the following formula of SPI = (WW−DS)/WW, where DS represents drought stress and WW represents well-watered conditions, following Schneider et al. (2020). The stress tolerance index (STI) of the barley root architectural traits for all genotypes was estimated by taking the phenotypic value under drought stress and well-watered conditions using the formula of STI = (DS × WW)/(WW)^2, where DS represents drought stress and WW indicates well-watered conditions, as formulated by Nourain et al. (2013). To determine the correlations between the means of the root traits under drought and control conditions, a heat map was constructed in R (The R Team for Statistical Computing R v. 4.2.3) using the "corrplot" package.

2.4 | SNP genotyping and population structure analysis

The barley diversity panel was genotyped using an Infinium iSelect Illumina 50K SNP chip (Illumina; San Diego; Bayer et al., 2017) and was selected for its diversity as a subset of a World Diversity Collection (Bouhlal et al., 2022). DNA extraction, SNP genotyping methodology, and Hapmap genotypic file are provided by Bouhlal et al. (2022). SNP data imputation was carried out in TASSEL 5.0 with LinkImpute (LD-kNNi) to eliminate missing SNPs with minor allele frequency of <5% (Siddiqui, Gabi, et al., 2023). To determine the population structure of the barley genotypes used in this study, a model-based clustering method using STRUCTURE 2.3.4 software (Dido et al., 2022; Pritchard et al., 2002) was run on the genotypic data.

After filtering, a total of 3900 random SNPs, at least 5 cM apart, were selected to estimate the population structure. Simulations were run by inferring K values from 2 to 10, with 20,000 iterations and burn-in period of 10,000 and 100,000 Markov chain Monte Carlo. The results were entered into the web-based program Structure Harvester (http://taylor0. biology.ucla.edu/structureHarvester) to obtain the delta K statistics of the panel (Earl & von Holdt, 2012). Using the ad hoc quantity (ΔK) metrics, the true value of K generated by the autocorrelation plot (ΔK vs. K) was ascertained by following

Evanno et al. (2005). The ancestry coefficients (Q-matrix), which were obtained from the population structure analysis, were printed out and utilized to visualize each genotype in the diversity set.

2.5 | Genome-wide association study

The RSA traits under different conditions (control and drought) and two indices, SPI and STI, for both seasons were used to conduct GWAS. It was performed following an MLM-PK approach, where root traits were considered as the phenotypes and the confounding effect of population stratification in the studied panel was accounted for by incorporating kinship matrix (K-matrix) and population structure (P-matrix) as covariates with five principal components (Kang et al., 2010; Price et al., 2010). Both P-matrix and K-matrix were calculated using TASSEL 5.0. Subsequently, GWAS was carried out in TASSEL 5.0, and the output was curated using the PROC MIXED macro program. The GWAS model was followed, as described by Oyiga et al. (2020). Due to Bonferroni correction at the 0.05 level, the threshold was highly conservative and may lead to false negatives (Gyawali et al., 2016; Zhao et al., 2011); therefore, a less stringent threshold of $-\log_{10}(P) > 4$ was selected to define the significant SNP-trait associations, followed by Li et al. (2019). GWAS results (Manhattan and Q–Q plots) from TASSEL 5.0 were displayed on CM plot using R program.

2.6 | Linkage disequilibrium (LD) and candidate gene analysis

Significant SNP markers of root architectural traits alone or with non-significant neighboring markers might regulate the traits. For this, the Plink data format (produced on TASSEL 5.0 software) was used to construct LD blocks in the Haploview 4.2 software. When a significant marker established an LD block with neighboring SNPs, the whole block region rather than alone was considered as a locus. Genes located in LD blocks were then considered as putative candidate genes. The significant SNPs that did not establish haplotype blocks, genes within 500 kbp up and downstream were considered as putative candidate genes, followed by Siddiqui, Teferi, et al. (2021).

For candidate gene analysis, the core sequences (120 bp) of the relevant SNPs were used in BLAST searches in the Barley Genome Gene-set database (BARLEX; https://apex.ipk-gatersleben.de/apex/f?p=284:10::::::) to identify putative candidate genes. Top gene hits were considered based on criteria of e-value < 1e-70 and identity scores >80% (Oyiga et al., 2020). We also looked for additional potential gene candidates 5 Mbp up- and downstream

TABLE 1 Descriptive statistics of phenotypic traits under drought and control conditions depicted in two different growing years 2020 and 2021.

Year	Traits	Control				Drought				ANOVA			
		Min.	Max.	Mean	CV (%)	Min.	Max.	Mean	CV (%)	H^2 (%)	G	T	G × T
2020	TRL	19.14	489.25	117.73	54.68	15.92	286.84	106.94	45.68	41.87	/	*	*
	PA	1.04	29.54	9.75	48.64	1.21	21.91	8.04	45.37	64.43	/	***	/
	RSA	4.71	92.80	30.98	47.46	3.80	68.82	25.29	45.20	63.40	/	***	/
	RAD	0.36	2.45	0.89	31.20	0.39	2.25	0.78	27.93	45.16	/	***	/
	RV	0.03	2.45	0.68	56.25	0.05	1.51	0.50	57.09	68.83	/	***	/
	NRT	54	1232	343.30	52.56	39	989	357.02	51.74	74.00	/	/	/
	NRF	76	2994	545.53	65.73	62	2116	509.35	56.52	52.39	/	/	.
	NRC	5	355	59.12	78.96	3	296	58.43	71.83	22.98	.	.	*
2021	TRL	35.39	606.31	214.19	50.57	5.16	450.15	102.39	72.14	75.03	/	***	/
	PA	2.56	55.04	15.55	58.44	0.48	25.20	4.83	75.82	78.22	*	***	/
	RSA	8.05	172.93	48.77	58.52	1.24	79.17	15.14	76.37	78.22	*	***	/
	RAD	0.33	2.07	0.72	28.42	0.18	2.65	0.50	42.75	59.15	/	***	/
	RV	0.10	6.33	0.93	78.81	0.006	1.108	0.19	87.43	73.75	*	***	.
	NRT	64	2479	589.38	59.81	19	1801	361.41	74.60	64.86	/	***	/
	NRF	122	5001	1149.83	69.12	11	2875	378.13	97.53	78.99	*	***	/
	NRC	6	88.91	125.42	69.12	1	396	56.78	103.9	77.13	/	***	/

Note: Significance codes (*p* value): 0: "***"; 0.01: "*"; 0.05: "."; 0.1: '/': not significant.

Abbreviations: ANOVA, analysis of variance; CV, coefficient of variation; G, genotype; GT, genotype treatment interaction; Max, maximum; Min, minimum; NRC, number of root crossings; NRF, number of root forks; NRT, number of root tips; PA, projected root area (cm^2); RAD, root average diameter (mm); RSA, root surface area (cm^2); RV, root volume (cm^3); SD, standard deviation; T, treatment; TRL, total root length (cm).

of the significantly associated SNP, as identified SNPs using GWAS may comprises components of a wider genomic region of linked genetic variants (van der Sijde et al., 2014). The IPK Barley Genome database (https://apex.ipk-gatersleben.de/apex/f?p=284:41:::NO:RP:P41_GENE_CHOICE:2) was used to obtain high-confidence genes with known annotations in the whole genome assembly of cultivar Morex V3 (IBGC, 2012) in these expanded SNP regions. The *Arabidopsis thaliana* orthologues were identified using *EnsemblPlants* database (https://plants.ensembl.org/Arabidopsis_thaliana), in which orthologs have previously been characterized; those were highlighted in this study.

3 | RESULTS

3.1 | Root architecture traits were significantly affected by drought stress and growing year

Two hundred barley genotypes were subjected to drought stress and control conditions to evaluate phenotypic variation in root architecture traits in 2020 and 2021 (Tables S2 and S3). Of the eight root traits measured, all showed significant differences between drought stress and control treatments in 2021, and five traits showed significant differences between

drought stress and control in both years (Table 1). In the year 2020, drought treatment significantly reduced PA (-10.72 cm^2), RSA (-5.69 cm^2), RAD (-0.11 mm), RV (-0.18 cm^3), and TRL (-111.8 cm) (Table 1). In 2021, all root traits showed strong significant effects of drought: TRL was decreased 111.8 cm, root projected area by 10.72 cm^2, RSA by 33.63 cm^2, RAD by 0.22 mm, RV by 0.74 cm^3, NRT by 227.97, NRF by 771.7, and NRC by 68.64 cm on average in drought-treated plants.

Phenotypic variation within each trait was assessed using the CV. The highest CV was observed in 2020 for NRC (79.0%) and in 2021 for RV (78.8%). The next most variable traits were NRF in 2020 (65.7%) and RSA in 2021 (71.3%). The lowest CV in both 2020 and 2021 was observed for RAD (31.2% and 28.4%, respectively). The heritability calculation revealed a moderate to high broad-sense heritability (H^2) for all of the studied traits in both growing seasons. The H^2 ranges from 23% to 74% and 59% to 79% in 2020 and 2021, respectively, indicating that the association panel harbors substantial genotypic diversity in root architecture traits under drought stress (Table 1).

Plantation year (2020 vs. 2021) had a significant effect on root traits (ANOVA, *p* < 0.001) (Table S4). Projected root area in 2020 was 8.0 cm^2 larger than in 2021. Significant soil moisture content and temperature differences were observed between the 2 years (Figure S1); for instance, the average
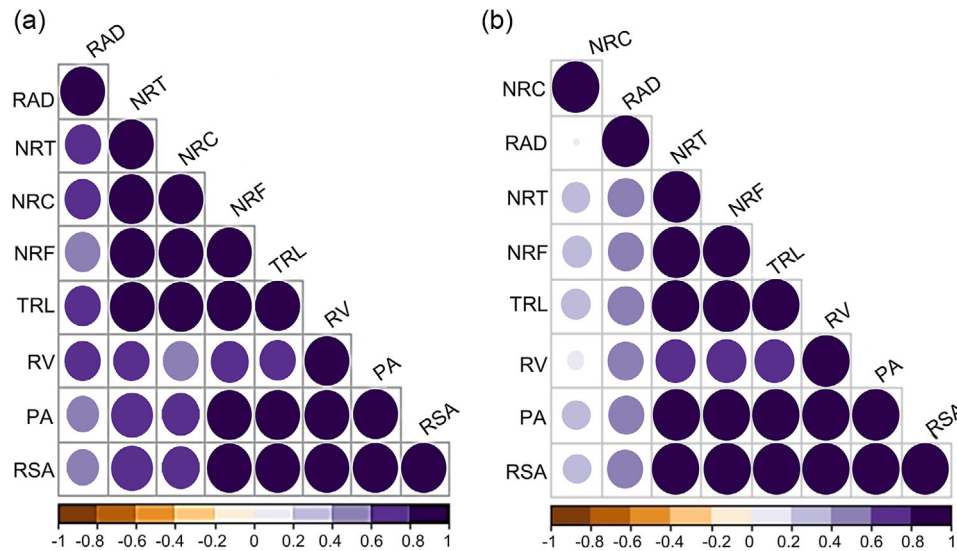
FIGURE 1 Correlations between root traits under drought stress. The violet color designates a positive correlation, the brown color designates a negative correlation, and the darkness of the color on both sides indicates the value of the correlation coefficient according to the legend. The absolute value of the correlation coefficient is designated by the size of the circle (the larger the circle, the greater the value). (a) Figure correlates to the data of the year 2020 treatments under drought and (b) Figure correlates to the data of the year 2021, treatments under drought. NRC, number of root crossings; NRF, number of root forks; NRT, number of root tips; PA, projected root area; RAD, root average diameter; RSA, root surface area; RV, root volume; TRL, total root length.

temperature was $0.3°C$ higher in 2020 as compared to 2021 (Figure S1b,d). A significant interaction effect (ANOVA, $p < 0.001$) was observed between treatment and year for all the measured traits.

The effect of genotype on root traits across both years was only significant for RAD out of all traits assessed ($p < 0.001$) (Table S4). However, the treatment-by-genotype interaction effect was significant for all traits ($p < 0.05$) except for RAD, RV, and NRT. The genotype-by-year interaction effect was also significant for four of the analyzed traits, mainly: PA, RSA, RV, and NRF ($p < 0.05$), but not for TRL, RAD, NRT, and NRC (Table S4). Overall, these results suggest that root architecture traits were less developed owing to drought stress when compared with well-water-treated plants, and substantial genetic variations in root architecture traits were observed in the barley mapping population in response to drought stress.

3.2 | Correlation between root phenotypic traits in response to drought stress

Correlation analysis was performed using the values of the eight traits under drought treatment. All the measured traits across both growing seasons under drought stress showed significant and positive correlations (Figure 1).

In 2020, positive pairwise correlations were observed between most traits. Total root length, PA, and RSA were all positively correlated ($p < 0.01$), and TRL showed a strong positive association also with NRT ($p < 0.01$), NRC ($p < 0.01$), and

NRF ($p < 0.01$). Positive but non-significant relationships were observed for RAD and RV with all measured traits (Figure 1a).

In 2021, highly positive significant ($p < 0.01$) correlations were found between almost all of the root traits (Figure 1b), except for number of root crossing and RAD, which showed weaker positive correlations ($p > 0.01$) with all root traits. As in 2020, TRL in 2021 showed similar strong positive associations ($p < 0.01$) with PA, RSA, NRT, and NRF.

3.3 | Population structure of the studied barley panel

Population structure analysis was conducted using STRUC-TURE software (version 2.3) for all 200 barley genotypes. SNP density across the seven chromosomes was highest on chromosome 3, followed by chromosomes 2 and 5, and lowest on chromosome 4 (Figure S2a). The population structure analysis showed a peak (ΔK) at $K = 2$ (Figure S2b), indicating that two sub-populations most likely exist in this association panel. These two sub-populations could be distinguished primarily based on their row types (two- vs. six-row barley) (Figure S2c,d).

3.4 | Associations between markers and root traits

GWAS analysis was performed using 23.8K high-quality SNP markers to identify genomic regions controlling root traits.

Using a significance threshold level of $-\log_{10}(P) > 4.0$, we identified a total of 186 loci associated with the eight root architecture traits under different conditions: 30 and 35 loci under control and drought conditions, respectively, 56 for the STI and 66 for SPI (Table S5). Of these 186 genetic loci, 57 loci were associated with NRC, 38 loci with RAD, 36 loci with RV, 15 loci with NRF, 14 loci with TRL, 10 loci with NRT, 8 loci with RSA, and 8 loci with root projected area (Table S5).

In particular, four significant SNPs surpassed the significant threshold level for TRL in 2020 (Figure 2a,b). One was located on chromosome 3, and other three SNPs were identified on chromosome 6 (Figure 2a,b). For TRL in 2021, two significantly associated SNPs were identified on chromosome 3 (Figure 2c,d). For RSA in 2020, two SNPs were identified on chromosome 6 (Figure 2e,f). Interestingly, these markers were syntenic with marker-trait association for TRL in the same year. In 2021, three significant SNPs for NRF (based on STI) were positioned on chromosome 3 (Figure 2g,h), whereas for STI on RV in 2021, seven candidate loci were found on chromosomes 2, 3, 4, 6, and 7 (Figure 2i,j). More importantly, we have identified several significant SNP-trait associations on chromosomes 3–6 for root system traits under control, drought, and indices (Table S5). Of these, a few SNPs were located in the vicinity (up- or down-stream) of the previously reported root QTL/genes (Table S6).

We further analyzed genetic loci simultaneously associated with multiple traits and found a total of 13 showed simultaneous multi-trait effect in RSA (Figure 3). One SNP on chromosome 6 was associated with four different root traits (TRL, PA, RSA, and NRF), while another SNP on chromosome 6 was associated with five traits under drought conditions (TRL, RSA, NRT, NRF, and NRC), and three SNPs on chromosome 3 were each associated with TRL, NRT, and NRF based on the STI calculation. Eight out of 13 candidate loci were concurrently linked with more than two different treatments (Figure 3; Table S7).

3.5 | Identification of drought-related candidate genes

We identified a total of 265 candidate genes underlying marker-trait associations, of which >50 genes had putative regulatory functions (mainly as transcriptional activator and in ion binding) based on the orthology of *A. thaliana* (Table S8). A total of 48 putative candidate genes were identified for the 13 loci that affect multiple traits simultaneously (Table S9). Of these, nine candidate genes possessed *A. thaliana* orthologues: most importantly, *SHT*, *PVA11*, *ACX3*, *PA200*, *DGK*, *APY*, and *EMB2656*, which have previously been reported and which, in turn, encode the following proteins: N-hydroxycinnamoyl transferase, vesicle-associated protein 1-1,

acyl-coenzyme A oxidase 3, a proteosome activator, a diacyl-glycerol kinase, a nucleoside phosphatase family protein, and an ARM repeat superfamily protein, respectively (Tables S8 and S9).

Next, one locus on chromosome 6 was associated with each of the following traits: PA, TRL, RSA, and NRF (Figure 4a; Table S5). Based on polymorphism in non-coding regions, two haplotypes could be established, which included this SNP marker (Figure 4b). Of these, Hap 2 showed significantly higher TRL under drought than Hap 1 (Figure 4c). This locus harbored a candidate gene, *HORVU3Hr6G0539460*, whose Arabidopsis reported ortholog is a protein encoding NERD1, NEW ENHANCER OF ROOT DWARFISM1. Polymorphism was detected in an intron region of this candidate gene, which showed annotated function in root development (GO:0048364) in terms of root hair growth, root meristem function, and cell elongation and uni-dimensional cell growth (GO:0009826) (Table S9). Another significant SNP on chromosome 2 was simultaneously associated with PA, RV, and RSA (Figure 5a; Table S7), with putative genetic linkage to candidate gene *HORVU3Hr2G0101980*. Two haplotypes were identified based on missense variation in the gene coding region (Figure 5b). Hap1 resulted in significantly higher RV in response to drought stress than Hap 2 (Figure 5c). Polymorphism in the coding region of this candidate gene was detected, and the gene showed annotated function in defense response (Table S9). Therefore, these genes might be fairly promising candidates for putative control of root trait development under drought, stress tolerance, and plasticity conditions, as their orthologues, especially *NERD1*, are reported to aid plants in root development to adapt to stress conditions.

4 | DISCUSSION

Drought is one of the leading constraints for agricultural production worldwide. Therefore, investigating root architectural traits under drought stress and identifying genetic control of these traits could provide useful information for breeders to improve plant performance under drought. The mapping panel used in our study is mostly issued from selected breeding lines from the Global Barley Breeding Program led by ICARDA. This breeding program is characterized by a wide use of genetic diversity, mostly due to its use of genetic resources from the ICARDA GenBank, the second largest in the world in terms of barley landraces and wild relatives, and the extensive international collaboration and global aim that characterizes this program. In fact, a recent study (Bouhlal et al., 2022) showed that a collection of ICARDA germplasm was able to capture most of the diversity of a larger global collection of varieties and genotypes. Most of the lines tested in the present study are issued from this collection. Moreover,
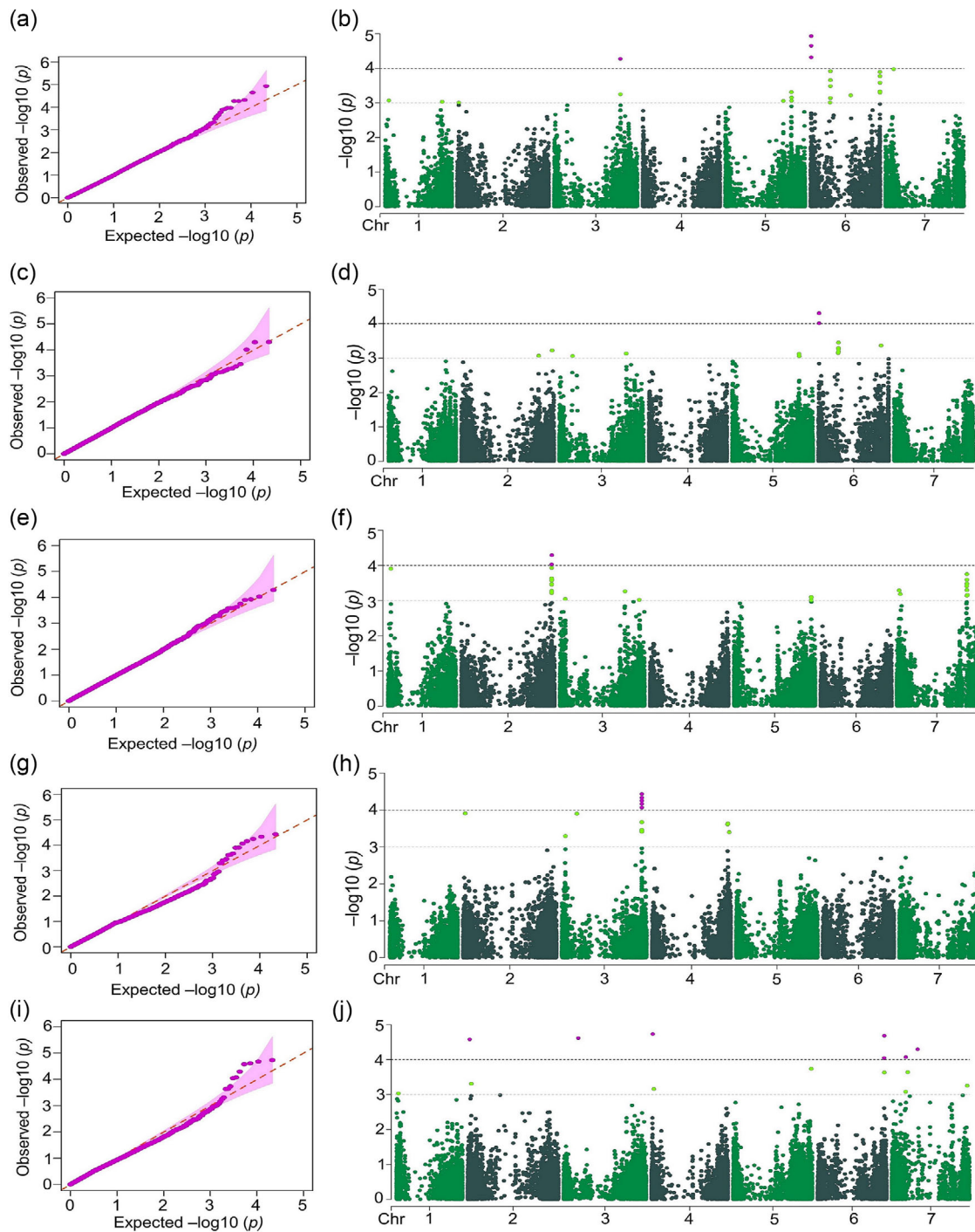
FIGURE 2 Marker-trait associations for root architectural traits under drought stress in the 2020 and 2021 growing seasons. (a) *Q–Q* plot displaying the deviation of the studied *p*-values from the expected associations for total root length (TRL) in 2020. (b) Local Manhattan plot showing the significant markers for TRL in 2020, according to the negative log10 (*p*-value) across seven chromosomes of barley. (c) *Q–Q* plot for TRL in 2021. (d) Local Manhattan plot for the root trait TRL in 2021. (e) *Q–Q* plot for root surface area (RSA) in 2020. (f) Local Manhattan plot for the root trait RSA in 2020. (g) *Q–Q* plot for number of root forks (NRF) in 2021. (h) Local Manhattan plot for the root trait NRF in 2021. (i) *Q–Q* plot for stress tolerance index (STI) of root volume (RV) in 2021. (j) Local Manhattan plot for the STI value of RV in 2021. The threshold is displayed with the bold dashed blackline at the position of the negative log10 value of 4. Every violet dot above this threshold represents a significant marker.
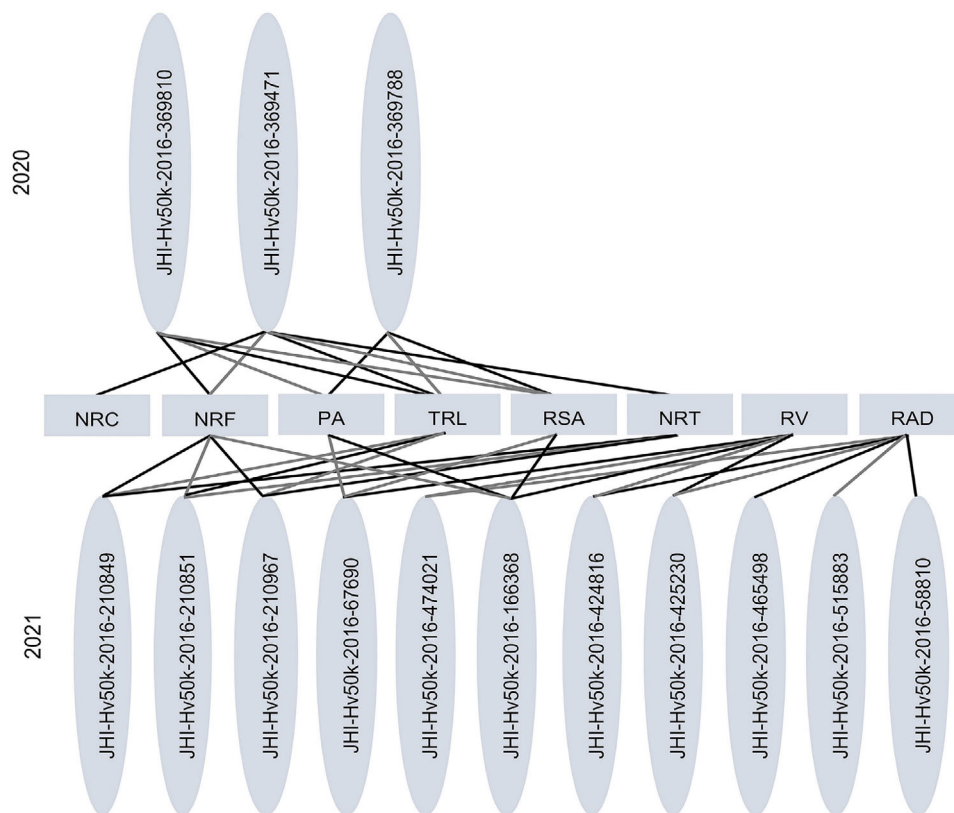
FIGURE 3 Network plot of multi-trait affecting SNPs and root traits. Lines indicate associations between a marker and a trait. NRC, number of root crossings; NRF, number of root forks; NRT, number of root tips; PA, projected root area; RAD, root average diameter; RSA, root surface area; RV, root volume; TRL, total root length.

the lines here evaluated have already been shown to contain diversity for stress inputs (limited fertilizer and moisture) in previous studies (Amezrou et al., 2018). Here, we determined root phenotypic responses under both natural rainfed and rain-out (drought) treatments at the complete flowering stage (BBCH51). All root traits investigated showed a high level of phenotypic variation (Table 1), which is especially important given the relatively small size of the population: field-based root screening can be unreliable and inefficient for large populations (Oyiga et al., 2020). Drought stress significantly reduced root growth and altered root architectural traits; genotype alone had little effect on most traits, but strong genotype by environment effects were also observed on root growth architecture (Table S4; Figure 1). Similar root phenotypic differences between drought and control treatments, particularly reduction in RSA, have also previously been observed in many crops (reviewed by Koevoets et al., 2016). Our data indicate that root architecture in barley is a complex trait controlled by multiple genomic loci with major and minor effects due to environmental factors and gene–environment interactions.

Using GWAS, we identified 186 candidate loci associated with root traits under different drought treatments. Using plasticity and STI to assess drought stress tolerance traits has

successfully been used to identify drought-resistant genotypes using GWAS in previous studies (Nouraein et al., 2013; Schneider et al., 2020). Previous studies have also reported QTL associated with different traits in barley, such as root and shoot architecture (Naz et al., 2014), low phosphorus tolerance (Gong et al., 2016), root system depth and root spreading angle (Jia et al., 2019), waterlogging, salinity, and drought tolerance (Borrego-Benjumea et al., 2021; Gill et al., 2017; Gudys et al., 2018), and nitrogen deficiency (Hoffman et al., 2012; Siddiqui, Kailash, et al., 2023). However, very few studies have reported root SNPs associated with drought in barley under natural field environments. Our target was to identify marker-trait association for a specific root trait that is simultaneously associated with multiple other traits and their allelic effect on natural drought stress. In the present study, we report numerous genomic regions mapped on chromosomes 3–6 associated with different root traits, which were located in the vicinity of the previously reported root QTL (Table S6). The SNP *JHI-Hv50k-2016-323438* associated with NRC was detected in the vicinity of the *qRA-5* and *RAQ2* reported for controlling barley seminal root traits (Robinson et al., 2016, 2018). Three SNPs (*JHI-Hv50k-2016-187463*, *SCRI_RS_211003*, and *JHI-Hv50k-2016-191436*) were identified in this study on chromosome 3 co-located with
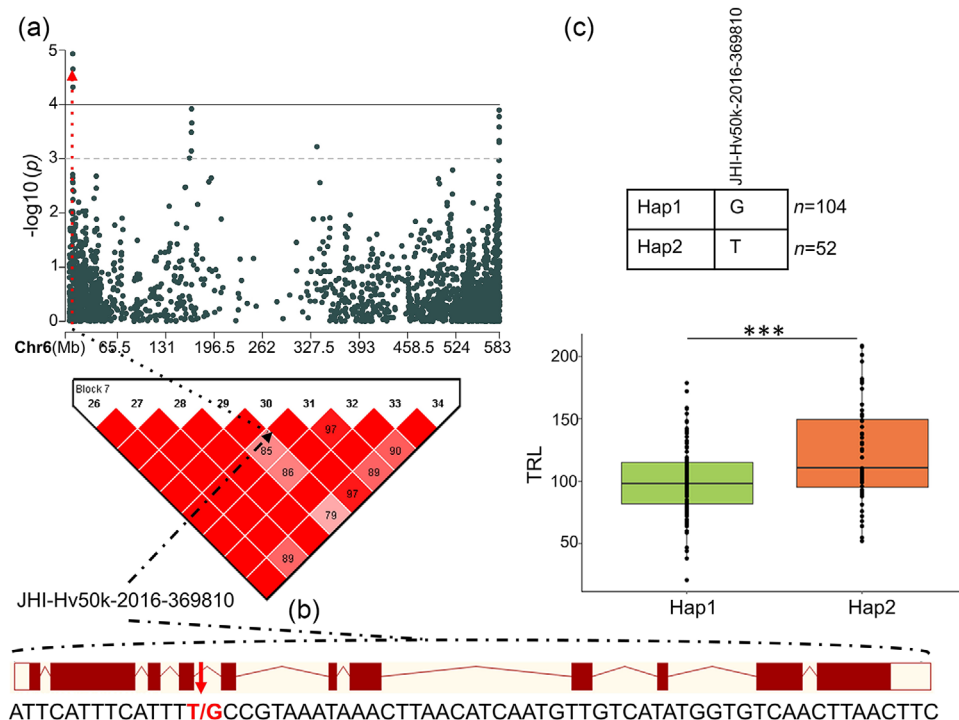
FIGURE 4 Linkage disequilibrium (LD) plot and haplotype analysis for total root length (TRL) under drought stress for the year 2020were generated using Haploview software. (a) The marker-trait association detected on chromosome 6 was showed by a Manhattan plot represented as harboring significant SNPs. The LD blocks are displayed as pairwise *D*' values that correspond to SNP pairs expressed as percentages (%), and LOD (log of the likelihood odds ratio) values to quantify confidence in the value of *D*' are shown within the respective squares. Shading represents the magnitude and significance of pairwise LD between SNPs, with a red-to-white color gradient reflecting higher-to-lower LD values. Higher *D*' values are indicated with a brighter red color (LOD = 2, *D*' = 1), and *D*' values of <1 indicated with white (LOD < 2, *D*' < 1). (b) Structure of the candidate gene *HORVU3Hr6G0539460* indicating the T/G sequence variation in the fourth intron. (c) Major haplotypes assembled from the major LD block on chromosome 6 are responsible for root length variation across genotypes (*n*); standard error bars are displayed. Statistical significance was calculated based on the one-way ANOVA: ***, $P < 0.001$.

previously reported QTL *QRl.S42.3H* influencing root length under drought stress (Arifuzzaman et al., 2014). Remarkably, we found a significant SNP mapped on chromosome 5 associated with RAD co-located with previously reported QTL gene *VRN1* that potentially regulates root system architecture in wheat and barley (Voss-Fels et al., 2018).

Interestingly, we identified 13 loci that were associated with two or more traits. For instance, NRF, PA, TRL, and RSA in 2020 were all associated with one single significant SNP marker located on chromosome 6 (Figure 3; Table S7), while another SNP was linked with NRC, NRF, TRL, NRT, and RSA also in 2020, and three additional markers were linked to different root traits in 2021 under drought conditions (Figure 3; Table S7). In support of a hypothesis that the SNP is controlling one specific trait that is associated to multiple other root traits, we found positive correlations between each of TRL, PA, and RSA under both drought and control treatments, and also a significant SNP, which was associated with each of these three traits. These root traits could be categorized together as "root growth" traits and maybe controlled by conserved genomic regions. Positive significant associa-

tions were observed between most of the root traits, including root area, RV, and root length (Reddy et al., 2020; Siddiqui et al., 2022), which may lead to multi-trait affecting genetic association. Pleiotropic loci for grain yield components were also identified under drought stress in winter wheat in previous studies (Koua et al., 2022). These loci relevant to multiple traits simultaneously can be considered for further breeding to improve root traits.

We identified a total of 26 SNPs particularly linked to drought effects, with varying significant levels (Table S4). Significant SNPs were associated with a set of candidate genes showing a wide range of biological activities (Table S8). Many candidate genes possessed orthologues in *Arabidopsis* with involvement in the response to drought and root growth, including a transcription cis-regulatory region binding protein, a Ca^{2+} binding domain protein, a zinc finger protein-like protein, a superfamily protein, and a K^+ channel outward rectifier (Table S8). In particular, *ATXR3* shows transcriptional activator activity that delays flowering (Yun et al., 2012), *ACX3* is associated with root length to confer drought tolerance (Shiraku et al., 2021), and GBF, as a bZIP
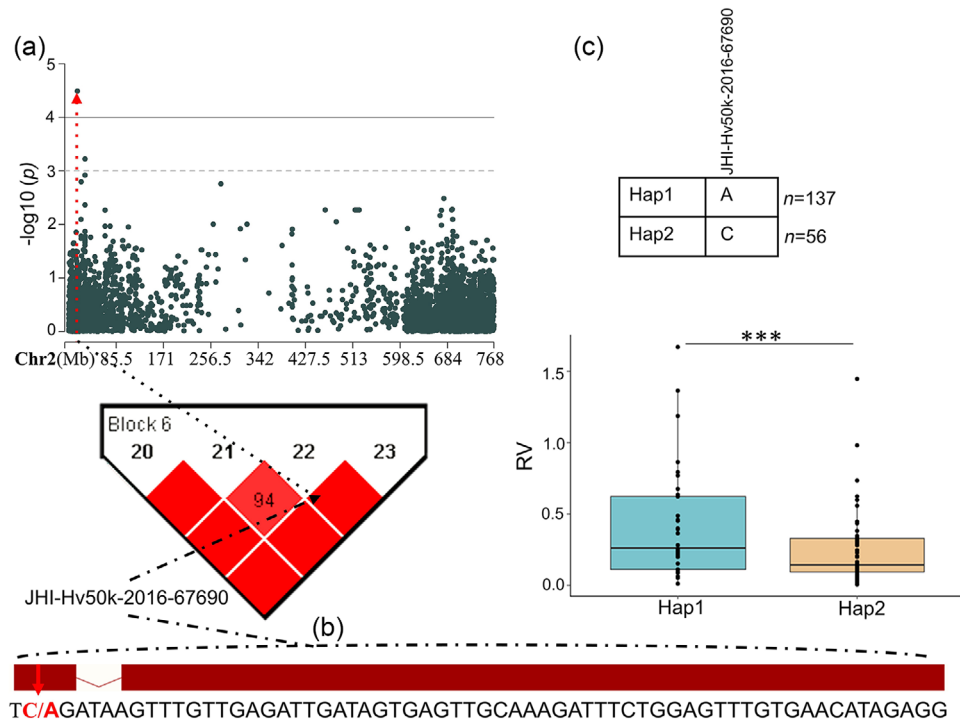
FIGURE 5 Linkage disequilibrium (LD) plot and haplotype analysis for total root volume (RV) for the year 2021, generated using Haploview software. (a) The marker-trait association detected on chromosome 2 showed by a Manhattan plot represented as harboring significant SNPs. The LD blocks are displayed as pairwise D' values that correspond to SNP pairs expressed as percentages (%) and LOD (log of the likelihood odds ratio) values to quantify confidence in the value of D' are shown within the respective squares. Shading represents the magnitude and significance of pairwise LD between SNPs, with a red-to-white color gradient reflecting higher-to-lower LD values. Higher D' values are indicated with a brighter red color (LOD = 2, $D' = 1$), and D' values of <1 are indicated with white (LOD < 2, $D' < 1$). (b) Structure of the candidate gene *HORVU3Hr2G0101980* indicating the C/A sequence variation in the first exon. (c) Major haplotypes assembled from the major LD block on chromosome 2, responsible for root volume variation across genotypes (n); standard error bars are displayed. Statistical significance was calculated based on the one-way ANOVA: ***, $P < 0.001$.

G-box binding factor is involved in developmental and physiological processes in response to hormones (Sibéril & Gantet et al., 2001). In addition, *A. thaliana* orthologue *FLA9* act as a component of secondary cell wall development (Ma et al., 2023), *NPF* is reported as a nitrate transceptor and root growth enhancer (Siddiqui, Kailash, et al., 2023), *GORK* and *SORK* as K⁺ channel rectifiers (Adem et al., 2020; Chen et al., 2021), *CDPK24* is involved in calcium-dependent plant development and nutrient and stress signaling (Dekomah et al., 2022), and *VAMPs* act as key proteins for abiotic stress tolerance (Gu et al., 2020). Hence, our study provides interesting candidate genes that may be involved in root drought stress response in barley for future validation and investigation.

One marker was simultaneously linked with multiple traits such as PA, TRL, RSA, and NRF, and its allelic haplotypes also differed significantly in TRL under drought stress (Figure 4). A candidate gene search found *HORVU3Hr6G0539460* that is known to be involved in root development and unidimensional cell growth functions (Table S9). The ortholog of this gene in model plant species *A. thaliana* is *NERD1*, which has already been reported to affect

root growth and cell expansion. Mutation of this gene leads to defects in root hair growth, root meristem function, and cell elongation, and it acts synergistically with the exocyst in root development (Cole et al., 2018). Molecular and biological functions and homologous study of this gene in *Arabidopsis* provide strong evidence for a causal association with PA, TRL, RSA, and NRF traits. The traits of PA, RV, and RSA were also linked to a single SNP with associated candidate gene *HORVU3Hr2G0101980* (associated with plant defense mechanism) in the year 2021 (Figure 5a,b). Hap1 for this locus resulted in significantly higher RV than Hap2 under drought (Figure c). Plants under stress develop different mechanisms to adjust to the stressful environment and ensure their survival (Gürel et al., 2016). Therefore, this gene might be promising for further functional analysis. Other genes with ATP binding factor, stress tolerance response, ion channel activity, and metal ion binding activities could also serve as a valuable resource in crop functional genomics research.

In summary, our study identified a strong positive correlation in phenotypic response to drought stress across

root traits. These positive correlations were corroborated by the identification of the effect pattern of allelic haplotypes of individual loci with multiple root traits under different treatments, including drought. Based on the loci-specific haplotype pattern of trait effects, we also provide high-confidence candidate genes and natural variation related to root architecture traits, which could be utilized as potential resources for marker-assisted genomic selection and/or genetic engineering of barley with enhanced drought tolerance. The identified drought tolerance allele could then be used in breeding programs. Further, we provide a set of candidate genes underlying loci significantly associated with root traits and with *A. thaliana* orthologs with reported functions related to drought response and root development that could be used in translational breeding programs. Additionally, we identified natural variation of a candidate locus harboring a *NERD1* ortholog, which exhibited increased root length under drought, thereby providing a feasible target for future functional verification of root-targeted drought tolerance in spring barley.

AUTHOR CONTRIBUTIONS

Md. Nurealam Siddiqui: Conceptualization; data curation; formal analysis; investigation; methodology; writing—original draft; writing—review and editing. **Melisa Jahiu**: Data curation; formal analysis; investigation; methodology. **Mohammad Kamruzzaman**: Formal analysis; investigation; writing—review and editing. **Miguel Sánchez García**: Resources; writing—review and editing. **Annaliese S. Mason**: Resources; supervision; visualization; writing—review and editing. **Jens Leon**: Funding acquisition; resources; software; supervision; validation; visualization; writing; review and editing. **Agim Ballvora**: Conceptualization; funding acquisition; project administration; resources; software; supervision; validation; writing—review and editing.

DATA AVAILABILITY STATEMENT

All data that support the findings in this study are available in this article and its supplementary files.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Md. Nurealam Siddiqui https://orcid.org/0000-0003-4604-3471
Melisa Jahiu https://orcid.org/0009-0007-2409-3837

REFERENCES

Adem, G. D., Chen, G., Shabala, L., Chen, Z. H., & Shabala, S. (2020). GORK channel: A master switch of plant metabolism? *Trends in Plant Science*, 25(5), 434–445. https://doi.org/10.1016/j.tplants.2019.12.012

Amezrou, R., Gyawali, S., Belqadi, L., Chao, S., Arbaoui, M., Mamidi, S., Rehman, S., Sreedasyam, A., & Verma, R. P. S. (2018). Molecular and phenotypic diversity of ICARDA spring barley (*Hordeum vulgare* L.) collection. *Genetic Resources and Crop Evolution*, 65, 255–269. https://doi.org/10.1007/s10722-017-0527-z

Arifuzzaman, M., Sayed, M. A., Muzammil, S., Pillen, K., Schumann, H., Naz, A. A., & Léon, J. (2014). Detection and validation of novel QTL for shoot and root traits in barley (*Hordeum vulgare* L.). *Molecular Breeding*, 34, 1373–1387. https://doi.org/10.1007/s11032-014-0122-3

Bayer, M. M., Rapazote-Flores, P., Ganal, M., Hedley, P. E., Macaulay, M., Plieske, J., Ramsay, L., Russell, J., Shaw, P. D., Thomas, W., & Waugh, R. (2017). Development and evaluation of a barley 50k iSelect SNP array. *Frontiers in Plant Science*, 8, 1792. https://doi.org/10.3389/fpls.2017.01792

Borrego-Benjumea, A., Carter, A., Zhu, M., Tucker, J. R., Zhou, M., & Badea, A. (2021). Genome-wide association study of waterlogging tolerance in barley (*Hordeum vulgare* L.) under controlled field conditions. *Frontiers in Plant Science*, 12, Article 711654. https://doi.org/10.3389/fpls.2021.711654

Bouhlal, O., Visioni, A., Verma, R. P. S., Kandil, M., Gyawali, S., Capettini, F., & Sanchez-Garcia, M. (2022). CGIAR barley breeding toolbox: A diversity panel to facilitate breeding and genomic research in the developing world. *Frontiers in Plant Science*, 13, 1034322. https://doi.org/10.3389/fpls.2022.1034322

Chen, Y., Peng, X., Cui, J., Zhang, H., Jiang, J., & Song, Z. (2021). Isolation and functional determination of SKOR potassium channel in purple osier willow, Salix purpurea. *International Journal of Genomics*, 2021, Article 6669509. https://doi.org/10.1155/2021/6669509

Cole, R. A., Peremyslov, V. V., Van Why, S., Moussaoui, I., Ketter, A., Cool, R., Moreno, M. A., Vejlupkova, Z., Dolja, V. V., & Fowler, J. E. (2018). A broadly conserved NERD genetically interacts with the exocyst to affect root growth and cell expansion. *Journal of Experimental Botany*, 69(15), 3625–3637. https://doi.org/10.1093/jxb/ery162

Comas, L. H., Becker, S. R., Cruz, V. M., Byrne, P. F., & Dierig, D. A. (2013). Root traits contributing to plant productivity under drought. *Frontiers in Plant Science*, 4, 442. https://doi.org/10.3389/fpls.2013.00442

Dekomah, S. D., Bi, Z., Dormatey, R., Wang, Y., Haider, F. U., Sun, C., & Bai, J. (2022). The role of CDPKs in plant development, nutrient

and stress signaling. *Frontiers in Genetics*, 13, 996203. https://doi.org/10.3389/fgene.2022.996203

Dido, A. A., Krishna, M. S. R., Assefa, E., Dawit, T. D., Singh, B. J. K., & Tesfaye, K. (2022). Genetic diversity, population structure and relationship of Ethiopian barley (*Hordeum vulgare* L.) landraces as revealed by SSR markers. *Journal of Genetics*, 101(1), 1–20. https://doi.org/10.1007/S12041-021-01346-7/TABLES/8

Earl, D. A., & von Holdt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4(2), 359–361. https://doi.org/10.1007/S12686-011-9548-7

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, 14(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Fan, Y., Gonzalo, M., Esteban, G. J., Robert, B., & Carlos, O. (2017). Hydrologic regulation of plant rooting depth. *Proceedings of the National Academy of Sciences*, 114(40), 10572–10577. https://doi.org/10.1073/PNAS.1712381114

Gill, M. B., Zeng, F., Shabala, L., Zhang, G., Fan, Y., Shabala, S., & Zhou, M. (2017). Cell-based phenotyping reveals QTL for membrane potential maintenance associated with hypoxia and salinity stress tolerance in barley. *Frontiers in Plant Science*, 8, 1941. https://doi.org/10.3389/fpls.2017.01941

Gitonga, V. W., Koning-Boucoiran, C. F., Verlinden, K., Dolstra, O., Visser, R. G., Maliepaard, C., & Krens, F. A. (2014). Genetic variation, heritability and genotype by environment interaction of morphological traits in a tetraploid rose population. *BMC Genetics*, 15(1), 1–14. https://doi.org/10.1186/s12863-014-0146-z

Gong, X., Wheeler, R., Bovill, W. D., & McDonald, G. K. (2016). QTL mapping of grain yield and phosphorus efficiency in barley in a Mediterranean-like environment. *Theoretical and Applied Genetics*, 129, 1657–1672. https://doi.org/10.1007/s00122-016-2729-8

Gu, X., Brennan, A., Wei, W., Guo, G., & Lindsey, K. (2020). Vesicle transport in plants: A revised phylogeny of SNARE proteins. *Evolutionary Bioinformatics*, 16, 1176934320956575. https://doi.org/10.1177/1176934320956575

Gudys, K., Guzy-Wrobelska, J., Janiak, A., Dziurka, M. A., Ostrowska, A., Hura, K., & Szarejko, I. (2018). Prioritization of candidate genes in QTL regions for physiological and biochemical traits underlying drought response in barley (*Hordeum vulgare* L.). *Frontiers in Plant Science*, 9, Article 769. https://doi.org/10.3389/fpls.2018.00769

Gürel, F., Öztürk, Z. N., Uçarlı, C., & Rosellini, D. (2016). Barley genes as tools to confer abiotic stress tolerance in crops. *Frontiers in Plant Science*, 7, 1137. https://doi.org/10.3389/fpls.2016.01137

Gyawali, S., Harrington, M., Durkin, J., Horner, K., Parkin, I. A., Hegedus, D. D., & Buchwaldt, L. (2016). Microsatellite markers used for genome-wide association mapping of partial resistance to *Sclerotinia sclerotiorum* in a world collection of *Brassica napus*. *Molecular Breeding*, 36, 1–13. https://doi.org/10.1007/s11032-016-0496-5

Hoffmann, A., Maurer, A., & Pillen, K. (2012). Detection of nitrogen deficiency QTL in juvenile wild barley introgression lines growing in a hydroponic system. *BMC Genetics*, 13(1), 1–15. https://doi.org/10.1186/1471-2156-13-88

Hufnagel, B., de Sousa, S., Assis, L., Guimaraes, C., Leiser, W., Azevedo, G., Negri, B., Larson, G., Shaff, J., Pastina, M., & Magalhaes, J. (2014). Duplicate and conquer: Multiple homologs of PHOSPHORUS-STARVATION TOLERANCE1 enhance phosphorus acquisition and sorghum performance on low-phosphorus soils. *Plant Physiology*, 166, 659–677. https://doi.org/10.1104/pp.114.243949

IBGC. (2012). International barley genome sequencing consortium: A physical, genetic and functional sequence assembly of the barley genome. *Nature*, 491(7426), 711. https://doi.org/10.1038/nature11543

Jia, Z., Liu, Y., Gruber, B. D., Neumann, K., Kilian, B., & Von Wirén, N. (2019). Genetic dissection of root system architectural traits in spring barley. *Frontiers in Plant Science*, 10, Article 400. https://doi.org/10.3389/fpls.2019.00400

Kadam, N. N., Tamilselvan, A., Lawas, L., Quinones, C., Bahuguna, R., Thomson, M., Dingkuhn, M., Muthurajan, R., Struik, P., & Yin, X. (2017). Genetic control of plasticity in root morphology and anatomy of rice in response to water deficit. *Plant Physiology*, 174, 2302–2315. https://doi.org/10.1104/pp.17.00500

Kang, H. M., Sul, J. H., Service, S. K., Zaitlen, N. A., & Kong, S. Y. (2010). Variance component model to account for sample structure in genome-wide association studies. *Nature Genetics*, 42, 348–354. https://doi.org/10.1038/ng.548

Kent, N. L., & Evers, A. A. (1994). *Kent's technology of cereals* (4th ed.). Elsevier Science Ltd.

Klein, S. P., Schneider, H. M., Perkins, A. C., Brown, K. M., & Lynch, J. P. (2020). Multiple integrated root phenotypes are associated with improved drought tolerance. *Plant Physiology*, 183(3), 1011–1025. https://doi.org/10.1104/pp.20.00211

Koevoets, I. T., Venema, J. H., Elzenga, J. T. M., & Testerink, C. (2016). Roots withstanding their environment: Exploiting root system architecture responses to abiotic stress to improve crop tolerance. *Frontiers in Plant Science*, 7, Article 1335. https://doi.org/10.3389/fpls.2016.01335

Koua, A. P., Oyiga, B. C., Dadshani, S., Benaouda, S., Sadeqi, M. B., Rascher, U., & Ballvora, A. (2022). Chromosome 3A harbors several pleiotropic and stable drought-responsive alleles for photosynthetic efficiency selected through wheat breeding. *Plant Direct*, 6(9), e438. https://doi.org/10.1002/pld3.438

Li, L., Peng, Z., Mao, X., Wang, J., Chang, X., Reynolds, M., & Jing, R. (2019). Genome-wide association study reveals genomic regions controlling root and shoot traits at late growth stages in wheat. *Annals of Botany*, 124(6), 993–1006. https://doi.org/10.1093/aob/mcz041

Lynch, J. P., & Brown, K. M. (2012). New roots for agriculture: Exploiting the root phenome. *Philosophical Transactions of the Royal Society*, 367, 1598–1604. https://doi.org/10.1098/rstb.2011.0243

Ma, Y., Shafee, T., Mudiyanselage, A. M., Ratcliffe, J., MacMillan, C. P., Mansfield, S. D., & Johnson, K. L. (2023). Distinct functions of FASCILIN-LIKE ARABINOGALACTAN PROTEINS relate to domain structure. *Plant Physiology*, 192(1), 119–132. https://doi.org/10.1093/plphys/kiad097

Maeght, J. L., Rewald, B., & Pierret, A. (2013). How to study deep roots-and why it matters. *Frontiers in Plant Science*, 4, Article 299. https://doi.org/10.3389/FPLS.2013.00299

Meister, R., Rajani, M. S., Ruzicka, D., & Schachtman, D. P. (2014). Challenges of modifying root traits in crops for agriculture. *Trends in Plant Sciences*, 19, 779–788. https://doi.org/10.1016/j.tplants.2014.08.005

Naz, A. A., Arifuzzaman, M., Muzammil, S., Pillen, K., & Léon, J. (2014). Wild barley introgression lines revealed novel QTL alleles for root and related shoot traits in the cultivated barley (*Hordeum vulgare* L.). *BMC Genetics*, 15, 1–12. https://doi.org/10.1186/s12863-014-0107-6

Newton, A. C., Flavell, A. J., George, T. S., Leat, P., Mullholland, B., Ramsay, L., Revoredo-Giha, C., & Russell, J. (2011). Crops that feed the world 4. Barley: A resilient crop? Strengths and weaknesses in the context of food security. *Food Security*, *3*, 141–178. http://doi.org/10.1007/s12571-011-0126-3

Nouraein, M., Mohammadi, S. A., Aharizad, S., Moghaddam, M., & Sadeghzadeh, B. (2013). Evaluation of drought tolerance indices in wheat recombinant inbred line population. *Annals of Biological Research*, *4*(3), 113–122. http://om.ciheam.org/article.php?IDPDF=801329

Oyiga, B. C., Janina, P., Tobias, W., Jonathan, P. L., Ali, A. N., Jens, L., & Agim, B. (2020). Genetic components of root architecture and anatomy adjustments to water-deficit stress in spring barley. *Plant Cell & Environment*, *43*(3), 692–711. https://doi.org/10.1111/pce.13683

Price, A. H., & Tomos, A. D. (1997). Genetic dissection of root growth in rice (*Oryza sativa* L.). II. Mapping quantitative trait loci using molecular markers. *Theoretical and Applied Genetics*, *95*, 143–152. https://doi.org/10.1007/s001220050542

Price, A. L., Zaitlen, N. A., Reich, D., & Patterson, N. (2010). New approaches to population stratification in genome-wide association studies. *Nature Reviews Genetics*, *11*, 459–463. https://doi.org/10.1038/nrg2813

Pritchard, J. K. (2002). *Documentation for structure software: Version 2*. http://pritch.bsd.uchicago.edu

R Core Team. (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Ram, M. (2014). *Plant breeding methods*. PHI Learning Pvt. Ltd.

Ramireddy, E., Hosseini, S. A., Eggert, K., Gillandt, S., Gnad, H., von Wirén, N., & Schmülling, T. (2018). Root engineering in barley: Increasing cytokinin degradation produces a larger root system, mineral enrichment in the shoot and improved drought tolerance. *Plant Physiology*, *177*(3), 1078–1095. https://doi.org/10.1104/pp.18.00199

Reddy, V. R. P., Aski, M. S., Mishra, G. P., Dikshit, H. K., Singh, A., Pandey, R., & Nair, R. M. (2020). Genetic variation for root architectural traits in response to phosphorus deficiency in mungbean at the seedling stage. *PLoS One*, *15*(6), e0221008. https://doi.org/10.1371/journal.pone.0221008

Robinson, H., Hickey, L., Richard, C., Mace, E., Kelly, A., Borrell, A., Franckowiak, J., & Fox, G. (2016). Genomic regions influencing seminal root traits in barley. *The Plant Genome*, *9*(1), 1–13. https://doi.org/10.3835/plantgenome2015.03.0012

Robinson, H., Kelly, A., Fox, G., Franckowiak, J., Borrell, A., & Hickey, L. (2018). Root architectural traits and yield: Exploring the relationship in barley breeding trials. *Euphytica*, *214*, 1–16. https://doi.org/10.1007/s10681-018-2219-y

Rogers, E. D., & Benfey, P. N. (2015). Regulation of plant root system architecture: Implications for crop advancement. *Current Opinion in Biotechnology*, *32*, 93–98. https://doi.org/10.1016/j.copbio.2014.11.015

Samarah, N. H. (2005). Effects of drought stress on growth and yield of barley. *Agronomy for Sustainable Development*, *25*(1), 145–149. https://doi.org/10.1051/agro:2004064

Samarah, N. H., Alqudah, A. M., Amayreh, J. A., & McAndrews, G. M. (2009). The effect of late-terminal drought stress on yield components of four barley cultivars. *Journal of Agronomy and Crop Science*, *195*(6), 427–441. https://doi.org/10.1111/j.1439-037X.2009.00387.x

Schneider, H. M., Klein, S. P., Hanlon, M. T., Nord, E. A., Kaeppler, S., Brown, K. M., Warry, A., Bhosale, R., & Lynch, J. P. (2020). Genetic control of root architectural plasticity in maize. *Journal of Experimental Botany*, *71*, 3185–3197. https://doi.org/10.1093/jxb/eraa084

Sehgal, A., Sita, K., Siddique, K. H. M., Kumar, R., Bhogireddy, S., Varshney, R. K., HanumanthaRao, B., Nair, R. M., Prasad, P. V. V., & Nayyar, H. (2018). Drought or/and heat-stress effects on seed filling in food crops: Impacts on functional biochemistry, seed yields, and nutritional quality. *Frontiers in Plant Science*, *9*, Article 1705. https://doi.org/10.3389/fpls.2018.01705

Shiraku, M. L., Magwanga, R. O., Cai, X., Kirungu, J. N., Xu, Y., Mehari, T. G., Hou, Y., Wang, Y., Agong, S. G., Peng, R., Wang, K., Zhou, Z., & Liu, F. (2021). Functional characterization of ghacx3 gene reveals its significant role in enhancing drought and salt stress tolerance in cotton. *Frontiers in Plant Science*, *12*, Article 658755. https://doi.org/10.3389/fpls.2021.658755

Sibéril, Y., Doireau, P., & Gantet, P. (2001). Plant bZIP G-box binding factors. Modular structure and activation mechanisms. *European Journal of Biochemistry*, *268*(22), 5655–5666. https://doi.org/10.1046/j.0014-2956.2001.02552.x

Siddiqui, M. N., Kailash, P., Suzan, K. B., Bahman, S., Michael, S., Miguel, S., Benjamin, S., Gabriel, S., Jeon, J., & Ballvora, B. (2023). Convergently selected NPF2. 12 coordinates root growth and nitrogen use efficiency in wheat and barley. *New Phytologist*, *238*, 2175–2193. https://doi.org/10.1111/nph.18820

Siddiqui, M. N., Schneider, M., Barbosa, M. B., Léon, J., & Ballvora, A. (2022). Natural selection under conventional and organic cropping systems affect root architecture in spring barley. *Scientific Reports*, *12*(1), Article 20095.

Siddiqui, M. N., Teferi, T. J., Ambaw, A. M., Gabi, M. T., Koua, P., Léon, J., & Ballvora, A. (2021). New drought-adaptive loci underlying candidate genes on wheat chromosome 4B with improved photosynthesis and yield responses. *Physiologia Plantarum*, *173*(4), 2166–2180. https://doi.org/10.1111/ppl.13566

Siddiqui, N., Gabi, M. T., Kamruzzaman, M., Ambaw, A. M., Teferi, T. J., Dadshani, S., & Ballvora, A. (2023). Genetic dissection of root architectural plasticity and identification of candidate loci in response to drought stress in bread wheat. *BMC Genomic Data*, *24*(1), 1–16. https://doi.org/10.1186/s12863-023-01140-7

Siddiqui, N. M., Léon, J., Ali, A. N., & Ballvora, B. (2021). Genetics and genomics of root system variation in adaptation to drought stress in cereal crops. *Journal of Experimental Botany*, *72*(4), 1007–1019. https://doi.org/10.1093/jxb/eraa487

Tuberosa, R., Sanguineti, M. C., Landi, P., Giuliani, M. M., Salvi, S., & Conti, S. (2002). Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. *Plant Molecular Biology*, *48*, 697–712. https://doi.org/10.1023/A:1014897607670

Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai, Y., Ono, K., & Kanno, N. (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature Genetics*, *45*, 1097–1102. https://doi.org/10.1038/ng.2725

van der Sijde, M. R., Ng, A., & Fu, J. (2014). Systems genetics: From GWAS to disease pathways. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1842*(10), 1903–1909. https://doi.org/10.1016/j.bbadis.2014.04.025

Voss-Fels, K. P., Robinson, H., Mudge, S. R., Richard, C., Newman, S., Wittkop, B., Stahl, A., Friedt, W., Frisch, M., Gabur, I., Miller-Cooper, A., Campbell, B. C., Kelly, A., Fox, G., Christopher, J., Christopher, M., Chenu, K., Franckowiak, J., Mace, E. S., … Hickey, L. T. (2018). VERNALIZATION1 modulates root system architecture in wheat and barley. *Molecular Plant*, *11*(1), 226–229. https://doi.org/10.1016/j.molp.2017.10.005

Vyver, C., & Peters, S. (2017). How do plants deal with dry days? *Frontiers for Young Minds*, *5*, 58. https://doi.org/10.3389/FRYM.2017.00058

Ye, H., Roorkiwal, M., Valliyodan, B., Zhou, L., Chen, P., Varshney, R. K., & Nguyen, H. T. (2018). Genetic diversity of root system architecture in response to drought stress in grain legumes. *Journal of Experimental Botany*, *69*(13), 3267–3277. https://doi.org/10.1093/jxb/ery082

York, L. M., Slack, S., Bennett, M. J., & Foulkes, M. J. (2018). Wheat shovelomics I: A field phenotyping approach for characterizing the structure and function of root systems in tillering species. *BioRxiv*, 280875. https://doi.org/10.1101/280875

Yun, J. Y., Tamada, Y., Kang, Y. E., & Amasino, R. M. (2012). Arabidopsis trithorax-related3/SET domain GROUP2 is required for the winter-annual habit of Arabidopsis thaliana. *Plant and Cell Physiology*, *53*(5), 834–846. https://doi.org/10.1093/pcp/pcs021

Zhao, K., Tung, C. W., Eizenga, G. C., Wright, M. H., Ali, M. L., Price, A. H., Norton, G. J., Islam, M. R., Reynolds, A., Mezey, J., McClung, A. M., Bustamante, C. D., & McCouch, S. R. (2011). Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. *Nature Communications*, *2*(1), Article 467. https://doi.org/10.1038/ncomms1467

Zhou, M. X. (2009). Barley production and consumption. In G. Zhang & C. Li (Eds.), *Genetics and improvement of barley malt quality* (pp. 1–17). Springer.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.