RESEARCH ARTICLE

Discovery of new genomic regions and candidate genes implicated in the natural variation of barley peduncle length and plant height

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Abstract The cereal plant barley (*Hordeum vulgare*) has high nutritional content and special characteristics that allow it to withstand adverse weather conditions. The peduncle's developmental properties under dry (drought and heat) conditions make it an ideal attribute for studying its function in tolerance and adaption processes. Thus, we intend to investigate the natural phenotypic and genetic variation of the peduncle length, as well as the genetic reasons underpinning its growth in dry (drought and heat) environments. Under natural drought and heat, the examined characteristic showed a large range of natural variation, showing its involvement in adaptation to such circumstances. A genome-wide association study (GWAS) was carried out to evaluate the relationship between 127,022 single nucleotide polymorphisms (SNPs) and peduncle length at maturity in 184 barley accessions from throughout the world. The genomic research suggest the possibility of 86 marker-trait associations (*p*-value: > FDR) in various genomic areas, five of which are physically situated on chromosome 3H. The presence of 23 candidate genes was discovered after predicting candidate genes underlying significant relationships. The RNA-sequencing expression profile of candidate genes revealed that the gene *HORVU.MOREX.r2.3HG0216310.1,* which was identified

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as a Polynucleotidyl transferase ribonuclease H-like superfamily protein, had the greatest level of expression, indicating its participation in peduncle development under these conditions. Quantifying the current natural variation in peduncles under dry environmental circumstances and identifying the genomic areas linked with the features will serve as the foundation for future genetic research to better understand its function in stress tolerance.

Introduction

Barley's (*Hordeum vulgare*) global production is paramount as it stands as one of the top major crops, serving as a staple food and an essential source of carbohydrates for communities worldwide (Geng et al. 2022). The high demand for cereal plants implies an urgent need to improve both the quality and quantity of crops in order to meet the food security goals for the global population. Nevertheless, the current trends of climate change in arid areas add more necessity to that urgency. Some of the main challenges that need to be confronted to fulfill such demand are overcoming drought and heat stress (Deihimfard et al. 2023). The production of some crop yields such as barley and wheat are foreseen to decrease by 4–7% for each 1 °C increase in temperature above the optimal temperature (Jacott and Boden 2020). Barley is known for its ability to tolerate harsh, arid conditions, especially when compared to its close relative, wheat. Given the predicted scenarios of global warming, research has identified barley as an ideal candidate for studying and potentially enhancing crop production's adaptation to climate change due to its drought tolerance (Dawson et al. 2015). Such ambition can be met through extensive research that dissects determining barley's genotypic characteristics, and determining the genetically rich regions and genes responsible for combating drought (Sallam et al. 2019).

Different developmental traits are assumed to contribute to drought tolerance in barley, among which are plant height, root length, and peduncle length (Sallam et al. 2019). The peduncle length is a developmental trait that presents the distance between the last internode connected to the spike. Its length is

proportional to plant height; the longer the peduncle, the higher the plant is. Thus, it is believed that the genetic makeup of the two traits contains some similarities (Liu et al. 2023). Both traits are desirable in cereal plants as they contribute to increased crop yield through improving photosynthetic efficiency, and lodging resistance. The peduncle is important in carbon sequence-ration in plants prior to and during grain filling where thereafter carbon is transferred to grains (Kong et al. 2010). Although all internodes could serve as carbohydrate reserves, the peduncle has the highest potential to contribute to the overall photosynthetic productivity in comparison to lower internodes as is exposed to sunlight (Torralbo et al. 2019; Gebbing 2003). Moreover, in a carbon dioxide-stressed environment, the peduncle was found to contribute to the leaf's photosynthetic productivity (Torralbo et al. 2019).

Understanding the genetic structure of these traits could pave the way for developing molecular tools that are able to enhance crop quality and quantity (e.g. Farooq et al. 2018). The genome-wide association study (GWAS) approach facilitated dissecting the genotypes that influence flowering time (Maurer et al. 2015), plant height (Alqudah et al. 2016), grain weight (Maurer et al. 2016), adaptation to fertilizers and environmental changes (Sharma et al. 2018; Herzig et al. 2018). Many of the associated genes and regions in these investigations showed pleiotropic effects that are linked to more than one trait. Moreover, GWAS has been implemented to improve the efficiency of breeding programs (Genievskaya et al. 2018). There is a limited number of investigations that provide insight into the relationship between genetic markers and peduncle characteristics in barley. Zahn et al. (2021) used GWAS and quantitative trait loci (QTL) to analyze the genetic markers influencing peduncle diameter and tissue thickness. In an experimental setting, two regions in chromosome 5 were found to be associated with peduncle length in barely plants exposed to drought stress, and in some cases, the same QTL region influenced more than one trait such as peduncle length and plant height. Interestingly, it was noted that the same trait can be promoted by different QTLs depending on environmental conditions (Jabbari et al. 2018). Furthermore, manipulating molecular pathways and gene expression in loci responsible for internode length was facilitated by Patil et al. (2019). Here, a disruption in one of the

genes responsible for promoting a stronger peduncle is applied through blocking the binding site of micro-RNA (mi172) in gene *APETALA2*. Mutated samples had lower mitotic division rates, shorter stem cells, and thus weaker peduncles. Such investigations facilitate the importance of employing genetic research in breeding programs and crop enhancement.

In this study, we aimed to understand the natural variation in the peduncle under harsh conditions and to detect the genetic markers associated with it in response to such conditions. In addition, significant single nucleotide polymorphisms (SNPs) and potential candidate genes were investigated. These results will maximize our genetic knowledge regulating peduncle development and further contribute to the genetic improvement of stress tolerance in barley.

Materials and methods

Data acquisition and Pre-processing

The EcoSeed Spring Barley Collection at IPK-Gatersleben (Germany) was screened under field conditions for peduncle length and plant height. More details on the EcoSeed panel are provided in Alqudah et al. (2021). A Field trial was conducted at the Leibniz Institute of Plant Genetics and Crop Plant Research (Gatersleben, Germany) during the 2018 growing season. This season has been described as the driest growing season for spring barley in middle

Germany in decades (Zscheischler and Fischer 2020). Each accession was sown in three independent plots, which comprised 1.2 m^2 (1 m long by 1.2 m wide), and contained six rows. Peduncle length and plant height were obtained from more than 10 biological replicates were recorded at maturity. Data distribution was plotted for each trait through a normal probability curve and histogram (supplementary material Fig. 1).

Genotypic information from 184 accessions yielded a total of 127,022 SNPs (Alqudah et al. 2021). This dataset was assembled by combining 4,809 SNPs from the Illumina 9K marker chip and 122,213 SNPs from the Genotyping-By-Sequencing technique (GBS). All used SNPs were subjected to a quality check where Minor Allelic Frequency (MAF) $\geq 5\%$. Details about the population structure and other molecular characterization of the population were published by Alqudah et al., (2021)

Data analysis

Data in this study were analyzed using R Studio version 2022. Correlation among phenotypic traits and Analysis of Variance (ANOVA) were tested at $p < 0.05$. The restricted maximum likelihood (REML) algorithm was applied for Best Linear Unbiased Estimators (BLUEs) calculations using the lme4 package (Bates et al., 2015) as described by Alqudah, et al. (2020) to estimate the mean value of each accession over the replicates. The graphics were generated
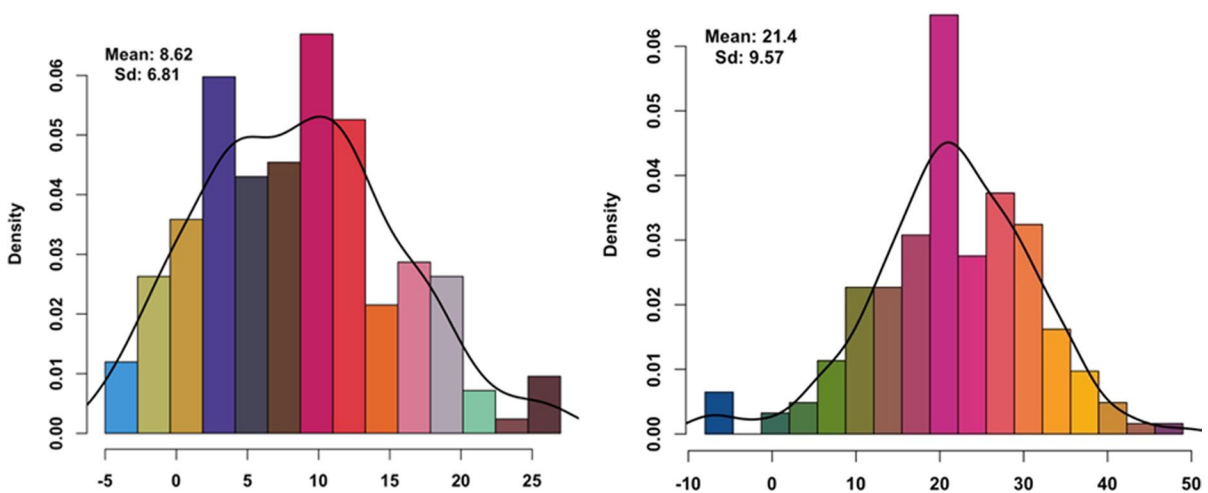

Fig. 1 Phenotypic distribution of the peduncle length (left) and plant height (right) over the study barley accessions

by the R version (RStudio-Team 2015) using the R package ggplot2 (Wickham 2016).

Genome-wide association study approach and candidate genes identification

FarmCPU was utilized in this analysis to ensure the best-fitted model recognizing the association between SNPs and the selected phenotype (Liu et al. 2016). We applied genome-wide analysis among BLUE and SNPs to calculate the associated SNPs with variation in peduncle length and plant height in our test individuals, this statistical approach had been commonly used in barley as described by Alqudah, et al. (2020). Manhattan plots were created by placing markers according to their physical position on the genome on the x-axis in correspondence to their -$\log_{10}$ (p-value) on the y-axis. While quantile–quantile (QQ) plots were constructed showing the expected values on the x-axis and observed values on the y-axis.

SNPs passed the false discovery rate (FDR) threshold of the –$\log_{10}$ (p-value) of SNP ≥ 4.93 were considered as significant associated quantitative trait nucleotides (QTNs) and used for further analysis.

Strong associated SNPs were further used to identify the high-confidence (HC) putative candidate genes underlying the natural variation of the traits within the linkage disequilibrium (LD) interval. The physical position of QTNs and candidate genes were defined using the barley database BARLEX of Morex version 2 (Colmsee et al. 2015).

Barlex database was used for molecular and cellular characterization and gene annotations of the candidate genes (https://apex.ipk-gatersleben.de/apex/f?p=284:10::::::).

Expression analysis for the candidate genes has been done through the RNA-Seq expression data from the Morex barley cultivar that includes the expression of genes at different stem development stages as TPM (Transcripts Per Kilobase Million).

Results

Phenotypic data

The peduncle length showed a bimodal distribution with two close-by peaks with a mean of 8.61 and wide data distribution (standard deviation = 6.79) indicating wide variability among tested individuals (Fig. 1). Likely, plant height showed broad variability among samples (mean = 21.4, standard deviation = 9.57) and a mono-peak normal distribution (Fig. 1).

Several significantly positive relations were found among phenotypic data with variant strengths of correlation, a strong positive correlation was observed in the correlation matrix between peduncle length and plant height at maturity which was 0.69. None of the tested related traits exhibited a negative correlation with one another. However, some were not significantly associated with each other (Fig. 2).

SNP-based GWAS and candidate gene discovery

The high density of SNPs was concentrated in chromosomes 3, 6, and 7 in locations between 170 to 430 Mb (Fig. 3). The total number of SNPs that are found to be significant ($p < 0.05$) is 145 distributed in chromosomes 1, 3, 5, 6, and 7 with 1, 131, 5, 5, and 3 SNPs, respectively. We found a total of 9438 genetic markers in chromosome 3. Based on GWAS analysis, five genomic regions with highly significant SNPs were detected to be associated with the traits of interest (Table S1). On the first region, the highest SNP (m_46750) was detected to be significantly associated with peduncle length and mapped on chromosome 3H with p = 2.05E-07 at position 631,337,837 bp which explained positive phenotypic variation by 2.57 (Table S1). In the same sense, the second region showed a significant SNP marker (m_31498) that was found to be associated with peduncle length with p = 6.75E-07 at position 265,648,599 bp (Table S1). Notably, the third genomic region showed the highest number of SNPs where most of the highly significant SNP markers with p-value ≥ 5 (Table S1).

QQ plots of p-values deviated toward the expected axis (Fig. 4). These results suggest the existence of an association between phenotypic and genotypic data in both traits.

Candidate genes

82 genes from the Barlex database Morex V2 that matched our SNPs on chromosome 3H with a high confidence level were identified for peduncle length and 2 for plant height (Table 1). Out of these, we highlighted 22 that contain markers (Table 1). The

Fig. 2 Heat map of Pearson's correlation coefficient between the studied traits at $p = 0.05$
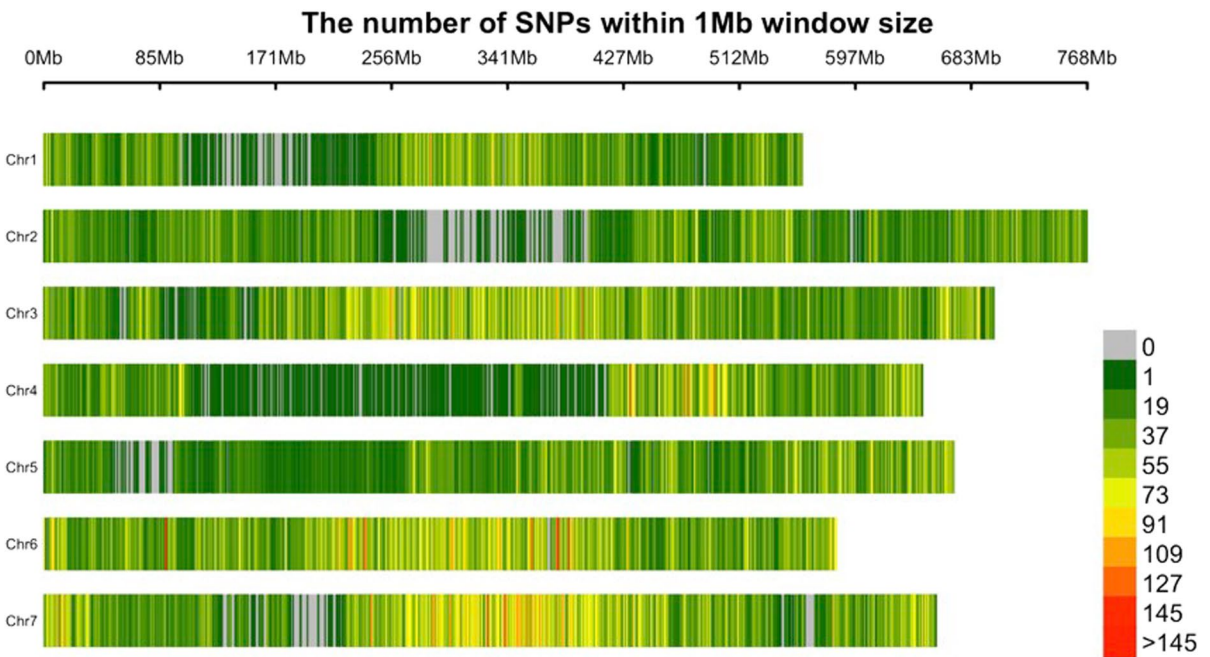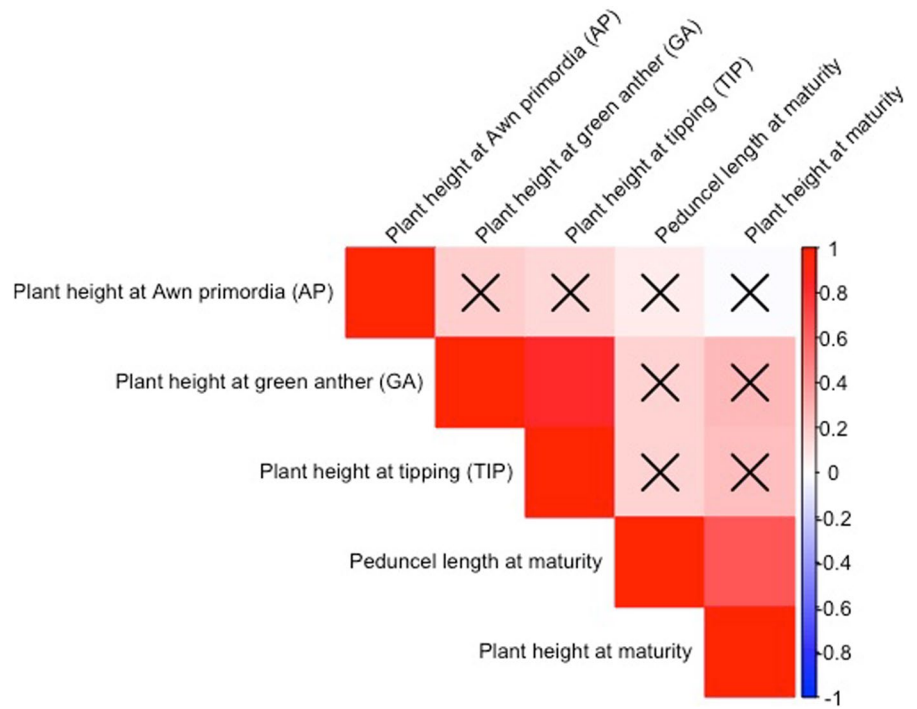

Fig. 3 Single nucleotide polymorphism (SNP) density plot chromosome-wise representing the number of SNPs within 1 Mb window size. The horizontal axis shows the chromosome length (Mb); the different color depicts SNP density
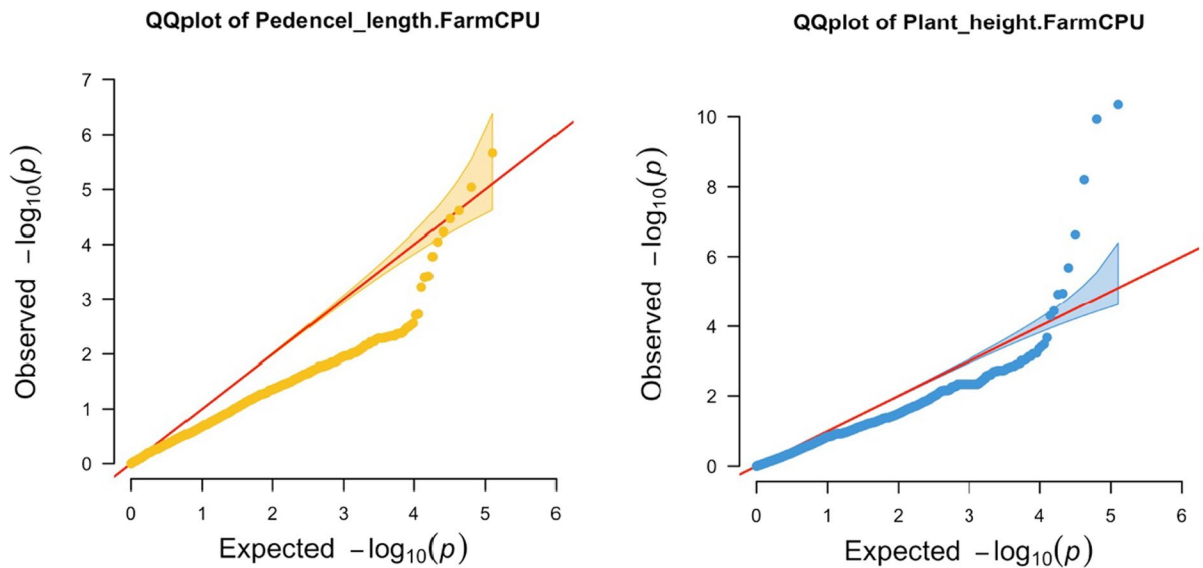
Fig. 4 Performed quantile–quantile plot on the peduncle length (left) and plant height (right) using the FarmCPU model

gene (*HORVU.MOREX.r2.3HG0215060.1* encoding Protein translocase subunit SecA) was the only gene that contained three markers, genes (*HORVU.MOREX.r2.3HG0215880.1* encoding Kinesin-like protein), (*HORVU.MOREX.r2.3HG0212540.1* encoding DNA gyrase subunit B) and (*HORVU.MOREX.r2.3HG0212600.1* encoding Ubiquitin-associated domain-containing protein 2), each had two markers while rest of the 18 candidate genes had 1 marker each. We examined the expression profile of each candidate gene and focused on the highly expressed NOD (Developing tillers, 3rd internode—42 DAP) and INF1 (Young developing inflorescences—5 mm) traits for further discussion in their association with the peduncle length and plant height in barley. RNA sequencing of these two traits revealed an expression range between 0.21 to 150.27 FPKM (Fragments Per Kilobase of transcript per Million mapped reads) for NOD and 1.76 to 105.70 FPKM for INF1. Altogether, our potential candidate genes can be used in breeding programs to develop cultivars with the desired stature, thus contributing to improved grain quality and yield under drought and heat-prone environmental conditions.

Discussion

In this study, we used genome-wide association to screen for the association between genetic markers and the peduncle length and plant height in spring barley. A total of 184 accessions were covered with 127,022 genetic markers through the Farm-CPU model. SNPs were well distributed across the genome with condensation in some regions in chromosomes 3H, 6H, and 7H suggesting an important genetic variation in these regions. After generating the Manhattan plot, it was evident that some significant SNPs on chromosome 3H may be associated with the peduncle length and plant height (Fig. 5). When investigating peduncle length, 25 SNPs were detected in this region. Out of these, three had decreased peduncle length while the rest had positive effects. Also, there was only one SNP for plant height that was found in region three (m_30085), the same SNP is shared with peduncle length resulting in decreases of both traits (Supplementary material Table S2). The statistical model used in this study (FarmCPU) suggested a significant correlation between the SNPs identified in 22 candidate genes on chromosome 3H and peduncle length. It should be noted that
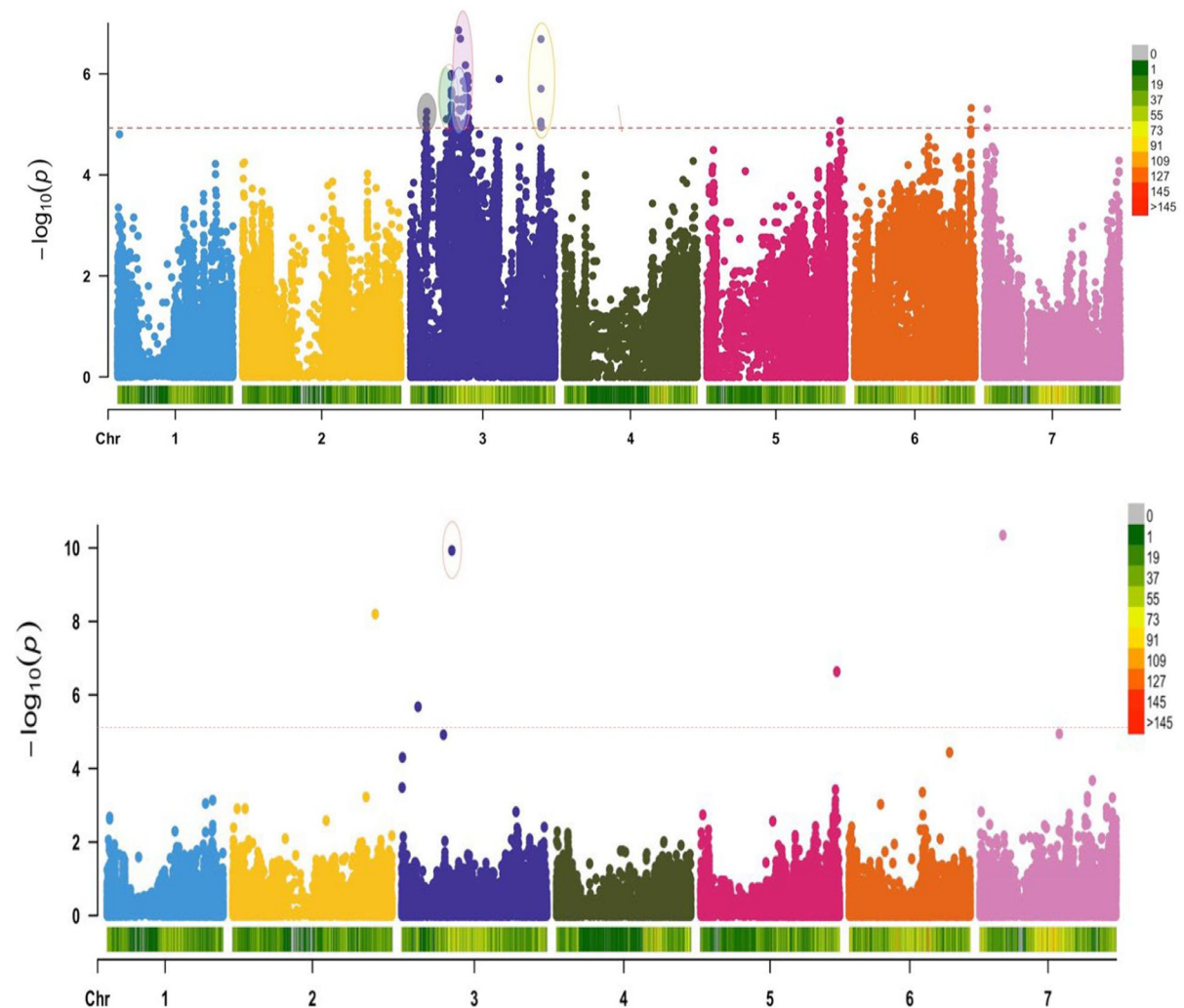
Fig. 5 Manhattan plot generated based on FarmCPU model showing the distribution of single nucleotide polymorphisms (SNPs) associated with peduncle length (top) and plant height (bottom) over the genome of barley. The annotated SNPs reflect selected regions. Threshold = 4.93

approximately 62.20% of candidate genes associated with peduncle length are located within region 3 (222,162,361–250,294,215 bp). This supports the previous statement on the existing correlation. Recently, Zahn et al. (2021) reported that important QTLs affecting peduncle length in barley have been located on chromosome 2H, which may influence the transport of nutrients and water to the developing grain, affecting yield and quality. As well, Mora et al. (2016) reported that important QTLs affecting peduncle length in barley have been located on chromosome 2H, which may influence the transport of nutrients and water to the developing grain, affecting

yield and quality. For plant height, Niu et al. (2022) reported significant QTLs on chromosome 2H related to plant height, including those that may be linked to the *sdw1* dwarfing gene. These genes are of interest for developing shorter, lodging-resistant cultivarss that are easier to harvest and can produce stable yields across different environments (Thabet et al. 2020a, b) (Table 1).

When scanning the expression profile of candidate genes, we targeted two traits that are correlated with the peduncle length, the developing tillers after 42 days (NOD; usually happens 40 days after planting) and young developing inflorescence after 5 mm

Table 1 Physical positions of candidate genes with annotation and markers obtained from the Barlex dataset—Morex V2 on chromosome 3H. The expression profile of two selected traits in each candidate gene is indicated. Expression values are graduating from high (red) to low expression (blue), and values in white have neutral expression values that are not significantly related to this gene.

No.	Region	Gene Name	AGP Start	AGP End	Annotation	Marker 1	Marker 2	Marker 3	RNA-seq (FPKM), NOD: Developing tillers, 3rd internode (42 DAP)	RNA-seq (FPKM), INF1: Young developing inflorescences (5mm)
1	2	*HORVU.MOREX.r2.3HG0217640.1*	265,623,498	265,648,470	Serine/threonine-protein phosphatase 4 regulatory subunit 3	JHI-Hv50k-2016-173000			40.0023	90.2075
2	3	*HORVU.MOREX.r2.3HG0214550.1*	223,827,719	223,855,159	Alkaline/neutral invertase	JHI-Hv50k-2016-171057			25.3797	10.9774
3	3	*HORVU.MOREX.r2.3HG0214620.1*	225,972,055	225,975,254	Kelch repeat protein, putative	JHI-Hv50k-2016-171193			31.9398	12.9903
4	3	*HORVU.MOREX.r2.3HG0214890.1*	228,945,299	228,948,711	F-box protein	JHI-Hv50k-2016-171267			58.8012	29.0222
5	3	*HORVU.MOREX.r2.3HG0215010.1*	230,484,558	230,487,361	**Cyclin**	JHI-Hv50k-2016-171314			**0.2349**	**15.5375**
6	3	*HORVU.MOREX.r2.3HG0215060.1*	231,666,907	231,737,811	Protein translocase subunit SecA	JHI-Hv50k-2016-171391	JHI-Hv50k-2016-171392	JHI-Hv50k-2016-171408	20.1204	21.1175
7	3	*HORVU.MOREX.r2.3HG0215290.1*	234,947,445	234,949,011	Cysteine desulfurase	JHI-Hv50k-2016-171627			15.9598	54.1818
8	3	*HORVU.MOREX.r2.3HG0215350.1*	235,450,384	235,463,085	Acyltransferase, putative	JHI-Hv50k-2016-171672			23.5826	11.2060
9	3	*HORVU.MOREX.r2.3HG0215400.1*	235,824,200	235,848,449	Sn1-specific diacylglycerol lipase alpha	JHI-Hv50k-2016-171704			37.6586	14.1840
10	3	*HORVU.MOREX.r2.3HG0215440.1*	236,184,956	236,188,273	Kinase family protein	JHI-Hv50k-2016-171745			8.4863	8.2790
11	3	*HORVU.MOREX.r2.3HG0215450.1*	236,188,830	236,202,215	Receptor-like protein kinase	JHI-Hv50k-2016-171742			33.5661	37.0236
12	3	*HORVU.MOREX.r2.3HG0215690.1*	241,991,378	242,007,005	Fimbrin-2	JHI-Hv50k-2016-171911			53.2813	55.7909
13	3	*HORVU.MOREX.r2.3HG0215820.1*	243,050,299	243,061,735	Lipase family protein	JHI-Hv50k-2016-17198			67.7074	37.9674
14	3	*HORVU.MOREX.r2.3HG0215880.1*	243,508,175	243,550,938	Kinesin-like protein	JHI-Hv50k-2016-172011	JHI-Hv50k-2016-172018		30.6711	28.1544
15	3	*HORVU.MOREX.r2.3HG0215960.1*	243,869,261	243,871,349	**Metal transporter, putative**	JHI-Hv50k-2016-172026			**4.0659**	**1.7601**
16	3	*HORVU.MOREX.r2.3HG0215990.1*	244,363,411	244,374,877	Chaperone protein dnaJ	JHI-Hv50k-2016-172074			38.0335	55.5613
17	3	*HORVU.MOREX.r2.3HG0216090.1*	245,827,476	245,865,136	**dentin sialophosphoprotein-like protein**	JHI-Hv50k-2016-172142			**86.9439**	**62.0503**
18	3	*HORVU.MOREX.r2.3HG0216150.1*	246,134,815	246,143,128	**Phosphate transporter**	JHI-Hv50k-2016-172160			**19.4640**	**11.1014**
19	3	*HORVU.MOREX.r2.3HG0216310.1*	247,735,155	247,741,963	**Polynucleotidyl transferase ribonuclease H-like superfamily protein**	JHI-Hv50k-2016-172220			**150.2722**	**105.6960**
20	4	*HORVU.MOREX.r2.3HG0212540.1*	197,923,066	197,943,561	**DNA gyrase subunit B**	JHI-Hv50k-2016-169612	SCRI_RS_141812		**0.2119**	**88.0202**
21	4	*HORVU.MOREX.r2.3HG0212600.1*	198,564,637	198,570,726	Ubiquitin-associated domain-containing protein 2	JHI-Hv50k-2016-169667	JHI-Hv50k-2016-169670		41.8371	19.0972
22	5	*HORVU.MOREX.r2.3HG0200080.1*	76,023,001	76,028,795	O-fucosyltransferase family protein	SCRI_RS_189045			16.6031	24.9708

(INF1). These traits can be used in barley as an adaptation mechanism to harsh conditions by manipulating its developmental cycle to speed up the flowering stage. In this study, the highest expressed gene in NOD and INF1 is polynucleotide transferase ribonuclease H-like superfamily protein with 150.27 and 105.70 FPKM, respectively. This protein family is known for its diverse roles in intracellular functions such as ribonuclease activity, nucleic acid binding, altering RNA, and mechanic responses such as reacting to biotic stimulus, and wound repairing. Polynucleotide transferase ribonucleases (PNTR) are enzymes involved in RNA metabolism, including the processing, editing, and degradation of RNA molecules (Walley et al. 2010a, b). These enzymes can influence various aspects of plant growth and development (Nishanth 2024). PNTR enzymes could affect the expression of genes that regulate peduncle length and plant height (Suzuki et al. 2015a, b).

For instance, genes involved in hormone biosynthesis, signaling pathways (like gibberellins, auxins, and brassinosteroids), and structural components of the cell could be impacted, indirectly influencing these growth traits. These enzymes might play a role in how plants respond to stress (e.g., drought, nutrient deficiency) that can, in turn, affect peduncle growth and overall plant height (Liang et al. 2009a, b). Efficient RNA turnover and processing can be crucial for the rapid adaptation of plants to changing environmental conditions, potentially affecting growth and development (Nishanth 2024). The activity of PNTR enzymes might affect the timing of developmental transitions and the rate of growth, which are critical for determining the final height of the plant and the length of the peduncle (Altmann et al. 2020a, b). Taken these findings together, PNTR is highly expressed under abiotic stress such as drought which is the case in our study.

Another highly expressed gene in NOD and INF1 stages is dentin sialophosphoprotein-like protein with (86.94) and (62.05) FPKM, respectively. The function of this gene is not explored yet in cereal

plants, however, in mammals, this gene is essential for biomineralization and teeth hardening development (Yamakoshi 2009; Shang et al. 2023). This aligns with the idea that it is used by barley to resist harsh environmental conditions that place strains on its structural development. Interestingly, DNA gyrase subunit B was under-expressed at NOD (0.21 FPKM) demonstrating the decreased DNA activity under stress conditions and highly expressed at INF1 (88.02 FPKM) where higher genetic activity was needed to support this stage of development. Manipulating molecular features is one of the mechanisms plants usually use to tolerate drought (Razi and Muneer 2023). We found the lowest expression rates at NOD (0.23 FPKM) and INF1 (15.54 FPKM) in Cyclin indicating downregulation when water is scarce. In *Arabidopsis thaliana*, observed levels of expression of Cyclin were negatively correlated with dehydration levels (Zhou et al. 2013). Another gene that we observed to be under-expressed is the Phosphate transporter (PTs) (19.46 and 11.10 FPKM and NOD and INF1, respectively). It was demonstrated in literature before that, plants' ability to absorb phosphorus drops over time under prolonged drought, thus, expression of phosphate transporter decreases (Karthikeyan et al. 2002; Bi et al. 2023). Similarly, water scarcity impacts the intracellular ionic profile by interrupting metal transportation activities mirroring the lower expression of gene metal transporter (4.07 and 1.76 FPKM for NOD and INF1, respectively) (Gill et al. 2021). Moreover, PTs might play a role in determining overall plant vigor and stature by ensuring that phosphate is efficiently distributed to growing tissues, including the stem (Cao et al. 2021b, a). PT activity might indirectly affect plant height by modulating the availability of phosphorus for biosynthetic pathways related to growth hormones (Vysotskaya et al. 2020a, b). Understanding the role of PTs in barley development is crucial for breeding programs aimed at improving nutrient use efficiency (Niu et al. 2013a, b). Altogether, cultivars with enhanced phosphate uptake and transport capabilities could maintain optimal growth and development, including desirable peduncle length and plant height, even under stress conditions.

Remarkably, there were two members of the Zinc finger CCCH domain protein on chromosome 3H but none of which was expressed in the developing tillers or inflorescence stages. This gene family is known for its significant role in drought tolerance in plants, hence, it controls several molecular pathways to reduce plant's sensitivity to dehydration (Ai et al. 2022). Perhaps, the lack of expression of these genes is due to the high drought tolerance in barley and not reaching the adequate stress level to trigger zinc finger CCCH expression, although there were other molecular signs of drought resistance. This emphasizes the need to further dissect chromosome 3 to locate more genotypic traits associated with the peduncle length and plant height in stress conditions in particular.

Conclusion

Our GWAS analysis revealed a strong association between peduncle length and SNPs in chromosome 3H. Further observations into candidate genes showed a strong alliance with RNA sequences of specific traits. Selected traits were linked to the flowering stage in barley directly and indirectly to stress tolerance. Our study is in favor of supposing a strong association between peduncle length and specific genotypic traits. We suggest further investigations to elaborate on how the expression profile of these genotypes influences peduncle length through different developmental stages.

Author contributions AMA designed the experiment. AMA, SS, CK, and EL did the field work and phenotyping. ZK and AMA analyzed the data. ZK, SGT, MDA, MS, AB, and AMA wrote and edited the manuscript. ZK, SG, MS, and AMA conceived the idea and participated in the interpretation of the results. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of interest I declare that the authors have no competing interests that might be perceived to influence the results and/ or discussion reported in this paper.

Ethical approval This article does not contain any research involving animals or human participants performed by any of the authors.

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