

Article

Genome-Wide Association Mapping of Seedling Vigor and Regrowth Vigor in Winter Wheat

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Abstract: Seedling vigor and regrowth ability are important traits for the forage production of winter wheat. The objectives of this study were to map quantitative trait loci (QTL) associated with seedling vigor and regrowth vigor traits using a genome-wide association mapping study (GWAS). Seedling vigor and regrowth vigor were evaluated with shoot length, the number of shoots per plant and shoot dry weight per plant 45 days after planting and 15 days after cutting. A large phenotypic variation was observed for all the traits studied. In total, 12 significant QTL for seedling vigor and 16 for regrowth vigor traits were detected on various chromosomes. Four QTL on chromosomes 2B, 4B, 5A and 7A for seedling vigor co-localized with QTL for regrowth vigor due to significant correlations between corresponding traits of the initial growth and regrowth. A BLAST search using DNA sequences of the significant loci revealed candidate genes playing roles in vegetative and reproductive development in different crop species. The QTL and single-nucleotide polymorphism (SNP) markers identified in this study will be further validated and used for marker-assisted selection of the traits during forage wheat breeding.

Keywords: coleoptile length; forage biomass; regrowth vigor; seedling vigor; winter wheat



Citation: Maulana, F.; Huang, W.; Anderson, J.D.; Kumssa, T.T.; Ma, X.-F. Genome-Wide Association Mapping of Seedling Vigor and Regrowth Vigor in Winter Wheat. *Crops* **2021**, *1*, 153–165. <https://doi.org/10.3390/crops1030015>

Academic Editors: Fulai Liu, Mohammad Anwar Hossain and Mohammad Golam Mostofa

Received: 19 October 2021
Accepted: 26 November 2021
Published: 30 November 2021

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1. Introduction

Seedling vigor and regrowth vigor are crucial traits for the forage production of winter wheat grown for cattle grazing. Rapid early development of seedling growth, denoted as seedling vigor, contributes to both high forage production and grain yield in the dual-purpose grazing-grain system [1,2]. Winter wheat pasture with vigorous early seedling growth also quickly covers the soil, enhancing water-use efficiency by reducing evaporative losses from the soil surface [1,3,4]. Good seedling vigor also improves light interception and the ability of the crop to compete with weeds for soil water and nutrients [4].

On the other hand, seedling regrowth vigor, exhibited in wheat pasture after being grazed, is an important trait of the sustainable production of forage biomass for multi-cycles of cattle grazing [5,6]. A good winter wheat pasture should be able to regenerate vigorously after initial and subsequent grazing for the continued production of forage biomass.

However, seedling vigor and regrowth vigor are genetically complex traits, controlled by minor genes spread across the genome, and they are associated with different plant growth and developmental traits, ranging from cell level to whole plant [7]. Seedling vigor correlates with coleoptile length, shoot length, number of shoots per plant and shoot dry weight [4,8–10]. Both seedling vigor and regrowth vigor are influenced by genotype, environmental conditions and genotype–environment interaction. Therefore, phenotyping seedling vigor and regrowth vigor using phenotypic selection is a time-consuming and costly challenge. Marker-assisted selection (MAS) may provide a viable alternative to phenotypic selection if diagnostic markers can be identified.

To date, some QTL studies have been conducted to map quantitative trait loci (QTL) for seedling vigor traits in wheat (*Triticum aestivum* L.) [11–13], sorghum (*Sorghum bicolor* L. Moench) [14] and rice (*Oryza sativa* L.) [15] using different types of mapping populations, including bi-parental mapping population. In wheat, QTL associated with seedling vigor traits, such as shoot length, number of shoots per plant and shoot dry weight have been found on chromosomes 1D, 2A, 2B, 2D, 3A, 4B, 6A, and 7D [10,16–18]. However, the regrowth vigor of wheat has rarely been studied.

Compared to traditional QTL mapping based on bi-parental populations, GWAS is an alternative tool for locating QTL of complex traits using diverse genotypes. Genome-wide association mapping can link genotype to phenotype in natural diversity populations for identifying natural allelic variations associated with traits of interest [19]. Over the years, GWAS has been successfully used to identify genomic regions associated with quantitative traits in different crop species, such as wheat [20–23], sorghum [24,25], rice [26–28], barley (*Hordeum vulgare* L.) [29,30] and maize (*Zea mays* L.) [31,32].

However, most of the GWAS reported have been based on single-locus mixed models, such as the mixed linear model (MLM) [33] and the compressed mixed linear model (CMLM) [34]. In single-locus mixed models, multiple tests are employed, with the test number undergoing a Bonferroni correction, which is often too conservative [35]. As a result, some significant loci can be eliminated. By contrast, multi-locus mixed models do not require the Bonferroni correction, leading to the identification of more significant marker-trait associations [36]. Recently, new multi-locus GWAS models, such as the multi-locus mixed linear model (MLMM) [37], have been developed. With MLMM, a forward-backward step-wise linear mixed-model regression is used during analysis, whereby associated markers are included in the model as covariates [37,38].

In this study, we characterized seedling vigor and regrowth vigor traits, including shoot length, the number of shoots per plant and the shoot dry weight per plant of a winter wheat diversity population, and performed GWAS on these traits using two single-locus mixed models, MLM [33] and CMLM [34], and one multi-locus GWAS model, MLMM. The objectives of this study were to map the QTL contributing to seedling vigor and regrowth vigor in winter wheat and to identify single-nucleotide polymorphism (SNP) markers associated with the traits for MAS during wheat breeding.

2. Materials and Methods

2.1. Plant Materials

This study was conducted using 200 diverse representative lines selected from the hard winter wheat association mapping panel (HWWAMP), consisting of 299 winter wheat lines originating from different geographical areas across the Great Plains of the United States [20,39]. The panel is archived in the T3 wheat database generated by the Triticeae Coordinated Agricultural Project (TCAP).

2.2. Phenotyping Seedling Vigor and Regrowth Vigor

The panel was grown and evaluated for seedling vigor and regrowth vigor traits under climate-controlled greenhouse conditions at the Noble Research Institute, LLC (Ardmore, Oklahoma, USA). The experiment was repeated three times. Each repeat was laid out in a randomized complete block design with a total of six plant replicates per line grown in two pots (4.5 inch), three plants per pot, for mimicking field planting density. The pots were filled with Metro Mix 360 growing medium. Data were collected on seedling vigor and regrowth vigor traits, including shoot length, number of shoots per plant and shoot dry weight per plant. The seedling vigor traits were collected 45 days after planting. After recording the seedling vigor data, the plants were then cut at the base one inch above the soil surface for regrowth. The regrowth vigor data of the same traits were collected 15 days after cutting (i.e., 60 days after planting).

For both the initial growth and regrowth, the shoot length was determined by measuring the length of the shoot from the base to the tip of the longest leaf. The number of

shoots per plant was recorded before cutting for biomass. The shoot dry weight per plant was the weight of tissue dry biomass harvested 45 after planting and 15 days after cutting for seedling vigor and regrowth vigor assessment, respectively.

2.3. Phenotypic Data Analysis

Phenotypic data analysis was conducted using the analysis of variance with PROC GLM in SAS v 9.3 [40]. The genotypes were considered fixed effects, whereas the experiments (runs) and the replicates nested within the experiment were considered random effects. The relationship between the seedling vigor and regrowth vigor traits was assessed by conducting Pearson correlation analysis in the R program [41]. A correlation scatterplot was generated using the R package Performance Analytics [42].

2.4. Genotyping and Population Structure

Genotyping was performed using the wheat 90K SNP array. A total of 15,574 SNPs with less than 10% missing data and more than 5% minor allele frequency (MAF) were used in this study [20,23]. The marker data file included only SNP markers with known chromosome numbers and chromosomal positions of the SNPs based on the 90K SNP consensus map [43] because many SNPs were not able to be mapped to the wheat reference sequence [44]. A genome-wide linkage disequilibrium decay curve for the panel is shown in Figure S1. The population structure was assessed using the principal component analysis with the princomp function in the R program and the results were reported in a previous publication [20].

2.5. Genome-Wide Association Mapping Analysis

A genome-wide association mapping analysis was performed in R package GAPIT [45] using two single-locus models, MLM [33] and CMLM [34], and one MLM [37,38]. For all the models, we included three principal components and familial relatedness (K-matrix) during the GWAS analysis as fixed and random-effect covariates, respectively, to reduce the detection of false positives. Firstly, we declared significant QTL and SNPs based on a false discovery rate (FDR) of <0.05 as a cut-off point. However, the FDR < 0.05 was too stringent in this study, so we eventually used a lower cut-off threshold, using an unadjusted *p*-value of <0.001 to declare significant QTL [20,46]. We used Manhattan plots, generated with the qqman R package [47], to visualize significant QTL and SNPs. To identify genes or related proteins with DNA sequences similar to those of significant SNP markers associated with seedling vigor and regrowth vigor traits detected in the present study, we conducted a basic local alignment search tool (BLAST) search using the URGI-INRA [48] platform.

3. Results

3.1. Phenotypic Data Analysis

The phenotypic variation of seedling vigor and regrowth vigor (i.e., regrowth after cutting) traits of the population are presented in Table 1. A large phenotypic variation was observed in all the traits. For shoot length, the mean value of initial growth measured 45 days after planting was 56.1 cm, ranging from 38.3 to 79.8 cm, while the mean shoot length of regrowth, measured 15 days after cutting, was 51.3 cm, ranging from 40.0 to 68.3 cm. For number of shoots, the mean value of initial growth per plant was seven, while it was six from regrowth. The number of shoots per plant ranged from 3 to 15 and 3 to 13 for initial growth and regrowth, respectively. For shoot dry weight per plant, the initial growth ranged from 0.35 to 1.10 g with the mean value of 0.84 g per plant, while regrowth ranged from 0.27 to 1.00 g, with a mean value of 0.71 g per plant.

Table 1. Phenotypic variation in seedling vigor and regrowth vigor traits of the winter wheat diversity population.

Trait	Seedling Vigor			Regrowth Vigor		
	Mean	Range	SD	Mean	Range	SD
Shoot length (cm)	56.1	38.3–79.8	6.6	51.3	40.0–68.3	5.6
Number of shoots per plant	7	3–15	2.4	6	3–13	1.9
Shoot dry weight per plant (g)	0.84	0.35–1.10	0.12	0.71	0.27–1.00	0.13

SD, Standard deviation.

3.2. Correlations between Seedling Vigor and Regrowth Vigor Traits

Pearson correlations of the traits are presented in Figure 1. Of the seedling traits measured from initial growth, shoot length (SL1) was negatively correlated ($r = -0.18$) with number of shoots (SN1), and positively correlated ($r = 0.25$) with shoot dry weight (SDW1). However, the three traits from regrowth (SL2, SN2 and SDW2) were positively correlated to each other with correlation coefficients $r = 0.22$ for SL2 and SN2, $r = 0.15$ for SL2 and SDW2, and $r = 0.10$ for SN2 and SDW2, although the last value is not significant (Figure 1).

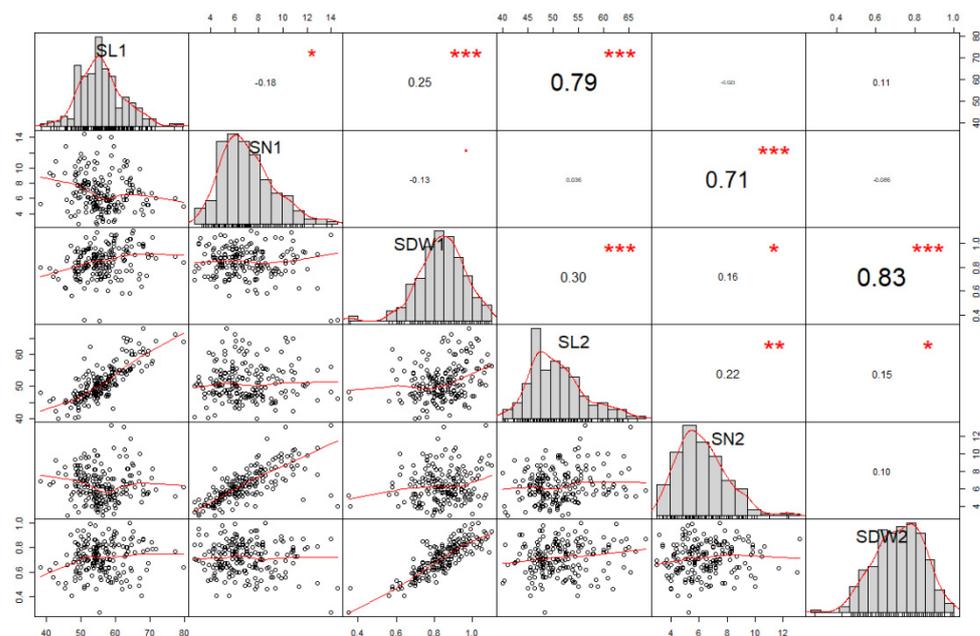


Figure 1. Correlation scatterplots of seedling vigor and regrowth vigor traits of the winter wheat diversity population. SL, SN and SDW denote shoot length, number of shoots, and shoot dry weight, respectively, for initial growth (1) and regrowth (2). For significance, the p -values of 0.001, 0.01, and 0.05, correspond to “***”, “**”, and “*”, respectively.

Furthermore, when the traits were compared between seedling vigor and regrowth vigor, significant correlations were observed between pairs of corresponding traits. Specifically, SL1 exhibited a significant and positive correlation ($r = 0.79$) with SL2. Similarly, significant correlations were observed for SN1 and SN2 ($r = 0.71$), and SDW1 and SDW2 ($r = 0.83$). In addition, SDW1 was positively correlated with SL2 ($r = 0.30$) and SN2 ($r = 0.16$) (Figure 1).

3.3. Genome-Wide Association Analysis

The GWAS results of the two single-locus mixed models, CMLM and MLM, were similar; therefore, only the results from MLM and MLMM are presented in this paper. The results of the genome-wide association mapping analyses are presented in Figures 2–4 for

the early vigor traits, and Supplementary Figures S1–S3 for the regrowth vigor traits. Using FDR < 0.05, significant SNPs were declared only for shoot length; however, significant SNPs were detected at an unadjusted p -value < 0.001 for all the traits evaluated. The QTL and SNP markers significantly associated with the seedling vigor and regrowth vigor traits are summarized in Table 2, with additional details presented in Supplementary Table S1.

Table 2. Significant QTL associated with seedling vigor and regrowth vigor traits of the winter wheat diversity population.

Trait	QTL Name	Peak SNP	Chr	Pos (cM)	MAF	MLM		MLMM	
						p -Value	R^2 (%)	p -Value	
Seedling vigor	Shoot length (cm)	<i>QSLeV.nri-1A</i>	IWB43660	1A	29	0.36		6.25×10^{-4}	
		<i>QSLeV.nri-2B</i>	IWB36210	2B	163	0.49	9.15×10^{-6}	9.3	2.82×10^{-4}
		<i>QSLeV.nri-4B.1</i>	IWB31302	4B	37	0.42			3.85×10^{-4}
		<i>QSLeV.nri-4B.2</i>	IWB7508	4B	55	0.07	2.43×10^{-5}	8.4	2.47×10^{-4}
		<i>QSLeV.nri-5A</i>	IWB43526	5A	86	0.25			6.81×10^{-4}
		<i>QSLeV.nri-7A</i>	IWB9062	7A	136	0.17	7.57×10^{-4}	5.2	4.45×10^{-4}
	Number of shoots	<i>QSNeV.nri-2A</i>	IWB28627	2A	143	0.13	6.44×10^{-4}	6.2	4.39×10^{-4}
		<i>QSNeV.nri-5A</i>	IWA2445	5A	53	0.43			8.99×10^{-4}
		<i>QSNeV.nri-7A</i>	IWB8896	7A	44	0.32	2.03×10^{-4}	7.3	1.17×10^{-4}
	Shoot dry weight (g)	<i>QSDWeV.nri-6A</i>	IWB50	6A	141	0.21			9.59×10^{-4}
<i>QSDWeV.nri-7A</i>		IWB42636	7A	228	0.11	6.72×10^{-5}	8.4	3.21×10^{-4}	
<i>QSDWeV.nri-7D</i>		IWB21364	7D	138	0.13	6.86×10^{-4}	6.0	4.71×10^{-4}	
Regrowth vigor	Shoot length (cm)	<i>QSLrV.nri-2A</i>	IWB70425	2A	126	0.12		7.67×10^{-4}	
		<i>QSLrV.nri-2B</i>	IWB36210	2B	163	0.49	1.46×10^{-4}	5.8	2.33×10^{-4}
		<i>QSLrV.nri-4B</i>	IWB35611	4B	57	0.42	2.72×10^{-6}	9.1	4.77×10^{-4}
		<i>QSLrV.nri-5A</i>	IWB38719	5A	89	0.17			1.30×10^{-4}
		<i>QSLrV.nri-6B.1</i>	IWB2837	6B	67	0.07	2.95×10^{-4}	5.3	6.76×10^{-4}
		<i>QSLrV.nri-6B.2</i>	IWB59306	6B	114	0.33			3.64×10^{-4}
		<i>QSLrV.nri-7B</i>	IWB8019	7B	54	0.20	3.21×10^{-4}	5.2	8.44×10^{-4}
	Number of shoots	<i>QSNrV.nri-4B</i>	IWB27735	4B	105	0.15	7.29×10^{-4}	6.0	5.04×10^{-4}
		<i>QSNrV.nri-6A</i>	IWB71326	6A	141	0.13	6.88×10^{-4}	6.0	4.72×10^{-4}
		<i>QSNrV.nri-7B</i>	IWA4802	7B	134	0.45	4.80×10^{-4}	6.4	3.15×10^{-4}
Shoot dry weight (g)	<i>QSDWrV.nri-1B</i>	IWB66973	1B	118	0.15	7.79×10^{-4}	6.1	5.42×10^{-4}	
	<i>QSDWrV.nri-2B</i>	IWB4865	2B	134	0.06	1.86×10^{-4}	7.6	1.06×10^{-4}	
	<i>QSDWrV.nri-4A</i>	IWA5968	4A	109	0.15	8.64×10^{-4}	6.0	6.10×10^{-4}	
	<i>QSDWrV.nri-4B</i>	IWB24289	4B	78	0.39			9.35×10^{-4}	
	<i>QSDWrV.nri-6A</i>	IWB12052	6A	26	0.22			9.85×10^{-4}	
<i>QSDWrV.nri-7A</i>	IWB42636	7A	228	0.11	7.76×10^{-4}	6.1	5.40×10^{-4}		

MAF: minor allele frequency; MLM: mixed linear model; MLMM: multi-locus mixed linear model. R^2 is phenotypic variance explained as a percentage.

3.3.1. QTL for Seedling Vigor

Shoot Length

Using the single-locus mixed linear model, MLM, three significant QTL for shoot length, represented by six SNPs, were found on chromosomes 2B, 4B and 7A based on an unadjusted p -value < 0.001 (Figure 2a and Table 2). The first QTL, *QSLeV.nri-2B*, was mapped at 163 cM on chromosome 2B and accounted for 9.3% of the total phenotypic variation in shoot length. The second QTL region, *QSLeV.nri-4B.2*, represented by four SNPs for an 8.4% variation of the trait, was mapped at 55–60 cM on chromosome 4B. The third QTL, *QSLeV.nri-7A*, was mapped at genetic distance position of 136 cM on chromosome 7A, explaining 5.2% of the total phenotypic variation of the trait. Overall, the most significant SNPs were IWB36210 and IWB7508 on chromosomes 2B (163 cM) and 4B (55 cM), respectively.

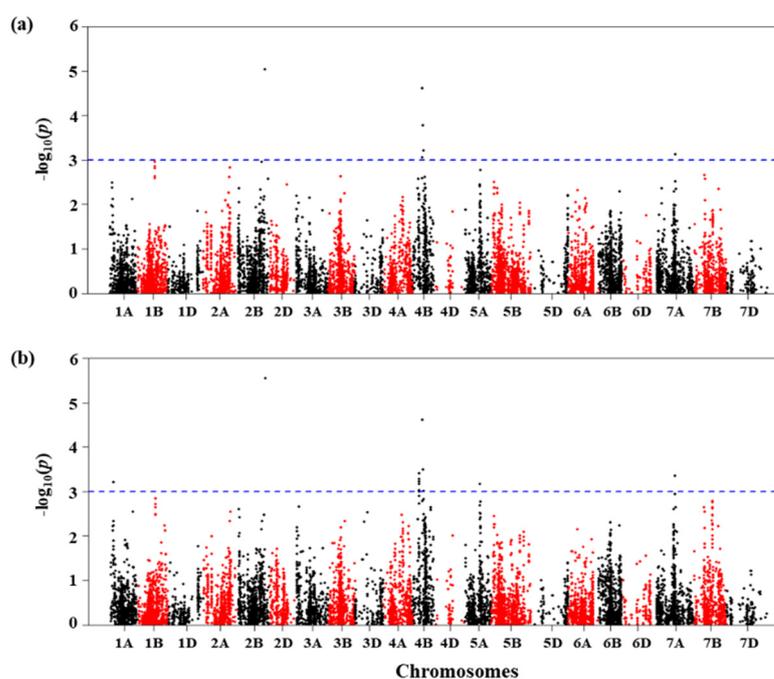


Figure 2. Manhattan plots of shoot length of initial growth detected by (a) MLM and (b) MLMM. The x-axis denotes chromosomes and the y-axis shows $-\log_{10}(P)$ values of marker-trait association. The dotted blue line represents the threshold of significant QTL.

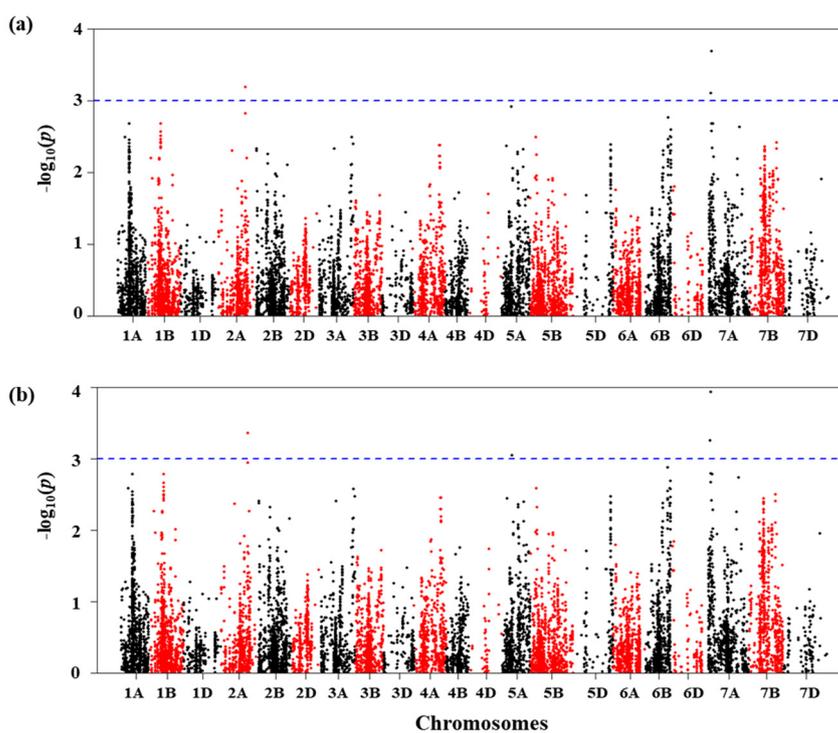


Figure 3. Manhattan plots of number of shoots of initial growth detected by (a) MLM and (b) MLMM. The x-axis denotes chromosomes and the y-axis shows $-\log_{10}(P)$ values of marker-trait association. The dotted blue line represents the threshold of significant QTL.

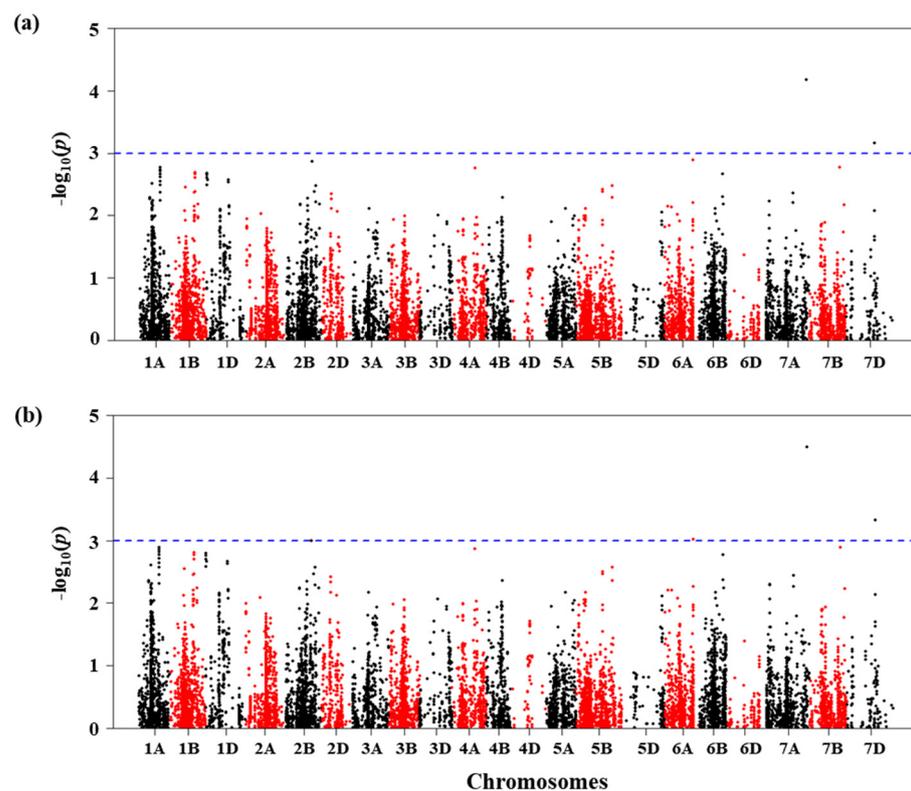


Figure 4. Manhattan plots of shoot dry weight of initial growth detected by (a) MLM and (b) MLMM. The x-axis denotes chromosomes and the y-axis shows $-\log_{10}(P)$ values of marker-trait association. The dotted blue line represents the threshold of significant QTL.

By contrast, using the multi-locus mixed linear model, MLMM, six QTL for shoot length were found on chromosomes 1A, 2B, 4B, 5A and 7A (Figure 2b and Table 2). On 1A and 2B, two QTL regions, *QSLeV.nri-1A* and *QSLeV.nri-2B*, were mapped at 29 and 163 cM, respectively. On 4B, two QTL, *QSLeV.nri-4B.1* and *QSLeV.nri-4B.2*, were found at the genetic positions of 37 and 55–60 cM, respectively. In addition, *QSLeV.nri-5A* and *QSLeV.nri-7A* were mapped at 86 and 136 cM on chromosomes 5A and 7A, respectively. Compared to MLM mapping, MLMM detected additional QTL on chromosomes 1A, 4B, and 5A. Again, with MLMM, the two most significant SNPs were the same SNPs detected by MLM.

Number of Shoots

Using MLM, two QTL significantly associated with number of shoots per plant were found on chromosomes 2A and 7A, according to an unadjusted p -value < 0.001 (Figure 3a and Table 2). The first QTL, *QSNeV.nri-2A*, at 143 cM on 2A, explained 6.2% of the total phenotypic variation in number of shoots per plant. The second QTL, *QSNeV.nri-7A*, was detected by two SNPs at 43–44 cM on chromosome 7A and caused a variation of about 7.3% in the number of shoots per plant.

Using MLMM, besides the same QTL for number of shoots per plant mentioned above, an additional QTL, *QSNeV.nri-5A*, at 53 cM on chromosome 5A was detected (Figure 3b and Table 2). Overall, SNP marker IWB8896, at 44 cM on 7A, was the most significant SNP detected by both MLM and MLMM.

Shoot Dry Weight

Using MLM, two QTL for shoot dry weight per plant were detected at 228 and 138 cM on chromosomes 7A and 7D, respectively, at an unadjusted p -value < 0.001 significance (Figure 4a and Table 2). The two QTL, *QSDWeV.nri-7A* and *QSDWeV.nri-7D*, explained 8.4% and 6.0% of the total phenotypic variation in shoot dry weight per plant, respectively.

Using MLMM, an additional SNP representing *QSDWeV.nri-6A* was mapped at 141 cM on 6A (Figure 4b and Table 2). Overall, the most significant SNP associated with shoot dry weight per plant was IWB42636 at 228 cM on chromosome 7A.

3.3.2. QTL for Regrowth Vigor

Shoot Length

Using MLM, four QTL significantly associated with shoot length were found on chromosomes 2B, 4B, 6B and 7B, based on an unadjusted p -value < 0.001 (Figure S2a and Table 2). On chromosome 2B, a QTL region, *QSLrV.nri-2B*, was mapped at a genetic distance position of 163 cM, explaining 5.8% of the total phenotypic variation of the trait. On chromosome 4B, *QSLrV.nri-4B* was mapped at 55–64 cM, and it was the most significant QTL, responsible for 9.1% of the total phenotypic variation in shoot length of regrowth. In addition, two QTL, *QSLrV.nri-6B.1* and *QSLrV.nri-7B*, were found on chromosomes 6B and 7B, which together accounted for 10.5% of the total phenotypic variance of the trait. Overall, the most significant representative SNPs were IWB35611 and IWB36210 on chromosome 4B (57 cM) and 2B (163 cM), which accounted for 9.1 and 5.8%, respectively, of the total phenotypic variance of the trait.

Using MLMM, seven QTL significantly associated with shoot length of regrowth were found on chromosome 4B based on an FDR of < 0.05 and on chromosomes 2A, 2B, 5A, 6B and 7B according to unadjusted p -value < 0.001 (Figure S2b and Table 2). The first QTL, *QSLrV.nri-2A*, was found at 126 cM on chromosome 2A. On chromosomes 2B, 4B and 5A, three QTL regions, *QSLrV.nri-2B*, *QSLrV.nri-4B* and *QSLrV.nri-5A*, were detected at 163, 57 and 89 cM, respectively. On 6B, two QTL, *QSLrV.nri-6B.1* and *QSLrV.nri-6B.2*, were mapped at the genetic distance positions of 66–67 cM and 114 cM, respectively. The seventh QTL, *QSLrV.nri-7B*, was identified at 54 cM on 7B. The QTL regions on 2B, 4B, 6B and 7B were detected with both MLM and MLMM, with an additional QTL by MLMM on 6B, while the QTL on 2A and 5A were only identified using MLMM. Again, the most significant SNP from MLMM analysis was IWB35611 on chromosome 4B.

Number of Shoots

Using MLM, three QTL were significantly associated with number of shoots per plant, based on an unadjusted p -value < 0.001 (Figure S3a and Table 2). These QTL, *QSNrV.nri-4B*, *QSNrV.nri-6A* and *QSNrV.nri-7B*, were mapped at 105, 141 and 134 cM positions on chromosomes 4B, 6A and 7B, respectively, and together accounted for 18.3% of the total phenotypic variation of the trait. The MLMM analysis detected the same QTL as the MLM results above (Figure S3b and Table 2).

Shoot Dry Weight

Using MLM, four QTL associated with shoot dry weight per plant were found on chromosomes 1B, 2B, 4A and 7A, according to p -value < 0.001 (Figure S4a and Table 2). *QSDWrV.nri-1B* was mapped at the genetic distance position of 118 cM on 1B, explaining 6.1% of the total phenotypic variation in shoot dry weight per plant. *QSDWrV.nri-2B* was located at 134 cM on 2B, accounting for 7.6% of the phenotypic variation. In addition, two QTL, *QSDWrV.nri-4A* and *QSDWrV.nri-7A*, were detected at 109 and 228 cM on chromosomes 4A and 7A, respectively, and each accounted for about 6.0% of the total phenotypic variation of the trait.

Using MLMM, six QTL significantly associated with shoot dry weight per plant were found on chromosomes 1B, 2B, 4A, 4B, 6A and 7A (Figure S4b and Table 2). On chromosome 1B, *QSDWrV.nri-1B*, represented by nine SNPs, was mapped at 118 cM. A further five QTL, *QSDWrV.nri-2B*, *QSDWrV.nri-4A*, *QSDWrV.nri-4B*, *QSDWrV.nri-6A* and *QSDWrV.nri-7A*, were located at 134, 109, 78, 26 and 228 cM, respectively, on their corresponding chromosomes. Among those, the QTL regions on 1B, 2B, 4A and 7A were detected by both MLM and MLMM.

4. Discussion

Seedling vigor and regrowth vigor are important traits of winter wheat for the increased and sustainable production of autumn-winter forage for cattle grazing. Breeding for improved seedling vigor and regrowth vigor is critical for sustainable forage production of winter wheat. However, the phenotyping of seedling vigor and regrowth vigor is difficult and time-consuming under field conditions. Marker-assisted selection could provide a complementary approach to address this limitation. Therefore, a GWAS was conducted to map QTL and identify SNP markers associated with seedling vigor and regrowth vigor traits for MAS during wheat breeding.

In the present study, two single-locus mixed models, MLM and CMLM, and one multi-locus mixed model, MLMM, were used to map QTL and identify SNP markers associated with seedling vigor and regrowth vigor traits. Morphological traits, such as shoot length, number of shoots and shoot dry weight, have been used previously to evaluate plant or seedling vigor in different crops, including wheat [10] and rice [15,26,27]. In the present study, winter wheat lines in the wheat diversity panel showed significant phenotypic variation in shoot length, number of shoots and shoot dry weight from both the initial growth and the regrowth of the plants. This result suggests that the panel can be used to mine desirable alleles, contributing to seedling vigor and regrowth vigor for forage wheat improvement.

Using MLM and MLMM, we identified multiple significant QTL associated with seedling vigor and regrowth vigor traits, including shoot length, number of shoots per plant and shoot dry weight per plant. Significant QTL for shoot length of initial growth, as a seedling vigor trait, were found on chromosomes 1A, 2B, 4B, 5A and 7A, with more QTL identified using MLMM. Similarly, significant QTL for shoot length of regrowth were found on chromosomes 2A, 2B, 4B, 5A, 6B and 7B. Shoot length has been shown to be positively correlated with coleoptile length, which is essential for early establishment, as reported in wheat [49] and barley [50]. Among the chromosomes with associated QTL in this study, chromosomes 2A, 2B, 4B, and 6B were also reported to harbor QTL for coleoptile length in a different study using the same diversity panel [51]. Furthermore, QTL for coleoptile length were also found on chromosomes 2B, 5A, 6B and 7B in a different wheat panel [49]. Similarly, under growth chamber conditions, nine significant QTL associated with coleoptile length were found on seven chromosomes, including 2B and 4B [51], which were associated with shoot length in this study. Chromosome 4B harbors the *Rht-B1* locus, which has a significant impact on morphological vigor traits [17]. The present study found additional QTL on chromosomes 1A and 7A (Table 2), which were not reported in the aforementioned studies.

A BLAST search revealed that some of the significant SNP markers feature high similarities with candidate genes involved in plant developmental processes in different crops, including wheat (Table S1). For example, on chromosome 1A, the significant SNP IWB43660 for shoot length features a 96% sequence similarity with E3 ubiquitin-protein ligase HERC2-like. This candidate gene plays an important role in plant development [52]. Similarly, on chromosome 4B, significant SNP IWB31302 for shoot length features a 97% sequence similarity with a candidate gene, aminopeptidase M1-B, which plays a role in embryonic, vegetative and reproductive development in *Arabidopsis thaliana* [53]. In addition, the significant SNP IWB8217 for shoot length on chromosome 4B features a 94% sequence similarity with 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, which regulates the early development of chloroplast in rice [54]. Chloroplast is one of the compartments in a plant cell that contains chlorophyll, which positively correlates with shoot length in rice [55].

Furthermore, we found QTL associated with number of shoots per plant of initial growth on chromosomes 2A, 5A and 7A. This result agrees with previous studies on wheat, which demonstrated QTL for number of shoots per plant and coleoptile length on the same chromosomes as found in the present study [17,51]. In addition, in this study, we

detected significant QTL for number of shoots from regrowth on chromosomes 4B, 6A and 7B, which were also mapped with QTL for coleoptile length in wheat [49,51].

This study also found significant QTL for shoot dry weight per plant from initial growth on chromosomes 6A, 7A and 7D, and from regrowth on 1B, 2B, 4A, 4B, 6A and 7A. Previously, QTL for shoot dry weight per plant were also found on chromosomes 4B, 6A and 7D, while chromosomes 2B, 4A, 4B and 6A were reported to harbor QTL associated with coleoptile length in wheat [10,17,49,51].

Overall, a total of 12 significant QTL for seedling vigor traits were found in this study. Shoot length featured the highest number (6) of QTL followed by number of shoots per plant (3) and shoot dry weight per plant (3), spreading across eight chromosomes. Chromosome 7A harbors three QTL for the three seedling vigor traits (Table 2 and Table S1). Increasing the marker density for this chromosome would increase the resolution to unravel genes associated with seedling vigor.

On the other hand, a total of 16 significant QTL on 10 chromosomes for regrowth vigor were found in this study, with the highest number (7) of QTL for shoot length followed by shoot dry weight per plant (6) and number of shoots per plant (3). In addition, three QTL involving all three regrowth vigor traits were commonly mapped on chromosome 4B. Overall, significantly more QTL were found with MLM than with MLMM, suggesting that MLMM increased the power of detecting additional significant QTL and, therefore, that it is the preferred method compared to MLM (Tables 2 and S1).

Furthermore, we identified four common QTL regions on chromosomes 2B, 4B, 5A and 7A for common traits of seedling vigor and regrowth vigor due to significant correlations between the corresponding traits of initial growth and regrowth (Tables 2 and S1). *QSLeV.nri-2B* and *QSLrV.nri-2B* for shoot length of initial growth and regrowth, respectively, were detected by the same SNP IWB36210 at 163 cM on 2B. Furthermore, for shoot length of initial growth and regrowth, *QSLeV.nri-4B.2* and *QSLrV.nri-4B* co-localized at 55–60 cM on chromosome 4B with several common SNP representatives, and *QSLeV.nri-5A* and *QSLrV.nri-5A* co-localized at 86–89 cM on 5A, although they were detected by different SNPs (IWB43526 vs. IWB38719). Similarly, for shoot dry weight of initial growth and regrowth, *QSDWeV.nri-7A* and *QSDWrV.nri-7A* were detected by the same SNP IWB42636 at 228 cM on chromosome 7A.

In addition, two common QTL regions were also detected for different traits between initial growth and regrowth. *QSNeV.nri-2A* for number of shoots per plant of initial growth was mapped with *QSLrV.nri-2A* for shoot length of regrowth at 126–143 cM on 2A. In addition, *QSDWeV.nri-6A* for shoot dry weight per plant of initial growth co-localized with *QSNrV.nri-6A* for number of shoots per plant of regrowth at 141 cM on 6A. The SNP markers tightly linked to these common QTL regions associated with traits of both seedling vigor and regrowth vigor are more important for MAS during forage wheat breeding.

5. Conclusions

This study identified QTL for seedling vigor and regrowth vigor traits on different chromosomes, spreading across the genome. Some QTL for seedling vigor traits were found on the same chromosomes previously reported to harbor QTL for seedling vigor in wheat. In addition, several QTL for seedling vigor co-localized with QTL for regrowth vigor due to significant correlations between corresponding traits of the initial growth and regrowth. A BLAST search using the DNA sequences of significant loci found in the present study revealed candidate genes known to play a role in vegetative, reproductive and physiological processes essential for plant development in different crop species. The QTL and SNP markers identified in this study will be further validated and used for MAS of the traits during wheat breeding.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/crops1030015/s1>. Figure S1: Genome-wide linkage disequilibrium decay curve for the panel; Figure S2: Manhattan plots of shoot length of regrowth detected by (a) MLM and (b) MLMM; Figure S3: Manhattan plots of number of shoots of regrowth detected by (a) MLM and (b) MLMM;

Figure S4: Manhattan plots of shoot dry weight of regrowth detected by (a) MLM and (b) MLMM; Table S1: BLAST hits of the significant SNPs detected in this study.

Author Contributions: Formal analysis, F.M.; investigation, F.M. and X.-F.M.; resources, W.H., J.D.A. and X.-F.M.; data curation, F.M., W.H., J.D.A. and T.T.K.; writing—original draft preparation, F.M.; writing—review and editing, F.M. and X.-F.M.; supervision, X.-F.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors sincerely thank Marilyn Cummins and Neal Fandek for critical reading of the manuscript. We thank the Triticeae Coordinated Agricultural Project (TCAP) for making the genotypic data publicly available. This study was supported by the Noble Research Institute, LLC.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Li, X.-M.; Chen, X.-M.; Xiao, Y.-G.; Xia, X.-C.; Wang, D.-S.; He, Z.-H.; Wang, H.-J. Identification of QTLs for seedling vigor in winter wheat. *Euphytica* **2014**, *198*, 199–209. [\[CrossRef\]](#)
- Botwright, T.; Condon, A.; Rebetzke, G.; Richards, R. Field evaluation of early vigour for genetic improvement of grain yield in wheat. *Aust. J. Agric. Res.* **2002**, *53*, 1137–1145. [\[CrossRef\]](#)
- ter Steege, M.W.; den Ouden, F.M.; Lambers, H.; Stam, P.; Peeters, A.J. Genetic and physiological architecture of early vigor in *Aegilops tauschii*, the D-genome donor of hexaploid wheat. A quantitative trait loci analysis. *Plant Physiol.* **2005**, *139*, 1078–1094. [\[CrossRef\]](#)
- Rebetzke, G.; Bruce, S.; Kirkegaard, J. Longer coleoptiles improve emergence through crop residues to increase seedling number and biomass in wheat (*Triticum aestivum* L.). *Plant Soil* **2005**, *272*, 87–100. [\[CrossRef\]](#)
- Maulana, F.; Anderson, J.D.; Butler, T.J.; Ma, X.-F. Improving dual-purpose winter wheat in the southern Great Plains of the United States. In *Recent Advances in Grain Crops Research*; Shah, F., Khan, Z., Iqbal, A., Turan, M., Olgun, M., Eds.; IntechOpen: London, UK, 2019; pp. 1–16.
- Kumssa, T.T.; Anderson, J.D.; Butler, T.J.; Ma, X.-F. Small grains as winter pasture in the Southern Great Plains of the United States. In *Grasses and Grassland Aspects*; Kindomihou, V.M., Ed.; IntechOpen: London, UK, 2019; pp. 1–13.
- Collard, B.C.; Jahufer, M.; Brouwer, J.; Pang, E. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica* **2005**, *142*, 169–196. [\[CrossRef\]](#)
- Spielmeyer, W.; Hyles, J.; Joaquim, P.; Azanza, F.; Bonnett, D.; Ellis, M.; Moore, C.; Richards, R. A QTL on chromosome 6A in bread wheat (*Triticum aestivum*, L.) is associated with longer coleoptiles, greater seedling vigour and final plant height. *Theor. Appl. Genet.* **2007**, *115*, 59–66. [\[CrossRef\]](#)
- Landjeva, S.; Lohwasser, U.; Börner, A. Genetic mapping within the wheat D genome reveals QTL for germination, seed vigour and longevity, and early seedling growth. *Euphytica* **2010**, *171*, 129–143. [\[CrossRef\]](#)
- Yang, Y.; Wan, H.; Yang, F.; Xiao, C.; Li, J.; Ye, M.; Chen, C.; Deng, G.; Wang, Q.; Li, A. Mapping QTLs for enhancing early biomass derived from *Aegilops tauschii* in synthetic hexaploid wheat. *PLoS ONE* **2020**, *15*, 1–17. [\[CrossRef\]](#)
- Maydup, M.L.; Graciano, C.; Guiamet, J.J.; Tambussi, E.A. Analysis of early vigour in twenty modern cultivars of bread wheat (*Triticum aestivum* L.). *Crop Pasture Sci.* **2013**, *63*, 987–996. [\[CrossRef\]](#)
- Moore, C.; Rebetzke, G. Genomic regions for embryo size and early vigour in multiple wheat (*Triticum aestivum* L.) populations. *Agronomy* **2015**, *5*, 152–179. [\[CrossRef\]](#)
- Bai, C.; Liang, Y.; Hawkesford, M.J. Identification of QTLs associated with seedling root traits and their correlation with plant height in wheat. *J. Exp. Bot.* **2013**, *64*, 1745–1753. [\[CrossRef\]](#) [\[PubMed\]](#)
- Upadhyaya, H.D.; Wang, Y.-H.; Sastry, D.V.; Dwivedi, S.L.; Prasad, P.V.; Burrell, A.M.; Klein, R.R.; Morris, G.P.; Klein, P.E. Association mapping of germinability and seedling vigor in sorghum under controlled low-temperature conditions. *Genome* **2016**, *59*, 137–145. [\[CrossRef\]](#) [\[PubMed\]](#)
- Singh, U.M.; Yadav, S.; Dixit, S.; Ramayya, P.J.; Devi, M.N.; Raman, K.A.; Kumar, A. QTL hotspots for early vigor and related traits under dry direct-seeded system in rice (*Oryza sativa* L.). *Front. Plant Sci.* **2017**, *8*, 286–300. [\[CrossRef\]](#)
- Landjeva, S.; Neumann, K.; Lohwasser, U.; Börner, A. Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. *Biol. Plant.* **2008**, *52*, 259–266. [\[CrossRef\]](#)
- Iannucci, A.; Marone, D.; Russo, M.A.; De Vita, P.; Miullo, V.; Ferragonio, P.; Blanco, A.; Gadaleta, A.; Mastrangelo, A.M. Mapping QTL for root and shoot morphological traits in a Durum Wheat × *T. dicoccum* segregating population at seedling stage. *Int. J. Genom.* **2017**, *2017*, 1–17. [\[CrossRef\]](#) [\[PubMed\]](#)

18. Sun, J.-J.; Guo, Y.; Zhang, G.-Z.; Gao, M.-G.; Zhang, G.-H.; Kong, F.-M.; Zhao, Y.; Li, S.-S. QTL mapping for seedling traits under different nitrogen forms in wheat. *Euphytica* **2013**, *191*, 317–331. [[CrossRef](#)]
19. Huang, X.; Han, B. Natural variations and genome-wide association studies in crop plants. *Annu. Rev. Plant Biol.* **2014**, *65*, 531–551. [[CrossRef](#)]
20. Maulana, F.; Ayalew, H.; Anderson, J.D.; Kumssa, T.T.; Huang, W.; Ma, X.-F. Genome-wide association mapping of seedling heat tolerance in winter wheat. *Front. Plant Sci.* **2018**, *9*, 1272–1287. [[CrossRef](#)] [[PubMed](#)]
21. Ayalew, H.; Liu, H.; Börner, A.; Kobiljski, B.; Liu, C.; Yan, G. Genome-wide association mapping of major root length QTLs under PEG induced water stress in wheat. *Front. Plant Sci.* **2018**, *9*, 1759–1768. [[CrossRef](#)] [[PubMed](#)]
22. Gahlaut, V.; Jaiswal, V.; Singh, S.; Balyan, H.; Gupta, P. Multi-locus genome wide association mapping for yield and its contributing traits in hexaploid wheat under different water regimes. *Sci. Rep.* **2019**, *9*, 1–15. [[CrossRef](#)]
23. Maulana, F.; Huang, W.; Anderson, J.D.; Ma, X.-F. Genome-wide association mapping of seedling drought tolerance in winter wheat. *Front. Plant Sci.* **2020**, *11*, 573786. [[CrossRef](#)] [[PubMed](#)]
24. Chopra, R.; Burow, G.; Burke, J.J.; Gladman, N.; Xin, Z. Genome-wide association analysis of seedling traits in diverse Sorghum germplasm under thermal stress. *BMC Plant Biol.* **2017**, *17*, 12–27. [[CrossRef](#)] [[PubMed](#)]
25. Marla, S.R.; Burow, G.; Chopra, R.; Hayes, C.; Olatoye, M.O.; Felderhoff, T.; Hu, Z.; Raymundo, R.; Perumal, R.; Morris, G.P. Genetic architecture of chilling tolerance in sorghum dissected with a nested association mapping population. *G3 Genes Genomes Genet.* **2019**, *9*, 4045–4057. [[CrossRef](#)] [[PubMed](#)]
26. Chen, K.; Zhang, Q.; Wang, C.-C.; Liu, Z.-X.; Jiang, Y.-J.; Zhai, L.-Y.; Zheng, T.-Q.; Xu, J.-L.; Li, Z.-K. Genetic dissection of seedling vigour in a diverse panel from the 3,000 Rice (*Oryza sativa* L.) Genome Project. *Sci. Rep.* **2019**, *9*, 1–15. [[CrossRef](#)]
27. Cordero-Lara, K.I.; Kim, H.; Tai, T.H. Identification of seedling vigor-associated quantitative trait loci in temperate japonica rice. *Plant Breed. Biotechnol.* **2016**, *4*, 426–440. [[CrossRef](#)]
28. Yu, J.; Zhao, W.; Tong, W.; He, Q.; Yoon, M.-Y.; Li, F.-P.; Choi, B.; Heo, E.-B.; Kim, K.-W.; Park, Y.-J. A Genome-wide association study reveals candidate genes related to salt tolerance in rice (*Oryza sativa*) at the germination stage. *Int. J. Mol. Sci.* **2018**, *19*, 3145. [[CrossRef](#)]
29. Abdel-Ghani, A.H.; Sharma, R.; Wabila, C.; Dhanagond, S.; Owais, S.J.; Duwayri, M.A.; Al-Dalain, S.A.; Klukas, C.; Chen, D.; Lübberstedt, T. Genome-wide association mapping in a diverse spring barley collection reveals the presence of QTL hotspots and candidate genes for root and shoot architecture traits at seedling stage. *BMC Plant Biol.* **2019**, *19*, 216–235. [[CrossRef](#)]
30. Sharma, R.; Draicchio, F.; Bull, H.; Herzig, P.; Maurer, A.; Pillen, K.; Thomas, W.T.; Flavell, A.J. Genome-wide association of yield traits in a nested association mapping population of barley reveals new gene diversity for future breeding. *J. Exp. Bot.* **2018**, *69*, 3811–3822. [[CrossRef](#)]
31. Wang, Q.-J.; Yuan, Y.; Liao, Z.; Jiang, Y.; Wang, Q.; Zhang, L.; Gao, S.; Wu, F.; Li, M.; Xie, W. Genome-wide association study of 13 traits in Maize seedlings under low phosphorus stress. *Plant Genome* **2019**, *12*, 1–13. [[CrossRef](#)]
32. Hu, G.; Li, Z.; Lu, Y.; Li, C.; Gong, S.; Yan, S.; Li, G.; Wang, M.; Ren, H.; Guan, H. Genome-wide association study identified multiple genetic loci on chilling resistance during germination in maize. *Sci. Rep.* **2017**, *7*, 1–11. [[CrossRef](#)]
33. Yu, J.; Pressoir, G.; Briggs, W.H.; Bi, I.V.; Yamasaki, M.; Doebley, J.F.; McMullen, M.D.; Gaut, B.S.; Nielsen, D.M.; Holland, J.B. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat. Genet.* **2006**, *38*, 203–208. [[CrossRef](#)]
34. Zhang, Z.; Ersoz, E.; Lai, C.-Q.; Todhunter, R.J.; Tiwari, H.K.; Gore, M.A.; Bradbury, P.J.; Yu, J.; Arnett, D.K.; Ordovas, J.M. Mixed linear model approach adapted for genome-wide association studies. *Nat. Genet.* **2010**, *42*, 355–360. [[CrossRef](#)]
35. Bradbury, P.J.; Zhang, Z.; Kroon, D.E.; Casstevens, T.M.; Ramdoss, Y.; Buckler, E.S. TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics* **2007**, *23*, 2633–2635. [[CrossRef](#)] [[PubMed](#)]
36. Li, C.; Fu, Y.; Sun, R.; Wang, Y.; Wang, Q. Single-locus and multi-locus genome-wide association studies in the genetic dissection of fiber quality traits in upland cotton (*Gossypium hirsutum* L.). *Front. Plant Sci.* **2018**, *9*, 1083–1099. [[CrossRef](#)] [[PubMed](#)]
37. Wang, S.-B.; Feng, J.-Y.; Ren, W.-L.; Huang, B.; Zhou, L.; Wen, Y.-J.; Zhang, J.; Dunwell, J.M.; Xu, S.; Zhang, Y.-M. Improving power and accuracy of genome-wide association studies via a multi-locus mixed linear model methodology. *Sci. Rep.* **2016**, *6*, 19444–19454. [[CrossRef](#)]
38. Segura, V.; Vilhjálmsson, B.J.; Platt, A.; Korte, A.; Seren, Ü.; Long, Q.; Nordborg, M. An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. *Nat. Genet.* **2012**, *44*, 825–833. [[CrossRef](#)] [[PubMed](#)]
39. Guttieri, M.J.; Baenziger, P.S.; Frels, K.; Carver, B.; Arnall, B.; Waters, B.M. Variation for grain mineral concentration in a diversity panel of current and historical Great Plains hard winter wheat germplasm. *Crop Sci.* **2015**, *55*, 1035–1052. [[CrossRef](#)]
40. SAS Institute. The SAS System for Windows. 9.3. SAS Institute, Cary, NC, USA. Available online: <http://support.sas.com/software/93/> (accessed on 16 May 2011).
41. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: <https://www.R-project.org/> (accessed on 20 August 2016).
42. Peterson, B.G.; Carl, P.; Boudt, K.; Bennett, R.; Ulrich, J.; Zivot, E.; Cornilly, D.; Hung, E.; Lestel, M.; Balkissoon, K. Package ‘PerformanceAnalytics’. Available online: <https://cran.r-project.org/web/packages/PerformanceAnalytics/> (accessed on 15 April 2018).

43. Wang, S.; Wong, D.; Forrest, K.; Allen, A.; Chao, S.; Huang, B.E.; Maccaferri, M.; Salvi, S.; Milner, S.G.; Cattivelli, L. Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotech. J.* **2014**, *12*, 787–796. [[CrossRef](#)]
44. IWGSC. Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science* **2018**, *361*, eaar7191. [[CrossRef](#)]
45. Lipka, A.E.; Tian, F.; Wang, Q.; Peiffer, J.; Li, M.; Bradbury, P.J.; Gore, M.A.; Buckler, E.S.; Zhang, Z. GAPIT: Genome association and prediction integrated tool. *Bioinformatics* **2012**, *28*, 2397–2399. [[CrossRef](#)]
46. Sukumaran, S.; Reynolds, M.P.; Sansaloni, C. Genome-wide association analyses identify QTL hotspots for yield and component traits in durum wheat grown under yield potential, drought, and heat stress environments. *Front. Plant Sci.* **2018**, *9*, 1–16. [[CrossRef](#)]
47. Turner, S.D. qqman: An R package for visualizing GWAS results using Q-Q and Manhattan plots. *J. Open Source Softw.* **2018**, *3*, 731. [[CrossRef](#)]
48. Alaux, M.; Rogers, J.; Letellier, T.; Flores, R.; Alfama, F.; Pommier, C.; Mohellibi, N.; Durand, S.; Kimmel, E.; Michotey, C. Linking the International Wheat Genome Sequencing Consortium bread wheat reference genome sequence to wheat genetic and phenomic data. *Genome Biol.* **2018**, *19*, 111–120. [[CrossRef](#)] [[PubMed](#)]
49. Ma, J.; Lin, Y.; Tang, S.; Duan, S.; Wang, Q.; Wu, F.; Li, C.; Jiang, X.; Zhou, K.; Liu, Y. A genome-wide association study of coleoptile length in different Chinese wheat landraces. *Front. Plant Sci.* **2020**, *11*, 677. [[CrossRef](#)]
50. Luo, H.; Hill, C.B.; Zhou, G.; Zhang, X.-Q.; Li, C. Genome-wide association mapping reveals novel genes associated with coleoptile length in a worldwide collection of barley. *BMC Plant Biol.* **2020**, *20*, 346–359. [[CrossRef](#)]
51. Sidhu, J.S.; Singh, D.; Gill, H.S.; Brar, N.K.; Qiu, Y.; Halder, J.; Al Tameemi, R.; Turnipseed, B.; Sehgal, S.K. Genome-wide association study uncovers novel genomic regions associated with coleoptile length in hard winter wheat. *Front. Genet.* **2019**, *10*, 1345–1358. [[CrossRef](#)] [[PubMed](#)]
52. Shu, K.; Yang, W. E3 ubiquitin ligases: Ubiquitous actors in plant development and abiotic stress responses. *Plant Cell Physiol.* **2017**, *58*, 1461–1476. [[CrossRef](#)]
53. Hosein, F.N.; Bandyopadhyay, A.; Peer, W.A.; Murphy, A.S. The catalytic and protein-protein interaction domains are required for APM1 function. *Plant Physiol.* **2010**, *152*, 2158–2172. [[CrossRef](#)]
54. Liu, X.; Cao, P.; Huang, Q.; Yang, Y.; Tao, D. Disruption of a rice chloroplast-targeted gene OsHMBPP causes a seedling-lethal albino phenotype. *Rice* **2020**, *13*, 1–11. [[CrossRef](#)]
55. Fukuda, A.; Terao, T. QTLs for shoot length and chlorophyll content of rice seedlings grown under low-temperature conditions, using a cross between indica and japonica cultivars. *Plant Prod. Sci.* **2015**, *18*, 128–136. [[CrossRef](#)]