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Original Article

Identification of QTLs associated with grain-filling duration and heading date in wheat

Identificação de QTLs associados à duração do enchimento do grão e à data de formação do espigão no trigo

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Abstract

An increase in genetic diversity of bread wheat caused by spring x winter forms leads to an alteration of genetic control of maturity time. Maturity time (MAT) is one of major yield components in wheat, which has two components: the heading date (HD) and grain-filling period (GFP). Using the Illumina Infinium 25k platform we analyzed the genetic control of the HD, GFP and MAT in the F_2 and $F_{2:3}$ populations from a cross between late-ripening spring/winter line 124-1 and spring wheat cultivar Novosibirskaya 31, possessing the same allelic composition of the *VRN1* and *PPD-D1* genes. The phenotypic evaluation of the populations studied was performed during three years. A total of 17 QTLs were mapped, out of which 4 QTLs for MAT or its components were confirmed over two years. Two common MAT and HD QTLs were identified on the 4A chromosome, and two loci controlling GFP and MAT were found on 6B chromosome. An environmentally stable HD QTL *QHd.icg-7B.1* was associated with the *FT-B1* gene having a non-synonymous polymorphism [G/C] in its coding region. A novel HD QTL was identified on 7D chromosome. QTL dissection allowed to propose putative genes for *QMat.icg4-A* and *QMat.icg6-B*, namely the *SPL* family gene (*TraesCS4A02G359500)* and the TCP transcription factor (*TraesCS6B02G462100)*, respectively. The results of this study provide information for further investigation into wheat development.

Keywords: common wheat, heading time, grain-filling, QTL mapping.

Resumo

O aumento na diversidade genética do trigo de pão, causado pela combinação de variedades de primavera e inverno, leva a uma alteração no controle genético do tempo de maturação. O tempo de maturação (MAT) é um dos principais componentes do rendimento do trigo, composto por dois elementos: a data de início (HD) e o período de enchimento do grão (GFP). Utilizando a plataforma Illumina Infinium 25k, analisamos o controle genético de HD, GFP e MAT nas populações F2 e F2:3 resultantes de um cruzamento entre a linhagem de primavera/inverno de maturação tardia 124-1 e a cultivar de trigo de primavera Novosibirskaya 31, que possuem a mesma composição alélica dos genes *VRN1* e *PPD-D1*. A avaliação fenotípica das populações estudadas foi realizada ao longo de três anos. Mapeamos um total de 17 QTLs, dos quais 4 QTLs relacionados ao MAT ou seus componentes foram confirmados ao longo de dois anos. Identificamos dois QTLs comuns para MAT e HD no cromossomo 4A, e dois loci controladores de GFP e MAT foram encontrados no cromossomo 6B. Um QTL HD ambientalmente estável, *QHd.icg-7B.1*, foi associado ao gene *FT-B1*, que possui um polimorfismo não sinônimo [G/C] em sua região codificadora. Um novo QTL HD foi identificado no cromossomo 7D. A análise dos QTL permitiu propor genes putativos para *QMat.icg4-A* e *QMat.icg6-B*, nomeadamente o gene da família SPL (*TraesCS4A02G359500*) e o fator de transcrição TCP (*TraesCS6B02G462100*), respectivamente. Os resultados deste estudo fornecem informações para investigações posteriores sobre o desenvolvimento do trigo.

Palavras-chave: trigo comum, data de início, período de enchimento do grão, QTL.

1. Introduction

Wheat development duration has a significant impact on its yield and is one of the most important characteristics of cultivated bread wheat varieties (*T. aestivum* L.). Since the growing regions in the world are a set of diverse environments, it is important to adjust the duration of wheat growth phases. The main phenophases contributing to wheat development duration are the days to heading (HD) and the days from heading to maturity, or the grain-filling period (GFP) that together comprise the maturity time (MAT). The duration is also influenced by wheat's

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genotype, sowing time and environmental conditions. Abiotic stresses (drought, low temperatures) occurring while heading and flowering have a negative impact on the grain number and lead to a decrease in yield (Brisson et al., 2010; Flohr et al., 2017). As for the sowing time, it depends on climatic factors and weather conditions, and changing its date can affect the duration of the developmental phases (Farhad et al., 2022; Khan et al., 2022; Tulayev et al., 2022).

The genetic factors affecting the HD flowering time (FLT) have been extensively studied in the last decades to demonstrate that the transition to heading and flowering in wheat is mainly controlled by vernalization requirement (*VRN*) and photoperiod sensitivity (*PPD*) loci, and earliness loci per se (Worland et al., 1998; Snape et al., 2001; Kamran et al., 2014).

Homoeologous *VRN1* genes (*Vrn-A1*, *Vrn-B1*, *Vrn-D1*) are located on chromosome group 5 (Yan et al., 2003). The recessive alleles at the all three *VRN1* loci determine the winter growth habit requiring an extended exposure to low temperature for flowering (Trevaskis et al., 2003; Yan et al., 2003). The *VRN1* down-regulate *VRN2* being the central flowering repressor (Yan et al., 2003). The *VRN2* affects flowering time by suppressing the expression of the *VRN3* (*FT*) gene (Yan et al., 2006), which is activated under long days and vernalization. Although *VRN3* genetic variability is low, several dominant alleles of the gene influencing FLT have been described (Yan et al., 2006; Chen et al., 2013; Berezhnaya et al., 2021).

The *PPD1* loci determine sensitivity to photoperiod. Long-day plants such as wheat start to flowering when the day length exceeds a certain threshold (called the critical day length), while short-day plants flower when daylight hours shorten. Wheat varieties can be responsive or irresponsive to photoperiod based on the allelic state at the loci. Of the three homoeologs located on chromosomes 2A, 2B and 2D, it is the *PPD-D1* gene that influences photoperiod sensitivity the most (Scarth and Law, 1984). While the photoperiod insensitive varieties possess a dominant *Ppd-D1a* allele, the photoperiod sensitive ones possess a recessive *Ppd-D1b* allele (Beales et al., 2007), so *PPD1* in wheat influences both flowering and maturity (Kamran et al., 2013; Chen et al., 2013; Perez-Lara et al., 2016).

Unlike the heading/flowering time that have been extensively investigated, including the effects of vernalization and photoperiod, the GFP has not been given that much attention. It is known that the GFP and FLT are influenced by independent genetic systems (May and van Sanford, 1992; Kajimura et al., 2011). Nguyen et al. (2015) observed that the GFP of the late-flowering individuals with recessive *Ppd-D1b* allele was significantly shorter than that in the early-flowering individuals with dominant allele. The GFP negatively correlated with heading time (HT), FLT and MAT in the two F_2 populations studied. Several other studies obtained similar results for wheat's vegetative period (days from sowing to anthesis) and days to ear emergence (Knott and Gebeyehou, 1987; Monpara, 2011; Kajimura et al., 2011).

There are few loci known to influence GFP in spring wheat. The *GRAIN PROTEIN CONTENT-1* or the *No Apical Meristem* (*GPC1*, *NAM1*) genes have a pleiotropic effect on the GFP and concentrations of protein and minerals (Uauy et al., 2006a, b; Cormier et al., 2015; Alhabbar et al., 2018a). The *NAM1* genes are located on chromosome group 6 and encode a NAC transcription factor (Uauy et al., 2006a; Avni et al., 2014). The wild-type allele of *NAM-B1* shortens grain filling due to earlier flag senescence (Uauy et al., 2006b). The *NAM-A1* homoeologue is also associated with GFP (Cormier et al., 2015; Alhabbar et al., 2018b; Harrington et al., 2019). Alhabbar et al. (2018b) concluded the varieties carrying functional copies of *NAM1* have shorter GFP and slightly lower yields. The *NAM-A1* alleles *c* and *d* in combination with the non-functional *NAM-B1* allele resulted in high-yielding phenotypes with prolonged GFP. Wang et. al. identified six QTLs for GFP on chromosomes 1A, 3B, 5D and 6D (Wang et al., 2009). The strongest QTL on chromosome 3B explained up to 15.72% of the phenotypic variation, though its GFP heritability was lower than those of the other traits studied (16.6%).

Early maturity is an important objective in breeding when one needs to adapt a wheat variety to shorter growing seasons. On the other hand, the kernel size and weight are determined during GFP, and the shorter duration of grain filling may negatively affect the yield. It has been demonstrated that in winter wheat, early heading and a prolonged GFP may result in higher yields due to evaded drought stress (Yang et al., 2019). At the same time, the development of early maturing genotypes is important to resist the frost and preharvest rain damage common for some growing regions (Iqbal et al., 2007; Kajimura et al., 2011). In other words, having wheat cultivars whose flowering/maturity times fit a given environment is highly important.

Cultivating the most suitable genotypes and adjusting the sowing date result in higher yield. That is why it is essential to discover new loci/genes determining the growth periods, especially those related with GFP, since the genetic factors determining this trait have not been sufficiently investigated.

Expanding the genetic diversity of wheat through various crossbreeding options leads to changing in maturity time. This can be predicted based on the major genetic factors contributing to this process (SB RAS, 2023). At the same time, when creating new wheat genotypes from crossing winter and spring forms, we have previously shown that the spring descendants of such a crossing (spring/winter lines), despite the presence of the same set of *VRN1* and *PPD-D1* genes, differ significantly (from 3 to 8 days, depending on growing conditions) from the original spring parental form (Stasyuk et al., 2017).

The present study was performed to identify novel QTLs controlling HD, GFD and MAT using the F_2 population derived from a cross between the late-ripening spring/winter line 124-1 and Novosibirskaya 31 (N31). The parental varieties carried the same allelic composition of the *VRN1*, *PPD1* and *NAM1* genes, yet had substantial differences in their heading and grain-filling periods. Therefore, such a germplasm recourse can be successfully utilized to dissect the novel loci and candidate genes for the abovementioned traits.

2. Materials and Methods

2.1. Plant materials and phenotyping

The F_2 mapping population was generated from a cross between late-ripening spring/winter line 124-1 and bread wheat cultivar Novosibirskaya 31. L124-1 obtained from a cross between winter wheat cultivar Filatovka and spring wheat variety Tulaikovskaya 10 (Stasyuk et al., 2017). The parental wheat varieties possessed the same allelic composition of the *VRN1* (*Vrn-A1a, vrn-B1, vrn-D1)* and *PPD-D1* genes, however L124-1 was characterized by later heading date and maturity times. One hundred and seventeen F_2 seeds along with parental cultivars were grown in a glasshouse under long day (16-hour day length) in 2019, the temperature was controlled at 18-20 °C.

Phenotyping of the $F_{2:3}$ families under field conditions was conducted in 2020 and 2021 in the experimental field of Institute of Cytology and Genetics of Siberian Branch of the Russian Academy of Sciences in Novosibirsk (54.9133° N, 82.9763° E). Each family was divided into two equal parts of approximately 18 seeds, one part was sown in 2020, and the other — in 2021, in two-rows plots of 50 cm in width with 30-cm spacing between the rows. The seeds were sown on the 10th and 12th of May, respectively.

The HD was measured as the number of days from seedling emergence (GS07) to the date of ½ of head emergence (GS55) using the Zadoks decimal growth scale (Zadoks et al., 1974). The MAT was determined when the glumes of main spike could no longer be dented by a thumbnail (GS92). The GFP was estimated as a difference between the MAT and HD.

In the 2020 and 2021 growing seasons the average monthly air temperature in May was higher than the long-term average (12.5 °C) by 3.1 and 1.6 °C, respectively. At the same time, the amount of precipitation in 2020 was 55% higher than the long-term average (34 mm), unlike 2021, whose precipitation was 26% below the norm. In June, the observation-period temperature was 2 °C lower than the long-term value (18.2 °C). In June 2020, 24.5 mm of precipitation fell to be 41% below the norm (59 mm). In June 2021, the amount of precipitation was 24% higher than the long-term value. The month of July was the period of heading and the beginning of GFP. The temperature of this month in 2020 and 2021 was in line with the long-term average (20.2 °C). The amount of precipitation in 2020 was 23% above the norm (69 mm). In July 2021, there was a precipitation deficit of 67% below the norm (only 22.4 mm rained). In August, the GFP continued and ripening began. Its average monthly temperature during the years of observation did not differ and amounted to 18.5 °C, which was 0.9 °C higher than the long-term average (17.6 °C). The amount of precipitation in 2020 and 2021 was above the long-term norm (53 mm) and amounted to 82.9 mm and 67.3 mm, respectively. In general, the weather conditions in 2020 were more favorable for spring wheat development than those of 2021. The long-term average temperature and precipitation data have been obtained from Hydrometeorological Center of Russia (2023). Actual monthly averages of temperature and precipitation in 2020 and 2021 were calculated according to the website Pogoda i Klimat (2023).

2.2. Analysis of allelic variation at the Ppd-D1, VRN1 and NAM1 loci

Allelic variation at the *PPD-D1* and *VRN1* loci in parental lines was determined using previously reported specific primers as described in [13]. The genotyping of *NAM-A1* allele was performed as described in Leonova et al. (2022).

2.3. Linkage maps construction and QTL analysis

The F_2 mapping population of 78 genotypes and two parents were genotyped with the Illumina Infinium 25k Wheat array (TraitGenetics, Germany). Genetic linkage map was constructed using the MultiPoint Ultra-dense software (MultiQTL Ltd., Israel) (Ronin et al., 2017). Prior to mapping, the markers with high missing data (>10%) and high segregation distortion (χ^2>6.4) had been eliminated. Clustering was performed at a recombination fraction (rf) threshold of 0.27. The Kosambi mapping function was used to convert the recombination fractions into centimorgans (cM). The markers violating the map's stability were detected through jackknife resampling and removed. The chromosomal assignment of linkage groups was based on the CerealsDB database (www.cerealsdb.uk.net).

The QTLs were detected based on the phenotyping results for the F_2 and $F_{2:3}$ populations in two environments by means of multiple interval mapping (MIM) using the MultiQTL software v.2.6 (MultiQTL Ltd., Israel). Thresholds for the logarithm of odds (LOD) values were estimated by computing a 1000 permutation test. The genetic maps were drawn in MapChart v.2.32 (WUR, 2023).

2.4. Identification of possible candidate genes

The physical intervals of the QTLs were identified using the flaking markers from IWGSC RefSeq v2.1. Functional annotation for the genes of interest were assigned according to IWGSC annotation v1.1. The expression profiles were analyzed using the wheat expVIP expression platform (Ramirez-González and Afshar, 2023) and Wheat eFP Browser (University of Toronto, 2023), applying the data from the developmental time-course of Azhurnaya (Ramírez-Gonzalez et al., 2018).

2.5. Statistical analysis

Analysis of the variance and correlation tests was carried out in RStudio with R v. 4.1.3. The broad-sense heritability was calculated as $H^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$, where σ_g^2 is the genetic variance, σ_e^2 is the environmental variance. The Shapiro-Wilk test was applied to assess the phenotypic data normality. The phenotypic values were compared using the Mann-Whitney U-test and Student's t-test. The correlations among the examined traits were estimated based on Pearson's correlations.

3. Results

3.1. Phenotypic data

In the glasshouse, parental varieties L124-1 and N 31 substantially differed in MAT and GFP for L124-1 maturing 7.5 days later ($p < 0.0001$). At the same time, the difference

in heading time between parents were only 0.8 days ($p < 0.05$). The F_2 progenies headed between 27 and 37 days and matured between 63 and 78 days (Table 1). The HD ($p < 0.0001$), GFP ($p < 0.0001$) and MAT ($p < 0.0001$) were significantly longer under field conditions than in the glasshouse (Table 1). In the glasshouse, the mean values of HD, GFP and MAT were higher in L124-1 than in N 31. The parents had greater differences in their HD under field conditions than in the glasshouse. All variables exhibited normal distribution in the F_2 and $F_{2:3}$ populations.

Both HD ($r = 0.64$, $p < 0.0001$) and GFP ($r = 0.81$, $p < 0.0001$) significantly correlated with MAT, but no correlation was found between HD and GFP. Broad sense heritability was 0.59 for HD, 0.90 for GFP, 0.83 for MAT.

A moderate negative correlation was found between HD and GFP in the $F_{2:3}$ population for both growing seasons r = -0.32 (2020), and r = -0.28 (2021). MAT was positively correlated with HD r = 0.62 (2020), r = 0.65 (2021) and GFP r = 0.53 (2020), r = 0.55 (2021). The p-value of all the statistical tests was < 0.0001.

3.2. F_2 genetic mapping

In this study we analyzed 78 plants of the L124-1/N 31 F_2 mapping population. Out of 24,146 SNP markers analyzed 6,752 were found to be polymorphic. After filtering out those with over 10% missing data and showing segregation distortion, 4,774 SNP markers were retained. A skeleton map, consisting of 814 markers, spanned a total length of 2,368 cM with average distance of 3.1 cM between the markers to. These markers formed 25 linkage groups (LG) to be assigned to 18 chromosomes, except for 3D, 4D, and 6D ones (Supplementary Material, Table S1). The linkage group length ranged from 8.5 cM (LG 17, chr. 5D) to 212.9 cM (LG 15, chr. 5A). The average distance between markers varied between 1.18 cM (LG 17, chr. 5D) and 8.77 cM (LG 6, chr. 2B).

3.3. QTL Identification

Multiple interval mapping identified a total of 17 QTLs associated with the three traits (HD, GFP and MAT) for all the environments. These QTLs were mapped on chromosomes 1D, 2A, 2B, 4A, 4B, 6A, 6B, 7B, 7D (Figure 1, Table 2).

Of the 17 loci identified, 12 had a LOD score higher than 3.0, therefore, were regarded as major.

The highest number of QTLs was detected for HD. In total, nine QTLs controlling HD on chromosomes 2A, 2B, 4A, 6B, 7B and 7D were identified. The phenotypic variance explained by these loci ranged from 9% (*QHd.icg-7B.2*) to 21% (*QHd.icg-6B*). Eight out of the nine QTLs were major, individually explaining from 10 to 21% of the phenotypic variance. The *QHd.icg-6B* locus on chromosome 6B had the highest phenotypic variation but was only significant in 2020 (Figure 1). Locus *QHd.icg-7B.1* on chromosome 7B was mapped based on the results of trait evaluation in both F_2 and $F_{2:3}$ generations, exhibiting 14% and 9% of the phenotypic variance, respectively (Figure 1).

Only two loci (*QGfp.icg-6A* and *QGfp.icg-6B*) were found to be associated with GFP both identified in 2021 on chromosomes 6A and 6B, explaining 21 and 14% phenotypic variance, respectively.

For MAT, seven QTLs were identified on chromosomes 1D, 4A, 4B, 5A, 6A, 6B, 7B, individually explaining from 14 to 23% of the phenotypic variance.

Additionally, the QTLs responsible for two different traits were found at the same or closely linked regions. The loci associated with HD and MAT were identified

Table 1. Phenotypic measurements (mean, standard error, range) of the F_2 population and parental cultivars under glasshouse conditions and F_3 population and parental cultivars under field conditions.

	HD (days)	GFP (days)	MAT (days)
L124-1 // N31 F_2 (glasshouse 2019)			
L124-1	35.1 ± 0.3*	41.5 ± 0.2***	76.6 ± 0.3***
N 31	34.3 ± 0.2	34.8 ± 0.2	69.1 ± 0.3
F_2 mean ± SE	31.8 ± 0.2	37.7 ± 0.3	69.5 ± 0.4
Range in F_2	27-37	32-47	63-78
L124-1 // N31 F_3 (field conditions 2020)			
L. 124-1	49.8 ± 0.61***	37.6 ± 0.50***	87.4 ± 0.60***
N 31	43.1 ± 0.6	35.8 ± 0.33	78.9 ± 0.36
F_3 mean ± SE	47.4 ± 0.2	37.8 ± 0.23	85.2 ± 0.35
Range in F_3	39-62	23-48	77-97
L124-1 // N 31 F_3 (field conditions 2021)			
L. 124-1	48.3 ± 0.80***	44.6 ± 0.69***	92.9 ± 0.91***
N 31	42.1 ± 0.31	39.3 ± 0.1	81.3 ± 0.29
F_3 mean ± SE	46.3 ± 0.21	42.8 ± 0.24	88.8 ± 0.35
Range in F_3	39-58	32-55	79-101

Asterisks indicate significant differences between L124-1 and N31 (*P <0.05, **P <0.01, ***P <0.001).

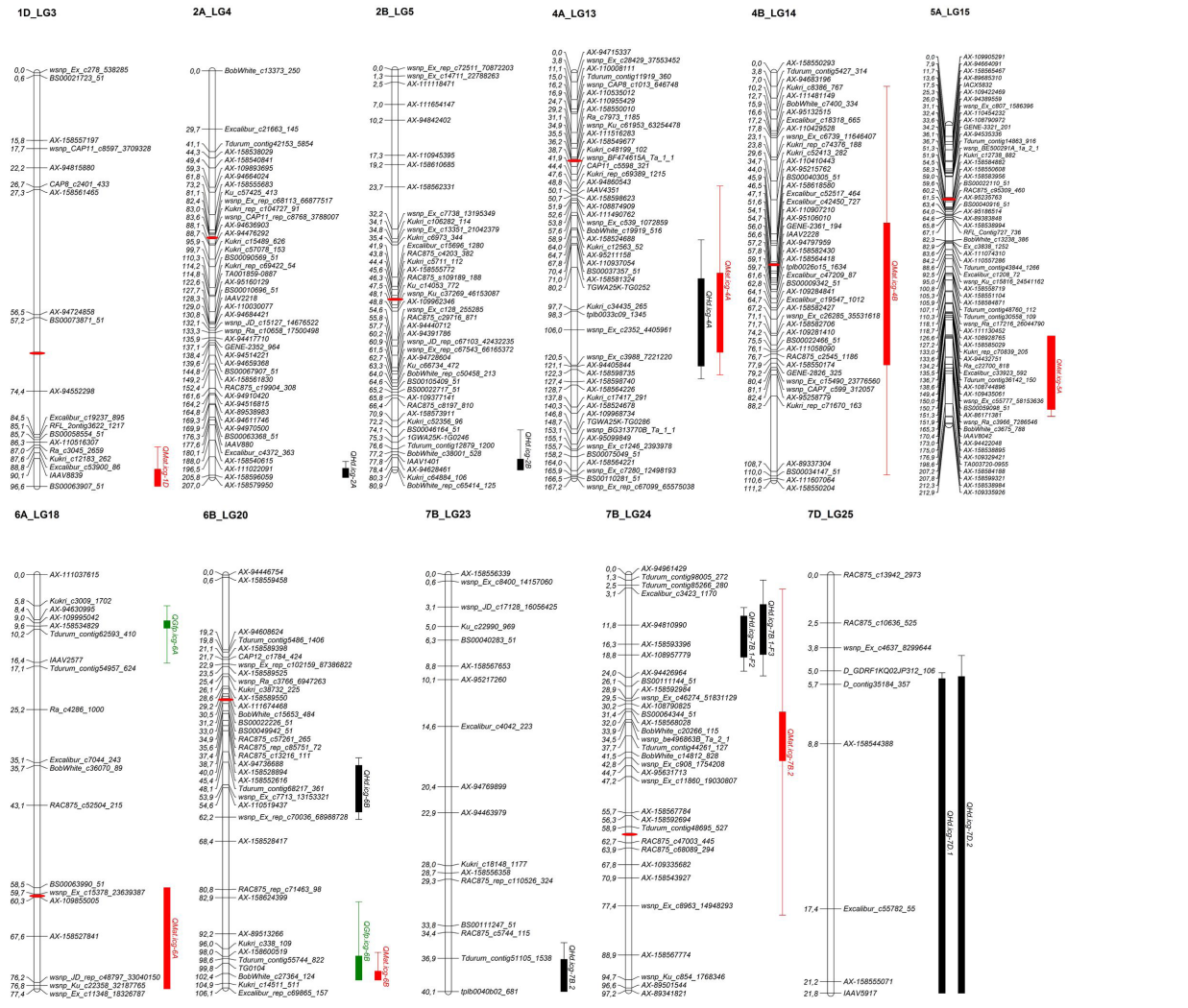

Figure 1. Linkage groups with QTLs for heading date (HD, black font), grain-filling period (GFP, green font) and maturity (MAT, red font) in the F_2 and $F_{2:3}$ populations. The horizontal red lines indicate the putative positions of centromeres. The vertical bars indicate the QTL confidence intervals.

on chromosome 4A, of which *QHd.icg-4A* was mapped in the 98.27 to 105.96 cM interval whereas *QMat.icg-4A* was detected between 80.18 and 97.65 cM. Another pair sharing the same confidence intervals was *QGfp.icg-6B* (GFP) and *QMat.icg-6B* (MAT).

4. Discussion

In Western Siberia heavy rainfalls and low temperatures overlap the end of wheat's vegetation period. The unfavorable conditions may negatively influence the yield; therefore, it is important to investigate the genetic mechanisms controlling the main development stages of the crop.

The phenotypic data demonstrated that in field conditions the HD and GFP were negatively correlated, which corresponds to the previously reported data (Knott and Gebeyehou, 1987; Monpara, 2011; Kajimura et al., 2011).

In this study, the MAT was positively correlated with both HD and GFP. According to the literature, the association of MAT and GFP is inconsistent, which might relate to the differences in growing conditions (Knott and Gebeyehou, 1987; Wiegand and Cuellar, 1981).

One of the main factors known to strongly influence the GFP is temperature, with every 1 °C increase shortening it by approximately three days (Wiegand and Cuellar, 1981). In this study, the GFP started in the beginning of July and completed by mid-August. In the two years of the experiment, the average daily temperature in the two months did not differ, however, the GFP of the second year was significantly longer (see the phenotypic results). In general, the weather conditions in 2020 were more favorable for spring wheat development than those of 2021. Probably, the lack of moisture in May, its excess in June and deficit in July of 2021 could have affected the duration of wheat development phases.

Table 2. QTLs associated with HD, GFP, and MAT detected in three environments in the F_2 and $F_{2:3}$ populations from the cross L124-1 × 'Novosibirskaya 31

QTL	Env.	Chr.[a]	Left marker (cM)	Right marker (cM)	LOD[b]	P.E.V.[c]	Add.[d]
Heading Time							
QHd.icg-2A	Field 2020	2A LG4	*AX-111022091* (196.50)	*AX-158596059* (205.77)	6.89	0.19	2.4
QHd.icg-2B	Field 2020	2B LG5	*AX-94628461* (78.42)	*Kukri_c64884_106* (80.31)	5.05	0.1	1.84
QHd.icg-4A	Glasshouse2019	4A LG13	*tplb0033c09_1345* (98.27)	*wsnp_Ex_c2352_4405961* (105.96)	3.76	0.12	2.02
QHd.icg-6B	Field 2020	6B LG20	*Tdurum_ contig68217_361* (48.05)	*wsnp_Ex_c7713_13153321* (53.91)	6.97	0.21	-0.21
QHd.icg-7B.2	Field2020	7B LG23	*Tdurum_ contig51105_1538* (36.89)	*tplb0040b02_681* (40.06)	4.63	0.09	0.21
QHd.icg-7B.1	Glasshouse2019	7B LG24	*AX-94810990* (11.77)	*AX-158593396* (16.30)	4.23	0.14	2.05
QHd.icg-7B.1	Field 2021	7B LG24	*AX-94810990* (11.77)	*AX-158593396* (16.30)	4.71	0.2	2.3
QHd.icg-7D.1	Glasshouse 2019	7D LG25	*D_contig35184_357* (16.18)	*D_GDRF1KQ02JPR1A_106* (16.80)	4.45	0.15	-2.23
QHd.icg-7D.2	Field 2021	7D LG25	*RAC875_ c10636_525* (19.31)	*RAC875_c13942_2973* (21.84)	3.36	0.15	-1.9
Grain-filling period							
QGfp.icg-6A	Field 2021	6A LG18	*AX-158527841* (9.86)	*AX-109855005* (17.08)	3.50	0.21	2.87
QGfp.icg-6B	Field 2021	6B LG20	*BobWhite_ c27364_124* (100.33)	*Kukri_c14511_511* (102.85)	2.67	0.14	2.14
Maturity time							
QMat.icg-1D	Glasshouse 2019	1D LG3	*IAAV8839* (90.07)	*BS00063907_51* (96.60)	3.00	0.18	2.51
QMat.icg-4A	Field 2021	4A LG13	*TGWA25K-TG0252* (80.18)	*Kukri_c34435_265* (97.65)	3.10	0.23	3.3
QMat.icg-4B	Field 2021	4B LG14	*GENE-2361_194* (55.98)	*IAAV2228* (56.60)	2.69	0.14	3.13
QMat.icg-5A	Field 2020	5A LG15	*wsnp_Ex_ c807_1586396* (31.10)	*AX-110454232* (32.35)	2.88	0.16	-1.79
QMat.icg-6A	Field 2021	6A LG18	*AX-94630995* (69.06)	*Kukri_c3009_1702* (71.59)	2.51	0.14	3.07
QMat.icg-6B	Field 2021	6B LG20	*Kukri_c14511_511* (102.85)	*Excalibur_rep_c69865_157* (104.10)	4.35	0.14	3.42
QMat.icg-7B	Field 2020	7B LG24	*BobWhite_ c20266_115* (33.91)	*wsnp_be496863B_Ta_2_1* (34.53)	2.72	0.14	2.86

[a]LG = stands for linkage group; [b]LOD = logarithm of the odds; [c]P.E.V. = proportion of variance explained by the QTL; [d]Add = additive effect of the QTL (in days), numbers given in parentheses indicate the position of the marker on the linkage group.

4.1. Comparing the QTLs against previously studied ones

In this study, a total of 17 loci associated with HD, GFP and MAT was identified. The HD and GFP did not coincide, which is in agreement with the previous results saying these development periods are under control of different genetic systems.

The mapping population did not segregate for the *PPD-D1* and *VRN1* genes, resulting in a high probability of finding novel QTLs.

The *QHd.icg-7B.1* QTL region on 7B chromosome is represented by the *FT-B1* gene having an SNP (G/C). The polymorphism was mapped by Brassac et al. (2021) for total spikelet number per spike (TSN). *FT-B1* was highly significant for TSN, but showed minor effect on heading time due to the dominating effects of a segregating *VRN-A1* gene. In this study, the QTL was major for HD and it was the only QTL consistently expressing both in glasshouse and field environments.

Within one confidence interval, *QHd.icg-4A* and *QMat.icg-4A* overlapped with the FLT-regulating *QFlt.dms-4A* QTL identified by Zou et al. (2017). While its interval was narrower and located within our confidence interval, the *QFlt.dms-4A* locus was environment-specific and explained less of the variance of the trait (8.5%) than did *QHd.icg-4A* (12%) and *QMat.icg-4A* (23%). Other two HD-regulating QTLs (*QHd.icg-6B* and *QMat.icg-4B)* were mapped near to the loci identified by Zou et al. (2017) (*QFlt.dms-6B* and *QMat.dms-4B,* respectively), with the distance between the loci in both cases being approximately 5 Mb (RefSeq v2.1).

The flanking marker *wsnp_Ex_c7713_13153321* of the *QHd.icg-6B (*position 693,547,220, RefSeq v2.1*)* was mapped closely to environmentally-stable HD-regulating QTL *QHd.cau-6B* (marker wsnp_Ex_rep_c70036_68988728, position 700,301,412, RefSeq v2.1) identified by Chen et al. (2020).

Only two GFP-regulating QTLs were identified, and none of them coincided with previously obtained results.

4.2. Probable candidate genes for HD, GFP and MAT

The search for possible candidate genes was focused on the stable and pleotropic QTLs, though some other major QTLs were investigated as well.

The *QHd.icg-7B.1* QTL on 7B chromosome was mapped to the well-known HD regulator gene *FT-B1* (*Vrn-B3*). L124-1 parental genotype contributed a non-synonymous mutation resulting in modifying aspartic acid (D) into histidine (H). According to Brassac et al. (2021), the mutation occurred in a highly conserved position and was widespread in hexaploid wheat. Notably, the authors found this mutation to be highly associated with the total spikelet number per spike, but its effect on heading and flowering time was small. In this study, the QTL explained up to 20% of the phenotypic variation, and was significantly associated with HD, so the plants homozygous for the mutant allele headed on average 2.2 days later than those with the non-mutant allele and heterozygotes (F= 6.515, p <0.001 ANOVA).

The *QHd.icg-6B* locus with the strongest HD effect in 2020 was mapped between the *Tdurum_contig68217_361* (48.05) and wsnp_Ex_c7713_13153321 (53.91) markers. The QTL interval of 9.32 Mb in the wheat physical map contained 101 genes (RefSeq v2.1). In this respect, gene *TraesCS6B02G404400* was one of interest for it encoded a histone H4 protein and might be involved in epigenetic control of heading (Supplementary Material, Table S2) (Simpson, 2004).

Both heading and grain-filling periods are the components of maturity time, so there were several QTLs influencing both MAT and HD/GFP.

QHd.icg-4A and *QMat.icg-4A* collocated on chromosome 4A with the confidence interval spanning 25.78 cM in length to produce a 23.42-Mb physical map interval harboring 333 genes (RefSeq v2.1). One probable candidate was the *TraesCS4A02G359500 SQUAMOSA promoter-binding protein-like* (*SPL*) gene encoding plant-specific transcription factors (Supplementary Material, Table S3). In *Arabidopsis*, SPL transcription factors was shown to regulate flowering by integrating photoperiod and gibberellic acid (GA) signals (Jung et al., 2012). The *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS* (*SOC1*) gene transcriptionally regulate *AtSPL3*, *AtSPL4* and *AtSPL5*, and the *FLOWERING LOCUS T* (*FT*) (orthologous to the *FT-B1* in wheat) in a complex with the bZIP transcription factor FD activates the *AtSPL3*, *AtSPL4* and *AtSPL5*, either directly or via *SOC1*. Under short day conditions, GA signals induce flowering by activating the *SOC1* gene. However, *SPL* genes in wheat have not been sufficiently studied. Zhu et al. (2020) identified 56 *SPL* genes in wheat genome and concluded that most of them regulate inflorescence and spike development. Based on the gene structure and motifs, the genes were divided into 8 subgroups. The *TraesCS4A02G359500* gene (*TaSPL016,* according to the authors' naming) belongs to group 7 that, together with group 10, possesses the largest number of exons. According to the data from the wheat expression browser website, *TaSPL016* exhibits high expression in spikes, like the majority (46 out of 56) of the *SPL* genes. Furthermore, using qRT-PCR, Chen et al. (2020) showed *TaSPL016* has high expression level in stems. All this suggests that the *SPL* genes, including *TaSPL016,* play an important role in the regulation of plant growth and development.

QGfp.icg-6B, one of the GFP-regulating *QTLs* coincided with the maturity time QTL *QMat.icg-6B* on the long arm of chromosome 6B. The corresponding physical map interval included 46 genes, and the genomic region harbored the *TraesCS6B02G462100* gene belonging to the TCP transcription factor family (Supplementary Material, Table S4). This gene was identified by Rees et al. (2022) as one of the orthologs of the CHE transcription factor (*At5g08330)* in *Arabidopsis*. CHE (CCA1 HIKING EXPEDITION) is a TCP family protein. CHE, being a part of a circadian network in *Arabidopsis*, is involved in a reciprocal regulation with *CCA1 (CIRCADIAN CLOCK ASSOCIATED 1)* by interacting with *TOC1* (*TIMING OF CAB EXPRESSION 1*) (Pruneda-Paz et al., 2009). The *TCP* family genes have not been extensively investigated in wheat until recently. Zhao et al. (2018) identified a total of 66 *TCP* genes in wheat, described their molecular structure and expression patterns. Wheat TCPs, based on their TCP domains, were divided into 2 classes: class I (PCF) and class II (CIN and CYC/TB1). The study suggests that the *TaTCP* genes play their role in a range of developmental

processes in wheat, including spike and grain development. The *TraesCS6B02G462100* (*TaTCP29-B*) gene found within the QTL peak in the present study is categorized as TCP class I. Studying the organ-specific expression of the *TCP* genes, Zhao et al. (2018) concluded that *TaTCP29-B* and its both homoeologs were most highly expressed in 2-4 days after pollination. This, and the results of QTL analysis, suggests that *TaTCP29* may be involved in early grain development.

5. Conclusions

In this study, 17 HD-, GFP- and MAT-regulating QTLs have been mapped on 10 wheat chromosomes (1D, 2A, 2B, 4A, 4B, 5A, 6A, 6B, 7B, 7D) using the F_2 mapping population obtained from a cross between late-ripening spring/winter line 124-1 and spring wheat cultivar Novosibirskaya 31. The reliability of the obtained results has been tested by the $F_{2:3}$ population grown in-field during two years. The *QHd.icg-7B.1* QTL was mapped to the *Vrn-B3* gene having a non-synonymous polymorphism [G/C] in its coding region. Five of the identified QTLs coincide with the previously reported QTLs, namely *QHd.icg-7B.1*, *QHd.icg-4A*, *QMat.icg-4A*, *QHd.icg-6B* and *QMat.icg-4B*. A novel QTL for HD was identified on 7D chromosome. Several possible candidate genes have been proposed for environmentally stable QTLs, including the *SPL* family gene *TraesCS4A02G359500* and the ortholog of the CHE transcription factor *TraesCS6B02G462100,* through further investigation is required to validate these suggestions. Further research is required to verify the candidate genes and their roles in wheat development.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Summary of the linkage map of the L124-1 × N31 F_2 population.

Table S2. High-confidence genes located within the interval of QHd.icg-6B.

Table S3. High-confidence genes located within the interval of QHd.icg-4A and QMat.icg-4A.

Table S4. High-confidence genes located within the interval of QGfp.icg-6B and QMat.icg-6B.

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