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Allelic variation and effects of earliness *per se (Eps)* genes in wheat cultivars of Pakistan

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Abstract
Optimization of the flowering time in wheat is an important breeding target for its adaptability in target environments. Flowering time is controlled by vernalization, photoperiod, and the relatively poorly characterized earliness *per se* (*Eps*) genes. When vernalization and photoperiod criteria are met, *Eps* genes account for the variance in flowering time. The objective of the study was to decipher the allelic variations for *Eps* genes in the wheat cultivars of Pakistan and draw their association with agronomic traits. The wheat cultivars released prior to 1965 had an average flowering duration of 82 days, whereas the cultivars released between 1965 and 2000 had an average flowering time of 79 days and 81 days, respectively. Kompetitive allele-specific PCR (KASP) markers were used to genotype all these cultivars for *TaElf3-B1*, *TaElf3-D1*, and *TaMOT1-D1* genes. For the gene *TaElf3-B1,* allele *Cadenza-type* had a frequency of 61.71%. For the gene *TaElf3-D1,* the proportion of its respective major alleles was recorded, i.e., deletion had a frequency of 72.94%, and *Savannah-type* was 86.04%. For the gene *TaMOT1-D1,* the allele *Wild-type* was found in 55.88% accessions. The gene *Elf3-B1* had a significant allelic effect for grain yield (GY), *TaElf3-D1* for grain length, and *TaMOT1-D1* for GY. Among the wheat cultivars, high percentage (56.89%) of the *Savanah-type* allele was associated with early flowering. However, the *Wild-type* alleles (43.1%) were observed to have low allelic frequency, and they were associated with late flowering. This study may allow wheat breeders to make genetic selection of wheat cultivars that are most suited to target environment, ensuring better yield and adaptability.

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Introduction

The amount of time the plants spend in the blossoming stage is referred to as 'flowering time'. It starts when the lights are set to a 12/12 cycle and concludes when the buds are ready to be harvested. Plants undergo significant physiological changes as they advance from vegetative to reproductive development (Jung and Müller, 2009). This transformation occurs as a result of the combination of several endogenous and exogenous cues that result in flowering. Extensive studies in model species have shown a complex network of regulatory links between proteins that transduce and integrate developmental and environmental variables to promote or prevent flowering (Amasino et al. 2005; Baumann et al., 2015; Bao, et al., 2019; Chávez-Hernández et al., 2020).

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Flowering is controlled by five different pathways that are genetically determined (Amasino et al. 2005). The vernalization pathway describes how a plant's flowering is accelerated after being exposed to cold for a long time (Kamran et al. 2014). Flowering is controlled by the photoperiod pathway (Borthwick and Hendricks, 1960), which responds to the length of the day and the quality of the light received. The gibberellin pathway suggests that gibberellic acid is required for normal flowering (Bao et al., 2019). Endogenous regulators that are unaffected by photoperiod and gibberellin pathways are referred to as independent pathways (Boss et al., 2004). A thorough investigation has been undertaken in *Arabidopsis thaliana* and other flowering plants to elucidate the molecular processes of these pathways (Srikanth and Schmid, 2011; Baumann et al., 2015; Chávez-Hernández et al., 2020). These pathways come together to control a group of genes known as 'floral integrators' (Moon et al., 2005; van Dijk and Molenaar, 2017) These genes aggregate the outputs of many pathways, and given the right circumstances, directly activate floral meristem identity genes.

Flowering in plants is controlled by vernalization, photoperiod, and the less well-defined *Eps* genes (Kamran et al. 2014; Zhao et al., 2023). Recent studies have suggested that even before the genes are cloned, marker assisted selection (MAS) of *Eps* effects is sufficient and helpful (Bapela et al., 2022). This means *Eps* genes can be characterized and cloned positionally in the same way as photoperiod and vernalization genes can. This validation investigation is the initial step toward detailed mapping, and eventually direct cloning of the genes in hexaploid wheat (Zikhali et al., 2014; Li et al., 2024). Thus, the objectives of the current study were: a) to identify alleles of major genes that underpin earliness *per se* in wheat, b) to narrow down wheat cultivars having characteristic of early maturity to ensure maximum productivity under projected heat stress environments, and c) to validate the phenotypic effect of genes controlling flowering time and drought tolerance in the historical wheat panel.

Materials and Methods

Germplasm

The germplasm of 174 historical wheat cultivars, obtained from Pakistan, contained traditional cultivars, modern cultivars and landraces. These cultivars are under cultivation since 1911 to 2018.

Field trials and phenotyping

During the 2019-2020 cropping season, field experiments were conducted at the National Agricultural Research Centre (NARC) in Islamabad (33.6701° N, 73.1261° E). All cultivar seeds were planted under long-day photoperiod (16 h light) and non-vernalizing temperatures (20-25 °C). Each entry was planted in a plot of 200 cm long row with a 30 cm distance between each of two rows and 12 cm between neighboring plants. Standard agronomic practices were used during the experimentation. Each plot was investigated for the data collection at the following growth stages of wheat i.e., heading, anthesis and maturity before harvesting.

Days to heading (DH) were documented as the number of days between 5th of December and the heading date. The heading date was documented for each entry according to the Zadoks scale (Zadoks et al., 1974) at stage 59 twice a week when nearly 50% of the plants in the plot showed full emergence of a spike from the flag leaf sheath. Also, the plants in the plots were monitored for the data for anthesis (stage 65) and maturity (stage 87). Grain yield (GY), thousand grain weight (TGW), plant height (PH), grain width (GW), grain diameter (GD), and grain length (GL) were other phenotypic traits documented.

Grains were separated from the husk, after harvesting. Insect-infested, diseased and physically damaged grains were discarded, and 200 healthy grains of each variety were taken for measuring kernel weight. Grain weight was measured in grams (g) using an electric balance.

DNA extraction and genotyping

DNA was extracted following the protocol described in the CIMMYT genotyping manual (Dreisigacker et al. 2013). DNA was visualized on 1 percent agarose gel and visualized on iBright TM CL 1000 gel doc. DNA was quantified using NanoDrop spectrophotometer. Genotypes for *TaElf3-B1, TaElf3-D1,* and *TaMOT-D1* were assessed using Kompetitive allele-specific PCR (KASP) assays described elsewhere (Zikhali et al. 2016; 2017). The KASP genotyping was conducted based on the fluorescence resonance energy transfer (FRET) method (Rasheed et al. 2016). The primers used for genotyping are given in **Table 1**. For a 5 µl of total reaction volume, an aliquot of 2.2 µl of the DNA sample was dispensed into 384-well microtiter plates. After that, DNA was dried for 50 minutes at 50 °C in an incubator. An aliquot of 2.5 µl of KASP mix (2x) and 2x KASP assay mix (including allele-specific and common primers) were dispensed into the DNA samples. Finally, 0.08 µl $MgCl_2$ and 2.4 µl PCR water were added.

Thermocycling conditions used were 94 °C for 15 min activation time, followed by 20 cycles at 94 °C for 10 sec, at 57 °C for 5 sec and 72 °C for 10 sec (temperature was dropped 0.6 °C per cycle), after that

18 cycles were run 94 °C for 10 s, 57 °C for 20 s and 72 °C for 40 s (Royo et al., 2020). Fluorescence was read at the end of the reaction mixture. KASP was conducted in a Bio-Rad CFX384TM Real-Time System using BioRad hard shell PCR plates (384-well). Kluster Caller was used to visualize and comprehend the results of the Real Time PCR reaction.

Table 1. List of genes with their primer sequences used to genotype historical wheat cultivars.

Gene	Marker	Sequence
	TaBradi2g14790_AL1	gaaggtgaccaagttcatgctCCTTGTCTCCGTCCCTG
TaELF3-D1	TaBradi2g14790_AL2	gaaggtcggagtcaacggattGACAGCTCCTCCCGAG
	TaBradi2g14790_C	TCGGTAATGTCTTCAGTGTTTTA
	TaELF3-B1_AL1	gaaggtgaccaagttcatgctCCCTTGCAGCTCGCT
TaELF3-B1	TaELF3-B1_AL2	gaaggtcggagtcaacggattCCCTTGCAGCTCGCC
	TaELF3-B1_C	CGACCCAACACTCACG
	TaELF3-D1-1_ AL1	gaaggtgaccaagttcatgctTGGAGACATGACGGGAACA
TaELF3-D1	TaELF3-D1-1_ AL2	gaaggtcggagtcaacggattTGGAGACATGACGGGAACG
	TaELF3-D1-1_ C	GGAAACCAGGCTTCACG
	TaELF3-D1-2_ AL1	gaaggtgaccaagttcatgctGCCTCAGAATCAGTGGCTT
TaELF3-D1	TaELF3-D1-2_ AL2	gaaggtcggagtcaacggattGCCTCAGAATCAGTGGCTC
	TaELF3-D1-2_ C	GTAGACGAACCCTTCCGA
	TaMOT1-D1_AL1	gaaggtgaccaagttcatgctGGCACATATAATGTAAGGATCAATCAT
TaMOT-D1	TaMOT1-D1_AL2	gaaggtcggagtcaacggattGGCACATATAATGTAAGGATGAATCAT
	TaMOT1-D1_C	AATATATAAGTTAACCATCTCATGAAAGTAAG

Statistical analysis

Basic statistical analysis was done using Microsoft Excel 2010. To analyze the effects of the alleles of a single gene, Jamovi version 2.0 was used. Variations in different phenotypic traits were shown in boxplots. Coefficient of correlation between phenotypic traits was calculated and visualized by Jamovi version 2.0. Allelic effects for KASP markers on phenotypes were tested for statistical significance using Welch's *t* test.

Results

In total, 174 historical wheat cultivars of Pakistan were included in this study released between 1911 to 2019. A subset consisting of 62 of them was used for phenotypic data collection over two years in 2019-20 and 2020-21. Based on the year of release, all 174 cultivars were grouped into three categories. The cultivars released before 1965 were 11, while cultivars released between 1965-2000 and post-2000 were 74 and 89, respectively. All these cultivars were subjected to the genotyping for *TaElf3-B1, TaElf3-D1,* and *TaMOT1-D1* genes using KASP markers.

Variation in phenological traits in wheat cultivars

The basic statistics describing the means, standard deviations, and range for GY, DH, GL, GW, TGW, PH, and GD is presented as **Table 2**. The average DH for the cultivars released in pre-1965 era was 82 days, ranging between 84 and 81 days. The average DH for the cultivars released during 1965-2000 era was 79.5 days, whereas the cultivars released after 2000 era had average DH of 81.7 days.

The PH was observed to lie between 79.8 cm to 119 cm and the PH was minimum for the cultivars released during 1965-2000 era with the mean of 99.2 cm and it was maximum for those released during pre-1965 era with a mean of 112 cm. The TGW had a range of 28.3 g to 39.6 g, and the minimum TGW was found in the cultivars of 1965-2000 era with a mean 32.5 g, and was found maximum for the cultivars of post-2000 era with a mean of 34.4 g. The minimum GL and GW was recorded in the cultivars released in pre-1965 era, however, the GL was maximum (2.23 mm) in the cultivars of post-2000 era and the GW was maximum (2.73 mm) in the cultivars of 1965-2000 era.

The phenotypic variation in GY, DH, PH, TGW, GL, GW and GD is shown in the boxplots in **Figure 1** and **Figure 2**, respectively. The plots showed the five-number overview of the 62 cultivars in the data set. The minimum, first quartile, median, third quartile, and maximum are the five numbers that make up the five-number summary.

Table 2. Descriptive statistics for key phenological traits in a set of historical Pakistani wheat cultivars released between 1911 and 2019.

Traits	Pre-1965		1965-2000	
	Mean ± SD	Range	Mean±SD	Range
GY	1.31±0.27	0.97-1.84	2.09±0.46	1.3-2.82
DH	82.7±1.03	81-84	79.5±2.52	76-84
PH	112±5.86	106-119	99.2±7.74	79.8-111
TGW	35.3±2.17	32.3-38.4	32.5±2.29	28.3-37.2
GL	1.25±0.08	1.11-1.36	1.45±0.2	1.16-2.04
GW	2.11±0.16	1.97-2.44	2.33±0.15	2.03-2.73
GD	6.16±0.28	5.86-6.65	6.34±0.21	5.89-6.65
Traits	**Post-2000**		**Average**	
	Mean±SD	Range	Mean±SD	Range
GY	2.44±0.29	1.87-3.22	2.19±0.49	0.975-3.22
DH	81.7±2.26	76-86	81.1±2.5	76-86
PH	95.6±6.28	84.6-109	98.6±8.29	79.8-119
TGW	34.4±2.61	28.9-39.6	33.9±2.61	28.3-39.6
GL	1.38±0.21	1.16-2.23	1.39±0.20	1.11-2.23
GW	2.25±0.13	2.02-2.52	2.26±0.15	1.97-2.73
GD	6.36±0.24	5.77-6.8	6.33±0.24	5.77-6.8

GY, Grain yield (t/ha); DH, Days to heading; PH, Plant height (cm); TGW, Thousand grain weight (g); GL, Grain length (mm); GW, Grain width (mm); GD, Grain diameter (mm).

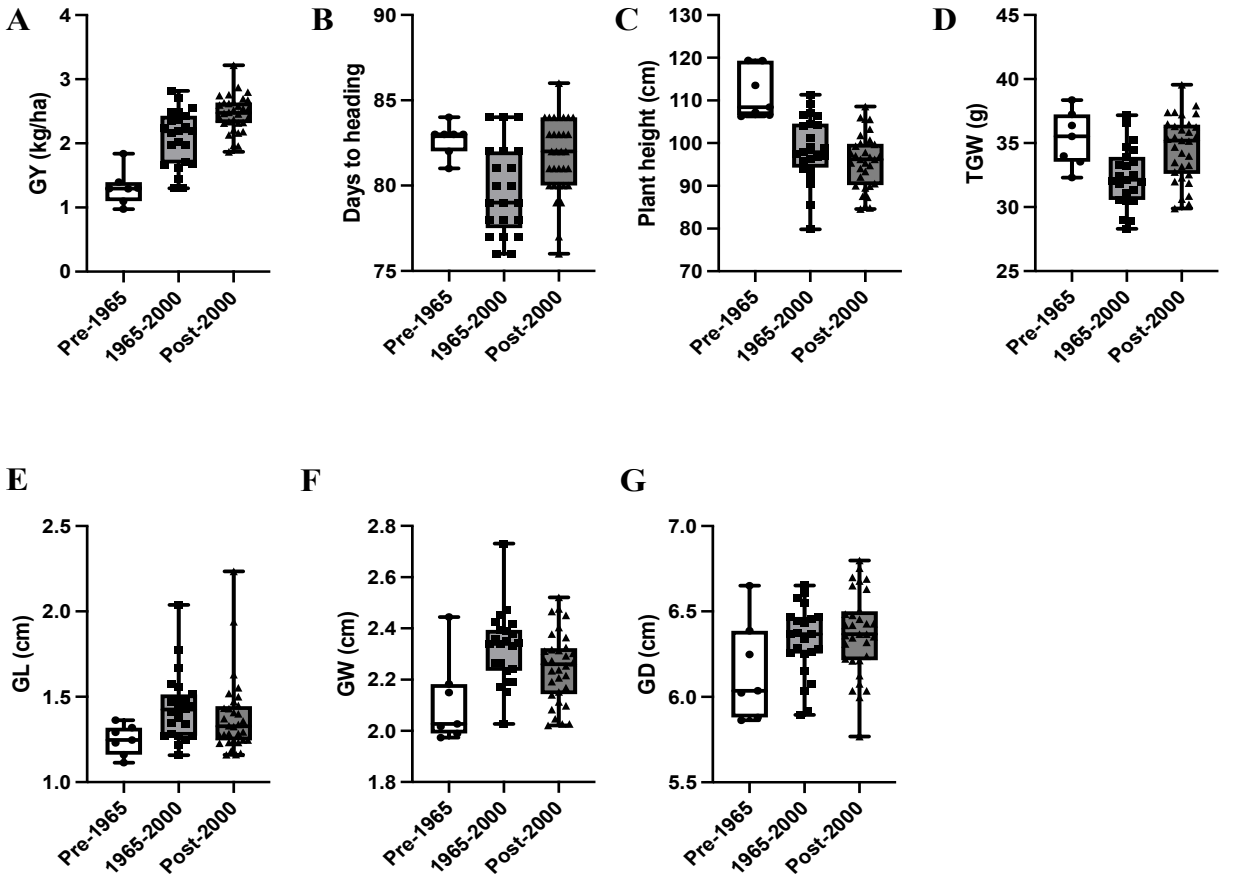

Figure 1. Box plot of 62 historical wheat cultivars of Pakistan showing (a) GY, (b) DH ,(c) PH and (d) TGW, (e) GL, (f) GW and (g) GD in three different breeding eras.

Coefficient of correlation between phenological traits in wheat cultivars

The coefficient of correlation was calculated between all the traits. The results are shown in **Figure 3**. The highest correlation was observed between GD and GY (r = 0.38), thus these traits were positively correlated. A positive correlation was also present between GW and GL (r = 0.37). The positive correlation was found between DH and TGW (r = 0.33), and between GD and TGW (r = 0.27). A negative correlation was found between DH and GL (r = -0.39), and between GW and TGW (r = -0.41) the correlation was observed to be negative. The correlation between TGW and PH was non-significant (r = 0.1), and the same was true between GL and PH (r = 0.01).

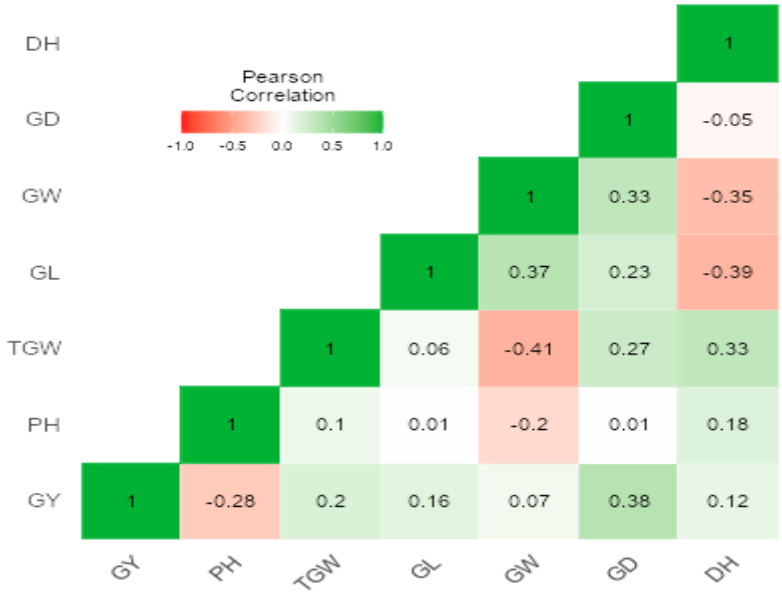

Figure 2. Coefficient of correlation between phenological traits in Pakistani historical wheat cultivars.

Allelic variation and effects of *Eps* genes on different phonological traits

To find out the allelic variations in wheat cultivars of Pakistan, KASP assays were used to genotype *Elf3-B1* alleles in the wheat cultivars. The marker TaELF3-B1 was used to identify alleles *Cadenza-type* and *Wild-type*, which were associated with late and early flowering, respectively. The *Cadenza-type* allele was identified in 108 wheat cultivars (61.71%), while the *Wild-type* allele was identified in 67 (38.28%) cultivars. The cultivars with the *Cadenza-type* allele had an average DH of 80.9 days, while the cultivars with the *Wild-type* allele had an average DH of 81.4 days. For GY, the allele *Cadenza-type* had an average of 2.08 t/ha and the allele *Wild-type* had an average of 2.35 t/ha, and the allelic effect for this trait was significant (**Table 3**).

An InDel in *TaElf3-D1* using marker TaBradi2g14790 was identified. It was observed that 124 wheat cultivars (72.94%) had deletion, while insertion was identified in 46 (27.05%) cultivars. The cultivars with deletion had an average DH of 81.1 days, while the cultivars with insertion had an average DH of 80.3 days.

Two more markers, TaELF3-D1-1 and TaELF3-D1-2, were used to distinguish between *Wild-type* and *Savanah-type* alleles. Using first marker, the *Savanah-type* allele was identified in 148 wheat cultivars (86.04%), while the *Wild-type* allele was identified in 24 cultivars (13.95%). The cultivars with the *Savanah-type* allele had an average DH of 81.2 days, while the cultivars with the *Wild-type* allele had an average DH of 80.2 days. For GY, the allele *Savanah-type* had an average of 2.19 t/ha and the allele *Wild-type* had an average of 2.09 t/ha.

Using marker TaELF3-D1-2, two alleles were identified, i.e., *Savanah-type* and *Wild-type.* The *Savanah-type* allele was identified in 99 cultivars (56.89%), while the *Wild-type* allele was identified in 75 cultivars (43.10%). The cultivars with the *Savanah-type* allele had an average DH of 81.4 days, while the cultivars with the *Wild-type* allele had an average DH of 80.7 days. For GY, the *Savanah-type* allele had an average of 2.26 t/ha and the *Wild-type* allele had an average of 2.08 t/ha.

For gene *TaMOT1-D1*, two alleles were identified, i.e., *Wild-type* and *Ria-type.* The *Wild-type* allele was identified in 95 wheat cultivars (55.88%), while the *Ria-type* allele was identified in 75 (44.11%) cultivars. The cultivars with the *Wild-type* allele had an average DH of 80.8 days, while the cultivars with the *Ria-type* allele had an average DH of 81.5 days. For GY, the *Wild-type* allele had an average of 2.3 t/ha and the *Ria-type* allele had an average of 2 t/ha, and the allelic effect for this trait was significant (**Table 3**).

Table 3. Description and associated phenotypes of KASP primers used to genotype *Eps* genes in the historical wheat cultivars

Gene	Polymorphism	Marker name	Call	Allele	Associated phenotype	Call	Allele	Associated phenotype
TaELF3-B1	A/G	TaELF3-B1	A	*Cadenza-type*	Late flower	G	*Wild-type*	Early flower
TaElf3-D1	T/A	TaBradi2g14790	T	*Deletion*	Early flower	A	*Insertion*	Late flower
TaElf3-D1	A/G	TaELF3-D1-1	A	*Savanah-type*	Early flower	G	*Wild-type*	Late flower
TaElf3-D1	T/C	TaELF3-D1-2	T	*Savanah-type*	Early flower	C	*Wild-type*	Late flower
TaMOT1-D1	C/G	TaMOT1-D1	C	*Wild-type*	Early flower	G	*Ria-type*	Late flower

Table 4: Allelic variation of genes *Elf3-B1*, *TaElf3-D1*, and *TaMOT1-D1* for their respective allelic types in the wheat collection from Pakistan and their association with phenological traits.

	Marker	**Elf3-B1**		**TaBradi2g14790**		**TaELF3-D1-1**		**TaELF3-D1-2**		**TaMOT1-D1**	
	Alleles	*Cadenza-type*	*Wild-type*	*Deletion*	*Insertion*	*Savanah-type*	*Wild-type*	*Savanah-type*	*Wild-type*	*Wild-type*	*Ria-type*
	Percentage	61.71	38.28	72.94	27.05	86.04	13.95	56.89	43.1	55.88	44.11
GY (t/ha)	Mean	2.08±0.53	2.35±0.35	2.17±0.50	2.26±0.38	2.19±0.48	2.09±0.57	2.26±0.44	2.08±0.53	2.3±0.40	2±0.56
	P-value	**0.02**		0.62		0.65		0.16		**0.04**	
DH	Mean	80.9±2.49	81.4±2.55	81.1±2.35	80.3±2.91	81.2±2.36	80.2±4.02	81.4±2.55	80.7±2.41	80.8±2.55	81.5±2.38
	P-value	0.52		0.36		0.41		0.29		0.31	
PH (cm)	Mean	99.1±9.15	96.7±5.51	98.4±8.24	96.3±5.18	97.1±7.29	107±9.25	98.5±7.33	97.4±8.77	96.9±7.16	99.6±8.87
	P-value	0.25		0.5		**0.006**		0.6		0.22	
GL (mm)	Mean	1.4±0.21	1.36±0.11	1.4±0.18	1.29±0.10	1.39±0.18	1.39±0.11	1.38±0.16	1.39±0.19	1.4±0.20	1.33±0.11
	P-value	0.34		**0.03**		0.99		0.92		0.13	

The values which are statistically significant are bold.

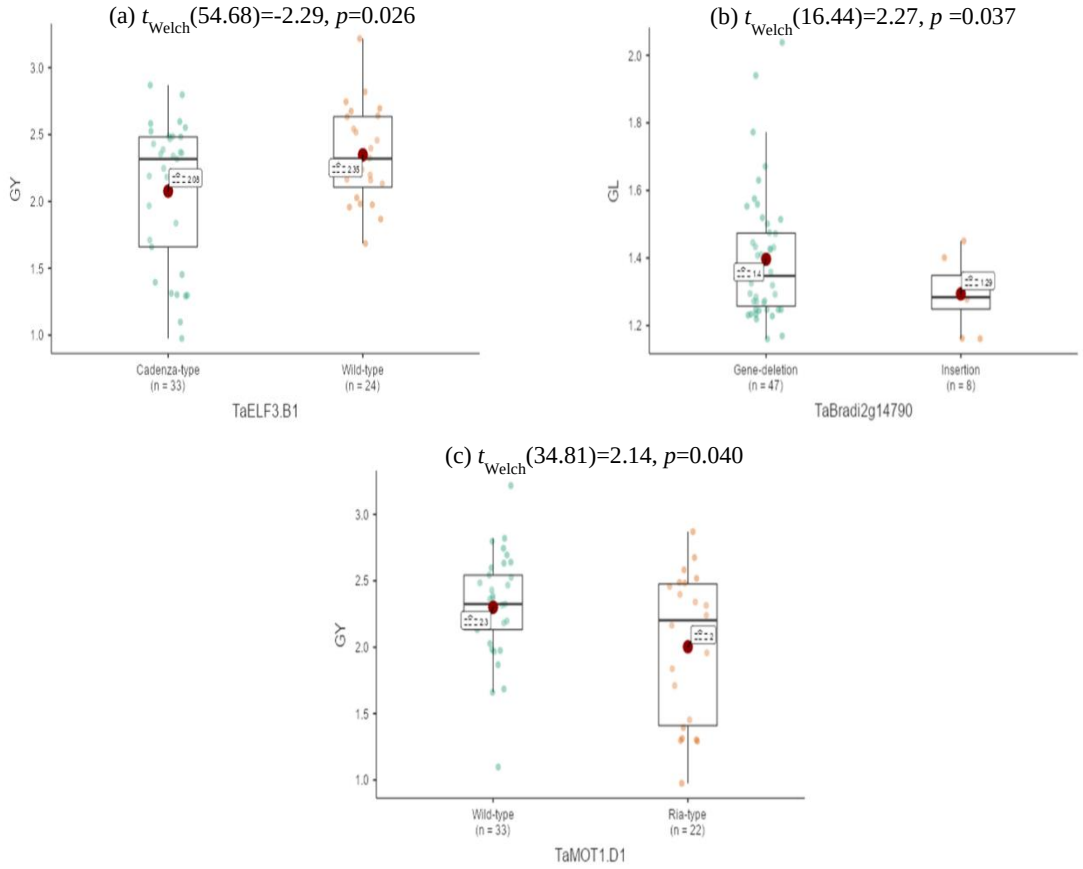

Figure 3. Allelic effect of genes on phenological traits in historical wheat cultivars of Pakistan, a) effect of *TaElf3-B1* on GY, (b) effect of *TaElf3-D1* on GL, and (c) effect of *TaMOT-D1* on GY.

Discussion

Heading time has a significant role in crop plants as it greatly affects the maturity of seed and adaptability of plants in target environments (Dubcovsky and Dvorak, 2008; Pin and Nilsson, 2012). Flowering time is of immense importance in wheat as it notably influences the grain yield. The timely switching from vegetative to reproductive stage, i.e., floral transition plays a vital role in reproductive success (Yamaguchi and Abe, 2012; Cheng et al., 2021). A continuous variation is exhibited by wheat due to different genetic systems controlling heading time (Kamran et al., 2014).

Understanding the phenotypic variance in modern wheat cultivars requires insight into genetic loci that have been selected throughout modern wheat breeding. This will allow wheat breedings to evolve into a knowledge-based activity, ultimately improving the rate of wheat genetic progress (Li et al., 2018; Bapela et al., 2022). The current study for the first time revealed the genetic architecture in Pakistani wheats for the genes underpinning earliness *per se*. Heading time in wheat is the most important for its adaptability and yield in different environmental conditions and is affected by three genetic systems, i.e., vernalization, photoperiodism and *Eps* genes (Kato and Yamagata, 1988; Benaouda et al., 2022).

In our study, wheat cultivars from Pakistan were evaluated for the variation in heading time. A negative correlation was found between the DH and GL, implying that the cultivars with early maturity are likely to have high GL. Also, a negative correlation was also observed between GW and TGW, indicating that the cultivars having high TGW, may have low GW. The cultivars released during the pre-1965 era (landraces) showed early flowering phenotypes. Most of the cultivars released during pre-1965 era had early DH ranging from 81-84 days. For these cultivars, the GY was lowest ranging from 0.97-1.84 t/ha and the TGW had a range of 32.3-38.4 g. Most of the cultivars released during the 1965-2000 era had early DH ranging from 76 to 84 days. For these cultivars, the GY had average values ranging from 1.3 to 2.82 t/ha and the TGW had a range of 28.3-37.2 g. Some cultivars released during post-2000 like Seher 2006 had DH (77 days), GY (2.79 t/ha) and TGW (37.22 g); Mairaj-2008 had DH (76 days), GY (2.45 t/ha) and TGW (30.21 g), and Gold-2016 had DH (81 days), GY (2.67 t/ha) and TGW (31.87 g). Most of the cultivars released during the post-2000 era had early DH ranging from 76 to 86 days. For these cultivars, the GY had highest values ranging from 1.87 to 3.22 t/ha and the TGW had high values with a range of 28.9-39.6 g.

The high-throughput KASP assay made it possible to genotype a large population with accuracy within a short period in comparison to the traditional genetic methods (Rasheed et al., 2016). Previously, allelic variation for flowering time and drought tolerance genes has been observed in a diversity panel of synthetic-derived wheats (Afzal et al., 2017; Ali et al., 2023). The study revealed the higher frequency of alleles deletion in *TaEfl-D1* was least frequent (46.15%) than that in *Wild-type* (78.85%). At *Elf3-B1*, the *Cadenza-type* allele was associated with the 'late flowering' and the *Wild-type allele* was associated with 'early flowering' in the wheat cultivars. The two alleles *Cadenza-type* and *Wild-type* for the marker Elf3-B1 were found to be significantly associated with GY.

The alleles that cause earlier flowering were found to be more common in the more recently discovered flowering time genes (*TaFT3-B1, TaFT3-D1, TaTOE-B1*, and *Eps-D1*). The *Avalon-type* allele had the highest allele frequency at *TaTOE-B1*, while the *Spark-type* allele had the lowest frequency at *TaFT3-B1*. The lines with a deletion at wheat chromosome 1DL (e.g., identified in the winter wheat lines Spark or Cadenza) were less common in the *Eps-D1* gene than the lines without the deletion. The lines carrying the *Avalon-type* allele at *TaTOE-B1* had a 4.7–5.0 percent higher yield and a 3.3–8.0 percent higher harvest index than the lines carrying the *Cadenza-type* allele, whereas the averages for biomass and physiological maturity were not significantly different as stated elsewhere (Dreisigacker et al., 2021).

The study conducted showed the high percentage of alleles (*Savanah-type)* that are associated with early flowering as compared to the alleles (*Wild-type*) associated with late flowering in the historical wheat cultivars of Pakistan. Wheat yield is expected to follow this trend in the next years as a result of global climate change. Due to the reduced danger of drought stress, early flowering wheat has a considerable advantage in conditions of impending terminal drought, and contemporary kinds are substantially more productive (Shavrukov et al., 2017). A quick vegetative stage may result in reduced plant biomass under favorable conditions due to the shorter time available for photosynthetic production and seed nutrient buildup. To conclude, early flowering is a viable strategy for improving grain yield in wheat cultivars.

Conclusion

The study showed a significant impact of *Eps* loci on DH and grain yield. Due to the continuously changing environment, there is a need for new allelic combinations that may be required for wheat adaptation. To develop new breeding strategies, it is important to continuously monitor the variation in flowering time and the genes affecting it. The findings will help imply our knowledge in wheat breeding and enable the plant breeders in genetic selection of cultivars that are well adapted in our region, ensuring a big step towards the goal of sustainable agriculture. The wheat adaptability can be improved using this information in targeted environments. Expedited research work should be done to understand gene pathways that can help future breeders to select such cultivars that can better adapt to target environments and produce high grain yield.

Author(s), Editor(s) and Publisher's declarations

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Contribution of authors
Conceived the idea and designed the experiment: AR, BI, HQ. KASP genotyping, BI, HQ, HMS. Phenotyping: HMS, AR, SuR. Data analysis: HK, KA, KT, MS, SZ. Drafting the paper: BI, SZ, AMK, AR.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent

All contributors have critically read this manuscript and agreed for publishing in IJAaEB.

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Declaration of generative AI and AI-assisted technologies in the writing process

It is declared that the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

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