QTL mapping for seed vigor-related traits under artificial aging in common wheat in two introgression line (IL) populations

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ABSTRACT

Background. Seed vigor recognized as a quantitative trait is of particular importance for agricultural production. However, limited knowledge is available for understanding genetic basis of wheat seed vigor.

Methods. The aim of this study was to identify quantitative trait loci (QTL) responsible for 10 seed vigor-related traits representing multiple aspects of seed-vigor dynamics during artificial aging with 6 different treatment times (0, 24, 36, 48, 60, and 72 h) under controlled conditions (48 °C, 95% humidity, and dark). The mapping populations were two wheat introgression lines (IL-1 and IL-2) derived from recipient parent (Lumai 14) and donor parent (Shaanhan 8675 or Jing 411).

Results. A total of 26 additive QTLs and 72 pairs of epistatic QTLs were detected for wheat seed-vigor traits. Importantly, chromosomes 1B and 7B contained several co-located QTLs, and chromosome 2A had a QTL-rich region near the marker Xwmc667, indicating that these QTLs may affect wheat seed vigor with pleiotropic effects. Furthermore, several possible consistent QTLs (hot-spot regions) were examined by comparison analysis of QTLs detected in this study and reported previously. Finally, a set of candidate genes for wheat seed vigor were predicted to be involved in transcription regulation, carbohydrate and lipid metabolism.

Conclusion. The present findings lay new insights into the mechanism underlying wheat seed vigor, providing valuable information for wheat genetic improvement especially marker-assisted breeding to increase seed vigor and consequently achieve high grain yield despite of further investigation required.

Subjects Agricultural Science, Plant Science

Keywords Wheat, Seed, Vigor, Artificial aging introgression lines, QTL mapping

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INTRODUCTION

Seed vigor is an important agronomic traits in determining wheat (*Triticum aestivum*) yields as in other crops (*Reed, Bradford & Khanday, 2022*). Generally, high-vigor seeds exhibit a high germination rate, leading to uniform seedlings and ultimately large yields.

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In contrast, low-vigor seeds show a low germination rate, resulting in unhealthy seedlings and eventually the reduced production (*Shi et al., 2020*). Therefore, it is so significant to elucidate the genetic basis of crop seed vigor and timely monitor the change of seed viability over the period of storage for ensuring stable and high yields of crops, maintaining economic benefits of agricultural production (*Rajjou et al., 2008*; *Schwember & Bradford, 2010*; *Ahmed, Yang & Fu, 2016*; *Zuo et al., 2018*).

For assessing seed vigor, the natural aging (NA) under normal conditions usually takes a long time complete, whereas artificial aging (AA) under high temperature and humidity is easily conducted for quicker examination of seed vigor-related traits (*Bentsink et al., 2000*; *Murthy, Kumar & Sun, 2003*; *Landjeva, Lohwasser & Börner, 2010*; *Zuo et al., 2018*). Thus, AA method is recommended for measuring seed vigor by the International Seed Testing Association (*Zheng et al., 2017*). Increasing reports showed that seed aging/deterioration is a complex biological trait involving a network consisted of biochemical, physiological, molecular, and metabolic processes (*Mcdonald, 1999*; *Rajjou et al., 2012*). The reported causes for seed deterioration during storage in various plant species include the loss of cellular membrane integrity, weak energy metabolism, production of reactive oxygen species (ROS) and their counter balance, lipid peroxidation, impaired RNA and protein synthesis, enzyme inactivation, and damage to DNA integrity (*Corbineau et al., 2002*; *Kibinza et al., 2006*; *Ahmed, Yang & Fu, 2016*; *Shi et al., 2020*). A number of genes were identified to be related to seed vigor. For instance, the increase of lipoxygenase (LOX) activity promoted oxidization of lipids, ROS burst, and seed deterioration (*Corbineau et al., 2002*). Overexpression of *ZmGOLS2* and *ZmRS* in *Arabidopsis* and maize increased seed vigor (*Li et al., 2017*). A rice small heat shock protein *OsHSP18.2* was found to enhance seed vigor by eliminating ROS in seeds under biotic stress (*Kaur et al., 2015*). Moreover, overexpression of *Heat Shock Factor A9* encoding a seed-specific transcription factor resulted in extension of seed longevity by enhancing levels of heat shock proteins in transgenic tobacco (*Nicotiana tabacum*) plants (*Prieto-Dapena et al., 2006*; *Kotak et al., 2007*). A lectin-like receptor protein kinase (*LecRK*) was also detected to contribute to innate immunity and seed germination in rice by upregulating α-amylase gene (*Cheng et al., 2013*). Mutation of *SNL1/2* (*SWI-INDEPENDENT3* (*SIN3*)*-LIKE*) in *Arabidopsis* resulted in the decrease of seed dormancy and a shortened seed longevity (*Wang et al., 2013*). These data demonstrated the complex genetic nature of seed vigor.

Phenotypic variations of seed vigor were detected among different crops and varieties (*TeKrony & Hunter, 1995*; *Soltani, 2001*; *Sun, Wang & Sun, 2007*), which were quantitative traits controlled by multiple genes (*Dickson, 1980*; *Arif & Börner, 2020*) and affected by environmental factors (*Arif et al., 2012*). A number of traits were measured to examine seed vigor, including germination rate (GR), germination energy (GE), germination index (GI), seedling length (SL), root length (RL), seed wet weight (SWW) and dry weight (SDW) as well as vigor index (VI). In view of quantitative heredity of those traits, QTL (quantitative trait loci) analysis was employed as an effective approach to dissect the genetic basis of seed vigor-related traits of crops in recent years (*Miura et al., 2002*; *Zeng et al., 2006*; *Singh et al., 2008*; *Xue et al., 2008*; *Nagel et al., 2009*; *Revilla et al., 2009*; *Nagel et al., 2011*; *Agacka-Moldoch et al., 2015*).

In wheat, QTLs were also identified for seed vigor. For example, 24 QTLs related to seed vigor were mapped to chromosomes 1D, 2D, 4D, 5D, and 7D using a set of common wheat lines carrying known D genome introgression segments (*Landjeva, Lohwasser & Börner, 2010*). 94 QTLs related to seed vigor traits in wheat were detected using 246 recombinant inbred lines derived from the cross between Zhou 8425B and Chinese Spring, with one of the stable QTL-rich regions on chromosome 2D flanked by *IWB21991* and *IWB11197* as a pleiotropic locus strongly affecting seed vigor-related traits after accelerated aging treatment (*Zuo et al., 2018*). QTLs of seed longevity in durum wheat were detected with a bi-parental mapping population consisited of 114 recombinant inbred lines, with four QTLs for germination energy on chromosomes 4B, 5A (2 QTLs), and 6B, and three significant QTLs for germination rate on chromosomes 5A and 7B (2 QTLs) under artificial aging (*Arif & Börner, 2019*). 24 QTLs related to wheat seed longevity were identified by a SNP based GWAS analysis (*Arif & Börner, 2020*). A total of 49 additive QTLs for seed vigor-related traits were mapped in wheat genome, with each accounted for 6.01–17.18% of the phenotypic variations (*Shi et al., 2020*). More recently, a total of 46 additive QTLs and 29 pairs of epistatic QTLs related to seed longevity and dormancy in bread wheat were identified using 7584 high-quality SNPs (*Arif et al., 2022*). Despite of such increasing reports on QTL analysis of wheat seed vigor-relate traits (*Ter Steege et al., 2005*; *Landjeva et al., 2008*; *Czyczyło-Mysza et al., 2014*; *Li et al., 2014*; *Moore & Rebetzke, 2015*; *Ayalew et al., 2018*; *Batool et al., 2018*; *Li et al., 2018a*; *Li et al., 2018b*; *Blackburn et al., 2021*), the genetic analysis of wheat seed vigor is still required (*Arif et al., 2012*; *Arif & Börner, 2020*) because it is hard to detect the same QTLs even within the same population under multiple conditions.

Different from other mapping populations, introgression line (IL) populations were more powerful in QTL mapping specially for small QTL detection by eliminating interference from the genetic background and improving the accuracy of mapping (*Kaeppler, 1997*; *Landjeva, Lohwasser & Börner, 2010*). Here, two IL populations and their parents were employed to identify QTLs related to seed vigor in wheat by AA method so as to better understand the genetic mechanism of wheat seed vigor.

MATERIALS & METHODS

Wheat materials

The test plant materials were two wheat IL populations (IL-1 and IL-2), and each of them contained 160 lines (BC_3F_9). IL-1 and IL-2 populations were derived from wheat cultivars Lumai 14 (recipient and recurrent parent) and Shaanhan 8675 (donor parent) or Jing 411 (donor parent), respectively. Lumai 14 is a winter wheat variety with better disease resistance, high yield, wide adaptability and early maturity (*Ge et al., 2013*). Shaanhan 8675 is a dryland wheat variety, which has strong stress resistance, wide adaptability, stable and high yield (*Dai et al., 2009*). Jing 411 is a wheat variety developed in the early 1990s, which has the advantages of strong cold and disease resistance, great tillering ability, high and stable yield, and wide adaptability (*Chen et al., 2009*). The IL-1 population was developed through an initial cross between Lumai 14 (female parent) and Shaanhan 8675 (male

parent) to produce the F_1 generation. Then, Lumai 14 was backcrossed with the F_1 for three generations to obtain the BC_3F_1, and then self-cross for many years. Similarly, the IL-2 population was constructed using the same approach, but with the male parent material being Jing 411 instead of Shaanhan 8675. The details of IL populations construction were described previously by *Chen et al. (2020)* and *Yan et al. (2019)*. These two IL populations were planted in the wheat experiment field (37°25′N, 112°25′E) of Shanxi Agricultural University (Taigu, China) in the growing season of 2019–2020. The field management was the same as used in the local production practice. The soil fertility of the test field before planting was as follows: total nitrogen 1.19 g/kg; total phosphorus 1.07 g/kg; total potassium 14.92 g/kg; available nitrogen 52.37 mg/kg; available phosphorus 13.39 mg/kg; available potassium 88.80 mg/kg. The application amount of nitrogen fertilizer in the experimental field was N 270 kg/hm (urea), with 50% applied as base fertilizer and 50% applied as topdressing during the greening jointing period. Water management was carried out in pre-wintering period, jointing period and mid-grouting period respectively, with irrigation volume of 650 m^3/hm^2 each time. The wheat seeds were collected in batches from each plant line at wax ripening stage for follow-up test.

Genetic background of IL populations

We use GGT2.0 (Graphical Genotype) software (https://ggt.software.informer.com/2.0/) to analyze the number, length, and proportion of imported fragments in each line's genome. In IL-1, some segments of the donor parent Shaanhan 8675 were introgressed into 160 lines. The number of introgressed segments ranged from 1 to 46, with an average of 8.04 cM, and the length of introgressed segments ranged from 12.80 cM-994.2 cM, with an average length of 133.70 cM. Within the 160 lines, the proportion of the donor parent's genome ranged from 0.50 to 38.70% (*Chen et al., 2020*). In IL-2, all 160 lines introgressed partial segments of the donor parent Jing 411. The number of introgressed segments ranged from 2 to 25, with an average of 6.85 cM, and the length of introgressed segments ranged from 23.12 cM–516.37 cM, with an average length of 155.68 cM. Within the 160 lines, the proportion of the donor parent's genome ranged from 0.90 to 20.10% (*Yan et al., 2019*).

Artificial accelerated aging test and standard seed germination test

An artificial aging (AA) test was carried out based on the method described by *Shi et al. (2020)*. In brief, after three months of storage at room temperature, the harvested wheat seeds were used for AA test. 950 healthy seeds from each wheat plant line were placed into the kraft paper bag, and then laid on the grid. 1,000 mL of distilled water was added at the bottom of the aging basin (30 cm in diameter). The basin was sealed with a cover and then placed on the grid, followed by putting the aging basin in an artificial climate incubator with a temperature of 48 °C and a relative humidity of 100% for artificial aging. Then, the seeds were respectively aged for 0 h (as the control), 24 h, 36 h, 48 h, 60 h, and 72 h, under dark conditions. After naturally drying at room temperature for three days, the seeds were used for the standard seed germination test. The treatment for each wheat plant line was conducted with three biological replicates.

The aged seeds were germinated according to the standard germination method. 50 seeds were selected for each treatment, with three replicates. Firstly, the seeds were disinfected

with 0.1% NaClO solution for 20 min, and washed with distilled water for 4 times, followed by immersion in a culture dish for 18 h (at this time point, 0 h-aged seeds (control) started to appear white). Secondly, the seeds were evenly placed on a seedling tray (31 cm long, 23 cm wide and 4.5 cm thick), and 1,000 ml distilled water were added into the tray. Thirdly, the seeds were placed in an artificial climate box, and then cultured under the conditions of temperature 25 °C, relative humidity 75%, light intensity 12000 LX, and a photoperiod of 16 h-light and 8 h-dark. An appropriate amount of distilled water was regularly added to ensure the humidity of seedling tray. Fourthly, after the seeds were immersed for three days, the normal seed germination was recognized when the protruding part of seed radicle reached to the half of seed length. Finally, on the seventh day of cultivation, five seedlings per treatment were randomly selected to measure the seedling lengh (SL), root length (RL), seedling fresh weight (SFW) and root fresh weight (RFW).

Calculation of traits related to wheat seed vigor

The calculation formulas were as follows: (*Shi et al., 2020*)

Germation energy (GE) = $(n_3/50) \times 100\%$

Germination rate (GR) = $(n_7/50) \times 100\%$

Germination index (GI) = $\Sigma Gt/Dt$

Germination pace (GP) = $n_7/ \Sigma(n \times g) \times 100$

Vigor index (VI) = GI × SL

Simple vigor index (SVI) = GR × SL

In these formulas, n_3 is the number of germinated seed on 3 d of culture while n_7 is the number of germinated seed on 7 d of culture. Gt means the number of germinated seed per day, and Dt refers to the corresponding germination days of Gt.

Data analysis and QTL mapping

Phenotypic data were statistically analyzed by Excel 2016 (Microsoft Corp., Redmond, USA), SPSS 25 (SPSS Inc., Chicago, USA), and Origin 2021 (OriginLab Corp., Northampton, MA, USA). The genetic map of the populations was integrated by our lab (*Yan et al., 2019*; *Chen et al., 2020*). The genetic linkage maps of the two IL populations were constructed using MapDraw software based on the integrated map of wheat high density SSR markers published by Somers (*Somers, Isaac & Edwards, 2004*). There were 187 polymorphic SSR markers in IL-1 population and 156 in IL-2 population. SSR amplicon sizes for these markers were detected using polyacrylamide gel electrophoresis (*Yan et al., 2019*). The total length of the linkage maps was 2569 cM for both two IL populations, and the mean distance between the markers was 13.73 cM for IL-1 population and 16.47 cM for IL-2 population.

QTLs were described by the nomenclature q trait-processing time-chromosome. QTL detection was performed using IciMapping 4.2 (http://www.isbreeding.net/) (Permutation times = 1,000, $P < 0.05$).

Table 1 Additive-effect QTLs for seed vigor traits detected in wheat IL-1 population.

Trait	Aging time	QTL	Chr.	Marker	LOD	Additive effect	PVE (%)
GR	0 h	*qGR-0h-3D*	3D	*Xwmc418*	3.26	−0.03	9.84
	72 h	*qGR-72h-5A*	5A	*Xwmc524*	3.23	0.13	9.02
GI	72 h	*qGI-72h-5A*	5A	*Xwmc524*	3.09	1.88	10
GP	48 h	*qGP-48h-7A*	7A	*Xcfa2019*	3.58	−1.5	11.47
	60 h	*qGP-60h-6A*	6A	*Xwmc807*	2.94	1.45	9.29
RL	0 h	*qRL-0h-1B*	1B	*Xwmc830*	2.89	0.88	7.47
	24 h	*qRL-24h-1B*	1B	*Xwmc830*	3.57	0.88	10.59
VI	36 h	*qVI-36h-7B*	7B	*Xgwm400*	2.78	−15.41	9.05
	60 h	*qVI-60h-6A*	6A	*Xwmc807*	3.09	17.59	10.07
SVI	36 h	*qSVI-36h-7B*	7B	*Xgwm400*	3.05	−0.83	9.65

Notes.

Positive values in additive effect indicate that 'Shaanhan 8675' alleles increase the corresponding trait, and, conversely, negative values indicate that 'Shaanhan 8675' alleles decrease the trait.

RESULTS

Phenotypic analysis of seed-vigor traits in two IL populations after artificial aging

After aging treatments, a total of 10 traits related to seed vigor were measured including GE, GR, GI, GP, SL, RL, SFW, RFW, VI and SVI. The results showed that there was progressive decline in all the 10 traits with the prolongation of aging time. In most cases, the absolute values of skewness and kurtosis coefficients of the traits in those two IL populations were less than one, representing the characteristic of normal distribution of the traits tested among these populations (Fig. 1 and Fig. S1). As evident from Tables S1 and S2, the variation of these seed vigor traits in the two mapped populations showed a continuous distribution. In the IL-1, the phenotypic value of both parents under different aging conditions were all within the range of variation of the genetic population for mapping, and in the IL-2, except for 72 h of aging, the traits of both parents under other aging conditions were all within the variation range of the population. The results indicated that these seed vigor traits were quantitative traits controlled by a few minor-polygenes, and the genes controlling these traits were effectively recombined. Therefore, these two wheat IL populations are suitable for QTL mapping of these seed vigor traits.

In addition, we also noticed that the donor parent Shaanhan 8675 was treated with 0 h aging except for SL and FRW, the other eight traits were lower than that of recipient parent Lumai 14, and the other nine traits of donor parent Jing 411 were lower than that of Lumai 14 except SFW, which indicated that the seed vigor of Lumai 14 was better than that of other two parents without aging treatment. After 72 h of aging, except for SL, the decline degree of the traits was lower in the Jing 411 than that in the Lumai 14, indicating a relatively good storability of the Jing 411. In the Shaanhan 8675, the decline degree of the six vigor traits including GE, GR, GI, GP, VI and SVI was lower than that in the Lumai 14, but the decline degree of SL, RL, SFW and RFW in the Shaanhan 8675 was higher than that in Lumai 14. This showed that Shaanhan 8675 was superior to Lumai 14 in seed germination

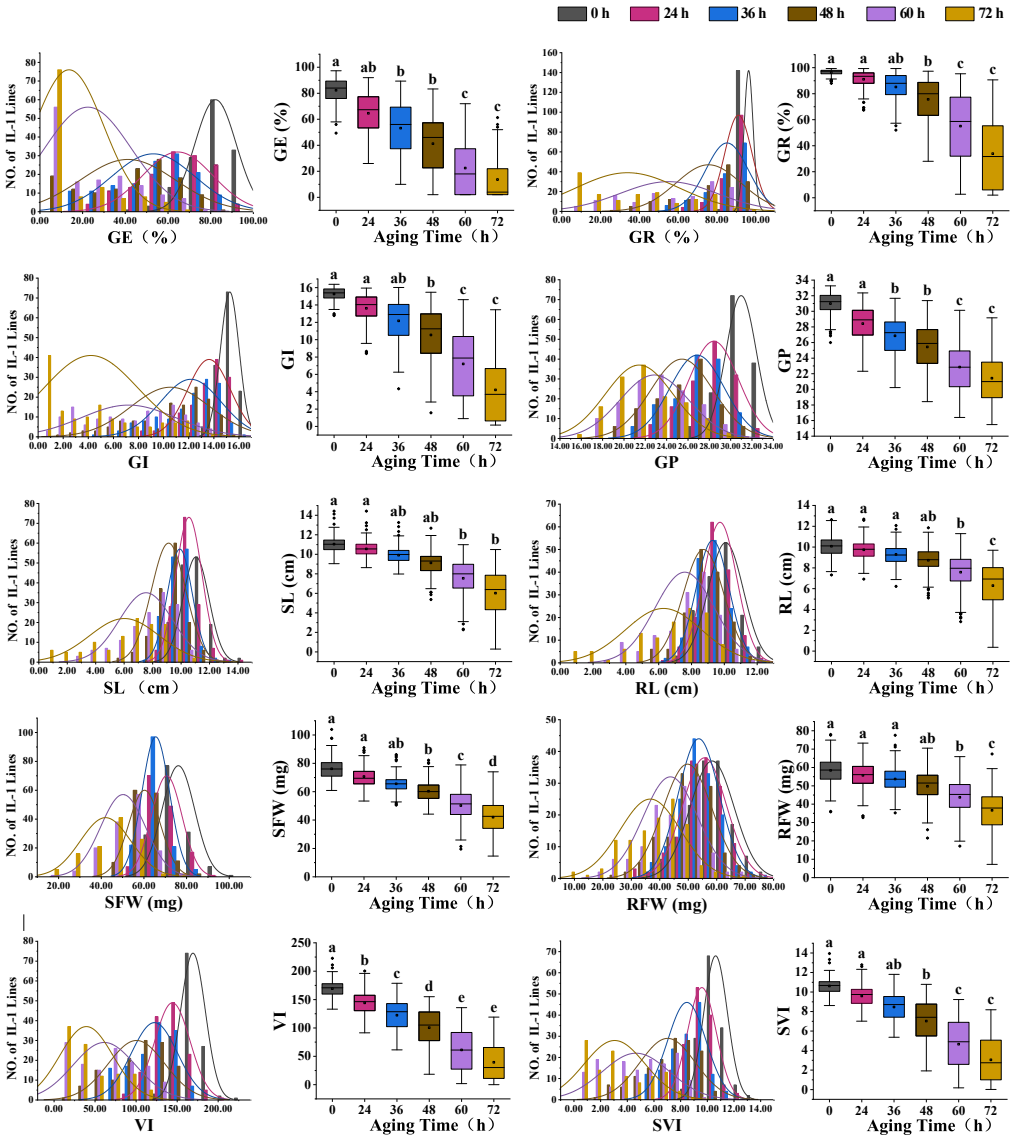

Figure 1 Frequency distributions and differences for wheat seed vigor-related traits under six different aging treatments in IL - 1 population. IL, introgression line, GE, gemination energy, GR, gemination rate, GI, germination index, GP, germination pace, SL, seedling lengh; RL, Root Length, SFW, seedling fresh weight, RFW, root fresh weight, VI, vigor index, SVI, simple vigor index. The same below. For the frequency distribution histogram, the *X*-axis represents the distribution range (interval) of phenotypic for each trait in IL-1 population, while the *Y*-axis corresponds to the number of lines in each intervals under 6 different treatments, The fitting curves of different colors reflect the distribution of phenotypic under the different aging treatment. For a boxpLot, the *X*-axis represents the six aging treatment for each trait, while the *Y*-axis corresponds to the phenotype. Different colors represent aging for 0 h, 24 h, 36 h, 48 h, 60 h and aging 72 h, respectively. Different lowercase letters showed significant difference ($P < 0.05$).

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vigor after extreme aging, but seedling vigor was weaker in Shaanhan 8675 than in Lumai 14 (Tables S1 and S2). Additionally, a correlation analysis was conducted for those 10 seed-vigor traits (Fig. 2 and Fig. S2). Consistently, a significant or extremely significant

Table 2 Additive-effect QTLs for seed vigor traits detected in wheat IL-2 population.

Trait	Aging time	QTL	Chr	Marker	LOD	Additive effect	PVE (%)
GE	60 h	*qGE-60h-7B*	7B	*Xwmc396*	4.85	0.14	21.35
GI	0 h	*qGI-0h-4B*	4B	*Xwmc47*	2.94	−0.51	9.38
	72 h	*qGI-72h-1B*	1B	*Xwmc406*	4.76	2.62	10.89
GP	36 h	*qGP-36h-5B*	5B	*Xwmc616*	4.19	1.14	9.91
	36 h	*qGP36h-6D*	6D	*Xbarc96*	3.51	0.92	8.27
	36 h	*qGP-36h-7D*	7D	*Xwmc671*	3.39	−1.79	798
SL	36 h	*qSL-36h-2A*	2A	*Xwmc667*	3.27	0.54	10.02
	48 h	*qSL-48h-2A-1*	2A	*Xwmc667*	4.99	0.82	13.16
	48 h	*qSL-48h-2A-2*	2A	*Xgwm359*	2.73	−0.49	6.8
	60 h	*qSL-60h-2A*	2A	*Xwmc667*	2.82	0.93	10.01
RL	0 h	*qRL-0h-1D*	1D	*Xgdm126*	2.59	0.46	7.49
	24 h	*qRL-24h-1D*	1D	*Xgdm126*	3.05	0.48	8.56
SFW	48 h	*qSW-48h-2A*	2A	*Xwmc667*	3.27	3.99	10.16
	60 h	*qSW-60h-2A*	2A	*Xwmc667*	2.8	5.11	10.12
VI	24 h	*qVI-24h-1D*	1D	*Xgwm458*	3.44	−10.55	12.58
	72 h	*qVI-72h-1B*	1B	*Xwmc406*	3.65	20.89	21.89

Notes.
Positive values in additive effect indicate that 'Jing 411' alleles increase the corresponding traits, and, conversely, negative values indicate that 'Jing 411' alleles decrease the trait.

correlation was detected between seed-vigor traits among these two IL populations under the same aging condition.

Detection of additive QTLs for wheat seed-vigor traits

Under six different aging treatments (six different lengths of aging time), a total of 26 additive QTLs for all the traits except RFW were identified from those two IL populations (Tables 1 and 2, Figs. 3 and 4). In IL-1 population, 10 additive QTLs were detected, with the LOD values of 2.78−3.58 and their phenotypic contribution rates from 7.47 to 11.47%. These additive QTLs were distributed on chromosomes 1B, 3D, 5A, 6A, 7A and 7B of wheat (Table 1), respectively. In IL-2 population, 16 additive QTLs were detected, with the LOD values of 2.59−4.99 and their phenotypic contribution rates were from 6.80% to 21.89%, as well as their distribution on chromosomes 1B, 1D, 2A, 4B, 5B, 6D, 7B and 7D (Table 2), respectively.

Additive QTL of seed germination energy (GE)

One additive QTL (*qGE-60h-7B*) of seed GE was identified in IL-2 population after 60 h of aging. This GE QTL was located near the marker Xwmc396 on chromosome 7B, with its favorable allele coming from Jing 411. This QTL contributed 21.35% of the phenotypic variation, with the additive effect of 0.14 and the LOD value of 4.85, indicating a high potential in wheat breeding to increase seed vigor.

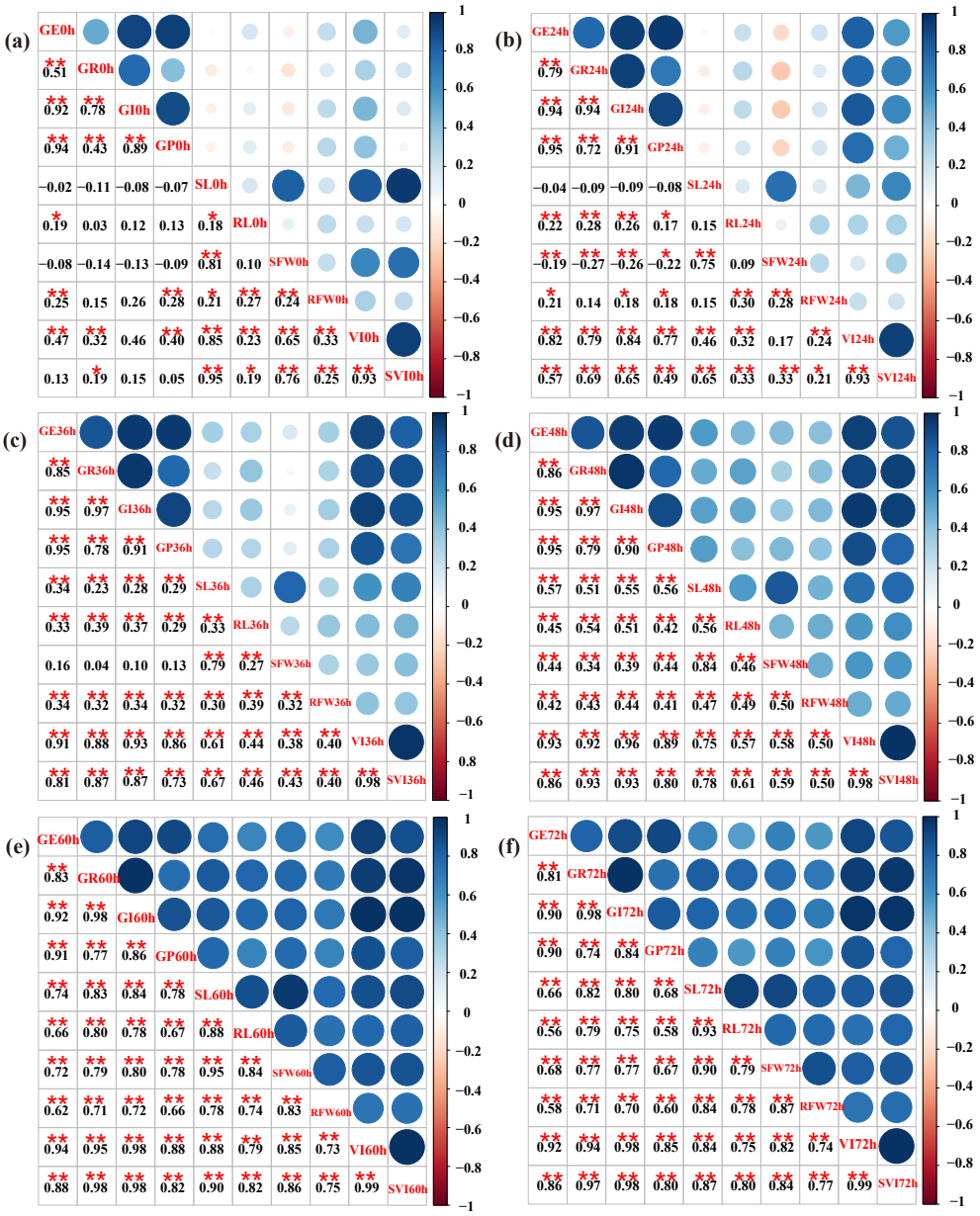

Figure 2 Heatmap of the correlations between wheat seed vigor-related traits in IL - 1 population. (A–F) Aging for 0 h, 24 h, 36 h, 48 h, 60 h and aging 72 h, respectively. Asterisks (*, **, ***) indicate that correlation is significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

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Additive QTL of seed germination rate (GR)

In IL-1 population, one additive QTL of GR was identified at 0 h and 72 h of aging, respectively. *qGR-0h-3D* detected at 0 h of aging was located near the marker Xwmc418 on chromosome 3D, with its synergetic alleles from Shaanhan 8675 and the additive effect of 0.13. *qGR-72h-5A* detected at 72 h of aging was mapped near the marker Xwmc524 on

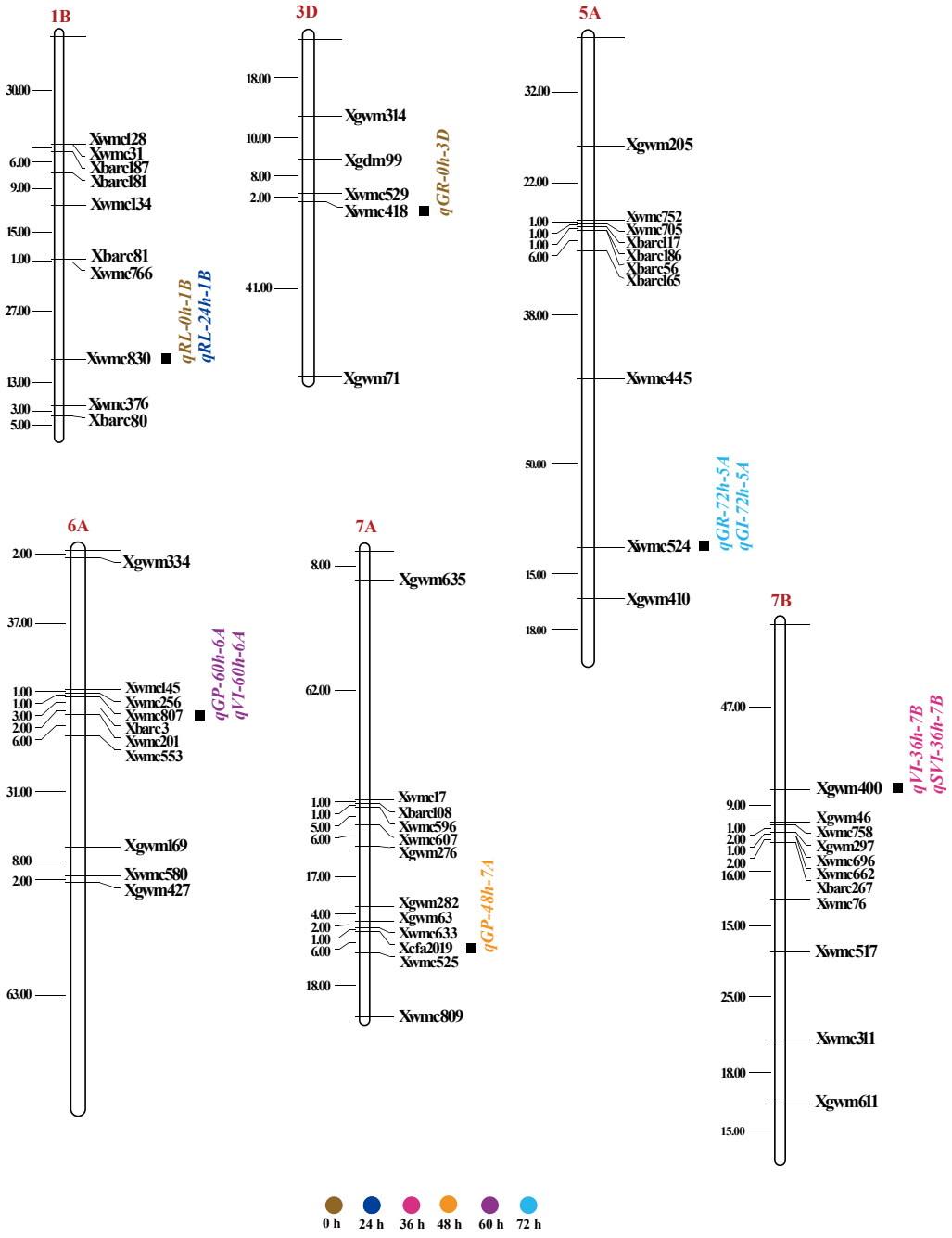

Figure 3 **Distribution of additive QTLs for wheat seed-vigor traits detected in IL - 1 population.** Different colors represent different aging time.

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wheat chromosome 5A. The contribution rate of *qGR-0h-3D* and *qGR-72h-5A* was 9.84% and 9.02% of the phenotypic variation, respectively.

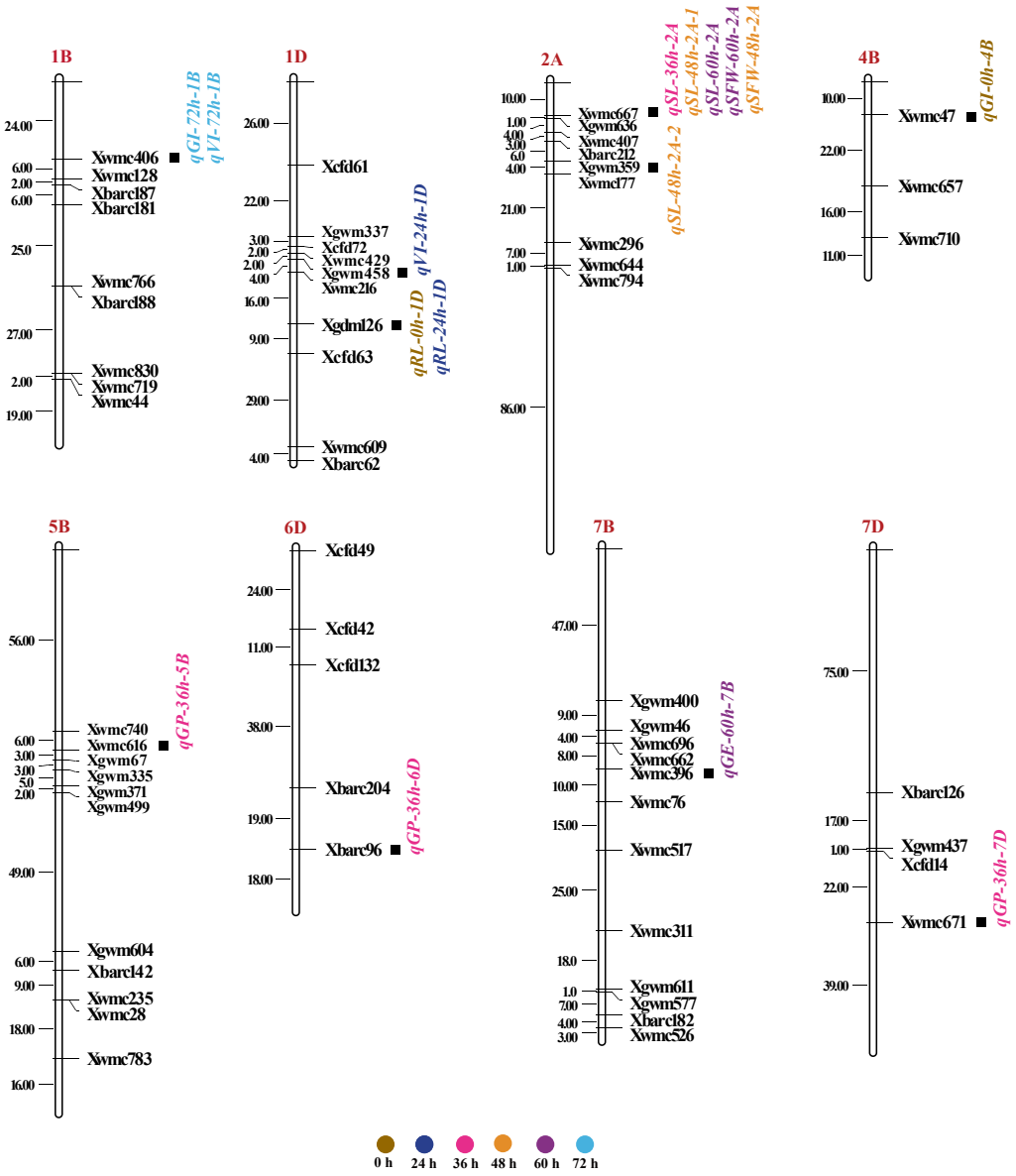

Figure 4 Distribution of additive QTLs for wheat seed-vigor traits detected in IL - 2 population. Different colors represent different aging time.

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Additive QTL of seed germination index (GI)

Three additive QTLs of GI were detected using these two IL populations, with their locations on chromosomes 1B, 4B and 5A. At 0 h of aging, an additive QTL of GI (*qGI-0h-4B*) was detected near the marker Xwmc47 on chromosome 4B using IL-2 population. After 72 h of extreme aging, an additive QTL of GI was detected, respectively, in the two populations, with *qGI-72h-5A* in IL-1 and *qGI-72h-1B* in IL-2. For these two loci, the synergistic alleles were Shaanhan 8675 and Jing 411, respectively. The additive effect was 1.88 and 2.62 for *qGI-72h-5A* and *qGI-72h-1B*, respectively.

Additive QTL of seed germination pace (GP)

In IL-1 population, an additive QTL of seed GP was detected at 48 h and 60 h of aging, respectively. *qGP-48h-7A* was mapped near the marker Xcfa2019 on chromosome 7A at 48 h of aging. This QTL was recognized as a major QTL responsible for wheat seed GP since it contributed 11.47% of phenotypic variation and its synergetic alleles were come from Lumai 14, with the additive effect of −1.50. For IL-2 population, three additive QTLs of GP were detected at 36 h of aging, locating on chromosomes 5B, 6D and 7D, respectively. With just 7.98%–9.91% of contribution to the phenotypic variation, they were minor QTLs for GP.

Additive QTL of wheat seedling length (SL)

Four additive QTLs for this trait were detected in IL-2 population. *qSL-36h-2A*, *qSL-48h-2A-1* and *qSL-60h-2A* were located near the marker Xwmc667 on chromosome 2A. Interestingly, these three QTLs are likely the same QTL locus, named *qSL-2A* detected under the three different aging treatments. Moreover, their contributions to the phenotypic variation were greater than 10, indicating that *qSL-2A* is the major QTL responsible for wheat SL under those three aging treatments. *qSL-48h-2A-2* was another additive QTL of SL on chromosome 2A, having its synergetic alleles provided by Lumai 14 and the additive effect of −0.49 as well as only accounting for 6.80% of phenotypic variation.

Additive QTL of root length (RL) of wheat seedling

Two additive QTLs for RL of wheat seedling were detected at 0 h and 24 h of aging, respectively. In IL-1 population, *qRL-0h-1B* and *qRL-24h-1B* were all located near the marker Xwmc830 on chromosome 1B. Their synergetic alleles were all derived from Shaanhan 8675, and the additive effect was 0.88. Thus, it's likely that they were located at the same QTL locus named as *qRL-1B* responsible for wheat seedling RT trait at 0 h and 24 h of aging. Similarly, in IL-2 population, *qRL-0h-1D* and *qRL-24h-1D* were mapped near the marker Xgdm126 on chromosome 1D. Their synergetic alleles were all derived from Jing 411 and their additive effects were 0.46 and 0.48, respectively. Therefore, these two QTLs were likely located at the same QTL locus named as *qRL-1D*. Since the QTLs of RL were detected in both IL populations under 0 h and 24 h of aging treatments, these two additive QTLs, *qRL-1B* and *qRL-1D* only affected the root length of wheat seedling under no aging or slightly aging, but had no significant impact on the root length after a long period of aging.

Additive QTL of seedling fresh weight (SFW)

One additive QTL for this trait was detected in IL-2 population at 48 h and 60 h of aging, repectively. *qSFW-48h-2A* and *qSFW-60h-2A* had the LOD value of 3.27 and 2.80, respectively. Since these two QTLs were located near the marker Xwmc667 on chromosome 2A, and their synergistic alleles were all derived from Jing 411, they should be the same QTL named as *qSFW-2A* responsible for this trait under the two aging treatments. This QTL accounted for the phenotypic variation by more than 10% and thus, *qSFW-2A* was a major QTL for this trait under two kinds of aging treatment, showing a certain utilization value in wheat breading to achieve stronger seed vigor.

Additive QTL of seed vigor index (VI)

After the wheat seeds were aged, two additive QTLs of seed VI trait were, respectively, detected in the two IL populations under aging treatments. In IL-1 population, *qVI-60h-6A* and *qVI-36h-7B* were detected to locate on chromosomes 6A and 7B, respectively, at 60 h and 36 h of aging. With the synergistic alleles derived from Shaanhan 8675 and the additive effect of 17.59, *qVI-60h-6A* was a major QTL for wheat VI trait due to its high contribution to the phenotypic variation (10.07%). Conversely, *qVI-36h-7B* was a minor QTL for this trait, with the synergistic alleles derived from Lumai 14 and the additive effect of −15.41, as well as a small contribution to the phenotypic variation (9.05%). In IL-2 population, two additive QTLs (*qVI-72h-1B* and *qVI-24h-1D*) of seed VI were located on chromosomes 1B and 1D, respectively. *qVI-24h-1D* had the synergistic alleles derived from Lumai 14 and the additive effect of −15.41 as well as a high contribution to the phenotypic variation (12.58%). *qVI-72h-1B* had the synergistic alleles derived from Jing 411 and the additive effect up to 20.89 as well as a much higher contribution to the phenotypic variation (21.80%) Collectively, both *qVI-24h-1D* and *qVI-72h-1B* were the major QTLs responsible for wheat seed VI under aging, with *qVI-72h-1B* having much greater impact and high value for use in wheat breeding program to enhance seed VI.

Additive QTL of simple vigor index (SVI) of seeds

In the IL-1 population, one additive QTL of SVI (*qSVI-36h-7B*) was detected after 36 h of aging. This QTL was located near the marker Xgwm400 on chromosome 7B, with its synergistic alleles provided by Lumai 14, the additive effect of −0.83, the LOD value of 3.05 and accounting for 9.65% of the phenotypic variation.

Detection of epistatic QTLs for wheat seed-vigor traits

A total of 72 pairs of epistatic QTLs responsible for eight seed-vigor traits (GR, GI, GP, SL, RL, SFW, VI and SVI) were detected using the two wheat IL population, with distribution on chromosomes 1A, 1B, 1D, 2A, 2B, 2D, 3B, 3D, 4D, 5A, 5B, 5D, 6A, 7A and 7B of wheat, respectively. 47 pairs of them were located on chromosome 4D (Tables S3 and S4). The LOD value of these epistatic QTLs ranged from 4.89 to 7.74. Their contributions to the phenotypic variation were from 0.20% to 15.92%, and their epistatic effects ranged from −16.42 to 16.57. Remarkably, there were 25 pairs of epistatic QTLs with parental effect greater than recombination effect (the interaction effect was greater than 0) and 47 pairs of epistatic QTLs with parental effect less than recombination effect (the interaction effect was less than 0). Moreover, 6 QTLs for wheat seed-vigor traits exhibited additive effect and epistatic effect, including *qGE-48h-7A*, *qSL-48h-2A-1*, *qSL-48h-2A-2*, *qSL-60h-2A*, *qRL-24h-1D*, and *qSW-48h-2A*.

In addition, several pairs of epistatic QTLs with pleiotropy were simultaneously mapped to two markers, conditionally designated marker 1 and marker 2. As shown in Tables S3 and S4, in wheat IL-1 population, five pairs of epistatic QTLs concurrently linked to SL, VI and SVI were located near the marker1 (Xwmc720) and marker2 (Xwmc752, Xgwm583, Xgwm63, Xwmc758 and Xwmc696), respectively. Likewise, ten pairs of epistatic QTLs located near the marker1 (Xwmc720) and the marker2 (Xwmc705, Xbarc56,

Table 3 Genetic information of additive QTL hotspots in IL population.

Gene ID	Chr.	Interval (bp)	Gene Annotation
TraesCS2A01G015800	2A	6997999..7000883	Cytochrome P450 family protein
TraesCS2A01G016500	2A	7687810..7692604	Pectate lyase
TraesCS2A01G017300	2A	8141118..8143112	F-box family protein
TraesCS2A01G017400	2A	8147731..8150606	FACT complex subunit SPT16
TraesCS2A01G018800	2A	8755966..8757713	Cytochrome P450

Xgwm400, Xgwm46, Xwmc662, Xwmc76, Xwmc766, Xwmc819, Xgwm148 and Xwmc41), respectively, were also simultaneously linked to VI and SVI. Another three epistatic QTLs located near the marker1 (Xwmc331) and the marker2 (Xgwm148, Xwmc41, Xgwm63), respectively, were all detected to affect VI and SVI traits. In wheat IL-2 population, three epistatic QTLs were detected near the marker1 (Xcfd9) and the marker2 (Xgwm261 and Xwmc112), respectively, with all of them affecting SL and SFW traits synchronously.

Prediction of candidate genes within QTLs responsible for wheat seed vigor

Five QTLs were detected near marker Xwmc667 on chromosome 2A in IL-2 population (Table 2, Fig. 4), including *qSL-36h-2A, qSL-48h-2A-1, qSL-60h-2A, qSW-48h-2A* and *qSW-60h-2A*. These five QTLs were detected to affect SL and SFW under different aging treatments with large contribution to the phenotypic variation (>10%).

The co-located region of these five QTLs on chromosome 2A was considered as an important hotspot region in association with wheat seed vigor. Based on the WheatOmics (http://202.194.139.32/jbrowse.html), marker Xwmc667 was located between 7,961,478 bp-7,961,536 bp on chromosome 2A. This physical region was then extended 1 Mb on the left and right sides, respectively, to be used as the target genome region (6,961,478 bp–8,961,536 bp) for mining the candidate genes. Based on the Chinese Wheat Complete Genome Reference Sequence (IWGSC RefSeqv1.0), a total of 37 genes (Table S5) were found in this region and gene annotation was performed by referring to the website (https://plants.ensembl.org/index.html). Of them, five genes Cytochrome P450 family protein (*TraesCS2A01G015800*), Pectate lyase (*TraesCS2A01G016500*), F-box family protein (*TraesCS2A01G017300*), FACT complex subunit SPT16 (*TraesCS2A01G017400*) and Cytochrome P450 (*TraesCS2A01G018800*) were found to be highly associated with seed vigor possibly (Table 3).

GO analysis was performed for these 37 candidate genes (Table S6). All the candidate genes were significantly enriched in two categories of GO function clustering, with seven terms in biological processes (BP) and three terms in molecular functions (MF) (Fig. 5). Importantly, two candidate genes Cytochrome P450 family protein (*TraesCS2A01G015800*) and Cytochrome P450 (*TraesCS2A02G018800*) were functionally clustered into four terms including single-organism process, metabolic process, catalytic activity, and binding. The

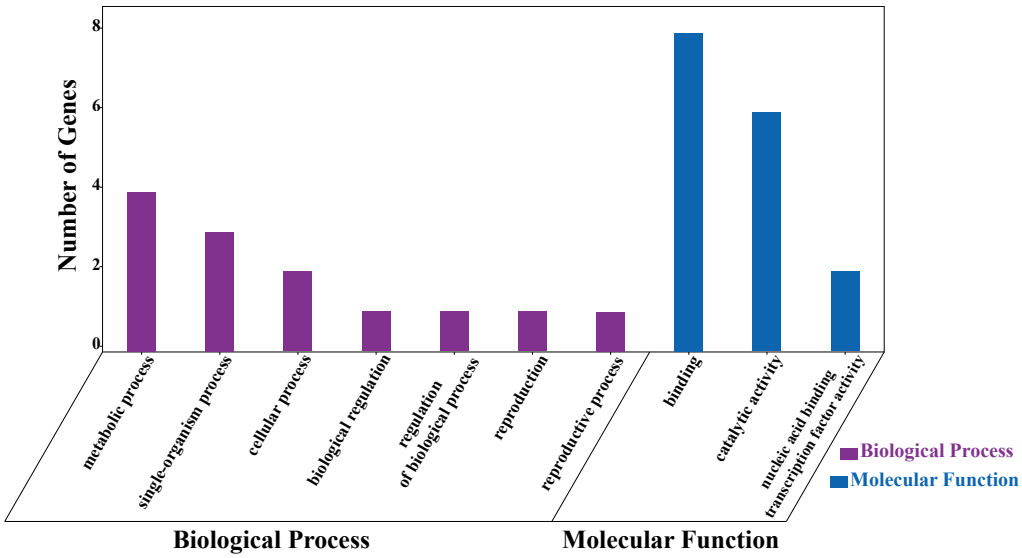

Figure 5 **GO annotation clustering map of the candidate genes.**
Full-size DOI: 10.7717/peerj.17778/fig-5

candidate gene pectate lyase (*TraesCS2A01G016500*) was grouped into metabolic process, catalytic activity, and binding.

KEGG analysis was employed to predict the pathways involved by the candidate genes (Table S7). Most of the candidate genes were enriched in metabolic pathway and biosynthesis of secondary metabolites (Fig. 6). Notably, Cytochrome P450 (*TraesCS2A02G018800*) was significantly enriched in five metabolic pathways including glucosinolate biosynthesis, 2-Oxocarboxylic acid metabolism, cyanoamino acid metabolism, metabolic pathways and biosynthesis of secondary metabolites. Pectate lyase (*TraesCS2A02G016500*) was significantly enriched in pentose and glucuronate interconversions, and metabolic pathways.

DISCUSSION

Phenotypic variation and genetic basis of seed-vigor traits

The level of seed vigor is mainly affected by genetic basis and environmental factors during seed development and seed storage (*Sun, Wang & Sun, 2007*). In order to effectively examine seed vigor, artificial aging is usually employed, which makes it possible to evaluate seed vigor in short time (*Rajjou et al., 2008*). During seed aging, a lot of physiological and biochemical processes occurred, which made it extremely complicated to measure seed vigor after artificial aging (*Corbineau et al., 2002*; *Ahmed, Yang & Fu, 2016*). Thus, it is required to determine appropriate parameters to measure the dynamics of seed vigor during seed aging. In the present study, 10 parameters representing different aspects of seed vigor were adopted to fully and precisely characterize the level of wheat seed vigor under six different aging conditions (0, 24, 36, 48, 60, and 72 h of aging), which is different from the previous reports where a few traits reflecting wheat seed vigor were measured under only one aging treatment (*Agacka-Moldoch et al., 2016*; *Arif et al., 2017*; *Zuo et al.,*

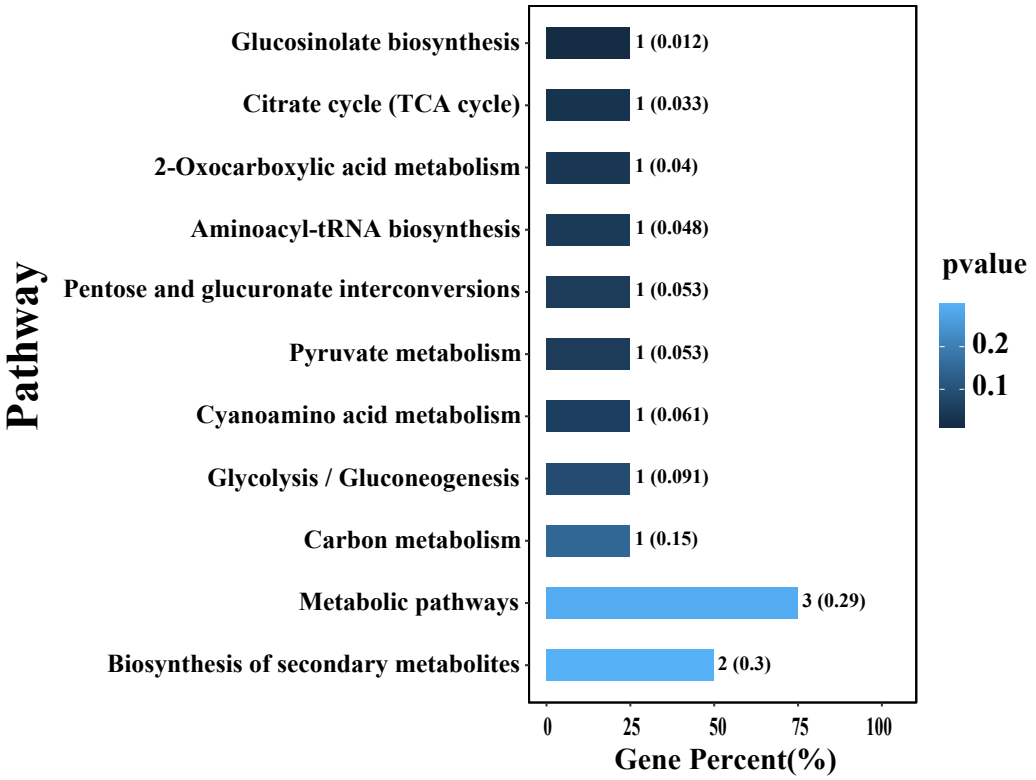

Figure 6 **Pathway distribution map of the candidate genes.**
Full-size DOI: 10.7717/peerj.17778/fig-6

2018). Our findings suggest that the numerical values obtained for these ten parameters (GE, GR, GI, GP, SL, RL, SRW, RFW, VI, and SVI) decreased with the extension of aging time with a continuous variation pattern (Figs. 1 and 2, Tables S1 and S2). Moreover, the absolute values of skewness and kurtosis coefficient of each trait in the two wheat IL populations evidenced the normal distribution of phenotypes of the traits examined (Fig. 1). This indicate that the ten parameters, together with two IL populations and six aging treatments tested here were very suitable for detecting QTLs responsible for wheat seed vigor.

In this study, a total of 26 additive QTLs and 72 pairs of epistatic QTLs were detected in two introgression lines under six different periods of aging, which affected the GE, GR, GP, GI, SL, RL, VI, SVI and other vigor traits in wheat. It shows that the traits could be improved by artificial selection effectively, thus achieving the target of improving seed vigor as well as the final yield increase. In addition to the RFW, additive QTLs were detected for the rest 9 seed vigor traits. As *qRL-0h-1B* and *qRL-24h-1B* were located near the marker Xwmc830 on chromosome 1B, *qRL-0h-1D* and *qRL-24h-1D* were located near the marker Xgdm126 on chromosome 1D, *qSW-48h-2A* and *qSW-60h-2A* were located near the marker Xwmc667 on chromosome 2A, *qSL-36h-2A*, *qSL-48h-2A-1* and *qSL-60h-2A* were located near the marker Xwmc667 on chromosome 2A (Tables S1, S2), they were the same QTL affected the corresponding traits at different aging periods, which suggested that these QTLs had

longer action period than other QTLs during seed storage or aging process, and should be paid more attention to in breeding practice.

A significant correlation was detected between the parameters tested here for assessing wheat seed vigor (Fig. 2), which is consistent with a previous reports (*Shi et al., 2020*). This suggests that the genetic loci controlling seed vigor may be pleiotropic and interacted with each other. Correspondingly, several chromosome regions (hotspots) were identified to contain multiple QTLs of wheat seed vigor in the present study. For example, *qGE-60h-6A* and *qVI-60h-6A* were detected near the marker Xwmc807 on chromosome 6A. *qVI-36h-7B* and *qSVI-36h-7B* located near the marker Xgwm400 on chromosome 7B. *qGI-72h-1B* and *qVI-72h-1B* were mapped near the marker Xwmc406 on chromosome 1B. Particularly, *qSL-36h-2A*, *qSL-48h-2A-1*, *qSL-60h-2A*, *qSW-48h-2A* and *qSW-60h-2A* were identified near the marker Xwmc667 (Tables 1 and 2). Clearly, these closely-located QTLs may have the pleiotropism on wheat seed vigor. Furthermore, a number of epistatic QTLs were also detected for wheat seed vigor here. For example, in IL-1 population, five pairs of epistatic QTLs were detected to simultaneously affect the SL, VI and SVI, while 13 pairs of epistatic QTLs were identified to synergistically influence VI and SVI. In IL-2 population, the epistatic QTLs located between the marker Xcfd9 and Xgwm261 or Xwmc112 were interacted in controlling the SL and SFW at the same time of aging (Tables S3 and S4). These additive QTLs with one cause and multiple effects, as well as epistatic QTLs with multiple effects, provide good molecular genetic evidence for significant phenotypic correlations between seed vigor traits.

Comparison of QTLs detected between the two IL populations of wheat

Theoretically, quantitative traits like seed vigor are controlled by minor-polygenes (*Bewley & Black, 1994*; *Zuo et al., 2018*), which makes it difficult to identify the same QTL even using the same population (*Schwember & Bradford, 2010*; *Arif et al., 2012*). Hence, it is necessary to identify QTL using different mapping populations, multiple traits and different aging treatments. In this study, QTL mapping was performed on 10 vigor traits of two wheat IL populations under six different aging times. The 10 traits represented different aspects in wheat seed germination and seedling morphogenesis, the six different aging times represented different degrees of seed aging/deterioration. It is useful to understand the vigor regulation of wheat seed in aging process. In order to precisely detect the real QTLs for wheat seed vigor, two IL populations derived from the cross between the common receptor parent Lumai 14 and different donor parents Jing 411 or Shaanhan 8675. Such populations have the common genetic background with difference in introgressive fragments, which makes it convenient to do comparative analysis of QTL effects.

Here, a total of 10 additive QTLs and 17 pairs of epistatic QTLs responsible for the seed vigor traits were identified using IL-1 population, with the additive QTLs mapped on chromosomes 1B, 3D, 5A, 6A, 7A and 7B of wheat (Table 1 and Table S3). Moreover, 16 additive QTLs and 55 pairs of epistatic QTLs were detected by IL-2 population, with the additive QTLs distributed on chromosomes 1B, 1D, 2A, 4B, 5B, 6D, 7B and 7D (Table 2 and Table S4). Most of these additive QTLs were found in A subgenome (11), followed by B (nine) and D (six) subgenomes. However, consistent QTLs for seed-vigor traits

were only detected on chromosomes 1B and 7B. The causes for just a few consistent QTLs detected could be due to genotype difference of donor parents, population size, introductions of donor fragments, molecular marker density and other factors of the two mapping populations. Therefore, it is still necessary to further use linkage or association mapping to analyze more diverse populations or germplasm so as to discover the stable QTLs related to seed vigor for wheat breeding to increase seed vigor or predict seed life under the multi-genetic background.

Comparison of QTLs detected with the QTLs reported previously in wheat

In view of importance of wheat seed vigor, some genetic loci related to seed germination or seedling vigor were identified by QTL analysis in recent years through different mapping populations (*Batool et al., 2018*; *Li et al., 2018a*; *Li et al., 2018b*; *Blackburn et al., 2021*). Notably, among the 26 QTLs detected in the present study, six QTLs were located in the same or adjacent areas on the chromosome as the seed-vigor related QTLs reported previously. In this study, our data showed that two QTLs of root length (*qRL-0h-1B* and *qRL-24h-1B*) were closely located at the marker Xwmc830 on chromosome 1B. Similarly, *Shi et al. (2020)* detected a QTL of SL (*QSLe1B*) between Xwmc44 and AX-108745931 on chromosome 1B using a doubled haploid population of wheat. According to the integrated genetic map of SSR molecular markers in wheat (*Somers, Isaac & Edwards, 2004*), the distance between Xwmc830 and Xwmc44 was only 2 cM. Thus, *qRL-0h-1B* and *qRL-24h-1B* detected here and *QSLe1B* obtained by *Shi et al. (2020)* may be the same QTL or co-located in a QTL cluster region responsible for wheat seed-vigor traits. Another example is that a QTL (*qVI-24h-1D*) of wheat seed vigor index was mapped near the marker Xgwm458 on chromosome 1D in our study. Coincidently, two QTLs (*QLi-mgt.ipk-1D* for the average germination time, and *QMgr.ipk-1D* for the average germination rate) were also detected near this marker on chromosome 1D by *Landjeva, Lohwasser & Börner (2010)*), with accounting for 15.3% and 16.2% of phenotypic variation, respectively. The third example is that a QTL (*qSL-48h-2A*) for wheat seedling length was detected near the marker Xgwm359 on chromosome 2A. Interestingly, a QTL (*QNDVIs-caas-2A.1*) controlling the vigor of wheat seedlings was detected near the marker Xwmc177 just having 0.96 cM apart from the marker Xgwm359 site on chromosome 2A (https://shigen.nig.ac.jp/wheat/komugi/maps/markerMap.jsp) by *Li et al. (2014)*. Therefore, *qSL-48h-2A* and *QNDVIs-caas-2A.1* were probably the same QTL for wheat seed-vigor related traits. The fourth example is that two QTLs (*qVI-60h-6A* and *qGP-60h-6A* for seed vigor index and germination pace, respectively) were detected at marker Xwmc807 on chromosome 6A. Consistently, *Li et al. (2014)* reported that *QGCs-caas-6A* and *QNDVIs-caas-6A* for wheat seedling vigor were detected at marker Xwmc256 within just 1 cM distance to the maker Xwmc807 on chromosome 6A (*Somers, Isaac & Edwards, 2004*). This indicates that *qVI-60h-6A, qGP-60h-6A, QGCs-caas-6A* and *QNDVIs-caas-6A* may be the same QTL locus (or form a QTL cluster). The fifth example is that a QTL (*qGP-36h-6D*) for seed germination rate was detected near marker *Xbarc96* on chromosome 6D in our study, while two QTLs (*QGCw-caas-6D* and *QGCs-caas-6D*) of wheat seedling vigor were also detected at this marker loci on chromosome 6D by *Li*

et al. (2014), representing that this locus plays a major role in the vigor of wheat seeds and seedlings. Collectively, the above six QTLs detected in the current study and previous reports were located in the same genome area (or hot-spot region), functioning importantly for wheat seed vigor though each of them affected one or more specific traits related to seed vigor. Compared to the known reports of QTLs for wheat seed vigor, the other 20 additive QTLs for wheat seed vigor detected in the present study might be all new loci.

High vigor seeds always have better field performance and higher yield (*Sun, Wang & Sun, 2007*). Seed vigor is also related to other agronomic traits (*Aparicio et al., 2002*; *Czyczyło-Mysza et al., 2014*; *Capo-chichi et al., 2021*). Correspondingly, QTLs of seed vigor may co-locate in the same genome area or adjacent region with QTLs for other traits. In agreement with this, six QTLs for wheat seed vigor detected in the present study were found to be co-located in the region adjacent to the QTLs for grain and other agronomic traits of wheat detected in our previous studies (*Chen et al., 2019*; *Yan et al., 2019*; *Chen et al., 2020*) using the same mapping population. Such co-located QTLs formed pleiotropic loci or gene cluster, functioning coordinately. For example, *qGP-36h-5B* for GP was mapped to the marker Xwmc616 on chromosome 5B in this study, while at the same locus, three QTLs were detected, including *qKL-5B-1* for grain length, *qKT-5B* for grain thickness, and *qLTR-5B-1* for the length-thickness ratio of grain by *Chen et al. (2019)*. Near the marker *Xbarc96* on chromosome 6D, *qGP-36h-6D* responsible for wheat seed GP in this study were located together with *qKW-6D* for grain width (*Chen et al., 2019*) and *QTgw-6D* for thousand seed weight (*Yan et al., 2019*). Near the marker Xwmc671 on chromosome 7D, *qGP-36h-7D* for wheat seed GP in this study were co-located with *QHd-7D* for wheat heading stage identified by *Yan et al. (2019)*. Moreover, both *qSL-48h-2A-2* for wheat seedling length by this study and *QSl-2A* for wheat spike length by *Yan et al. (2019)* were mapped near Xgwm359 on chromosome 2A. Near the marker Xgdm126 on chromosome 1D, there exist two QTLs (*qRL-0h-1D* and *qRL-24h-1D*) for root length in this study, and three QTLs (*QNt-1D* for effective tillers, *Gwp-1D-2* for yield per plant and *QFsn-1D* for fertile spikelets) detected by *Yan et al. (2019)*. Furthermore, *qGP-60h-6A* for wheat seed GP in this study was located near the marker Xwmc807 on chromosome 6A, together with other five QTLs detected by *Chen et al. (2020)* including *qPH-6A* for plant height, *qSL-6A* for spike length, *qTGW-6A-1* for thousand seed weight, *qNT-6A* for the number of effective tillers and *qFSN-6A* for the number of fertile spikelets on the left and right side of Xwmc807 (within a 4 cM region). Clearly, these six QTLs formed a QTL cluster, affecting not only wheat seed germination pace, but also other agronomic traits of wheat. In addition to QTL mapping results by our lab, *Batool et al. (2018)* detected a QTL affecting wheat GR, GI, VI and RL located between Xwmc177 and xbarc19 on chromosome 1B using a RIL population derived from a cross of wheat Pasban 90 × Frontana. This QTL was co-located with another QTL of seedling length detected in the present study near the marker Xgwm359 closely linked to Xwmc177 (within a distance of 4 cM), revealing that this genomic region may play an important role for wheat seed vigor and seedling growth.

From the point of wheat breeding, several major QTLs or hot-spot genome region described above can be used in molecular marker-assisted breeding for pyramiding excellent agronomy traits to develop new wheat varieties with stronger stress resistance and

storage tolerance. In this regard, priority should be given to the use of those pleiotropic loci or gene clusters identified during the breeding practices, also paying attention to the coordination between the traits controlled by the QTL-rich regions.

Candidate genes related to wheat seed vigor

Seed vigor is a quantitative trait involved in multiple metabolite processes such as carbohydrates, lipids, proteins, and secondary substances. Here, 37 genes were mined from the genomic region (6,961,478 bp–8,961,536 bp) of five QTL-colocation on wheat chromosome 2A (Table S5). Five candidate genes were screened to be closely related to seed vigor according to the function annotation by GO and KEGG (Figs. 5 and 6).

Two candidate genes Cytochrome P450 family protein (*TraesCS2A01G015800)* and Cytochrome P450 (*TraesCS2A01G018800*) were functionally annotated as encoding cytochrome P450 family proteins. CYP450 family have multiple functions invlovled in biosynthesis and catabolism (*Xu, Wang & Guo, 2015*; *Pandian et al., 2020*; *Singh et al., 2021*). For example, a rice P450 (*CYP724B1*) functioning crucially in plant architecture, panicle development, and seed germination (*Tong et al., 2018*). Rice *CYP96b4* mutant exhibited pleiotropic-unusual phenotype such as dwarft plant, delayed seed germination and enhanced drought resistance (*Tamiru et al., 2015*). An *Arabidopsis, CYP77A4*, was examined to catalyze epoxidation of fatty acids during seed germination (*Xiang et al., 2023*). Soybean *GmCYP78A10* regulated seed size and weight as well as pod number (*Xiang et al., 2023*). According these reports, we speculate that Cytochrome P450 family protein (*TraesCS2A01G015800*) and Cytochrome P450 (*TraesCS2A01G018800*) may function importantly in wheat seed vigor. The candidate gene pectate lyase (*TraesCS2A01G016500*) was annotated to encode a pectate lyase. This enzyme regulated cell relaxation and expansion through controlling pectate content in cell wall, and thus, affected plant development process (*Wagner & Kohorn, 2001*; *Peaucelle et al., 2011*; *Xu et al., 2022*). The increased expression of pectate lyase family genes in the auxin-treated *Arabidopsis* roots resulted benefiting lateral root regeneration (*Laskowski et al., 2006*). A rice dwarf and early-senescence leaf1 (*del1*) was identified to encode a pectate lyase (*PL*) precursor, with its high expression in elongating tissues (*Leng et al., 2017*). Another example for pectate lyase function is that inhibition of pectate lyase gene expressions in strawberry and tomato fruits altered peel cell size, number and thickness, leading to enhancement of fruit firmness and storage capacity (*Benítez-Burraco et al., 2003*; *Sesmero, Quesada & Mercado, 2007*; *Santiago-Doménech et al., 2008*; *Posé et al., 2015*; *Uluisik et al., 2016*). These examples provide some evidences that pectate lyase (*TraesCS2A01G016500*) may involve in wheat seed vigor since cell division and elongation affected by pectate lyase are the typical features during seed germination. The candidate gene FACT complex subunit SPT16 (*TraesCS2A01G017400*) was annotated to encode the subunits (SSRP1 and SPT16) of FACT (facilitates chromatin transcription) (*Lolas et al., 2010*) *Arabidopsis* mutant plants of *AtSSRP1* or *AtSPT16* displayed various defects in vegetative and reproductive development including early bolting and less seed production (*Grasser, 2020*). *AtSSRP1* was also identified to modulate the transition from seed dormancy to germination and from vegetative to reproductive development by focilitating expression of *DOG1* and *FLC* genes,

respectively (*Michl-Holzinger, Mortensen & Grasser, 2019*; *Grasser, 2020*). The candidate gene F-box family protein (*TraesCS2A01G017300*) was annotated to encode a F-box family protein. Several members of F-box family proteins were detected to mediate phytohormone signaling pathway, flower development, seed germination, and lateral branching (*Saxena, Negi & Sharma, 2023*). For example, *AtFOA2* (*AT3G16740*) controlled seed germination by regulating biosynthesis of GA and ABA (*He et al., 2016*). Overexpression of rice F-box protein gene *OsFBK12* delayed seed germination, and enlarged grains (*Chen et al., 2013*). Remarkably, one of wheat F-box proteins was found to be related to seed germination and other processes. Over expression of a F-box protein gene *TaFBA1* in tobacco plants resulted in the reduced sensibility to ABA treatment during seed germination but increase of tolerance against abiotic stresses including drought, salt, heat and oxidative stress, and this wheat protein could regulated multiple target genes to adjust plant growth and development (*Zhou et al., 2014*; *Kong et al., 2016*; *Zhao et al., 2017*; *Li et al., 2018a*; *Li et al., 2018b*).

Collectively, five wheat genes mined from the QTL region in this study might function importantly for wheat seed vigor. In future investigation, the candidate genes among all the major QTLs identified here should be mined and then functionally characterized in detail, including transgenic assays and mutant examinations so as to comprehensively elucidate the complex gene network underlying the regulation of seed vigor in wheat.

CONCLUSIONS

In the present study, a total of 26 additive QTLs and 72 pairs of epistatic QTLs for 10 seed-vigor traits were detected under six AA treatments using two wheat introgression line populations derived from cultivars Lumai 14 (recipient/recurrent parent) and Shaanhan 8675 (donor parent) or Jing 411 (donor parent), respectively. Of them, 10 additive QTLs distributed on chromosomes 1B, 1D, 2A, 3D, 4B, 5A, 5B, 6A, 6D, 7A, 7B and 7D were identified as new QTLs for wheat seed vigor. Importantly, several major QTLs were identified to control multiple seed-vigor traits such as *qSL-2A* responsible for both wheat seedling length and weight at 36 h, 48 h and 60 h of aging treatments. Particularly, five QTLs (*qSL-36h-2A*, *qSL-48h-2A-1*, *qSL-60h-2A*, *qSW-48h-2A* and *qSW-60h-2A*) near the marker Xwmc667 on chromosome 2A formed a closely-located QTL-rich region, exhibiting pleiotropic effects on wheat seed vigor. Moreover, several QTL clusters detected here were, respectively, located in the same genomic regions as QTLs for wheat seed-vigor traits detected previously using other populations, indicating that they might be the consistent/stable QTLs for wheat seed vigor. A number of candidate genes mined from the co-located region of the five QTLs were predicted to mediate carbohydrate and lipid metabolism, transcription regulation and cell division, with Cytochrome P450 family protein (*TraesCS2A01G015800*), Cytochrome P450 (*TraesCS2A01G018800*), Pectate lyase (*TraesCS2A01G016500*), FACT complex subunit SPT16 (*TraesCS2A01G017400*) and F-box family protein (*TraesCS2A01G017300*) functing importantly in wheat seed vigor. The present findings including stable QTLs, related molecular markers and candidate genes provide valuable scientific reference for molecular marker-assisted selection in wheat

breeding to improve seed vigor and storage life. The epistatic QTLs detected here should be also considered in wheat breeding towards high grain yield and excellent quality despite epistasis is so complicated and remains to be further investigation.

ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Zhenrong Yang conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Jirong Wu performed the experiments, prepared figures and/or tables, and approved the final draft.
- Qiyu Wang conceived and designed the experiments, performed the experiments, prepared figures and/or tables, and approved the final draft.
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- Ruilian Jing conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the Supplementary Files.

Supplemental Information

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