A Novel QTL Controlling Flag Leaf Width Located on Chromosome Arm 7AS in Bread Wheat (Triticum Aestivum L.)

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Abstract

**Background:** Wheat is an important cereal crop and improving wheat production is essential for meeting the food demand from the growing population worldwide. Flag leaf width (FLW) is an important trait affecting plant architecture and contributing to grain yield. To detect loci conferring FLW, we assessed a population of recombinant inbred lines (RILs) from a cross of EGA Wylie/Sumai 3 in different environments.

**Results:** A total of six QTL were detected from the population. Two of them located on chromosome 2B and the other four located on chromosomes 2D, 4B, 7A, and 7B, respectively. The percentage of phenotypic variation (PEV) explained by these loci ranged from 14.6% to 33.8%, with LOD scores varying from 3.01 to 7.81. Of them, the locus located on chromosome arm 7AS is likely novel. Significant effects of this locus were detected in multiple trials conducted and the PEV explained by this QTL varied from 14.6% to 19.8% among the different trials. An orthologous analysis based on rice and *Arabidopsis* identified 3 putative genes underlying this potentially novel locus.

**Conclusion:** This study identified a stable potentially novel QTL in multiple environments and predicted three candidate genes of it, which laid the foundation for further fine-mapping and cloning the gene(s) underlying *QFlw.WS-7A* with the contribution to grain yield.

Background

Common or bread wheat (*Triticum aestivum* L.) is one of the most important food crops and provides about 20% of calories consumed by humans [1]. By 2050, the world population is anticipated to reach 9.6 billion which is 34% higher than that of today. To meet the demands of food requirement, the annual crop production should rise to 3 billion tons [2]. Producing enough wheat for the growing population is one of the vital tasks for food security.

Yield is a complex trait which is affected by many factors. The foundation of improving yield is to improve the accumulation of organic matter in grains. Almost 90%-95% dry material in plant is formed through photosynthesis, and leaves are the main organs for photosynthesis [3]. The flag leaf is an important morphological trait in wheat and other cereal crops. It influences plant architecture, absorbs the most irradiation light, and contributes to grain yield. The photosynthetic ability of flag leaf has a close relationship with grain yield [4]. In wheat, the flag leaf is the primary photosynthetic organ for grain filling which is the key period for kernel development [5, 6]. The flag leaf translocated almost all of the carbohydrate to grain directly and contributed up to one third of grain yield [7–9]. Important characteristics of flag leaf included length, width (FLW), area, and angle. There is a significant positive relationship between FLW and grain yield [10–14]. The genetic control of FLW is quantitative. The trait is regulated by multiple loci and influenced by environments [15–17]. With the development of molecular technique, more and more QTL/genes regulating FLW have been reported. Putative loci controlling FLW have been located on each of the 21 common wheat chromosomes [12–21]. For example, *TaFLW1* is a major QTL that has
been fine-mapped to 0.2 centimorgan (cM) interval on the long arm of chromosome 5A [20]. And Yan et al. (2020) [17] used two related populations of introgression lines to identify the locus on 6A (QFLW-6A).

Conventional breeding methods are time-consuming, while marker-assisted selection (MAS) is faster and more efficient. MAS, as an optimal method in wheat breeding, mainly depends on the genes/QTL and their linkage markers. QTL mapping lay the foundation for MAS. QTL mapping as a popular and efficient method has a long story. Sax (1923) [22] first suggested the basic idea for studying QTL through linkage markers, and the idea was put into practice by Thoday (1961) [23]. Paterson et al. (1988) [24] proved for the first time that the QTL mapping using molecular markers worked well, which opened the door to QTL mapping in many traits. Afterward, many researchers have contributed to the improvement of QTL mapping methods for different conditions by different models or algorithms [25–30]. Now, QTL mapping has become a powerful tool and it is widely used in many species [31–35]. Many QTL associated with every aspect of traits were detected using QTL mapping in wheat [36–48]. The availability of the high throughput molecular markers [49–54] and the high-quality genome reference IWGSC RefSeq v1.0 [55] have resulted in more precise identification of QTL via the use of dense genetic maps.

Following the identification of QTL conferring FLW using a RIL population with an existing genetic map consisting of Diversity Arrays Technology (DArT) markers, we identified candidate genes for a novel locus through orthologous analysis. These results are reported in this publication.

Results

Phenotypic variation of flag leaf width in the mapping population

FLW of Sumai 3 was significant wider than that of EGA Wylie (Fig. 1). FLW was measured against the RIL population under for different environments. Significant correlations were detected for results from these trials, with correlation coefficients ranging from 0.352 to 0.861 (p < 0.01) (Table 1). Two of these are conducted in the field environments at CSIRO Research Station in Queensland in 2018, one was located at 27°32'16.4"S 152°20'14.6"E (designated as E1), and the other at 27°33'56.9"S 152°19'49.4"E (designated as E2). FLW ranged from 1.17 to 2.08 in E1 and from 0.99 to 1.73 in E2. The mean value was significantly higher in E1 (1.66) than that in E2 (1.34), while the phenotypic diversity index was significantly higher in E2 (0.93) than that in E1 (0.88). The other two trials were conducted in glasshouses at the Queensland Bioscience Precinct (QBP) in Brisbane, Australia, one in 2017 and the other in 2018 (designated as E3 and E4, respectively). FLW varied from 0.95 to 2.10 in E3 and from 1.00 to 1.80 in E4. The mean values and the phenotypic diversity indexes of FLW in E4 were higher than that in E3. BLUP values of FLW from these trials ranged from 0.92 to 1.75 with a mean of 1.44 and the phenotypic diversity index being high as 0.96. The estimated $h^2$ for FLW from these trials was 0.96 (Table 2), suggesting that genetic effects were the major determinant for the phenotypic variance of this trait. The numbers of RILs for different FLW followed the normal distribution in all the four trials (Fig. 2).
Table 1
Pearson correlation coefficients for flag leaf width among the four trials conducted

<table>
<thead>
<tr>
<th>Trials</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
<th>BLUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E2</td>
<td>0.528**</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E3</td>
<td>0.352**</td>
<td>0.535**</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E4</td>
<td>0.364**</td>
<td>0.693**</td>
<td>0.518**</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>BLUP</td>
<td>0.673**</td>
<td>0.861**</td>
<td>0.718**</td>
<td>0.841**</td>
<td>1</td>
</tr>
</tbody>
</table>

E1 and E2 represent the two field, and E3 and E4 the two glasshouse trials; BLUP, the best linear unbiased prediction

Table 2
Variations of flag leaf width among the RILs in the EGA Wylie/Sumai 3 population in different trials

<table>
<thead>
<tr>
<th>Trials</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>STDEV</th>
<th>CV</th>
<th>H'</th>
<th>h^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>1.17</td>
<td>2.08</td>
<td>1.66</td>
<td>0.19</td>
<td>0.11</td>
<td>0.88</td>
<td>0.96</td>
</tr>
<tr>
<td>E2</td>
<td>0.99</td>
<td>1.73</td>
<td>1.34</td>
<td>0.14</td>
<td>0.11</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>E3</td>
<td>0.95</td>
<td>2.10</td>
<td>1.41</td>
<td>0.25</td>
<td>0.18</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>E4</td>
<td>1.00</td>
<td>1.80</td>
<td>1.37</td>
<td>0.21</td>
<td>0.15</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>BLUP</td>
<td>1.20</td>
<td>1.75</td>
<td>1.44</td>
<td>0.13</td>
<td>0.09</td>
<td>0.96</td>
<td></td>
</tr>
</tbody>
</table>

STDEV, Standard deviation; CV, Coefficient of variation; H', The Shannon-Weaver diversity index; h^2, The broad sense heritability

E1 and E2 represent the two field trials, and E3 and E4 the two glasshouse trials; BLUP, the best linear unbiased prediction

The identification of QTL for flag leaf width

To identify QTL for FLW in the RIL population, the trait was evaluated in each of the four trials and BLUP values from these trial results were also obtained and used. A total of 6 QTL were detected, two of them located on chromosome 2B, and the other four located on chromosomes 2D, 4B, 7A, and 7B, respectively (Table 3, Fig. 3). The two QTL located on chromosome 2B (named as QFlw.WS-2B.1 and QFlw.WS-2B.2, respectively) were detected in E2 and also with the use of the BLUP values. They were mapped in the region of 25.87 cM to 31.29 cM and 56.02 cM to 57.79 cM, respectively. QFlw.WS-2B.1 had LOD scores of up to 7.1 with the percentages of phenotypic variation (PEV) varied from 15.4–31.1%. The LOD scores of QFlw.WS-2B.2 ranged from 3.12 to 6.99, and its PEV was up to 31.4%. Similarly, the locus on 7B (QFlw.WS-7B) was detected in E2 and with the use of the BLUP values. The LOD score of this locus was up to 7.39
and its PEV reached 33.8%. The locus on 2D (QFlw.WS-2D) was only detected in one of the trials (E1). This locus was in the interval of 25.26 cM to 25.69 cM on the chromosome 2D. The loci on 4B (QFlw.WS-4B) and 7A (QFlw.WS-7A) were two stable QTL. Both were detected in all trials excepted E1. The locus on 4B (QFlw.WS-4B) was located in the region of 52.08 cM to 54.16 cM. LOD scores of this locus ranged from 3.16 to 7.81, and its PEV was up to 33.8%. QFlw.WS-7A was in the region of 17.92 cM to 18.04 cM on the short arm of this chromosome. The LOD score of this locus varied from 3.01 to 4.05 and its PEV ranged from 14.6–19.8%.

The prediction of candidate genes for QFlw.WS-7A

QFlw.WS-7A was in the interval of 24.37 Mb to 26.04 Mb on chromosome arm 7AS based on the reference genome of Chinese spring (CS). There are 31 high-confidence genes in the interval. Among them, 19 genes were detected in the region of 18.86 Mb to 20.26 Mb on chromosome arm 7AS in the wild emmer wheat (Fig. 4). According to the Chinese Spring reference RefSeq v1.0 (IWGSC) and RefSeq Annotation v1.1 [55], the 31 high-confidence genes in the region of QFlw.WS-7A were selected for collinearity analysis with Arabidopsis and rice. Based on the function of their orthologous genes, we predicted three candidate genes that may be associated with FLW. They were TraesCS7A02G050900, TraesCS7A02G051200, and TraesCS7A02G052000, which were involved in melatonin degradation, substances and energy metabolism, and leaf development that affected the leaf width directly and indirectly.
Table 3
The details of QTL detected for flag leaf width in the mapping population in each of the four trials conducted

<table>
<thead>
<tr>
<th>QTLs</th>
<th>Detected condition</th>
<th>Interval position (cM)</th>
<th>Leaf marker</th>
<th>Right marker</th>
<th>LOD</th>
<th>PEV (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BLUP</td>
<td>27.08–31.29</td>
<td>1117617</td>
<td>F</td>
<td>0</td>
<td>3021168</td>
<td>F</td>
</tr>
<tr>
<td>QFlw.WS-2B.2</td>
<td>E2</td>
<td>56.02–57.79</td>
<td>1037396</td>
<td>F</td>
<td>0</td>
<td>1122548</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>BLUP</td>
<td>56.63–57.79</td>
<td>1117142</td>
<td>F</td>
<td>0</td>
<td>1122548</td>
<td>F</td>
</tr>
<tr>
<td>QFlw.WS-2D</td>
<td>E1</td>
<td>25.26–25.69</td>
<td>1112617</td>
<td>F</td>
<td>0</td>
<td>1294025</td>
<td>F</td>
</tr>
<tr>
<td>QFlw.WS-4B</td>
<td>E2</td>
<td>52.08–54.16</td>
<td>1131412</td>
<td>F</td>
<td>0</td>
<td>100004321</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>E3</td>
<td>52.08–52.90</td>
<td>1131412</td>
<td>F</td>
<td>0</td>
<td>1112165</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>E4</td>
<td>52.08–52.90</td>
<td>1131412</td>
<td>F</td>
<td>0</td>
<td>1112165</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>BLUP</td>
<td>52.08–54.16</td>
<td>1131412</td>
<td>F</td>
<td>0</td>
<td>100004321</td>
<td>F</td>
</tr>
<tr>
<td>QFlw.WS-7A</td>
<td>E2</td>
<td>17.92–18.04</td>
<td>1106129</td>
<td>F</td>
<td>0</td>
<td>1076278</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>E3</td>
<td>17.92–18.04</td>
<td>1106129</td>
<td>F</td>
<td>0</td>
<td>1076278</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>E4</td>
<td>17.92–18.04</td>
<td>1106129</td>
<td>F</td>
<td>0</td>
<td>1076278</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>BLUP</td>
<td>17.92–18.04</td>
<td>1106129</td>
<td>F</td>
<td>0</td>
<td>1076278</td>
<td>F</td>
</tr>
<tr>
<td>QFlw.WS-7B</td>
<td>E2</td>
<td>50.08–50.55</td>
<td>100035195</td>
<td>F</td>
<td>0</td>
<td>1056979</td>
<td>F</td>
</tr>
</tbody>
</table>

PEV, the percentage of phenotypic variation explained

E1 and E2 represent the two field sites (one at 27°32'16.4"S 152°20'14.6"E and the other at 27°33'56.9"S 152°19'49.4"E); and E3 and E4 represent the two glasshouse trials conducted in 2017 and 2018, respectively. BLUP, the best linear unbiased prediction

*, the potentially novel QTL
<table>
<thead>
<tr>
<th>QTLs</th>
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<th>Interval position (cM)</th>
<th>Leaf marker</th>
<th>Right marker</th>
<th>LOD</th>
<th>PEV (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLUP</td>
<td>50.08–50.55</td>
<td>100035195/F</td>
<td>0</td>
<td>1056979/F</td>
<td>0</td>
<td>3.75–6.23</td>
<td>18.2–29.5</td>
</tr>
</tbody>
</table>

PEV, the percentage of phenotypic variation explained

E1 and E2 represent the two field sites (one at 27°32’16.4”S 152°20’14.6”E and the other at 27°33’56.9”S 152°19’49.4”E); and E3 and E4 represent the two glasshouse trials conducted in 2017 and 2018, respectively. BLUP, the best linear unbiased prediction

*, the potentially novel QTL

**Discussions**

**The phenotype in multiple environments**

FLW is an important agronomical trait affecting plant architecture and contributing to grain yield. To identify loci controlling this trait, it was measured in four trials. A strong correlation was detected among the results from these trials (Table 1). Genetic effects were the major determinant of the phenotypic variance on FLW as the estimated $h^2$ was high. Arbelbide et al. (2006) [56] reported there were many factors determining the power of QTL mapping, one of them was heritability. The high $h^2$ of FLW detected in this study laid the foundation for QTL identification.

**QTL analysis**

The population used in this study was derived from EGA Wylie and Sumai 3. Both parents were commercial varieties of great values and they performed well in agronomical and morphological traits. Identifying and utilizing the elite FLW QTL from these varieties could be an effective way to contribute to grain yield. We identified six QTL associated with FLW by assessing this population. These loci were all detected in multiple conditions except for QFlw.WS-2B.1, QFlw.WS-2B.2, QFlw.WS-2D, and QFlw.WS-7B. The physical location of QFlw.WS-2B.1 was 159.81 Mb to 337.91 Mb, which was co-located with the reported locus QFlw.aww-2B [41]. QFlw.WS-2B.2 was mapped on the physical map of 627.32 Mb to 679.90 Mb. It covered the reported locus of QFlw-2B [21]. QFlw.WS-2D was a minor QTL. It was in the region between 22.44 Mb to 23.30 Mb on chromosome 2D, sharing a similar location with QFlw.nau-2D [12]. QFlw.WS-7B was located in the region of 50.08 cM to 50.55 cM at the long arm of chromosome 7B. The reported QTL associated with SNP marker BE518436_7_B_Y_671 [57] was co-located with QFlw.WS-7B. QFlw.WS-4B and QFlw.WS-7A were two stable QTL. Both of them were detected in three of the four environments assessed and with the use of the BLUP values. By comparing the physical position with reported QTL associated with FLW on chromosome 4B, we found this locus overlapped with QFlw-4B [21]. QFlw.WS-7A was in the region of 17.92 cM to 18.04 cM on the chromosome 7A and its physical location was between 24.37 Mb and 26.04 Mb. Compared with the reported QTL for FLW on short arm of chromosome 7A, the nearest one was a locus linked closely with the SSR marker Xwmc139 [19]. The physical location of Xwmc139 was
around 19.90 Mb, thus these two loci were separated by a physical interval of around 4.47 Mb. We analyzed the RIL population and their parents using Xwmc139 and found that the marker was not polymorphic. We thus believe that QFlw.WS-7A was a different locus from those loci reported previously.

Many QTL have been reported but few have been utilized in breeding programs. One of the important factors determining the useful of a locus was its hereditary. In this study, the detected $h^2$ for FLW was high, which could lay the foundation for the identification of major and stable QTL. Based on the presence/absence of the two different alleles at QFlw.WS-7A, the population was classified as two groups, one with EGA Wylie allele and the other Sumai 3 allele. The FLW of Sumai 3 type was significantly higher than that of Wylie type in each of the trials (Fig. 5). The presence of the Sumai 3 allele increased the FLW by an average of 16.6% based on the results from the two field trials, by an average of 16.1% based on results from the two glasshouse trials, and by an average of 18.6% based on results from the BLUP values. These results suggested that the allele of QFlw.WS-7A from Sumai 3 is a stable and effective QTL for breeding programs.

The analysis of candidate genes underlying QFlw.WS-7A

Three candidate genes were identified for QFlw.WS-7A. They were TraesCS7A02G050900, TraesCS7A02G051200, and TraesCS7A02G052000. These genes were involved in melatonin degradation, substances and energy metabolism, and leaf development that affected the leaf width directly and indirectly. Both TraesCS7A02G050900 and TraesCS7A02G051200 are homologous to rice gene 2ODD11 (2-oxoglutarate-dependent dioxygenase 11) that was involved in melatonin degradation [58]. According to the reports of Arnao and Hernández-Ruiz (2015) [59], the absorption of melatonin can increase leaf size. It has also been reported that 2ODD was involved in several structural modifications in the biosynthesis of gibberellins and it played a key role in many growth and developmental processes including leaf expansion [60]. Function analysis also showed that 2ODD11 participated in the dioxygenase activity, L-ascorbic acid binding, and metal ion binding. TraesCS7A02G052000 is aligned with rice gene SDH8A (Succinate dehydrogenase subunit 8A). GO annotation analysis showed that this gene was involved in the pathway of tricarboxylic acid cycle, which is the important hub of substances and energy metabolism. The carbohydrate metabolism and photosynthesis involved in the tricarboxylic acid cycle provide the substances and energy for leaf development, which affecting FLW indirectly. Based on the report of Zhao et al. (2015) [61], amino acids and organic acids participated in the tricarboxylic acid cycle were significantly different in metabolite levels between two rice accessions with different leaf width. There could be a relationship between leaf width and the tricarboxylic acid cycle [61–62].

Orthologs for these genes were also found in Arabidopsis. TraesCS7A02G050900 and TraesCS7A02G051200 were orthologous with Arabidopsis gene ANS and SRG1, respectively. Both genes belong to the iron/ascorbate-dependent oxidoreductase family. ANS was involved in the flavonoid biosynthetic process and regulation of jasmonic acid mediated signaling pathway [63]. SRG1 was involved in leaf senescence [64].

Conclusion
To detect QTL for FLW, a RIL population consisting of RIL was assessed in different environments. We identified 6 QTL in these trials. They were located on chromosomes 2B, 2B, 2D, 4B, 7A, and 7B, respectively. Of them, QFlw.WS-4B was a stable major QTL with a PVE of up to 33.8%. Compared with the location of previously reported QTL associated with FLW, QFlw.WS-7A was potentially novel. Based on an orthologous analysis with rice and Arabidopsis, we identified three candidate genes for this locus. They were involved in the regulation of plant growth, substances and energy metabolism, and leaf development. The QTL mapping and the candidate genes prediction laid the foundation for cloning the gene(s) underlying QFlw.WS-7A.

Methods

Plant materials

A population of 92 RILs derived from a cross of EGA Wylie/Sumai 3 [44] was used in this study to detect QTL for FLW. EGA Wylie is a commercial variety widely grown in Australia and Sumai 3 was a variety released some four decades ago in China. The population was developed by Zheng et al. (2014) [44].

Collection and analysis of phenotypic data

The population was planted in two field environments at CSIRO Research Station in Queensland in 2018, one located at 27°32′16.4″S 152°20′14.6″E (designated as E1), and the other at 27°33′56.9″S 152°19′49.4″E (designated as E2). For each of the two trials, each RIL was planted with 3 replications. For each of these replicates, ten seeds for each line were evenly planted in a row of 1 m with a 0.3 m between rows. Both field trials were well watered and managed following the standard local practices. Five plants from the middle of each line were randomly selected during the grain-filling stage to measure the flag leaf width (FLW). The population was also assessed twice in a glasshouse at the Queensland Bioscience Precinct (QBP) in Brisbane, Australia, one in 2017 and the other in 2018 (designated as E3 and E4, respectively). In the glasshouse, the population was grown at day/night temperature of 25/16 (°C) and relative humidity of 58%/72%. Plants were well watered and managed. FLW was measured from three plants for each line during the grain-filling stage.

BLUP values were estimated based on the linear model using the lme4 package in the R program [65]. The broad-sense heritability (\(h^2\)) estimates for FLW was calculated across all the trials with the formula \(h^2 = \frac{V_G}{V_G + V_E}\) using the lme4 package [65], where \(V_G\) and \(V_E\) are the genotypic and environmental variances, respectively [66]. The Shannon-Weaver diversity index (\(H'\)) was calculated to reflect the phenotypic variation [67]. The analysis of variance (ANOVA), Pearson correlation coefficient, and the \(t\)-test were all carried out using the software SPSS 20.0 (IBM Corp., Armonk, NY, USA). The histograms of FLW were drawn using the ggplot2 package in the R program [68].

The genotyping and QTL mapping

The genetic map for the population used in this study was generated using DArT Pty Ltd as described in an earlier study [44]. The QTL analysis was carried out using software MapQTL 5.0 through MQM
mapping [69]. For each trial, the significant LOD threshold was determined by a test of 1000 permutations with the whole genome scanning of 0.05 level. QTL detected in multiple trials was considered as a stable QTL. The genetic maps were drawn with MapChart 2.32 (https://www.wur.nl/en/show/MapChart-2.32.htm). Comparison of QTL detected in this study and those reported earlier was conducted based on the Chinese Spring reference RefSeq v1.0 [55].

The analysis of candidate genes

Identification of candidate genes for the novel QTL was conducted based on an orthologous analysis. Firstly, we delineated the physical intervals of novel QTL based on the wheat reference genome RefSeq v1.0 of Chinese Spring [55]. Gene sequences in the targeted interval were extracted using TBtools [70] and used to blast against the wild emmer (T. turgidum ssp dicoccoides) genome [71] and protein database SWISS-PROT [72] using TBtools [70] with default parameters. Functions of the candidate genes were extracted from their homologs in Oryza sativa and Arabidopsis.

Abbreviations

FLW
flag leaf width; RILs: recombinant inbred lines; MAS: marker-assisted selection; DArT: Diversity Arrays Technology; STDEV: standard deviation; CV: coefficient of variation; $H'$: the Shannon-Weaver diversity index; $h^2$: the broad sense heritability; BLUP: best linear unbiased prediction; PEV: the percentage of phenotypic variation; cM: centimorgan; CS: Chinese spring.

Declarations

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Availability of data and materials

All data are fully available within the manuscript and without restriction.

Authors’ contributions
D.X. and X.Y. conceived the study; X.Y., J.L., Z.Z. and H.Z. collected and analyzed data; X.Y. and D.X. prepared the manuscript with contribution from other authors. And all authors have read and approved the manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**


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**Figures**
Figure 1

The flag leaf width of parents. The Wylie and Sumai 3 are maternal and paternal, respectively.
Figure 2

The distribution of flag leaf width in the Wylie/Sumai 3 recombinant inbred lines (RILs) population. E1 and E2 indicate the population was planted in the field environments at 27°32’16.4”S 152°20’14.6”E and 27°33’56.9”S 152°19’49.4”E at CSIRO Research Station at Gatton in Queensland in 2018; E3 and E4 indicate the glasshouse in 2017 and 2018; BLUP, the best linear unbiased prediction.
Figure 3

The locations of QTL for flag leaf width on the genetic map in the population. E1 and E2 indicate the population was planted in a glasshouse in 2017 and 2018; E3 and E4 indicate the field environments at 27°32′16.4″S 152°20′14.6″E and 27°33′56.9″S 152°19′49.4″E at CSIRO Research Station at Gatton in Queensland in 2018; BLUP, the best linear unbiased prediction
Figure 4

The physical position for QFlw.WS-7A and their predicted genes
The effect of QFlw.WS-7A on the flag leaf width in all trials. The Sumai 3 and Wylie type indicates two different groups based on the flanking markers of QFlw.WS-7A. The Sumai 3 type carrying QFlw.WS-7A had significant higher FLW than Wylie type without QFlw.WS-7A. ***, significant at p < 0.0001; ****, significant at p < 0.00001. FLW, flag leaf width. E1 and E2 indicate the population was planted in the field environments at 27°32’16.4”S 152°20’14.6”E and 27°33’56.9”S 152°19’49.4”E at CSIRO Research Station at Gatton in Queensland in 2018; E3 and E4 indicate the glasshouse in 2017 and 2018; BLUP, the best linear unbiased prediction.