Identification and mapping of QTL associated with some traits related for drought tolerance in wheat using SSR markers

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Abstract
Background: Wheat is the most important crop around the world. Drought stresses affect wheat production and their characterization. Most of the traits that are affected by drought are quantitative traits, so detection of the quantitative trait's loci (QTLs) related to these traits is very important for breeder and wheat producers. In this trend, 285 F2 individuals from crosses between four bread wheat genotypes (Triticum aestivum L.), i.e., Sakha93, Sids1, Sakha94, and Gemmiza9, were used for identified QTLs associated with plant height (PH) and leaf wilting (LW). Single marker analysis and composite interval mapping (CIM) were used.

Results: A total of 116 QTLs loci were detected which covered 19 chromosomes out of the 21 chromosomes of wheat. PH and LW had 74 and 42 QTLs loci, respectively. On the other hand, chromosome 7A showed to bear the highest number of QTLs loci (15 loci). While chromosome 1A beard the highest number of QTLs related to PH (10 loci), chromosome 2B and 7A beard the highest number of QTLs related LW. We highly recommend our finding to help breeders in wheat breeding programs to improve plant height and leaf wilting.

Conclusion: Our investigation concluded that SSR markers have high efficiency in the identification of QTLs related to abiotic stress; also the CIM method had more advanced priority for QTLs mapping.

Keywords: Wheat, QTLs, Mapping, Composite interval mapping (CIM), Plant height, Leaf wilting, Simple sequence repeats, SSR

1 Background
Wheat is the most important crop that contributes to nutritional and food security around the world. Wheat is one of the strategic crops in Egypt, and the wheat breeding program to produce superior varieties is one of the important breeding programs that many researchers are concerned with. Despite this importance, there is relatively little research in the field of identifying QTLs responsible for some yield-related traits, especially by using the Molecular markers technique. Therefore, this research is an important step to identify the QTLs associated with some drought-affected traits in order to contribute to the development of drought-tolerant wheat cultivars. Although many QTLs related to plant height (PH) were detected in wheat, no QTL related to wilting was detected. On the other hand, abiotic stresses (drought, cold, heat, and salt) affected wheat productivity, while drought stress affects about 1 billion hectares of global agricultural soil including sodic and saline soils [1].

Among the environmental stresses, drought is the important one that affects the development and growth of crops. Drought still to be a major challenge to researchers and breeders. Factors that affect responses of plants to drought stress include genotype, stage of...
growth, duration and stress severity, physiological process of growth, different genes expression patterns, the different activity of respiration patterns and photosynthesis activity, and environmental factors [2–5]. A drought had effects on genes expression, so various responsive genes related to drought were featured [6]. Gene’s role could be featured by gene expression to high levels of resistance between varieties (Ouward et al., 1995). Drought influence plants in levels of protein, production of antioxidant, osmotic adjustment, the composition of the hormone, depth and extension of the root, stomata closing and opening, photosynthesis inhibition, chlorophyll decreasing content, transpiration reduction, and growth inhibition [7–10]. Drought can also cause pollen sterility, loss in grain yield, and abscisic acid accumulation [11].

Recent techniques like molecular methods must be appropriate useful identification tools for some clonal variation, stress tolerance, and genetic stability establishment [12–16].

The main goal of quantitative trait loci (QTL) analysis is to answer the question of whether phenotypic differences are depending on a few loci with quite large effects, or to many loci, each with midget effects. Remington and Purugganan [17] said, “It appears that a substantial proportion of the phenotypic variation in many quantitative traits can be explained with few loci of large effect, with the remainder due to numerous loci of small effect” [17–19].

QTLs can be categorized to constitutive QTL, that detected with most environments (their effects are stable across environmental conditions); and adaptive QTL, that detected with specific conditions of the environment (expression increasing with a level of environmental factor) like QTL that increases drought tolerance [20]. The sensitivity to environmental stress could be explained due to the regulations response (e.g., transcription) of the QTL gene to hint of environment. Meanwhile, response differences may cause by an indirect effect (e.g., larger root systems genotypes will be less affected by water or nutrient deficit, so genes controlling root development may underpin QTLs defined by stomatal conductance, or biomass accumulation). On the other hand, QTLs that caused an alteration in flowering time often affect yield against water or nutrient deficit because the duration of the crop life cycle affects the timing and intensity of the stress experienced by the plants [21].

Many QTLs and molecular markers are related to genes responsible for drought tolerance or sensitivity [22]. Advances in genomic and molecular technologies develop molecular markers which could be useful for QTLs identification. DNA markers based on the polymerase chain reaction (PCR) were the most notable ones among markers that used in studying the genetic characterization of wheat, sequence tagged microsatellite sites (STMSs) and/or simple sequence repeats (SSRs) [23], amplified fragment length polymorphisms (AFLP) [15], and chloroplast simple sequence repeats (cpSSR) [24]. SSR markers had an advantage in wheat molecular studies because it has a co-dominant type of inheritance, a large number of genomes, reproducibility, locus specificity, and high informational content. Moreover, their high polymorphism ratio, chromosome specificity, multiallelic nature, and wide distribution throughout the wheat genome are observed [25, 26].

SSR markers used to identify QTLs related to yield traits such as harvest index and thousand-grain weight [27], to study D genome-based genetic diversity in terms of drought tolerance [28], to study the physiological and genetic characterization wheat genotypes against the drought and temperature tolerance [29], and to detect the quantitative trait loci (QTL) for various traits [30, 31]. The aim of our investigation is to construct QTLs mapping for some traits related to drought tolerance in wheat.

### 2 Methods

#### 2.1 Wheat materials

A total of 285 F2 individuals from crosses between four bread wheat genotypes (*Triticum aestivum* L.), i.e., Sakha93, Sids1, Sakha94, and Gemmiza9, were used for QTL analysis of four traits related to drought tolerance. The parents were chosen from a previous study [32], as representing a wide range of diversity for several agronomic characters. The parents were supplied by Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Table 1 presents the Parent’s name, pedigree, and drought stress.

| No | Genotype   | Cross name and pedigree                                              | Drought stress response |
|----|------------|-----------------------------------------------------------------------|-------------------------|
| 1  | Sakha93    | Sakha 92/TR810328 5.8871-15.25-15.05                                      | Tolerant                |
| 2  | Sids1      | HD2172/Pavon “S”/1158.57/Maya74 “S”/6d46-45d-25d-15d-05d               | Tolerant                |
| 3  | Sakha94    | Opata / Rayon // Kauz CMBW90Y3180-0TOPM-3Y-010M-010M-0100-10GM-015Y-010Y-015Y-00-0AP-05 | Sensitive               |
| 4  | Gemmiza9   | Ald“S”//Huac/Cmih74A 630/Sx CGMA583-5GM-1GM                               | Sensitive               |
2.2 Field experiment and drought tolerance assessment
The four parental wheat varieties were sown at the Experimental Farm of Genetics Department, Faculty of Agriculture, Mansura University (31.0449° N, 31.3537° E). Then, these varieties were crossed to produce possible crosses, i.e., Cross 1 (H1) = (Sakha93 × Gemmiza9), Cross 2 (H2) = (Sakha94 × Gemmiza9), and Cross 3 (H3) = (Sakha93 × Sids1) according to Habiba et al. [32]. F2 and their parents were evaluated for drought tolerance at two drought treatments. They were sown in pots (25 cm) containing sand and clay (2:1 v/v). Irrigation was given as normal irrigation for control and one irrigation 45 days after planting irrigation, i.e., two irrigations were given through the whole season for drought treatment. Pots were fertilized with P₂O₅, in one dose during soil preparing and Nitrogen was added by ammonia injection in one dose after soil preparing and before 4 days from planting. The trial was arranged in randomized complete blocks design with three replications. The experiment was conducted with 13/11 day/night photoperiod, 20/15 °C day/night temperature, and relative humidity of about 85%. Data were recorded on plant height (PH in cm) and leaf wilting (LW = per day to wilting).

2.3 DNA extraction and SSR markers amplification
DNA extracted from green leaves from each genotype was collected from ten-day seedlings germinated from seeds of each genotype according to Khaled and Esh [33] and Khaled et al. [16]. A set of 143 SSRs from the Wheat database (BARC, CFA, CFM, CFD, GWM, WMC, WMSX, BARC, XGWM, XPSP, and XWMC) and new 52 SSRs from the Cotton database (JESPR) involve the 21 chromosomes of wheat (References). Out of the 530 SSR primers, 195 (143 of wheat primers and 52 of cotton primers) have polymorphism to distinguish the genotypes and are used for mapping. Amplification was performed as follows, 94 °C for 1 min (one cycle); 94 °C for 20 s, 50–55 °C for 35 s, 72 °C for 45 s (35 cycles), and final extension at 72 °C for 45 s (one cycle). Then hold at 4 °C (infinite). The PCR products were conducted to electrophoresis at 90 V, in 2% agarose gel containing 0.5 μg/ml ethidium bromide for approximately 2 h, using 0.5 × TBE buffer, along with a DNA ladder. The gel was visualized under UV.

2.4 Linkage map and QTLs analysis
Single marker analysis (SMA) and composite interval mapping (CIM) were used to localize the QTL associated with drought tolerance in wheat using 285 plants of an F2 population derived from crosses between four bread wheat genotypes using QTL IciMapping v4.2.5.3 software [34] depending on Kosambi mapping function. The logarithm of odds (LOD) threshold of higher than 3 was used. Segregation ratios of the genotypes classes at each locus were tested using the chi-square test (p < 0.01).

The linkage mapping was compared with previous maps. The QTL analysis was also performed using IciMapping v4.2.5.3 software by combined analysis of adjusted means of the phenotypic trait value and genotyping data via inclusive composite interval mapping (ICIM) algorithm for additive gene effect with function inbuilt in the software. The walking speed chosen for all QTLs was 1 cM and the LOD threshold was calculated by 1000 permutation and p = 0.05.

2.5 Statistical analysis
The collected data were subjected to analysis of variance of the split-plot design and significant differences were estimated according to Bernardo [35]. The analyses of variance (ANOVA) were calculated using SPSS v25 and MS-Excel v365. Values of means, standard deviation, correlation coefficients, and plots showing the distribution of phenotypic data for different traits were determined using SPSS v25 and MS-Excel365. QTLs map was constructed using QTL IciMapping v4.2.5.3 software [34].

3 Results
3.1 Phenotypic evaluation
The phenotypic variations between parents and their hybrids (i.e., Sakha93 (S93), Sids1(Sids), Sakha94 (S94), Gemmiza9 (G9), H1, H2, and H3) were evaluated for plant height (PH) and leaf wilting (LW). Mean, standard error, standard deviation, and coefficient of variance (CV %) are presented in Table 2. Analysis of variance and correlation are presented in Table 3.

Data presented in Table 2 and Fig. 1 illustrated that genotypes (parents and their crosses) exhibited significant variations among studied traits. Due to the results of agronomic traits, Sakha93 and Sids1 were considered drought-tolerant genotypes; and Gemmaza9 and Sakha94 were the sensitive ones. While Sakha93 and Sids1 surpass the others in LW and PH, their cross (H3) was on average. In general, significant variation between tolerant and sensitive genotypes was observed.

Figure 1 and Table 2 reveal that Sakha93 recorded the highest value of PH within parents, while Sids1 surpassed all genotypes and hybrids for their survival against drought treatment (LW value). On the other hand, the cross H1 had the highest PH among parents and their crosses, while H1 was in average LW. Variations for all the traits were significantly observed for treatments, genotypes, and genotype × treatments under drought conditions (p < 0.05).

The coefficient of variation (CV) was lower for all traits, while LW was the highest among them. Because mean is used in calculating CVs, increasing mean were expected
Table 2  Mean, standard error, standard deviation, and coefficient of variance of parents and their crosses for plant height (PH) and leaf wilting (LW) traits

|        | Mean PH | Standard error PH | Standard deviation PH | CV% PH | Mean LW | Standard error LW | Standard deviation LW | CV% LW |
|--------|---------|-------------------|-----------------------|--------|---------|-------------------|-----------------------|--------|
| G9     | 11.737  | 1.067             | 1.509                 | 12.856 | 39.313  | 2.313             | 3.270                 | 27.864 |
| S94    | 12.100  | 1.100             | 1.556                 | 12.856 | 42.500  | 2.500             | 3.536                 | 29.219 |
| Sids   | 32.263  | 2.933             | 5.735                 | 12.856 | 98.813  | 5.813             | 8.220                 | 25.478 |
| S93    | 46.013  | 4.183             | 5.916                 | 12.856 | 90.313  | 5.313             | 7.513                 | 16.328 |
| H1 (S93 x G9) | 44.605 | 4.055             | 5.735                 | 12.856 | 79.581  | 4.681             | 6.620                 | 14.842 |
| H2 (S94 x G9) | 29.755 | 2.705             | 3.825                 | 12.856 | 64.633  | 3.802             | 5.377                 | 18.071 |
| H3 (S93 x Sids) | 48.290 | 4.390             | 6.208                 | 12.856 | 88.152  | 5.185             | 7.333                 | 15.186 |

PH: plant height, and LW: leaf wilting

Table 3 Analysis of variance (ANOVA) of plant height (PH) and leaf wilting (LW)

| Source of variation | SS   | Df  | MS   | F    | p value | F crit |
|---------------------|------|-----|------|------|---------|--------|
| Plant height (PH)   |      |     |      |      |         |        |
| Genotypes           | 2864.4790 | 6.0000 | 477.4132 | 121.0000 | 0.0000 | 4.2839 |
| Drought treatments  | 119.2879 | 1.0000 | 119.2879 | 30.2334 | 0.0015 | 5.9874 |
| Error               | 23.6734 | 6.0000 | 3.9456 |       |         |        |
| Total               | 3007.4402 | 13.0000 | 3.9456 |       |         |        |
| Leaf wilting (LW)   |      |     |      |      |         |        |
| Genotypes           | 6731.0279 | 6.0000 | 1121.8380 | 289.0000 | 0.0000 | 4.2839 |
| Drought treatments  | 250.4372 | 1.0000 | 250.4372 | 64.5159 | 0.0002 | 5.9874 |
| Error               | 23.2908 | 6.0000 | 3.8818 |       |         |        |
| Total               | 7004.7558 | 13.0000 | 3.8818 |       |         |        |

Fig. 1 Response of different parental genotypes to drought stress, i.e., the effect of drought on plant height (cm) and leaf wilting (days) of Wheat genotypes
to produce smaller coefficients of variation. Phenotypic correlations ranged widely among traits under drought conditions and control. Correlations were significantly positive \( (p < 0.05) \) between genotypes and both PH and LW.

### 3.2 Construction of linkage map

A genetic map was constructed for plant height and leaf wilting using 195 SSR markers of that 79 SSRs on A chromosomes were mapped, 69 SSRs on B chromosomes, and 47 SSRs on D chromosomes. Chromosomes 1A and 2B beard highest markers that coverage of 19 SSRs, and the chromosome 7D had the lowest one that 3 SSRs coverage it. The genetic length that the linkage map covered was 5057.4858 cM and the average inter marker distance was 25.9358 cM (Fig. 2).

The number of QTLs covered by each chromosome is presented in Tables 4, 5, and 6. Data in Table 4 revealed that QTLs related to plant height (PH) and leaf wilting (LW) were distributed among all chromosome sets except chromosomes 20 and 21.

The nineteen chromosomes were shown to bear 116 QTLs where plant height had 74 QTLs loci and 42 loci for leaf wilting. Out of the observed 116 QTLs, chromosome 19 (7A = 15) beard the highest QTLs number for both the studied traits, followed by chromosome 5 (2B = 14) and chromosome 1 (1A = 13). Among the two traits (plant height and leaf wilting), chromosome 1(1A) exhibited the highest number of QTLs that related to plant height trait (= 10) followed by chromosome 3 (1D) and 19 (7A) which recorded 7 QTLs loci, while chromosomes 5 (2B) and 19 (7A) showed the highest number of QTLs related to leaf wilting.

### 4 Discussion

Traits such as plant height and leaf wilting, historically, have been subjected to strong selective natural and artificial pressure, to improve the adaptation of bread wheat to different climatic conditions and to increase the grain yield \[36–39\]. However, these same traits are not only important for increasing crop yield potential, but they are also useful in determining the adaptation to climate change \[40\]. In the present work, the genetic control of two traits was investigated to identify associated QTLs. Variations for all the traits were significantly observed for treatments, genotypes, and genotype × treatments under drought conditions \( (p < 0.05) \). The coefficient of variation (CV) was lower for all traits, while LW among them was the highest. Because mean used in calculating CVs, increasing mean were expected to produce smaller coefficients of variation. Phenotypic correlations ranged widely among traits under drought conditions and control. Correlations were significantly positive \( (p < 0.05) \) between genotypes and plant height, similarly between plant height and LW. However, significant negative correlations \( (p < 0.05) \) were exhibited between LW and genotypes. This was consistent with previous reports in wheat and also in other cereal species such as rice and barley, indicating a high response to selection of these traits \[41–43\]. Continuous distribution or absence of discrete segregating classes for PH and LW suggested that its inheritance is either determined by a large number of genes with small effects or by a few major genes with substantial environmental effects. The presence of transgressive segregants in all traits investigated suggested that each of the parental cultivars had desirable and undesirable alleles in various proportions for loci governing these traits.

A total of 530 high-quality SSR markers were used to build the genetic map, and as expected, most of them were placed on genomes A and B, in line with previous results \[44, 45\]. Wen et al. \[46\] showed that the D genome had fewer markers than the A and B genomes in the high-density consensus map in common wheat. A total of 28 and 10 QTLs were found in the F2 populations. The comparative QTL analysis of genomes A and B between F2 populations showed that 55 QTLs for PH could be considered to be adjacent and nearly overlapping. Pearson rank between the assessed traits revealed that PH was correlated with the LW, in agreement with Rabbi and Hisam \[45\], Bilgrami et al. \[47\], and Mecha et al. \[48\]. In our study, several QTLs for PH and LW co-localized on the same chromosome, suggesting that they were not distributed evenly in the wheat genome, but they tended to cluster in particular chromosome regions (Table 4).

### 5 Conclusions

Most of the traits that are affected by drought are quantitative traits, so detection of the QTLs related to these traits is very important for breeder and wheat producers. In this trend, QTLs for plant height (PH) and days to wilting (W) were studied. A total of 116 QTLs loci were detected which covered 19 chromosomes out of the 21 chromosomes of wheat. Chromosome 7A showed to bear the highest number of QTLs loci (15 loci). While chromosome 1A beard the highest number of QTLs loci related to PH (10 loci), chromosome 2B and 7A beard the highest number of QTLs related to surviving (days to wilting). We highly recommend our finding to help breeders in wheat breeding programs to improve plant height and survival (days to wilting). SSR markers are useful for the detection of QTLs related to abiotic stress like drought. Liu et al. \[49\] detected seven QTLs related to PH on chromosomes 1B, 4B (two regions), 6A (two regions), 6D and 7A; on the other hand, Wang et al. \[50\] identified two major QTLs related to PH on chromosomes 4B and 6D.
Fig. 2 Linkage maps revealed the position and distribution the QTLs related to plant height and leaf wilting among Wheat chromosomes. QTL name on the right side and centimorgan (cM) distance on the left. A colored bar represents the CI (confidence interval) of QTL identified through single-locus analysis.
Fig. 2 continued
Table 4  Chromosomes ID and their QTLs loci related to plant height and leaf wilting for nineteen chromosomes out of twenty-one wheat chromosomes

| Chrom. ID | QTLs | Plant height | Leaf wilting | Total | Mean |
|----------|------|--------------|--------------|-------|------|
| -Ch1-1A  |      | 10           | 3            | 13    | 8.67 |
| -Ch2-1B  |      | 4            | 1            | 5     | 3.33 |
| -Ch3-1D  |      | 7            | 4            | 11    | 7.33 |
| -Ch4-2A  |      | 6            | 3            | 9     | 6.00 |
| -Ch5-2B  |      | 6            | 8            | 14    | 9.33 |
| -Ch6-2D  |      | 2            | 3            | 5     | 3.33 |
| -Ch7-3A  |      | 4            | 1            | 5     | 3.33 |
| -Ch8-3B  |      | 1            | 1            | 2     | 1.33 |
| -Ch9-3D  |      | 1            | 2            | 3     | 2.00 |
| -Ch10-4A |      | 3            | 3            | 6     | 4.00 |
| -Ch11-4B |      | 0            | 1            | 1     | 0.67 |
| -Ch12-4D |      | 2            | 0            | 2     | 1.33 |
| -Ch13-5A |      | 2            | 0            | 2     | 1.33 |
| -Ch14-5B |      | 6            | 1            | 7     | 4.67 |
| -Ch15-5D |      | 5            | 0            | 5     | 3.33 |
| -Ch16-6A |      | 3            | 0            | 3     | 2.00 |
| -Ch17-6B |      | 3            | 1            | 4     | 2.67 |
| -Ch18-6D |      | 2            | 2            | 4     | 2.67 |
| -Ch19-7A |      | 7            | 8            | 15    | 10.00 |
| Mean     |      | 3.89         | 2.21         | 6.11  | 4.07 |
| Total    |      | 74           | 42           | 116   |      |
| Chromosome | Position | Left marker | Right marker | LOD   | PVE (%) | Add |
|------------|----------|-------------|--------------|-------|---------|-----|
| -Ch1-1A    | 29       | XWMC605     | XWMC304      | 34.4606 | 0.9496  | 0.0102 |
|            | 40       | XWMC304     | XGWM610      | 33.8512 | 0.9572  | 0.0055 |
|            | 261      | JESPR311    | JESPR7       | 38.0261 | 0.8465  | -0.0055|
|            | 276      | JESPR7      | WM1C65       | 33.7177 | 0.8451  | -0.0100|
|            | 280      | WM1C65      | WM5024       | 36.4749 | 0.843   | 0.0041 |
|            | 298      | WM1C65      | WM5024       | 36.085  | 0.8498  | 0.0011 |
|            | 303      | WM5024      | WM304        | 38.5963 | 0.8532  | 0.0293 |
|            | 320      | WM5024      | WM304        | 34.9425 | 0.8466  | 0.0031 |
|            | 390      | WM5030      | WM5198       | 40.7933 | 0.8392  | -0.0085|
|            | 393      | WM5198      | WM5337       | 40.1553 | 0.8495  | -0.0067|
| -Ch2-1B    | 8        | XGWM484     | WM5493       | 27.2976 | 0.9367  | -0.0055|
|            | 145      | JESPR292    | JESPR284     | 36.5342 | 0.8558  | 0.0052 |
|            | 169      | JESPR284    | JESPR19      | 40.0445 | 0.8551  | 0.0010 |
|            | 192      | JESPR284    | JESPR19      | 36.952  | 0.9561  | -0.0163|
| -Ch3-1D    | 98       | WM1C70      | JESPR287     | 36.2982 | 0.9524  | -0.0011|
|            | 107      | JESPR287    | GW2M271      | 39.0537 | 0.9532  | -0.0001|
|            | 132      | JESPR287    | GW2M271      | 41.4678 | 0.951   | 0.0050 |
|            | 140      | GW2M271     | XGWM93       | 41.6785 | 0.9556  | 0.0004 |
|            | 174      | GW2M271     | XGWM93       | 34.5048 | 0.9617  | -0.0220|
|            | 187      | XGWM93      | XGWM130      | 33.928  | 0.9609  | -0.0238|
|            | 218      | XGWM93      | XGWM130      | 39.2011 | 0.9562  | -0.0153|
| -Ch4-2A    | 30       | WM5C07      | JESPR28    | 24.0876 | 0.9642  | -0.0172|
|            | 83       | GW2M57      | JESPR13      | 35.7785 | 0.8563  | -0.0446|
|            | 152      | JESPR293    | JESPR300     | 32.2305 | 0.9634  | -0.0264|
|            | 228      | GW2M19      | BARC101      | 30.7708 | 0.9618  | 0.0200 |
|            | 242      | BARC101     | CFD65        | 26.2169 | 0.953   | 0.0276 |
|            | 477      | WM5054      | WM5148       | 26.4074 | 0.9607  | 0.0042 |
| -Ch5-2B    | 170      | BARC124     | WM5325       | 34.6034 | 0.9596  | 0.0364 |
|            | 187      | WM5325      | WM5297       | 46.0389 | 1.0697  | 0.0773 |
|            | 190      | WM5297      | WM5193       | 48.4367 | 1.0739  | 0.0825 |
|            | 297      | WM5164      | WM5144       | 51.8691 | 1.1331  | 0.0507 |
|            | 301      | WM5144      | WM5144       | 52.1626 | 1.1341  | 0.0486 |
|            | 338      | WM5144      | WM5144       | 43.3711 | 1.1476  | -0.0264|
| -Ch6-2D    | 204      | WM5C154     | GW2M292      | 41.742  | 0.8497  | 0.0142 |
|            | 207      | GW2M292     | JESPR6       | 39.0355 | 0.8485  | 0.0139 |
| -Ch7-3A    | 12       | WM5C28      | JESPR309     | 24.3793 | 0.9356  | -0.0136|
|            | 303      | WM5011      | JESPR288     | 33.0059 | 0.9451  | -0.0024|
|            | 311      | JESPR288    | GW1M48       | 29.471  | 0.8896  | 0.0093 |
|            | 333      | GW1M48      | XGWM389      | 28.0939 | 0.9657  | 0.0016 |
| -Ch8-3B    | 96       | WM5C04      | WM5C245      | 28.7844 | 0.9661  | -0.0006|
| -Ch9-3D    | 3        | JESPR308    | JESPR302     | 41.7766 | 1.0162  | -0.0162|
| -Ch10-4A   | 46       | JESPR4      | JESPR296     | 48.1947 | 1.2901  | -0.0150|
|            | 180      | XGWM332     | XWMC107      | 34.6088 | 0.9524  | -0.0102|
|            | 218      | XWMC182     | JESPR11      | 25.6417 | 0.9619  | -0.0001|
| -Ch12-4D   | 73       | Xgwm1302    | JESPR310     | 21.3911 | 0.8453  | -0.0044|
|            | 86       | JESPR310    | Xgwm194      | 25.5333 | 0.9638  | -0.0022|
| -Ch13-5A   | 31       | JESPR286    | XGWM099      | 23.3193 | 0.9656  | -0.0195|
|            | 76       | WM5218      | WM5765       | 36.0515 | 0.8476  | 0.0000 |
Table 5 (continued)

| Chromosome | Position | Left marker | Right marker | LOD   | PVE (%) | Add     |
|------------|----------|-------------|--------------|-------|---------|---------|
| -Ch14-5B  | 68       | WMCS32      | WMS060       | 29.1324 | 0.9597  | 0.0092  |
|            | 106      | WMS060      | XWMC603      | 38.4724 | 0.8466  | -0.0050 |
|            | 114      | XWMC603     | CFD38        | 38.8464 | 0.9508  | -0.0107 |
|            | 129      | XWMC603     | CFD38        | 39.614  | 0.9535  | -0.0209 |
|            | 137      | CFD38       | GWM294       | 39.7165 | 0.8489  | 0.0013  |
|            | 159      | CFD38       | GWM294       | 36.638  | 0.9537  | -0.0016 |
| -Ch15-5D  | 7        | WMS261      | JESPR297     | 32.7536 | 0.9607  | 0.0148  |
|            | 135      | XGWM011     | JESPR280     | 39.4811 | 0.8469  | 0.0009  |
|            | 138      | JESPR280    | WMS340       | 39.8181 | 0.8478  | -0.0070 |
|            | 168      | WMS340      | JESPR282     | 33.824  | 0.95    | -0.0080 |
|            | 185      | WMS340      | JESPR282     | 32.8221 | 0.9507  | 0.0030  |
| -Ch16-6A  | 43       | WMCS16      | WMS118       | 37.929  | 0.8452  | 0.0030  |
|            | 47       | WMS118      | XGWM108      | 40.1389 | 0.8504  | -0.0023 |
|            | 73       | WMS118      | XGWM108      | 38.4625 | 0.9541  | -0.0014 |
| -Ch17-6B  | 5        | XGWM186     | XPSP3200     | 38.204  | 0.9564  | -0.0190 |
|            | 39       | XGWM186     | XPSP3200     | 36.8637 | 0.8546  | -0.0215 |
|            | 44       | XPSP3200    | JESPR9       | 36.7797 | 0.8585  | -0.0235 |
| -Ch18-6D  | 59       | WMS058      | WMC235       | 29.0349 | 0.9574  | 0.0221  |
|            | 73       | WMC235      | XWMC233      | 27.5574 | 0.9571  | 0.0188  |
| -Ch19-7A  | 120      | XWMC009     | GW181        | 35.1153 | 0.9549  | -0.0035 |
|            | 151      | XWMC009     | GW181        | 31.242  | 0.9585  | 0.0080  |
|            | 160      | GW181       | JESPR290     | 31.5985 | 0.8614  | 0.0412  |
|            | 163      | GW181       | JESPR290     | 31.6545 | 0.9482  | 0.0114  |
|            | 223      | JESPR290    | JESPR304     | 32.1148 | 0.8729  | 0.0646  |
|            | 264      | WMC177      | WMS043       | 152.458 | 6.5384  | 1.0027  |
|            | 271      | WMS043      | WMS044       | 144.937 | 6.5372  | 1.0032  |
Table 6  Position, characters, and distribution the QTLs related to leaf wilting among nineteen chromosomes out of twenty-one wheat chromosomes

| Chromosome | Position | Left Marker | Right Marker | LOD  | PVE (%) | Add  |
|------------|----------|-------------|--------------|------|---------|------|
| -Ch1-1A    |          | XWMC304     | XGWM610      | 17.4069 | 1.4801 | 0.0521 |
|            | 259      | JESPR311    | JESPR7       | 25.6445 | 1.4736 | -0.0308 |
|            | 394      | WMS198      | WMS337       | 28.8588 | 1.4826 | -0.0584 |
| -Ch2-1B    | 119      | WMS130      | JESPR294     | 15.5173 | 1.4733 | 0.0358 |
| -Ch3-1D    | 61       | WMC083      | WMC170       | 19.5631 | 1.5536 | -0.0081 |
|            | 139      | GW271       | XGWM493      | 24.2811 | 1.4685 | 0.0499 |
|            | 192      | XGWM493     | XGWM130      | 16.9353 | 1.5755 | 0.0080 |
|            | 218      | XGWM493     | XGWM130      | 22.9043 | 1.4934 | 0.0029 |
| -Ch4-2A    | 60       | JESPR2      | JESPR7       | 21.0829 | 1.4521 | -0.0257 |
|            | 229      | GW219       | BARC101      | 20.7341 | 1.5709 | 0.0967 |
|            | 241      | BARC101     | CFD65        | 16.8518 | 1.5776 | 0.1122 |
| -Ch5-2B    | 172      | BARC124     | WMS252       | 48.1412 | 2.1650 | 0.1868 |
|            | 179      | WMS252      | WMS297       | 40.9734 | 2.1790 | 0.1843 |
|            | 191      | WMS297      | WMS193       | 26.859  | 1.5677 | 0.1432 |
|            | 220      | WMS193      | WMS165       | 30.6749 | 1.9623 | 0.1286 |
|            | 337      | WMS144      | WMC144       | 25.9318 | 1.513  | 0.0143 |
|            | 345      | WMC144      | WMC167       | 23.2376 | 1.4579 | 0.0052 |
|            | 359      | WMC144      | WMC167       | 22.3533 | 1.4586 | -0.0399 |
|            | 432      | WMC445      | XGWM273      | 17.488  | 1.7303 | 0.1001 |
| -Ch6-2D    | 208      | GW292       | JESPR6       | 23.6446 | 1.4494 | -0.0133 |
|            | 229      | GW292       | JESPR6       | 24.7027 | 1.4517 | -0.0232 |
|            | 234      | JESPR6      | WMS106       | 26.735  | 1.4583 | -0.0281 |
| -Ch7-3A    | 134      | GW148       | XGWM289      | 11.8946 | 1.5697 | 0.0263 |
| -Ch8-3B    | 109      | WMC044      | WMC245       | 21.0153 | 1.4801 | -0.0107 |
| -Ch9-3D    | 87       | JESPR18     | JESPR15      | 17.3147 | 1.4543 | -0.0231 |
|            | 93       | JESPR15     | WMC333       | 23.7691 | 1.4605 | -0.0264 |
| -Ch10-4A   | 22       | JESPR12     | WMC018       | 18.9897 | 1.5582 | 0.0009 |
|            | 41       | WMC018      | JESPR4       | 27.6101 | 1.9431 | 0.0079 |
|            | 46       | JESPR4      | JESPR296     | 38.4845 | 1.9785 | 0.0033 |
| -Ch11-4B   | 271      | WMS109      | XGWM350      | 8.3317  | 1.5451 | 0.0391 |
| -Ch12-5B   | 105      | WMS060      | XWMC603      | 22.9912 | 1.4643 | 0.0136 |
| -Ch13-6B   | 83       | JESPR9      | JESPR8       | 20.0978 | 1.4532 | -0.0056 |
| -Ch14-6D   | 61       | WMS058      | XWMC235      | 16.8681 | 1.5493 | 0.0933 |
|            | 73       | WMC235      | XWMC235      | 19.853  | 1.5985 | 0.0760 |
| -Ch15-7A   | 70       | WMS095      | XGWM626      | 16.0521 | 1.618  | -0.0311 |
|            | 112      | XGWM626     | XWMC009      | 21.471  | 1.4436 | -0.0188 |
|            | 119      | XWMC009     | GW181        | 24.079  | 1.4653 | -0.0075 |
|            | 152      | XWMC009     | GW181        | 22.432  | 1.5547 | 0.0712 |
|            | 162      | GW181       | JESPR290     | 22.8931 | 1.5316 | 0.0929 |
|            | 222      | JESPR290    | JESPR304     | 26.2245 | 1.5366 | 0.1215 |
|            | 229      | JESPR304    | WMC177       | 26.1425 | 1.5501 | 0.1487 |
|            | 278      | WMS043      | WMS044       | 81.141  | 5.268  | 0.9462 |
Abbreviations
AFLP: Amplified fragment length polymorphisms; CIM: Composite interval mapping; cM: Centimorgan; SSR: Chloroplast simple-sequence repeats; CV: Coefficient of variance; DNA: Deoxyribonucleic acid; ICM: Inclusive composite interval mapping; LOD: Logarithm of odds; LW: Leaf withering; PCR: Polymerase chain reaction; PH: Plant height; QTLs: Quantitative trait loci; SMA: Single marker analysis; SSR: Simple-sequence repeats; STMS: Sequence-tagged microsatellite; TBE: Tris-borate-EDTA.

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Authors' contributions
All authors were involved in conceptualization and methodology; KAMK, MHA, and RMMH helped in application of drought treatments and DNA extraction from all samples; KAMK contributed to PCR and bioinformatics analysis, writing—original draft preparation; MHA, RMMH, and JAB were involved in review and editing. All authors have read and approved the final manuscript.

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The authors declare no competing interest.

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