RESEARCH ARTICLE

Mapping QTLs of flag leaf morphological and physiological traits related to aluminum tolerance in wheat (*Triticum aestivum* L.)

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Abstract Genetic improvement of aluminum (Al) tolerance is one of the cost-effective solutions to improve plant productivity in acidic soils around the world. This study was performed to progress our understanding of the genetic mechanisms of aluminum tolerance underlying wheat (*Triticum aestivum* L.) flag leaf morphological and physiological traits. A recombinant inbred line population derived from SeriM82 and Babax was used for mapping quantitative trait loci (QTL) in wheat for tolerance to Al toxicity through 477 DNA markers. Based on a single-locus analysis, 48 QTLs including 16 putative and 32 suggestive QTLs were identified for all studied traits.

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Individual QTL explained 4.57–11.29% of the phenotypic variance in different environments during both the crop seasons. These QTLs located unevenly throughout the wheat genome. Among them, 52.08%, 29.17%, and 18.75% were in the A, B, and D genomes, respectively. Based on two-locus analysis, 54 additive QTLs and 6 pairs of epistatic effects were detected, among which 29 additive and 5 pairs of epistatic QTLs showed significant QTL × environment interactions. The highest number of stable QTLs was identified on genome A. Determining a number of QTL clusters indicated tight linkage or pleiotropy in the inheritance of different traits. The stable and major QTLs controlling traits in this research can be applied for verification in different environments and genetic backgrounds and identifying superior allelic variations in wheat to increase the performance of selection of high yielding lines adapted to Al stress in breeding programs.

Keywords Aluminum stress · Epistatic QTL · QTL × environment · *Triticum aestivum*

Introduction

Stable production of cereal crops is mostly influenced by environmental stresses, such as heavy metals, resulting in a significant decrease in crop yields (Ramegowda and Sen-thil-Kumar 2015). Aluminum (Al) toxicity presents a major limitation to crop production worldwide, particularly in tropical and subtropical zones which are predominated by acidic soils (Vitorello et al. 2005). According to Jaskowiak et al. (2018), more than 50% of the world's arable land used for wheat production in developing countries are prone to Al toxicity. Further, industrial pollution and the

use of ammonia- and amide-containing fertilizers intensify soil acidification worldwide. Trivalent Al cation (Al^{3+}) is the most toxic of all Al species available to plants, which grows under acidic conditions (Merino-Gergichevich et al. 2010). Plants undergo diverse morphological, physiological, and biochemical changes to adapt to Al stress. Some of their adaptation strategies include reduction of stomata opening, which in turn has a negative effect on crop growth and grain yield (Vitorello et al. 2005). On the other hand, the expansion of root systems enhances plants' accessibility to water during the periods of stress, while also presenting water potential and contributing to opening of the stomata (Zhang et al. 2018). However, there has been a major difficulty in investigating the differences between cultivars in root architecture with regards to Al tolerance. On the contrary, it is easier to investigate the physiological status of the plant aerial region which could indicate plant accessibility to water and nutrient resources. Aluminum tolerance is a complex quantitative trait with genetic control which is significantly influenced by the environment (Dai et al. 2013). Over the years, mapping of the quantitative trait loci (QTL) has been employed as an important and useful tool for dissecting genetic and physiological bases of Al tolerance in wheat via associable genomic information with complementary phenotypic data. The resulting information is vital for breeders as it can help them to develop Al stress tolerant cultivars (Langridge and Reynolds 2015). Molecular markers may provide new resources for identifying Al-tolerance genes in breeding populations. Marker-assisted selection (MAS) will decrease or can even eliminate the need for phenotypic assays (Ma et al. 2005). In recent years, acceptable numbers of QTLs have been introduced in diverse wheats for different traits under various environmental conditions. For example, QTLs have been reported for flag leaf length and width (Xue et al. 2013; Wu et al. 2015), flag leaf area (Fan et al. 2015), relative water content and canopy temperature (Gupta et al. 2017), cell membrane stability (Talukder et al. 2014), water soluble carbohydrates (McIntyre et al. 2010), grain yield (Shukla et al. 2015), and aluminum concentration in wheat (Sasaki et al. 2004) and rice (Ma et al. 2002). However, very few studies have been conducted for mapping QTLs of physiological traits and their co-location with impact on yield under Al stress. Several evidences has reiterated that important genetic components for quantitative traits constitute both epistasis and QTL $\times$ environment (QE) interactions (Yu et al. 2002; Liu et al. 2006). Therefore, identification of epistatic QTLs is essential for the development of marker-assisted selection (MAS) program in the genetic dissection of stress adaptive responses (Govindaraj et al. 2009). However, only a few QTL studies have been conducted on detection of epistasis and QE interaction for adaptation to different stresses in

SeriM82 $\times$ Babax population (Pinto et al. 2010, Tahmasebi et al. 2016). Numerous mapping studies have been reported for Al tolerance in wheat (i.e. Sasaki et al. 2004; Raman et al. 2005) under controlled environmental conditions (e.g. hydroponics and greenhouse). However, the results observed under controlled conditions may not correspond with those observed under field conditions due to prevailing soil conditions including soil physiochemical properties coupled with other environmental interactions. Such problems have been reported for other abiotic stresses, such as salinity (Munns and James 2003; El-Hendawy et al. 2009). Therefore, this study was conducted (1) to evaluate the effects of aluminum stress on some flag leaf morpho-physiological traits in a wheat recombinant inbred line (RIL) population under field conditions, (2) to map major and stable marker-QTLs linkages involved in tolerance to Al stress, (3) to discover the additive and epistatic effects of QTLs, and (4) to check the interactions of the additive and epistatic QTL with environment.

Materials and methods

Plant materials

A wheat recombinant inbred line (RIL) population consisting of 167 lines produced from a cross between two semi-dwarf spring wheat varieties with high yield potential, SeriM82 and Babax was used in this study. SeriM82 is distinguished by moderate tolerance to drought and environmental stresses with Pedigree (MX 196-97 M 31IBWSNS- 1). While, Babax with Pedigree (CM92066-J-OY-OM-OY-4 M-OY-OMEX-48BBB-OY) usually identified for its drought and environmental stresses tolerance (Oliveres-Villegas et al. 2007; McIntyre et al. 2010).

Field trials

The parental lines, together with the RIL population, were evaluated at the Darab Agricultural Research Station ($28^{\circ}47'N$, $57^{\circ}17'E$, 1110 m Altitude) located in the south of Iran. Totally, four managed field trials, including normal and aluminum stress in each of 2014–2015 (NO15 and AL15, respectively) and 2015–2016 growing seasons (NO16 and AL16, respectively) were considered as environments to perform QTL analysis of the SB population. In each trial, parental cultivars and RILs were planted in a 13×13 alpha lattice design with two replications. Each experimental plot was 2 m long comprising of three rows spaced 20 cm.

In aluminum stress experiments, aluminum chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, Merck KGaA) was applied in concentrations of 800 μM at three stages; tillering, stem

elongation and flowering. For this purpose, the pH of the solutions was adjusted to 4.00 by using 1 N (one normal) HCl (hydrochloric acid) to solubilize aluminum in the treatment solution. After shaking for 12 h, dissolved Al in the form of Al^{3+} was added to the irrigation water as a solution with the specified volume in each plot at morning time (8:00–10:00 a.m.). In each stage, 62 kg h^{-1} of aluminum chloride was applied to each stress trial in both replicates (183.5 g per plot).

Accordance with standard crop management procedures, diseases and weeds were controlled in all trials.

Data collection

Using a portable infrared thermometer (Sixth Sense LT300 IRT), the canopy temperature (CT_g) was measured during the grain filling stage at midday (between 11 am and 1 pm) on clear, sunny days with minimal wind. Likewise, canopy temperature depression (CTD) was estimated which was defined as the difference between air temperature (°C) and the average temperature of the leaf canopy (°C) at the grain filling stage. We observed that when the canopies were warmer than the air, estimated values for the CTD were negative. 15 days after flowering, ten flag leaves were randomly picked from the center row of each plot and used for measuring traits related to the flag leaves, which were carried out at the research laboratory of the Faculty of Agriculture and Natural Resources of Darab, Iran. Moreover, Flag leaf length (FLL) was measured as the distance (cm) from the base of ligula to tip of the leaf while flag leaf width (FLW, cm) was determined from the widest part of the leaf. Also, flag leaf area (FLA, cm^2) was quantified by the leaf area meter (Delta.T, England) using the WinDIAS3 software. According to methods reported by Costes et al. (2006), relative water content (RWC) was measured while leaf samples were dissected into pieces and weighed (FW = fresh weight, g) using the Shimadzu analytical balance (four decimal places, AUW220D, Japan). Afterwards, leaf pieces were left to immediately float on distilled water for 24 h and reweighed after paper towels were used to dry additional surface water so as to obtain the turgor weight (TW, g). Dry weights (DW, g) of the leaf pieces were then measured after they were oven-dried using an Eyela, model NDS-450D, Japan at 75 °C for 48 h. The RWC was calculated using the following formula:

$$\text{RWC} (\%) = [(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100.$$

Cell membrane stability (CMS) assay was also performed following the methods of Sairam et al. (2002) with the aid of an EC meter (ST3100C-B, American). Leaf samples of 2.5 cm length were rinsed with double-distilled water before being placed in a falcon tube containing 20 mL double-distilled water. The samples were stored at

room temperature for 24 h. After shaking the samples for 10 s by vortex shaker (Genious3, IKA, Germany), initial electrical conductivity (EC_1 , dS/m) of sample solution was measured. Then samples were placed in a shaking water bath (Eyela, Japan) at 100 °C for 1 h. After cooling, the samples to room temperature, conductivity (EC_2 , dS/m) was measured for second time. The CMS then was estimated using the following formula:

$$\text{CMS} (\%) = \text{EC}_1 / \text{EC}_2 \times 100.$$

For carbohydrate content measurement, sample was prepared based on the modified method of Irigoyen et al. (1992). 0.10 g of the fresh leaf was weighed and placed in a falcon tube, subsequently 5 mL of 96% ethanol was added to it and placed in a shaking water bath at 85 °C for 1 h. After cooling, the sample on ice for 10 min, the mixture was mixed by vortex shaker for 15 s. This was followed by centrifuging the mixture for 10 min at 6000 rpm using a centrifuge (Universal 320 R, Hettich, Germany). Finally, 100 μL of the falcon extract was removed and diluted with 900 μL of 96% ethanol. The Gen5 software was used to record water soluble carbohydrate concentration (WSC) from a microplate reader (Epoch, BioTek, USA) based on the modified technique of Masuko et al. (2005). Methodically, an initial 100 μL of concentrated sulfuric acid (98%) was added into the wells of a 96-well plate, followed by addition of 30 μL of sample extract or glucose standards and 20 μL of 5% phenol in water respectively. The mixture was heated for 10 min at 80 °C in a shaking water bath where the plate was carefully floated. The plate was then placed on ice for 10 min and kept at room temperature for 20 min. Absorption of the samples at 490 nm were recorded and contents of soluble carbohydrate were determined using glucose standard curve and expressed in mg g^{-1} fresh weight (LFW mg g^{-1}). The glucose standard curve provided a linear function ($y = 0.1081x - 0.0201$, $R^2 = 0.992$) to calculate the concentration of carbohydrates in each sample.

The aluminum concentration (Al in mg kg^{-1} , $\lambda = 237 \text{ nm}$) of plant samples was also determined by an atomic absorption spectrophotometer with graphite furnace (GF-990, England) and recorded using a AAWin software. The acid digestion method for sample preparation was employed (Saiyed and Yokel 2005; Tabande et al. 2013).

Grain yield (GYLD) was determined as gm^{-2} in each plot when grains were dried at about 4–5% humidity, and weighing the grains.

Data analyses

Variance analyses of data collected from four trials were done using the software GenStat V. 15 (Payne et al. 2012). Combined analysis of data across trials was carried out

using the Restricted Estimation of Maximum Likelihood (REML) procedure (Virk et al. 2009). Descriptive statistics (means, minimum, maximum, standard deviation and coefficient of variation) were calculated using the SAS_{9.2} software (SAS Institute, Inc. 2002). Adjusted means (Best Linear Unbiased Estimates; BLUEs) were also computed using the REML method, in which lines were treated as fixed effects. BLUEs were used as phenotypic data for estimations of simple correlation coefficients for all traits across environments in SPSS₂₄ software and QTL analyses (Smith et al. 2001; Rattey et al. 2009).

Map construction and QTL analysis

A number of 475 markers consisting of 120 simple sequence repeat (SSR), 211 amplified fragment length polymorphism (AFLP), and 144 diversity array technology (DArT) distributed over 29 linkage groups (LGs) were previously available for SB population (McIntyre et al. 2010; Lopes et al. 2013). The data from 475 previously available markers and the two newly SSR and EST–SSR markers were combined to construct a new map using JoinMap 3.0 by Tahmasebi et al. (2016). A total of 477 DNA markers were used to construct linkage groups that covered 1619.6 cM of the genome, with an average distance of 3.39 cM between adjacent markers. This map can be applied for practical targets in breeding programs. Within each linkage group, the best order of markers was distinguished according to maximum likelihood method and the distance between markers in centi Morgan (cM) was computed by the Kosambi (1943) equation. As illustrated by Lopes et al. (2013), segregation ratio for each marker within a LG was tested χ^2 test and p value < 0.01 . Single markers exhibiting a segregation distortion from a 1:1 ratio were removed if their presence affected marker order or marker distances in the linkage group (McIntyre et al. 2010). Chromosome numbers were followed by a suffix (a, b, c, or d) if more than one LG was determined to a chromosome. QTL analysis was performed using a constructed molecular map. For each trait evaluated in 2014–2015 and 2015–2016, variance analyses were performed and only BLUEs with significant genotypic variances were used for QTL analysis.

Single-locus QTL analysis

Single-locus QTL analysis involving detection of main effect QTL (M-QTL) was performed with Composite Interval Mapping (CIM) using Windows QTL Cartographer 2.5 software for traits in each trial. In the CIM method, Model 6 (standard model) and the forward–backward regression method were used with five background markers, a window size of 10.0 cM, and a walk speed of

2 cM. The QTLs were named according to a catalog of gene symbols (McIntosh et al. 2003). The QTLs where two or more linked markers were detected at $2.0 < \text{LOD} < 3.0$ were defined as suggestive QTLs. While, putative QTLs were defined as two or more linked markers associated with a trait at $\text{LOD} > 3.0$ (McIntyre et al. 2010). Also, QTLs were categorized based on the proportion of the phenotypic variation explained by a QTL (based on the R^2 value), as minor QTLs with R^2 values of less than 10%, and major QTLs with a minimum $R^2 = 10\%$. MapChart 2.2 software was used for graphical presentation of the linkage map and QTLs (Voorrips 2002).

Two-locus QTL analysis

A two-locus (QTL $\times$ QTL) analysis by mixed model-based composite interval mapping (MCIM) was carried in the software QTL Network 2.1 (Yang et al. 2008). In this model, main additive effect QTLs (M-QTL), epistatic QTLs (E-QTLs), additive $\times$ environment interactions (AEI), and epistasis $\times$ environment interaction (AAE) were identified. The CIM analysis was undertaken using forward–backward stepwise, multiple linear regression with 1-cM walking speed, 2D genome scan. The critical F-value was determined by 1000-permutation tests with $p \leq 0.05$ as an experimental-wise significance level for putative QTL identification and determination of QTL effects (Masoudi et al. 2015).

Results

Analysis of variance (ANOVA) and mean comparison

The results of REML analysis used for identifying the differences between lines across trials are shown in Table 1. Differences among the lines in all trials were significant for CMS, WSC, Al, and GYLD. For CTgf, FLL, FLW, FLA, and RWC, there was no significant difference between lines in AL15, NO15, AL16, NO15, and NO16, respectively. CTDgf showed no significant difference between inbred lines grown under normal and stress conditions in 2015–2016 growing season. As compared to the normal trial, GYLD had a decrease of 17.77% and 18.86% under stress conditions in the first and second years, respectively. In comparison to the normal trials, WSC increased as 59.86% and 19.89% in AL15 and AL16, respectively. Similarly, Al concentration in leaf samples compared to normal trials increased as 67.74% and 56.00% in AL15 and AL16, respectively. In second year, a decrease in FLL, FLW, FLA, and CMS values was observed under stress condition compared to normal condition. RWC and

Table 1 REML analysis for studied traits in SeriM82/Babax population in 2014–2015 and 2015–2016 growing seasons

Trait	Wald statistic				
	(2014–2015)		(2015–2016)		2014–2015 and 2015–2016
	Normal	Stress	Normal	Stress	All environments
CTgf	227**	185 ^{ns}	247**	240**	234**
CTDgf	182 ^{ns}	186 ^{ns}	285**	253**	201*
FLL	165 ^{ns}	240**	228**	274**	357**
FLW	350**	238**	243**	175 ^{ns}	461**
FLA	198 ^{ns}	227**	225**	252**	346**
RWC	255**	252**	169 ^{ns}	295**	286**
CMS	247**	244**	646**	1169**	574**
WSC	1236**	2426**	1514**	1207**	1979**
Al	604**	1652**	1293**	940**	1514**
GYLD	281**	286**	236**	237**	298**

CTgf canopy temperature at grain filling stage (°C), CTDgf Canopy temperature degradation at grain filling stage (°C), FLL flag leaf length (cm), FLW flag leaf width (cm), FLA flag leaf area (cm²), RWC relative water content (%), CMS cell membrane stability (%), WSC water soluble carbohydrate concentration (LFW mgg⁻¹), Al aluminum amount (mgkg⁻¹), GYLD grain yield (gm⁻²)

*and **statistically different from zero at $p \leq 0.05$ and $p \leq 0.01$, respectively

^{ns}not statistically different from zero

CTgf indicated an increase of 4.82% and 21.57% in AL15 and AL16 than normal trials, respectively (Online Resource 1).

Combined REML analysis of variance showed a significant difference ($p < 0.01$) between inbred lines and years for all traits. Environments were highly significant for all traits except RWC. The line $\times$ environment interactions ($L \times E$) were also significant ($p < 0.01$) for CTDgf, CMS, WSC, and Al concentration traits, indicating the differential performance of wheat lines over different environments. The line $\times$ year interaction was significant for all traits except FLL, FLW, FLA, and RWC. The year $\times$ environment interaction was significant for all traits except CTgf, FLL, and GYLD. Line $\times$ environment $\times$ year interaction was significant for CTgf, CTDgf, CMS, WSC, Al concentration, and GYLD traits (Online Resource 2).

Phenotypic variation and correlation

SeriM82 and Babax differed significantly in the measured traits, so that, the phenotypic values of SeriM82 was higher for CTDgf, FLL, FLW, FLA, and RWC under Al stress and, for CTgf under normal conditions than those of Babax. Babax was superior to SeriM82 for CMS in two environments. On the other hand, SeriM82 had higher WSC, Al concentration, and GYLD than Babax under both normal and stress conditions. The 167 RIL populations indicated a wide range of phenotypic variation among the measured traits. Some lines had higher or lower values than

the parents under both conditions, indicating transgressive segregation, although the average values of RIL lines for those traits were intermediate between the parental values (Online Resource 3). In addition, traits showed considerable phenotypic variation and continuous distributions, representing their quantitative nature. Test statistics for skewness and kurtosis were commonly less than 1.0 (data not shown), implying polygenic inheritance and suitability of the data for QTL analysis.

The phenotypic correlations (r_p) between all traits for 2-year combined analysis of normal and stress trials showed that the traits were significantly correlated with each other under both conditions. Under both normal and stress conditions, GYLD showed significantly positive correlation with CTgf, RWC, and WSC. Furthermore, GYLD was negatively correlated with CTDgf, FLL, FLW, FLA, CMS, and Al concentration. There was significantly a positive association between Al concentration with CTDgf, FLL, FLW, and FLA, and also, a negative association between Al concentration with CTgf, RWC, and WSC. Under stress conditions, CMS indicated positive r_p with CTDgf, FLL, FLW, FLA, Al concentration, and GYLD; however, its correlations with CTgf and WSC were negative (Online Resource 4).

Single-locus QTL analysis

The results of CIM analysis are shown in Online Resource 5. Generally, among 48 QTLs, 32 suggestive and 16 putative QTLs were detected which explains estimates of

4.57–11.29% of the phenotypic variation with a LOD of 2.06–4.40 for all traits in four trials. In the normal condition, two putative QTLs were responsible for low canopy temperature at the grain filling stage with decreased alleles derived from SeriM82 in the vicinity of 6A-aca/cac-4 and 6A-agg/cat-6 markers, respectively. For canopy temperature degradation at grain filling stage, two QTLs were detected in NO16 trial with increased CTDgf which was conferred by the SeriM82 alleles. Only one putative QTL, *QCTDgf-3B.NO16*, was mapped on linkage group (LG) 3B. This study identified five QTLs for flag leaf length with additive effects ranging from 0.60 to 1.09 cm, which explained 6.10–11.29% of the R^2 . Among the FLL-QTLs, two major and putative QTLs, *QFLL-1D-a.NO16* and *QFLL-3B.AL16* (at 84.70 cM) exhibited positive additive effects indicative of the favorable alleles derived from SeriM82. We found four QTLs for flag leaf width, out of which only one QTL, *QFLW-6B.AL15*, was related to the AI stress with QTLs indicative of 10.19% of the FLW R^2 . The *QFLW-1D-a.NO15* exhibited major effects on FLW. Another putative QTLs for FLW was detected on LGs 1D-a, and 4A. Among three stress-specific QTLs associated with the flag leaf area, two putative QTLs were mapped on LG 3B, and they were designated by *QFLA-3B.AL16*. The *QFLA-5A.AL15* linked to 5A-aac/ctc-12 marker had the largest effect on FLA. Among the five QTLs recorded for relative water content, three (on LGs 1D-a, 5A, and 6A-b) were found in stress trials. The only one putative QTL, *QRWC-5A.AL15*, was mapped to the 5A-barc0001-5A-aac/ctc-12 interval. All of the increasing RWC alleles except one were derived from SeriM82. For cell membrane stability, eight identified QTLs were distributed on LGs 1A, 1D-a, 4A, 4B, 5A, 6B, and 7A. Likewise, most CMS-increasing alleles were derived from SeriM82. Three putative QTLs, *QCMS-1A.AL16*, *QCMS-4A.AL16*, and *QCMS-5A.AL15* were identified for CMS in stress trials. A total of five QTLs were identified for water soluble carbohydrate concentration in NO15 and AL16. Amongst them, *QWSC-2B.AL16* with the highest R^2 had the largest additive effects. This study detected six QTLs affecting AI concentration, three in stress trials (on 1A, 2D, and 7A) and three in normal trials (on 1A, 2B, and 5A). Linkage group 7A (*QAI-7A.AL15*) showed the strongest additive effects contributed by the SeriM82 allele and explained the highest variation of AI. Out of eight QTLs associated with grain yield, two putative QTLs, *QGYLD-4A.NO16* (at 19.20 cM) and *QGYLD-7B.AL16* were detected with high GYLD alleles originating from both parents. Each GYLD QTL demonstrated variations that ranged between 6.04 and 10.68%.

Main additive QTLs, epistatic QTLs, and their interaction with environment

Presented in Tables 2 and 3 are the results of the MCIM analysis for detecting main additive and epistatic QTLs, respectively. A total of 54 M-QTLs and six pairs of E-QTLs were identified on linkage groups for different traits across four trials. Out of seven canopy temperature QTLs, six QTLs located on LGs 2A-a, 2B, 3B, 6A-a, and 6B showed significant AEI. All the additive effects were related to the SeriM82 alleles except the QTL located on LG 2A-a. The CTgf M-QTL located on LG 6A-a, made the largest contribution to phenotypic variance (Tables 2). For canopy temperature degradation at the grain filling stage, four M-QTLs were identified on LGs 2A-a, 3B, and 6A-a. Amongst these loci, two QTLs on LGs 2A-a, and 3B (at 119.40 cM) had a significant AEI effect. Moreover, all the additive effect emanated from the SeriM82 alleles except the M-QTL on LG 2A-a (Tables 2). More so, it is important to mention that there was no E-QTL for CTgf and CTDgf traits. We detected seven M-QTLs for flag leaf length. One FLL QTL positioned on LG 3B showed significant AEI (Table 2). The highest additive effect and phenotypic variation was explained by the FLL M-QTL on LG 2D. No E-QTL was found for FLL. For flag leaf width, two M-QTLs were identified on LGs 2D and 7A (Tables 2) while there was no AEI and E-QTL for this trait. Among the two QTLs recorded for flag leaf area, one (on LG 3B) was involved in AE interactions and was contributed by SeriM82 allele. The largest additive effects belonged to FLA M-QTL on LG 2D in the gdm035-acg/cta-6 interval (Table 2). Among four QTLs associated with relative water content, three QTLs on LGs 4A (at 109.60 cM), 5A, and 7A were influenced by AEI effects. The SeriM82 parent carried QTL alleles that increased RWC values. The additive effect and AEI explained 1.72% and 3.52% of RWC variation, respectively (Tables 2) while no E-QTL was found for FLA and RWC M-QTLs. Both parents contributed alleles for increased cell membrane stability. All CMS QTLs showed significant AEI, except QTL on LG 4A. For CMS, one pair of QTLs located on LGs 4A/6B was also involved in epistatic effects which had significant AAE. M-QTLs, E-QTLs and AAE effects were resolved to 2.67, 0.13, and 0.91% of phenotypic variation, respectively (Tables 2, 3). We found eight QTLs for water soluble carbohydrate concentration, with additive effect from either SeriM82 or Babax. These loci explained 4.35% of the phenotypic variation. Of these loci, three QTLs on LGs 2D (at 8.10 cM), 5B and 7D-b had a significant AEI effect. We also detected one pair of E-QTLs for WSC, which were located on LGs 5B/7D-b. WSC E-QTLs showed significant AAE effect (Tables 2 and 3). For AI concentration, four of six QTLs positioned on 3A-a, 5A, 7A, and 7D-b were

Table 2 Estimated additive (A) and additive $\times$ environment interaction (AE) effects of M-QTLs for flag leaf morpho-physiological traits in SeriM82/Babax wheat population under normal and aluminum stress conditions in 2014–2015 and 2015–2016 growing seasons

Plant traits	Linkage group	Markers interval	QTL position ^a	Additive effect ^b	H ² (A, %) ^c	AE1	AE2	AE3	AE4	H ² (AE, %) ^d
CTgf	2A-a	2A-acc/ctg-8-2A-gwm636	0.00	– 0.12	0.08	–	–	–	0.20*	0.18
	2B	2B-acc/ctg-4-2B-acc/ctc-10	25.20	0.05	0.03	–	–	–	– 0.28**	0.22
	2B	2B-agg/ctg-2-2B-agg/cta-3	51.10	0.01	0.02	–	–	0.25*	0.23*	0.35
	3B	3B-wPt-6047-3B-wmc0307	119.40	0.001	0.001	–	0.26**	–	–	0.34
	6A-a	6A-aca/cac-4-6A-agg/cat-6	12.60	0.18	0.14	–	0.23*	–	–	0.22
	6B	6B-agg/cat-8-6B-wPt-4218	64.50	0.03	0.01	–	–	0.20*	–	0.28
	7A	7A-agg/cag-9-7A-act/ctc-10	11.30	0.13	0.12	–	–	–	–	–
CTDgf	2A-a	2A-acc/ctg-8-2A-gwm636	0.00	– 0.12	0.05	–	– 0.20*	–	0.24**	0.21
	3B	3B-agg/cat-3-3B-gwm389	90.90	0.18	0.14	–	–	–	–	–
	3B	3B-wPt-6047-3B-wmc0307	119.40	0.03	0.001	–	0.42**	–	–	0.41
	6A-a	6A-aca/cac-4-6A-agg/cat-6	13.60	0.16	0.08	–	–	–	–	–
FLL	1A	1A-aag/cta-8-1A-agg/cac-6	39.90	– 0.23	0.25	–	–	–	–	–
	2A-a	2A-acc/ctg-8-2A-gwm636	0.00	0.40	0.59	–	–	–	–	–
	2A-d	2A-wPt-3281-2A-agg/cat-15	26.90	– 0.27	0.28	–	–	–	–	–
	2D	2D-gdm035-2D-agg/cta-6	8.10	– 0.47	0.88	–	–	–	–	–
	3A-b	3A-aca/cta-13-3A-acc/ctg-12	3.10	0.24	0.43	–	–	–	–	–
	3B	3B-wPt-2757-3B-barcl47	86.80	0.15	0.001	–	–	– 0.32*	0.43**	0.67
	6A-a	6A-wmc0256-6A-acc/ctg-6	68.80	– 0.20	0.23	–	–	–	–	–
FLW	2D	2D-gwm102-2D-wmc0018	64.50	– 0.02	0.43	–	–	–	–	–
	7A	7A-cfa2049-7A-wPt-4553	46.40	0.02	0.57	–	–	–	–	–
FLA	2D	2D-gdm035-2D-agg/cta-6	9.10	– 1.04	0.62	–	–	–	–	–
	3B	3B-wPt-2757-3B-barcl47	86.80	0.09	0.00	–	–	–	0.90*	0.50
RWC	4A	4A-gwm397-4A-barcl236	35.70	0.001	0.63	–	–	–	–	–
	4A	4A-wPt-4487-4A-wPt-4620	109.60	0.001	0.61	– 0.01*	–	–	–	1.11
	5A	5A-barcl360-5A-aac/caa-8	61.20	0.002	0.04	–	–	–	0.01*	1.10
	7A	7A-aca/cag-10-7A-cfa2123	78.60	0.007	0.44	–	–	–	– 0.02**	1.31

Table 2 continued

Plant traits	Linkage group	Markers interval	QTL position ^a	Additive effect ^b	H ² (A, %) ^c	AE1	AE2	AE3	AE4	H ² (AE, %) ^d
CMS	1B	1B-agc/cta-3-1B-wPt-0944	0.00	– 0.02	0.34	–	– 0.03*	–	–	0.89
	3B	3B-aac/cta-6-3B-wPt-7186	84.70	0.01	0.29	–	0.04*	–	–	1.06
	4A	4A-wmc048c-4A-act/cag-5	0.00	0.03	0.32	–	–	–	–	–
CMS	5D-a	5D-gdm063-5D-wPt-5870	25.00	– 0.02	0.26	–	– 0.04*	–	–	1.06
	6B	6B-agc/cta-5-6B-wPt-7662	17.00	0.03	0.81	–	0.08**	–	–	1.70
	7A	7A-wPt-4553-7A-wPt-6931	58.50	0.03	0.65	–	0.04*	–	–	0.71
WSC	2A-a	2A-acc/ctg-8-2A-gwm636	0.00	0.67	0.39	–	–	–	–	–
	2A-d	2A-wPt-7901-2A-wPt-6687	0.00	0.73	0.59	–	–	–	–	–
	2D	2D-gdm035-2D-agc/cta-6	8.10	– 0.55	0.001	–	1.25**	– 1.00**	–	1.69
	2D	2D-wmc0018-2D-wPt-0298	68.70	0.86	1.22	–	–	–	–	–
	4D	4D-cfd023-4D-cfd071	12.30	– 0.88	0.82	–	–	–	–	–
	5B	5B-wPt-5737-5B-wPt-9814	0.00	0.36	0.17	–	–	0.72*	–	0.57
	7A	7A-barc0049-7A-barc121	95.40	0.65	1.05	–	–	–	–	–
	7D-b	7D-acc/cat-10-7D-cfd0014	21.70	0.10	0.11	–	1.52**	– 1.57**	–	1.32
AI	1D-a	1D-wPt-7946-1D-acc/ctc-1	19.40	0.03	0.52	–	–	–	–	–
	2D	2D-wPt-0298-2D-gwm0448	70.80	– 0.03	0.36	–	–	–	–	–
	3A-a	3A-wPt-4407-3A-agc/cag-13	46.10	0.04	0.51	–	–	0.05**	–	0.70
	5A	5A-barc100-5A-barc040	47.40	0.02	0.50	–	–	0.06**	–	1.47
	7A	7A-aca/cag-10-7A-cfa2123	78.60	0.03	0.69	–	–	0.05**	–	1.40
	7D-b	7D-gwm437-7D-gdm0046	78.60	– 0.03	0.77	–	–	– 0.03*	–	0.84
GYLD	1D-a	1D-cfd0027-1D-wPt-1799	77.60	– 18.01	0.32	–	–	–	–	–
	2B	2B-wPt-9668-2B-wPt-7320	0.00	– 11.35	0.21	–	–	–	–	–
	4A	4A-gha44-4A-gwm397	21.20	– 7.10	0.02	26.10**	– 26.34**	–	–	0.67
	4A	4A-aac/caa-6-4A-aac/caa-3	65.90	4.01	0.00	–	13.45*	–	–	0.22
	6A-b	6A-agc/cta-5-6A-wPt-7204	44.00	1.27	0.03	15.07*	–	–	–	0.33
	6B	6B-agg/cat-8-6B-wPt-4218	64.50	– 11.92	0.24	–	–	–	–	–
	7A	7A-gwm0276-7A-barc0049	94.10	– 11.39	0.25	–	–	–	–	–

Table 2 continued

Plant traits	Linkage group	Markers interval	QTL position ^a	Additive effect ^b	H ² (A, %) ^c	AE1	AE2	AE3	AE4	H ² (AE, %) ^d
	7B	7B-acg/cta-9-7B-gwm132c	15.10	5.85	0.03	–	–	–	16.00*	0.41

E1: Normal environment in first year (2014–2015); E2: Normal environment in second year (2015–2016), E3: Stress environment in first year (2014–2015) and E4: Stress environment in second year (2015–2016)

CTgf canopy temperature at grain filling stage (°C), *CTDgf* canopy temperature degradation at grain filling stage (°C), *FLL* flag leaf length (cm), *FLW* flag leaf width (cm), *FLA* flag leaf area (cm²), *RWC* relative water content (%), *CMS* cell membrane stability (%), *WSC* water soluble carbohydrate concentration (LFW mgg⁻¹), *Al* aluminum amount (mgkg⁻¹), *GYLD* grain yield (gm⁻²)

*and** Significant ($\alpha = 5\%$) and highly significant ($\alpha = 1\%$), respectively

^aQTL position expressed in cM, from origin of the linkage group (end of shortarm)

^bA positive and negative values indicate that the Serim82 and Babax alleles increased trait, respectively

^cContribution of phenotypic variance explained by putative main-effect QTL or additive effect

^dContribution of phenotypic variance explained by additive $\times$ environment interaction effect

Table 3 Estimated epistatic (AA) and epistatic $\times$ environment interaction (AAE) effects of E-QTLs for flag leaf morpho-physiological traits in Serim82/Babax wheat population under normal and aluminum stress conditions in 2014–2015 and 2015–2016 growing seasons

Plant traits	Markers interval_i	LG (position_i)	Markers interval_j	LG (position_j)	AA ^a	H ² (AA, %) ^b	AAE	H ² (AAE, %) ^c
CMS	4A-wmc048c-4A-act/cag-5	4A (0.00)	6B-acg/cta-5-6B-wPt-7662	6B (17.00)	– 0.01	0.13	AAE2 (– 0.06**)	0.91
WSC	5B-wPt-5737-5B-wPt-9814	5B (0.00)	7D-acc/cat-10-7D-cfd0014	7D-b (21.70)	– 0.62	0.27	AAE3 (– 1.28*)	0.40
Al	1D-wPt-7946-1D-acc/ctc-1	1D-a (19.4)	5A-barc100-5A-barc040	5A (47.40)	0.016	0.21	AAE3 (0.04*)	0.85
	1D-wPt-7946-1D-acc/ctc-1	1D-a (19.4)	7A-aca/cag-10-7A-cfa2123	7A (78.60)	0.018	0.27	AAE3 (0.05**)	1.31
GYLD	1D-cfd0027-1D-wPt-1799	1D-a (77.60)	7A-gwm0276-7A-barc0049	7A (94.10)	– 22.44	0.59	–	–
	7A-gwm0276-7A-barc0049	7A (94.10)	7B-acg/cta-9-7B-gwm132c	7B (15.10)	5.40	0.09	AAE3 (– 13.63*)	0.29

CMS cell membrane stability (%), *WSC* water soluble carbohydrate concentration (LFW mgg⁻¹), *Al* aluminum amount (mg kg⁻¹), *GYLD* grain yield (gm⁻²)

*and ** Significant ($\alpha = 5\%$) and highly significant ($\alpha = 1\%$), respectively

E1: Normal environment in first year (2014–2015); E2: Normal environment in second year (2015–2016), E3: Stress environment in first year (2014–2015) and E4: Stress environment in second year (2015–2016)

^aA positive and negative values indicate that the Serim82 and Babax alleles increased trait, respectively

^bContribution of phenotypic variance explained by epistatic QTL or additive $\times$ additive effect

^cContribution of phenotypic variance explained by additive $\times$ additive $\times$ environment interaction effect

involved in AEI effect and together explained 4.41% of the Al variation. Also, two pairs of Al-QTLs (on LGs 1D-a/5A and 1D-a/7A) with epistatic effects were detected with significant AAE interaction (Tables 2 and 3). For grain yield, four environmentally stable QTLs were detected on LGs 1D-a, 2B, 6B, and 7A, with increasing alleles from Babax parent. Also, four GYLD M-QTLs on LGs 4A (2), 6A-b, and 7B were significantly involved in AEI. Two pairs of E-QTLs were observed for GYLD, which were placed on LGs 1D-a/7A and 7A/7B, and AAE interaction was only observed for the 7B/7B E-QTL (Tables 2 and 3).

Discussion

Single-locus QTL

In total, 48 QTLs were detected using composite interval mapping analysis. Of these, 20 and 28 QTLs were detected in normal and Al stress conditions, respectively. The highest number of QTLs in Al stress condition was identified for CMS and GYLD. The highest numbers of putative QTLs were detected for FLW and CMS, respectively. Among the traits studied in four trials, the *QFLL-1D-*

a.NO16 had the highest R^2 and LOD values. The most important chromosomal regions were 1A, 1D-a, 2B, 3B, 4A, 5A, 6A-a, 6B, and 7A which were associated with major and putative QTLs. For all studied traits, QTL frequency was maximum in the A genome with 25 QTL (52.08% of the total number of QTLs). Other QTLs including 14 (29.17%) and 9 (18.75%) were found in genomes B and D, respectively. The high genetic coverage of the A and B genomes compared with the D genome were previously reported in some other studies such as in Shi et al. (2017). This occurrence could be due to the superior diversity demonstrated by the A and B genomes relative to the D genome, which exhibited less diversity (Ling et al. 2013).

QTL mapping of flag leaf morpho-physiological traits associated with Al tolerance

Breeding progress for tolerance to abiotic stress is accelerated if physiological, biochemical, and morphological characteristics are integrated as selection criteria (Reynolds et al. 2012). Canopy temperature is an easy, fast, and indirect selection tool for stress tolerance traits such as root depth and function in breeding programs under field condition (Mason and Singh 2014). In the present study, CTgf-QTLs were found on LG 6A. CT QTL on this chromosome has been reported previously (Diab et al. 2008, Lopes et al. 2013; Tahmasebi et al. 2016). Detection of QTL for CT is extremely difficult due to the complex phenotypes and genetic mechanisms of this trait for adaptation to stress (Reynolds et al. 2012). High CTD has been widely applied as a breeding tool to improve adaptation to abiotic stress such as drought, heat, and aluminum and has been associated with yield enhancement across wheat (*Triticum aestivum* L.) genotypes (Zia et al. 2013). The genetic studies of CTD should be extended as until now very little has been done on it. The results demonstrated that CTDgf QTLs were positioned on 3B and 6A-a, which was congruent with the results of Mason et al. (2013), while Paliwal et al. (2012) reported putative QTLs for CTD on LG 7B in a wheat RIL population under heat stress. The flag leaf represents the ultimate leaf before the emergence of the spike and serves as the primary source of assimilates for grain filling and thus grain yield in wheat (Yang et al. 2016). The entirety of carbohydrates stored in the flag leaf is transposed towards the grain while those from other leaves are only released in part to grain (Ali et al. 2010). Morphological traits related to flag leaf (length, width, and area) are generally quantitative traits which are controlled by many genes or quantitative trait loci (QTL) and are influenced by environmental stresses such as heavy metals (Tian et al. 2017). Since the flag leaf size affects wheat canopy morphology and photosynthetic efficiency, thus it

is important in wheat breeding. In the present study, genomic regions associated with the three-flag leaf morphological traits (FLL, FLW and FLA) were detected on LGs 1D, 4A, and 7A under normal conditions and on LGs 2D, 3B, 5A, 6B, in Al stress. Previous studies also reported that QTLs for FLL, FLW, and CTD were located on LGs 1B, 2D, 3B, and 5A in a wheat population under drought stress in the field (Mason et al. 2011). Liu et al. (2018) identified 23 putative QTLs for FLL, FLW, and FLA on LGs 1B, 2B, 3A, 4B, 5A, and 6B, using a SSR genetic linkage map in a bread wheat population. Estimation of flag leaf RWC is the most important growth/physiological parameter revealing the stress intensity in assessment of wheat response to environmental stresses (Saleem et al. 2018). The utilization of RWC genetic control could assist enhance breeding for Al stress tolerance. In this study, the existence of RWC-QTLs on the LGs 5A, 1D-a, and 6A under Al stress, has suggested the possible link of these chromosomes with Al tolerance. Further investigation is required to explore these chromosomes for Al tolerance. Diab et al. (2008) found only one QTL for RWC on the LG 2B close to marker gwm108 in a RIL population of durum wheat under drought stress conditions in field. Using a population of 108 spring wheat, Ahmad et al. (2014) also detected three RWC-QTLs on 2A under the drought stress in field. Among the diverse heavy metals-stress and water-stress tolerance traits targeted in crop breeding, CMS is regarded as a physiological parameter which is mostly affected (Singh et al. 2016). QTLs for CMS have been previously reported on LGs 1D, 4B, 5A, 6B, and 7A for drought tolerance across different wheat populations under field conditions (Elshafei et al. 2013; Talukder et al. 2014; Sohrabi et al. 2018), which were in accordance with our results. Variations in WSC content are largely genetically determined. Therefore, identification of genomic position, molecular mechanism and genotypic variation in WSC is necessary for understanding yield-limiting factors and also for improving yield potential in wheat (Dong et al. 2016). Pinto et al. (2010) evaluated Seri/Babax RIL population under drought and heat stress and identified major QTLs for WSC on LGs 1A-b, 1B-a, 3A-b, 3B-b, 4A-a, 4A-b, and 5A-a. The QTLs found for WSC on LGs 1D-a, 2A-d, 2B, 4D, and 6B in the present study were confirmed by the results of Snape et al. (2007), Yang et al. (2007), and McIntyre et al. (2010) in wheat Seri/Babax and DH populations under drought stress conditions. Membrane transporters encoded by the *TaALMT1* and *TaMATE1B* genes which are responsible for the malate and citrate efflux from the root apices respectively constitute an important Al resistance mechanism in wheat (Tovkach et al. 2013; Pereira 2018). So far, no QTLs have been reported for aluminum concentration of leaf in bread wheat. However, several studies have been released for concentrations of

other heavy metals in some parts of plants (Reuscher et al. 2016; Liu et al. 2017). In our study, the strongest QTL associated with Al concentration was detected on LG 7A in stress condition and flanked by markers gwm282 and aca/cag-10. In rice grains (*Oryza sativa* L.), six putative QTLs controlling Cd concentration have been reported on chromosomes 3, 6, and 8 (Ishikawa et al. 2010). Eleven QTLs were detected for Arsenic (As) concentration in the leaves, bracts, stems, and kernels tissues using SSR markers in a maize RIL population (Ding et al. 2011). The grain yield of bread wheat is a quantitatively inherited complex trait which is highly influenced by interaction of genetic and environmental factors (Guan et al. 2018). The QTLs affecting the GYLD on LGs 1A, 2B, 4A, 6A-a, and 7B have been reported previously in wheat populations (Zhang et al. 2010; Pinto et al. 2010; Lopes et al. 2013). In our study, the largest additive effect of GYLD-QTL was detected on LG 1A in AL15 trial. Two of the GYLD-QTLs identified in our study were positioned at the same distances as reported by Tahmasebi et al. (2016) on LGs 1A (linked to aca/cta-2 and wmc097) and 4A (linked to gha44 and gwm397) under stress and normal conditions, respectively.

As shown by this present study, using single-locus QTL analysis, no stable QTL was found. In other words, for all traits under studied conditions, diverse QTLs were obtained due to differences in their location.

QTLs with additive $\times$ environment interaction and epistatic effects

Intensities of expression of quantitative traits are affected by the epistatic and QTL $\times$ environment interactions effects which are important genetic components (Li et al. 2014). Epistasis and AE studies have been conducted in wheat for CT and GYLD (Tahmasebi et al. 2016), FLM-related traits (Yang et al. 2016), CMS, and WSC (Sohrabi et al. 2018). In most of the previous studies carried out on QTL mapping in bread wheat, the associated interactions (AA, AE, and AAE) were not totally investigated (e.g. McIntyre et al. 2010; Pinto et al. 2010). However, in this research, we studied a RIL population and progenitor in four trials. A total of 54 QTL with additive effects, were found for all studied traits, which all had uneven distributions in the wheat genome. Among them, 27 (50.00%), 15 (27.78%) and 12 (22.22%) were in the A, B and D genomes, respectively. Also, among all studied traits, WSC M-QTL in the 2D-wmc0018-2D-wPt-0298 interval justified the highest phenotypic variance. Out of 54 additive QTLs, 29 QTLs showed interactions with the environment (AEI). AEI effects can be a result of contrasting influence of the parent alleles across environments. On a general note, a QTL with little or no AE interaction enables its

utilization in varieties of environments while the presence of a QTL with significant AEI limits their usage as they can only be utilized in specific environments where they are located (Zhao and Xu 2012). In two-locus QTL analysis, main-effect QTLs with no AE interaction were stable. Significant AEI effects were detected for all traits except FLW. In other words, two FLW-QTLs were stable. Other stable QTLs included 1, 2, 6, 1, 1, 1, 5, 2, and 4 QTLs for the CTgf, CTDgf, FLL, FLA, RWC, CMS, WSC, Al, and GYLD, respectively. These QTLs with remarkable stability can be employed for QTL pyramiding which could serve as a potentially useful approach for the genetic improvement of stress adaptability in wheat. In a previous study by Fan et al. (2015), two stable QTLs for FLL on LGs 4B and 6B, and one for FLA on LG 5B were detected in eight environments. Yang et al. (2016) also reported 20 stable QTLs related to flag leaf morphology. It is also important to mention that the failure to take epistasis into consideration would account for overestimation or underestimation of QTL effects which could in turn affect the efficiency and accuracy of MAS (Bocianowski 2013). In this present study, six pairs of epistatic QTLs were identified across four trials for CMS, WSC, Al, and GYLD traits. Identification of AEI for a QTL can be correlated to the performance of the epistatic AAE in which the QTL was involved (Zhuang et al. 2002). Five out of the six E-QTL detected pairs exhibited significant AAE effects. The GYLD E-QTL pair located on chromosome 1D-a/7A acted in favor of the parent and exhibited the largest effect with a contribution a of 22.44 GYLD, which in the phenotypic variance accounted for about 0.59%. In all of the QTLs with significant AEI and AAE effects, the percentage of phenotypic variation explained by the additive and epistatic effects was less than the percentage of variations explained by AE and AAE components. In addition, our results linked all epistatic effects to QTLs that revealed additive effects which were detectable. The highest number of E-QTLs was related to grain yield and Al concentration which uniquely represented the epistatic importance for the observed traits to the genetic architecture of Al and GYLD in the SB population. Generally, as derived from statistical efficiency, additive effects had more contributions to the phenotypic variations of traits investigated when compared to the effects of epistasis. Similarly, Tahmasebi et al. (2016) found that the main additive effects had a greater importance as compared to epistasis in the SB population.

Findings from this present study were compared to previous studies related to Al tolerance in wheat. The major *QFLL-1D-a.NO16*, *QFLW-1D-a.NO15*, *QGYLD-1A.AL15*, and the putative *QCMS-1A.AL16* were determined by the CIM method, likewise the stable QTLs for FLL (on LG 1A), CMS (on LG 1B), and GYLD and Al (on LG 1D-a) were determined by the MCIM method and

presented in our study. These QTLs were probably associated with the *TaMATE2* gene located on the long arm of homoeologous group 1 chromosomes (1A, 1B, and 1D) in bread wheat, which encodes a citrate transporter and increased Al tolerance (Garcia-Oliveira et al. 2018). The efflux of malate anions from the root apices, which typifies an important mechanism for Al tolerance, is encoded by *TaALMT1*, a gene located on chromosome 4DL (Navakode et al. 2014). In our study, the *QWSC-4D.AL16* (CIM method), and stable WSC-QTL (MCIM method) positioned on LG 4D probably contained gene *TaALMT1* for Al tolerance in wheat. Dai et al. (2013) also reported QTLs for Al tolerance on the LGs 4DL and 3BL in a RIL population of wheat for hematoxylin stain score and net root growth, which agreed with our result for the *QCTDgf-3B.NO16*, *QFLL-3B.AL16*, *QFLA-3B.AL16*, and *QRWC-4B.NO15*.

In single-locus QTL analysis, in contrary to the adapted line Babax, the moderate adapted line SeriM82 contributed more positive alleles (31 vs. 17) proposing that the moderate adapted germplasm has a considerable potential either to enhance available gene pool for flag leaf morphological and physiological traits or to improve wheat yield under Al stress condition. For four major loci located on 1D-a, 3B, 6A-a, and 7A, the moderate adapted parent SeriM82 contributed the alleles for increased FLL and decreased CTgf and Al concentration. On the other hand, the alleles for increased FLW and GYLD at three major loci mapped on 1A, 1D-a, and 4A were contributed by Babax; the adapted parent. In another category, the alleles for increased CTDgf, FLL, FLW, RWC, CMS, and GYLD, and decreased CTgf at the putative QTLs (12 out of 31) identified on 1D-a, 3B, 4A, 5A, 6A-a, 7A, and 7B were contributed by SeriM82. While, the alleles for increased FLW, CMS, and GYLD at four (out of 17) putative QTLs located on 1A, 1D-a, and 4A were contributed by Babax. It can be inferred that the useful alleles which are necessary for not only the reduction of Al toxicity, but also the enhancement of yield were contributed by both parental lines. This could open a new attitude to merge these favorable alleles by modern genomic tools for realizing appropriate flag leaf morphological and physiological traits to enhance grain yield. In two-locus QTL analysis, the moderate adapted line SeriM82 and adapted line Babax had approximately the same contribution (13 and 12, respectively) of beneficial positive alleles for stable QTLs. Overall, the composition of superior alleles from both parental lines can corroborate the identification of some RILs accompanied with improved phenotypic yield.

Comparison of two-locus and single-locus analyses

The results of single-locus and two-locus analyses were contrasted for QTL detection. In total, 48 and 52 QTLs were detected using single locus CIM and two-locus MCIM, respectively. Results revealed that some of the QTLs were found in both analyses. About 4% of the M-QTLs were as well as recognized by single locus CIM analysis in the same and/or near marker intervals. The inconsequential differences related to the position and the marker interval of the QTLs could be ascribed to different procedures and software used. Also, QTLs expression is changeable, due to AE interactions. So, QTL effects discovered by CIM often do not resolve the network interactions involving AA, AE or AAE effects (Gowda et al. 2011). Of 54 QTLs, two QTLs including CMS and GYLD located on LGs 7A and 2B, respectively, as well as detected by single locus CIM analysis in the same position and marker interval.

Co-location of QTLs in single-locus and two-locus analyses

Co-localizations of QTLs for different traits are very often observed in the SB population (e.g. McIntyre et al. 2010; Tahmasebi et al. 2016). Yang et al. (2016) found co-localization of QTLs associated with flag leaf related traits on LGs 1B and 2B. Using single-locus QTL analysis, the co-location of QTLs for several traits as investigated in this study were clearly observed in some chromosomal intervals (Fig. 1). At map position 84.70 cM on LG 3B, an Al stress-specific FLL QTL was co-located with a QTL for FLA at the *aac/cta-6-wPt-7186* interval in AL16, that confirmed their significant positive correlations. Four RWC QTLs at 116.90, 110.10, 60.40, and 38.30 cM position on LGs 1D-a, 4A, 4B, and 5A linked to *wPt-5888*, *gwm350*, *aac/cta-9*, and *barc0001* markers were co-located with CMS QTLs in AL15, NO15, NO15, and AL15, respectively. In normal and stress trials, the co-location of RWC and CMS QTLs coupled with the similar allelic effects could be due to both correlation and pleiotropy. Pinto et al. (2010) reported co-locations of CT, WSC, and GYLD QTLs in the same genomic regions using a wheat SB population under heat and drought conditions.

In two-locus QTL analysis, QTL co-location was found in six marker intervals, suggesting the possible presence of pleiotropy or gene linkage. The co-located QTLs for CTgf, CTDgf, and WSC were identified at 0.00 cM distance of LG 2A-a. Among QTLs with additive-effects, CTgf QTLs at map positions 119.40 and 64.50 cM were co-located with CTDgf and GYLD QTLs on LGs 3B and 6B, respectively. Also, FLL QTLs at 8.10 and 86.80 cM distance on LGs 2D and 3B were co-located with WSC and

Fig. 1 The map of co-located QTLs in SeriM82/Babax wheat population. For each QTL, the inner interval (e.g. 1-LOD) is shown as a rectangle and the outer interval (2-LOD) is shown as line

FLA QTLs, respectively. One of RWC M-QTLs on LG 7A was co-located with AI QTL in 7A-aca/cag-10-7A-cfa2123 marker interval.

Conclusion

According to results presented in this study, many QTLs and linked markers were found for different traits in normal and AI stress trials. In summary, we identified 25 stable QTLs for various traits which can be employed for QTL pyramiding relative to the genetic improvement of AI tolerance in wheat. This study detected major or stable AI-tolerance QTLs on LGs 1A, 1D-a, 2A-a, 2A-d, 2B, 2D, 3A-b, 3B, 4A, 4D, 6A-a, 6B, and 7A controlling different traits. The closely linked molecular markers in these loci enjoy a significant potential value in marker-assisted selection to improve grain yield and different traits in wheat when MAS breeding is employed following validation. The findings from this study revealed that the epistatic effects contributed to values of CMS, WSC, AI, and GYLD traits; however, E-QTLs were less influential than M-QTLs. Likewise, we observed that LGs 1D-a, 4A, 5A, and 7A via CIM method and LGs 2D, 3B, 4A, and 7A via MCIM method were more involved than other chromosomes in controlling traits, which can be considered as

segments. Co-located QTL bars and related linked markers are shown in same color in each chromosome

main chromosomes for different traits of QTLs under AI stress. In both methods, D genome carried the minimum number of QTLs, which might be due to relatively few markers located on this genome. Several QTL cluster regions, including 1D-a, 3B, 4A, 4B, and 5A by CIM method and 2A-a, 2D, 3B, 6B, and 7A by MCIM method were indicative of strong linkage or pleiotropy in the inheritance of examined traits. Fine-mapping or cloning major stable QTL and QTL regions with pleiotropic effects would advance our conception of the underlying molecular mechanisms involved in aluminum tolerance in wheat. According to this study, common genomic regions found for different variations of traits implied that a group of linked and (or) co-located QTLs affected physiological, morphological, and grain yield-related traits. We have also identified a number of QTLs which highly interacted with the environments. These QTLs could provide essential insights into the agents crucial for the adaptation of bread wheat to specific environmental conditions. The results of this study indicated that the SeriM82/Babax population combines useful alleles, from both parental lines, and could be a perfect resource in researches geared towards the development of wheat genotypes and improved grain yield for AI stress environments. Most of the QTL regions detected in SB population have not been reported previously and was novel in wheat. However, further

evaluations in other genetic backgrounds and environments are required to confirm and validate these QTLs.

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Authors contributions BAF, ST, NMN and AM designed the research and edited the manuscript. Thanks to ST for the help in statistical analysis. SF performed the experiments, analyzed the data, and wrote the primary draft of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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