Multi-environment QTL analysis using an updated genetic map of a widely distributed Seri × Babax spring wheat population

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Abbreviations:

QTL Quantitative trait locus

RIL Recombinant inbred line

SNP Single nucleotide polymorphism

PVE Phenotypic variation explained by QTL

Abstract

Seri/Babax spring wheat linkage mapping population was developed to minimize the confounding effect of phenology in the genetic dissection of abiotic stress traits. An existing linkage map (< 500 markers) was updated with 6,470 polymorphic Illumina iSelect 90K array and DArTseq SNPs to a genetic map of 5576.5 cM with 1748 non-redundant markers (1165 90K SNPs, 207 DArTseq SNPs, 183 AFLP, 111 DArT array, and 82 SSR) assigned to 31 linkage groups. We conducted QTL mapping for yield and related traits phenotyped in seven major wheat growing areas from Egypt, Sudan, Iran and India, and nine environments (heat, drought, heat plus drought, and yield potential) in Obregon, Mexico. The current study confirmed QTLs from previous studies and identified novel QTLs. QTL analysis identified 39 (LOD: 2.5-23.6; PVE: 4.8-21.3%), 36 (LOD: 2.5-15.4; PVE: 2.9-21.4%), 30 (LOD: 2.5-13.1; PVE: 3.6-26.8%), 39 (LOD: 2.7-14.4; PVE: 2.6-15.9%), and 22 (LOD: 2.8-4.8; PVE: 6.8-12.9%) QTLs for grain yield, thousand-grain weight, grain number, days to heading, and plant height, respectively. QTL analysis based on high-yielding and low-yielding environment clusters identified 11 additional QTLs (LOD: 2.6-14.9; PVE: 2.7-19.7%). The updated map thereby provides a better genome coverage (3.5-fold) especially in D genome (4-fold), higher density (1.1-fold) and a good collinearity with the IWGSC RefSeq v1.0 genome, thus increased the number of detected QTLs (5-fold) compared with the earlier map. This map provides a useful genomic resource for further genetic analyses of important traits in this wheat population that was widely distributed around the world.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the staple food crops worldwide, contributing ~ 28% of the global grain production and 20% of the calories and protein consumed by human (FAOstat 2018). With a predicted world population of nine billion by 2050, the demand for wheat is expected to increase (Rosegrant and Cline 2003). As the most widely cultivated crop geographically, wheat covers a large variance of climate and soil conditions. Abiotic stresses such as drought, temperature, salinity, and nutrient imbalances reduce wheat yield in many environments (Trethowan and Mujeeb-Kazi 2008). Improvement of grain yield in these target environments is the primary target of wheat research considering climate change (Reynolds et al., 2009).

Grain yield (GY) is a complex quantitative trait that is strongly influenced by interacting genetic and environmental factors (Quarrie et al. 2006). GY can be dissected into direct components: thousand-grain weight (TGW) and grain number (GN) per unit area of land. Both TGW and especially GN are quantitatively inherited and not easy to improve simultaneously through the conventional approaches due to the tradeoff between them (Yano and Sasaki 1997; Griffiths et al. 2015). Quantitative trait loci (QTL) mapping in bi-parental populations has provided an effective approach to dissect quantitative traits into component loci to study their relative effects on a specific trait of interest (Doerge 2002). A large number of QTLs for yield and yield components have been identified to date on almost all wheat chromosomes (Bennett et al. 2012; Golabadi et al. 2011; Kirigwi et al. 2007; Liu et al. 2019b; Maccaferri et al. 2008; Pinto et al. 2010; Tahmasebi et al. 2016). However, the identification of QTL for yield may be confounded by flowering time and plant height (PH), whereby genes of major effects have been shown to mask the identification of minor effects, especially under abiotic stress where the onset of stress treatments will occur at different growth stages depending on the genotypes phenological pattern (Reynolds et al. 2009). Therefore, effects of phenology and PH may be restricted in the population to identify QTLs for major traits without the confounding effect of phenology (Lopes et al. 2013; Pinto et al. 2010; Reynolds and Tuberosa 2008).

The narrow range of PH and flowering time of the Seri/Babax recombinant inbred line (RIL) population makes it ideal for genetic and physiological studies. Many QTLs have been identified for agronomic and physiological traits in the Seri/Babax population under water limiting and heat stress conditions over the past decade (Lopes et al. 2013; Mathews et al. 2008; McIntyre et al. 2010; Pinto et al. 2016; Pinto et al. 2010; Tahmasebi et al. 2016). Seri/Babax population is still widely used by researchers around the world for genetic and physiological studies of different traits in various environments and some of the lines have been valuable in breeding. Genetic linkage map is

the foundation for mapping QTLs and subsequent marker-assisted selection or map-based cloning. The existing genetic map of Seri/Babax was primarily based on 475 markers comprising SSR (Simple Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism), and DArT (Diversity Arrays Technology) markers (Lopes et al. 2013). With the rapid development of new genotyping technologies, numerous molecular makers for wheat are available now. Therefore, a new linkage map is necessary for researchers to compare their results with peers. By genotyping the Seri/Babax population with the wheat 90K Illumina iSelect array and DArTseq platform, the objectives of the study was 1) to update the genetic linkage map of the Seri/Babax population by integrating 90K and DArTseq markers to the existing linkage map; 2) to identify QTLs for yield and yield-related traits with phenotypic data from multiple environments, combining studies not used earlier and from earlier published data; and 3) to compare the properties of the updated genetic map with the existing linkage map.

MATERIALS AND METHODS

Wheat population and phenotyping

The Seri/Babax population comprising 156 RILs was derived from a reciprocal cross between two elite spring bread wheat, Seri M82 (a released line from the "Veery" cross, KVZ/BUHO// KAL/BB) and Babax (a line derived from the cross "Babax", BOW/NAC//VEE/3/BJY/COC) (Olivares-Villegas et al. 2007). Seri M82 carries the 1BL.1RS translocation and was characterized by moderate tolerance to drought conditions and high yield potential. Babax is without the 1BL.1RS translocation, and is highly tolerant to severe drought (Mathews et al. 2008). Seri M82 and Babax both have the same photoperiod-insensitive allele at *Ppd-D1*, and spring-type alleles for vernalization (*Vrn-B1* and *Vrn-D1*) loci (Pinto et al. 2010). Hence, this population is characterized by its narrow range of plant height and flowering time (ca. 17 cm, 10-15 days across environments as reported by Lopes et al. (2013)), and is ideal for genetic mapping and physiological studies. The phenotyping was conducted in eight major wheat growing regions from five countries around the world (**Fig. 1a**), including ten environments (Darab in Iran, Sohag in Egypt, Dongola and Wad Medani in Sudan, Ludhiana and Karnal in India, and yield potential, drought stress, heat stress, and heat plus drought stress in Obregon, Mexico in 2008-2009) reported in an earlier study (Lopes et al. 2013), and six new environments (one from Shandaweel, Egypt in 2008-2009, and five from different stresses in Obregon, Mexico in 2004-2005 and 2005-2006). The sowing and harvest date, temperature, precipitation, total amount of water applied by irrigation, relative humidity, and evapotranspiration during the growing season were reported in an earlier study (Lopes et al.

2013). In Mexico, the experiments were conducted at the Campo Experimental Norman E. Borlaug (CENEB), Cd. Obregon, where the environments were designated as yield potential, drought stress, heat stress, and heat plus drought stress. Yield potential (YP) was applied by normal sowing (late November) with optimum irrigation (total water supply > 700 mm); drought stress (D) was applied by normal planting (late November) with significantly reduced irrigation (total water supply < 300 mm); heat stress (H) was applied by late sowing (late February) with supplementary irrigation (total water supply > 700 mm) to avoid the effect of drought; and the combined heat plus drought stress (HD) was applied by delayed planting date (late February) with reduced irrigation (total water supply < 400 mm). By late sowing, the average maximum temperature during wheat growing cycle was above 32°C, almost 8°C higher than that in normal sowing cycles (Liu et al. 2019b; Pinto et al. 2010), a typical method for applying heat stress in Obregon that has been demonstrated to be successful in generating germplasm for heat stress environments such as ME5 (Reynolds et al. 1994). The experimental design was randomized lattice with two replications. Seeds were sown on raised beds of 2 m length with 2 rows (25 cm between rows) and 80 cm between the beds, with seed rates of 120 kg/ha. Five agronomic traits were measured: grain yield (GY; t ha^{-1}), thousand-grain weight (TGW, g), grain number (GN; estimated as: GY (t ha^{-1})/ TGW (g) × 100 × 1000), days to heading (DTH, i.e. the number of days from emergence when 50% of the spike were emerged), and plant height (PH, cm from the soil surface to the tip of the spike without awns).

Phenotypic data analysis

The adjusted means for each genotype in individual environment were calculated using the MIXED procedure in SAS as described by Lopes et al. (2013). Cluster of environments based on yield performance was generated using the "cluster" package in R (Maechler 2019).

For the individual environment, the broad sense heritability (H^2) was estimated as:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2/r}$$

where σ_g^2 and σ_e^2 were genotype and error variance, and r was the number of replications.

For the combined analysis of high-yielding and low-yielding clusters, the H^2 was estimated as:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{ge}^2/l + \sigma_e^2/rl}$$

where σ_{ge}^2 was the genotype by environment interaction variance, and l was the number of environment.

100

101 **Genotyping, map construction and QTL mapping**

102 A genetic map with 475 markers (120 SSR, 211 AFLP, and 144 DArT) distributed over 29 linkage groups (LGs) had
103 been previously constructed for this population (Lopes et al. 2013). Recently, the Seri/Babax RIL population was
104 genotyped with the wheat 90K Illumina iSelect array and DArTseq platform (data available at
105 https://data.cimmyt.org/dataset.xhtml?persistentId=hdl:11529/10548196). After quality control (removing markers
106 with minor allele frequency < 5%, missing data > 15%, and segregation distortion $p < 0.05$), an additional 5386- 90K
107 SNP and 609 DArTseq SNP -markers were added to construct the linkage map. A new genetic map was constructed
108 using QTL IciMapping v4.1 (Meng et al. 2015), which combined the map with the previous 475 markers and the new
109 SNP markers. The 90K and DArTseq markers were assigned to chromosomes based on the IWGSC RefSeq v1.0
110 genome. Within each chromosome, genetic distance was determined using the Kosambi mapping function (Kosambi
111 2016). Chromosomes were split into LGs and reordered when the gap between two adjacent markers were > 50 cM.

112 QTL mapping was performed using QTL IciMapping v4.1 software (Meng et al. 2015). Inclusive composite
113 interval mapping of additive (ICIM-ADD) function was selected as mapping method to detect additive QTL. The
114 LOD threshold for QTL detection was determined by using 1000 permutations at $P < 0.05$. Putative QTLs were
115 declared using a LOD of 2.5. QTLs detected across environments were considered the same if the distance between
116 the peak positions was less than 10 cM. Stable QTLs were declared when QTLs were identified in at least two
117 environments.

118

119 **RESULTS**

120 **Phenotypic variations across environments**

121 The phenotypic traits of the Seri/Babax RIL population showed a wide range of variation across environments
122 (**Supplementary Table 1**). The highest mean GY was found in Sohag (7.5 t ha^{-1}) and Shandaweel (7.5 t ha^{-1}, H^2 =
123 0.45) in Egypt, while the lowest mean GY was found in Obregon under heat stress in 2006 (1.1 t ha^{-1}, $H^2 = 0.79$; **Table**
124 **1**). Cluster analysis of the 16 environments for GY generated two main groups: the high-yielding group (> 5.5 t ha^{-1},
125 $H^2 = 0.35$) comprising Sohag and Shandaweel in Egypt, Ludhiana in India, Dongola in Sudan, and two yield potential
126 environments in Obregon, Mexico. The low-yielding group (< 4.5 t ha^{-1}, $H^2 = 0.37$) encompassed Karnal in India,
127 Darab in Iran, Wad Medani in Sudan, and all drought, heat, and heat plus drought environments in Obregon, Mexico

(**Fig. 1b**). The correlation analysis for GY was consistent with the results of cluster analysis, with Sohag and Shandaweel in Egypt showing the highest correlation. Most of the environments showed positive correlations with each other, while Wad Medani was negatively correlated with Obregon drought stress in 2004-2005 and 2005-2006 growing seasons (**Fig. 1c**).

Similar to GY, the highest average TGW (45.4 g, H^2 = 0.54) was observed in Sohag and Shandaweel in Egypt, while the lowest TGW (26.6 g, H^2 = 0.78) was found in Obregon heat plus drought stress environment (**Table 1**). The highest GN (18558, H^2 = 0.88) was found in Ludhiana, India with a large range, and the lowest GN (3405, H^2 = 0.48) was observed in Obregon drought stress in 2005-2006. The phenology range was 6 to 18 days across environments. In Darab, Iran, the Seri/Babax population had the longest time-112 days-for days to heading; while in Obregon heat plus drought environment, the mean days to heading was 53 days. The Seri/Babax population had the highest PH in Sohag and Shandaweel (107.5 cm), and lowest mean PH (71 cm) for the population in Obregon heat plus drought condition. The largest and smallest range for PH was observed in Wad Medani (a range of 40 cm) and Obregon heat stress in 2009 (a range of 15.5 cm), respectively.

Trait associations across environments

Across the 16 environments, GY was significantly positively correlated with TGW (*r* range: 0.29-0.77), GN (0.48-0.94), DTH (0.32-0.69), and PH (0.24-0.73) in most of the environments with a few exceptions: GY was weakly negatively correlated with TGW under Obregon heat stress (2005, 2006; *r* = -0.03), with GN under Obregon drought stress (2005-2006; *r* = -0.03), and with DTH in Shandaweel of Egypt (*r* = -0.13; **Supplementary Fig. 1**). TGW and GN was positively correlated with each other in 10 environments (*r* range: 0.19-0.55) except in Shandaweel of Egypt (*r* = -0.63), Obregon yield potential (2008-2009; *r* = -0.62), drought (2005-2006; *r* = -0.36), and heat (2005, 2006; *r* ranges from -0.32 to -0.46) conditions, where negative correlations were observed. TGW was positively correlated with DTH (*r* range: 0.87-0.96) except in Shandaweel of Egypt (*r* = -0.33). TGW was positively correlated with PH (*r* range: 0.90-0.97) except in Darab, Iran. GN was positively correlated with DTH (*r* range: 0.14-0.61) and PH (*r* range: 0.90-0.97) in all environments.

Genetic linkage map construction

We used 6470 markers comprising SSR (120), AFLP (211), DArT (144), 90K SNP (5386), and DArTseq SNP (609) markers to construct the genetic map. Discarding redundant markers at the same loci, the final genetic map was 5576.5 cM with 1748 markers (82 SSR, 183 AFLP, 111 DArT, 1165 90K SNPs, and 207 DArTseq SNPs) arranged to 31 LGs (**Fig. 2, Supplementary Table 2**). The total length of the updated map increased 3.5 folds than that of the previous one Lopes et al. (2013). The A, B and D genomes harbored 768 (44%), 648 (37%) and 332 (19%) markers with a total length of 1959.5, 1961.9, and 1655.1 cM, respectively. The current map provided a better coverage of all the 21 chromosomes than the previous one, in which only 11% of markers were distributed on D genome and no markers were assigned to 3D. On the updated map, the longest LG was 4A (352.5 cM), followed by 6A (351.6 cM) and 1D (350.6 cM). The shortest LG was 7Dc (4.7 cM), followed by 3Ab (7.2 cM). Marker density was lowest in 3D (16.2 cM per marker), followed by 1Ba (14.1 cM per marker), and highest in 7Dc (0.9 cM per marker), followed by 1A (1.6 cM per marker, **Table 2**). We obtained the physical position of 1059 mapped markers on the linkage map and projected our linkage map to the IWGSC RefSeq v1.0 genome. Results showed there was a good collinearity between the updated genetic map and the physical map (**Fig. 3**).

Of the 1748 mapped markers, significant segregation distortion (CHITEST, $p < 0.001$) was found in 68 (4%) markers including 28 DArT, 25 AFLP, 9 SSR, and 6 DArTseq markers. Fifty-eight and ten markers showed distortion with alleles biased towards parent Babax and Seri, respectively. The markers that showed segregation distortion in favor of the Babax parent were mainly distributed on 1Bb (35.6-104.9 cM, 43 markers), while those in favor of the Seri parent were mainly assigned to 3Aa (142-153 cM, 8 markers).

Clusters of markers from the same marker type on the map were frequently observed (Fig. 2). Of the 1748 mapped markers, 1270 markers (73%) were adjoined with markers from the same type. Marker intervals with a distance < 0.5 cM between two adjacent markers from the same type were observed in 326, 26, 6, 4, and 1 intervals for 90K, DArTseq, AFLP, DArT, and SSR markers, accounting for 34%, 29%, 8%, 15%, and 7% of their total intervals (**Supplementary Fig. 2**). The frequency of clustering was primarily related to the number of markers from different types.

We also built a linkage map only using the 90K SNPs to a map length of 5333 cM with 1222 non-redundant 90K markers. While there were many large gaps on the map with 90K SNPs (**Supplementary Table 3**), indicating the necessity to include different types of markers. As was shown in **Fig. 2**, DArTseq, DArT, SSR, AFLP markers could cover most of the large gaps.

QTLs identified in individual environments

A total of 166 QTLs with LOD scores from 2.5 to 23.7 were identified for GY, TGW, GN, DTH, and PH in the 16 environments with the updated genetic map (**Supplementary Table 4**). The number of QTLs was higher than the earlier map where 31 QTL were detected for the same traits (**Table 2**). The largest number of QTLs was identified on chromosome 4A (23), followed by 7Da (22). No QTL was identified on 1Ba, 2Da, 3Ab, 3Ba, 5Db, 7Bb, 7Db, and 7Dc (**Supplementary Fig. 3**).

QTLs for GY were identified on chromosome 1A, 1Bb, 1D, 2Db, 3Aa, 3Bb, 4A, 4B, 5A, 5Da, 6A, 6B, 6Da, 7A, and 7Da (LOD: 2.6-23.6; PVE: 4.8-21.3%; **Supplementary Table 4, Supplementary Fig. 3**). Seven stable QTLs on chromosomes 3Aa (128-134 cM), 3Bb (228-232 cM), 4A (268 cM, 280-286 cM, 304-308 cM), 6B (206-208 cM), and 7Da (124 cM) were detected in at least two environments (**Table 3**). The stable QTL on chromosome 4A (304-308 cM, **Fig. 4**) was detected across the yield potential (2005-2006, 2008-2009), drought (2008-2009), and heat plus drought (2009) conditions in Obregon, Mexico. QTLs for TGW were identified on chromosome 1A, 1D, 2B, 3Bb, 4A, 4B, 5A, 5B, 5Da, 6A, 6B, 7A, 7Ba, and 7Da (LOD: 3-15.4; PVE: 3-21.4%). Six stable QTLs on chromosomes 2B (44-46 cM), 3Bb (44-48 cM), 6A (218 cM), 7Ba (26-32 cM), and 7Da (108-116 cM, 124 cM) were identified (**Table 3**). The QTL on chromosome 7Da (108-116 cM) was detected in six environments across Karnal in India, Darab in Iran, and Obregon yield potential (2005-2006, 2008-2009) and drought (2005-2006, 2008-2009) conditions.

QTLs for GN were identified on chromosome 1A, 1Bb, 1D, 2A, 2B, 3Aa, 3Bb, 4A, 4B, 4Db, 5A, 6B, 6Db, 7A, and 7Da (LOD: 2.5-13.1; PVE: 3.6-26.9%). Three stable QTLs were identified on chromosomes 1Bb (72-80 cM), 4A (308 cM, **Fig. 4**), and 6B (214 cM) in Obregon, Mexico (**Table 3**), of which the QTL on 6B (214 cM) was a heat adaptive QTL. QTLs for DTH were located on chromosome 1Bb, 1D, 2A, 2B, 3D, 4B, 4Da, 5A, 5Da, 6B, 6Db, 7A, 7Ba, and 7Da (LOD: 2.7-14.4; PVE: 2.6-15.9%). Eight stable QTLs were identified on chromosome 2A (158-160 cM), 4B (70 cM, 186 cM), 5Da (38 cM), 6B (206 cM), 7Ba (20-22 cM), and 7Da (110-112 cM, 120-124 cM) across environments. QTLs for PH were identified on chromosome 1D, 2B, 3Aa, 4A, 4B, 4Db, 5A, 6A, 7Ba, and 7Da (LOD: 2.8-4.8; PVE: 6.9-12.9%). Four stable QTLs on chromosomes 2B (84 cM), 3Aa (166 cM), 4A (268-272 cM), and 4B (192-194 cM) were identified across environments (**Table 3**).

QTLs identified for high-yielding and low-yielding clusters

A QTL analysis based on the high- and low-yielding clusters identified 32 QTLs (LOD range: 2.6-14.9, PVE: 2.7-19.7%) for GY, TGW, GN, DTH, and PH, of which 11 QTLs were new and absent in individual environments (**Supplementary Table 5**). Six QTLs on chromosomes 1A, 1Bb, 2B, 4A, and 4Db were specific for high-yielding cluster, 16 QTLs on chromosomes 1A, 1Bb, 4A, 4B, 4D, 6B, 7A, 7Ba, and 7Da were specific for low-yielding cluster, and five QTLs on chromosomes 1D, 3Aa, 7Ba, and 7Da were common between the two clusters (**Fig. 5**).

QTLs for GY were found on chromosomes 1Bb (28 cM, new) and 4A (26 cM) in high-yielding cluster; on 4A (4 cM, 282 cM) and 4B (186 cM) in low-yielding cluster; and the common QTL was located on 1D (26 cM, new). For TGW, the QTLs for low-yielding cluster were identified on chromosomes 1A (242 cM) and 7Ba (26 cM), and the common QTL was identified on 7Da (114-116 cM). For GN, QTLs were located on chromosomes 1A (86 cM, new) and 4A (2 cM, new) in high-yielding cluster, and on 1Bb (74 cM), 4A (158 cM, new; 268 cM), 4Db (90 cM), 6B (218 cM), and 7A (60 cM, new) in low-yielding cluster. For DTH, common QTLs were identified on 1D (30-32 cM) and 7Ba (20 cM); high-yielding cluster specific QTL was located on chromosome 4Db (102 cM, new); and low-yielding cluster specific QTLs were located on chromosomes 4B (70 cM), 6B (206 cM), 7A (264 cM), 7Ba (20 cM), and 7Da (114 cM). For PH, QTL in high and low yield cluster was identified on chromosomes 2B (64 cM, new) and 7Ba (130 cM), respectively. The common QTL for PH in both clusters was located on chromosome 3Aa (122 cM, new).

Pleiotropic QTL

Pleiotropic QTLs were observed in this study (**Fig. 6**). Among the 21 QTL hotspots for multiple traits, nine were independent from DTH and/or PH QTL. Co-location of QTL for GY, TGW, and GN was on chromosome 1D at 194-198 cM, where the favorable allele for GY and TGW was contributed by Babax, while for GN was from Seri. Co-locations of QTLs for GY and TGW were observed on chromosomes 3Bb (228-236 cM) where the favorable allele was contributed by Babax, and on chromosome 5A (26 cM) where the favorable allele was contributed by Seri. Co-location of QTLs for GY and GN were on chromosomes 1A (120 cM) where the favorable allele was from Seri, and on chromosome 1Bb (68-92 cM), 4A (56-62 cM, 280-286 cM, 304-308 cM), and 4B (32 cM) where favorable alleles were contributed by Babax.

DISCUSSION

238 The rapid advances in genotyping technologies exponentially increase the number of markers available for genetic
239 studies. Genetic maps are very useful tools in the identification of molecular markers closely linked to QTLs of
240 interest, isolation of genes via map-based cloning, comparative mapping, and genome organization studies (Varshney
241 et al. 2007). In the present study, with the addition of the 90K SNP and DArTseq SNP markers, the number of mapped
242 markers (1748) on the genetic map of Seri/Babax increased 3.5 folds compared with the previous map of 475 markers
243 (Lopes et al. 2013). The final size of the linkage map was 5576.5 cM, almost 3.5-fold the length of the previous one
244 (Lopes et al. 2013). The current map provides a better coverage of the 21 chromosomes of wheat than the previous
245 one where linkage group 3D was absent (Lopes et al. 2013; McIntyre et al. 2010). Around 19% of markers were
246 assigned to D genome, which was higher than the earlier D genome with 11% of markers. Though the updated map
247 showed a better genome coverage, gaps with a distance > 50 cM still remained on chromosome 1B, 2D, 3A, 3B, 4D,
248 5D, 6D, 7B, and 7D, where small LGs were generated. Gaps of distances > 20 cM were observed almost on all LGs
249 and need to be filled with more markers for the non-centromeric regions. Of the 1748 mapped markers, 11 markers
250 were assigned to 3D, confirming the difficulty in identifying polymorphic markers for 3D, perhaps due to the large
251 common regions of 3D between the Seri and Babax (McIntyre et al. 2010). In addition, the lower frequency of
252 polymorphic markers in D genome compared with AB genome in wheat makes it difficult to have a good coverage of
253 D genome (Allen et al. 2011; Chao et al. 2009). The final map comprised of markers from AFLP, SSR, DArT, 90K,
254 and DArTseq, and clustering of markers from the same marker platform on the map were frequently observed. The
255 frequency of marker clustering was primarily related to the number of markers from different types. For example,
256 clustering of 90K markers was more frequent than other types, which was not surprising since the number of 90K
257 markers were more abundant than others. The occurrence of clusters are very common when different kinds of markers
258 are integrated into a single linkage map (Peleg et al. 2008; Semagn et al. 2006). Overall, the updated genetic map
259 provided a higher density (1.1-fold) map with more markers (3.5-fold) and a good collinearity with the IWGSC RefSeq
260 v1.0 genome, representing a step forward in mapping analysis and also in comparing QTLs with other studies using
261 90K genetic map.

262 With the updated integrated genetic map, the number of identified QTLs was increased by five-fold compared
263 with the previous study (Lopes et al. 2013) and 25 QTLs were common between the two studies (**Supplementary**
264 **table 6**). Since the Seri/Babax population was widely used for QTL studies (Mathews et al. 2008; McIntyre et al.
265 2010; Pinto et al. 2016; Pinto et al. 2010; Tahmasebi et al. 2016), we managed to locate all the reported QTLs on the

updated map and found that the QTLs were clustered on several big chromosome regions (**Supplementary table 6**). For example, on chromosome 1Bb at 70-105 cM, there was a big cluster of QTLs for GY, TGW, GN, DTA, PH, NDVI, CT, SPAD, harvest index, rate of senescence, and percentage of greenness lost. On chromosome 2B (24-84 cM), QTLs for GY, TGW, GN, DTH, PH, CT, maturity, grains per spike, leaf rolling, and spikelet compactness were co-located. On chromosome 4A at 265-300 cM, QTLs for GY, GN, and PH in the present study were co-located with QTLs for CT, NDVI, grain-filling rate, and water-soluble carbohydrates. On chromosome 5A (69-139 cM), QTLs for GY, GN, DTH, PH, spikelet per spike, spikelet compactness, flowering time, maturity, and leaf rolling were clustered. On chromosome 6B at 182-216 cM, QTLs for GY, TGW, GN, DTH, grain-filling rate, spikelet per spike, spikelet compactness, grains per spike, and percentage of greenness lost at mid grain-filling stage were frequently identified. Pinto et al. (2010) reported six common QTLs for drought and heat stress on chromosome 1B-a, 2B-a, 3B-b, 4A-a, 4B-b, and 7A-a in the Seri/Babax population in Obregon, Mexico environments, while in the present study, the QTL on chromosome 1B-a can be detected in Obregon heat stress in 2006 (71-75 cM on 1Bb in the present study); the QTL on chromosome 2B-a was detected in Wad Medani, Dongola, Darab, and Obregon heat stress in 2009 (39-47 cM on 2B); the QTL on chromosome 3B-b was detected in Obregon yield potential (2005-2006), drought (2004-2005), and heat (2006) conditions (219-235 cM on 3Bb); the QTL on chromosome 4A-a was detected across Obregon heat stress (2006, 2009), Ludhiana, Shandaweel, and Sohag (265-269 cM on 4A); the QTL on chromosome 4B-b was identified in Obregon heat plus drought stress (2009), Darab, Dongola, and Ludhiana (189-195 cM on 4B), the QTL on 7A-a was detected in Wad Medani and Karnal (265-267 cM on 7A). These results suggested that the experiments and breeding conducted in drought and heat stress environments in Obregon, Mexico could be used to predict the performance of the germplasm in other parts of the world. Reynolds et al. (1994) also reported that the typical heat stress in Obregon was demonstrated to be successful in generating germplasm for heat stressed environments such as ME5 comprising India and Sudan (Hodson and White 2007; Rajaram et al. 1993).

Typically, there is a trade-off between grain weight and grain number, making it difficult to improve yield through increasing grain weight and grain number at the same time (Griffiths et al. 2015; Sukumaran et al. 2018a; Sukumaran et al. 2018b). However, in the present study, TGW and GN were observed to be positively correlated, and both TGW and GN were positively correlated with GY in most environments. Several QTLs co-located for GY and GN were found on chromosome 1A (120 cM), 1Bb (68-92 cM), 4A (56-62, 280-286, 304-308 cM), and 4B (32 cM), where QTLs for GY and GN shared the favorable allele from the same parent. QTLs co-located for GY and TGW

were on chromosome 3Bb (228-236 cM) and 5A (26 cM), where the same parent contributed the favorable allele for both GY and TGW. Among the QTLs co-located for TGW and GN, most of the favorable alleles for TGW and GN were contributed by different parents, while the QTL on chromosome 6B (194-200 cM) shared the favorable allele from the same parent for TGW and GN, indicating it is possible to improve yield through TGW QTL complemented by adding correlated GN QTL (Groos et al. 2003).

The stable heat adaptive QTL on chromosome 1D (84 cM) for GY could be projected to 484-493 Mb on the IWGSC RefSeq v1.0 genome, where genes were located for disease resistance, heat shock family protein, and early flowering. The QTL on chromosome 2Db (110 cM) for GY was previously identified for photochemical reflectance index (PRI) in a CIMMYT spring wheat population (WAMI) under yield potential condition (Liu et al. 2019a). The QTL on chromosome 3Bb (228-236 cM) for GY and TGW was very close to a locus associated with multiple traits (GY, maturity, and chlorophyll content at vegetative state) in the temperate irrigated environments in spring wheat (Sukumaran et al. 2015). The QTL on chromosome 4A (308 cM) for GY and GN could be mapped to 531-535 Mb in physical map, which contains 34 genes including heat-shock protein genes. The TGW QTL on chromosome 7Ba (26-32 cM) was located at 3.7-5.9 Mb on the physical map, which harbored 33 genes. The GN QTL on chromosome 1A (86 cM) was associated with ground cover in winter wheat (Gao et al. 2016). The GN QTL on chromosome 6Db (8 cM) was co-located with a QTL for DTH in a synthetic derived RIL population (Liu et al. 2019b).

The Seri/Babax population was initially developed in Obregon, Mexico, so the lowest range of DTH (8 days) and PH (15-23 cm) was observed in the Obregon environments in this study. When grown in international trials, longer DTH and higher PH with a wider range were observed in most environments. For DTH, the QTL on chromosome 4Da was previously reported for maturity in spring wheat (Sukumaran et al. 2015). The QTL on chromosome 5D (38 cM) was located at a distance (about 60 Mb) from the *Vrn-D4* locus (Yoshida et al. 2010). The stable QTL for DTH on chromosome 7Ba (20-22 cM) was co-located with a previously reported QTL for kernel number per spike (Gao et al. 2015). Projecting this QTL to the physical map, it was located at the *Vrn-B3* locus (Yan et al. 2006), which could accelerate flowering and bypass the vernalization requirement of its dominant allele if present. This QTL was contributed by the parent Seri, and has never been reported before by other studies using Seri/Babax. The other QTLs for DTH are most likely to be associated with earliness *per se* genes that act independently of environmental signals, and are usually responsible for fine-tuning flowering time (Flood and Halloran 1984). For PH, the locus on chromosome 4B (192-194 cM) was associated with GY in a CIMMYT spring wheat panel (Sukumaran et al. 2013).

This locus can be projected to 3.5-4.7 Mb on the physical map, which was very close to the *Rht-B1* gene (Pearce et al. 2011). The PH QTL on chromosome 5A (72 cM) was also associated with *Cephalosporium* stripe resistance in wheat (Quincke et al. 2011).

This study updated the genetic map of the widely used Seri/Babax RIL population with the 90K and DArTseq SNPs. The updated genetic map has a good genome coverage, higher marker density, and a good collinearity with the physical map, and increased the number of QTLs compared with the earlier studies. A larger number of QTLs were identified with this genetic map across various environments, and some of them were stable in different environments. QTLs that are unique to the current study, verify the importance of an updated genetic map for QTL detection. This updated genetic map is expected to serve as a platform for further genetic analyses of important quantitative traits, map-based cloning, and marker-assisted selection studies using Seri/Babax RIL population.

AUTHOR CONTRIBUTIONS

SS designed the current study, ML and MR provided the phenotypic data, CS contributed with the explanation of DArTseq data, SD and MR genotyped the population, CL, SM and SS created the genetic maps and analyzed the data, CL and SS wrote the manuscript. All authors reviewed and accepted the contents.

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CONFLICT Of INTEREST

The authors declare no conflict of interest.

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465

Tables and figures

Table 1. Adjusted means and range of grain yield (GY, t ha^{-1}), thousand grain weight (TGW, g), grain number (GN), days to heading (DTH, d), and plant height (PH, cm) collected on the Seri/Babax RIL population comprising 156 entries in 16 environments from five countries (Egypt, India, Iran, Mexico, and Sudan) during 2004-2005, 2005-2006, and 2008-2009 growing seasons.

Abbreviations	Country & Location	Year	Mean (Range)				
			GY (t ha^{-1})	TGW (g)	GN	DTH (d)	PH (cm)
EgySha09	Egypt Shandaweel	2008-09	7.5 (6.6-8.3)	45.4 (42.9-48.4)	16808 (15026-18674)	94 (87-102)	108 (97-119)
EgySoh09	Egypt Sohag	2008-09	7.5 (4.9-9.7)	45.4 (36.1-54.8)	16697 (10754-23453)	94 (87-103)	108 (94-122)
IndKar09	India Karnal	2008-09	4.1 (2.3-5.9)	35.1 (26-47)	11697 (6570-17688)	93 (86-102)	100 (88-109)
IndLud09	India Ludhiana	2008-09	6.6 (2.4-10.4)	35.8 (29.7-44.3)	18558 (5945-29194)	91 (85-103)	99 (78-111)
IraDar09	Iran Darab	2008-09	4.5 (2.5-5.9)	34.6 (15-79.8)	13229 (6551-29540)	112 (109-114)	-
MexObrD05	Mexico Obregon_Drought	2004-05	3.6 (2.8-4.2)	35.2 (29.8-42.8)	10268 (9018-11363)	-	-
MexObrD06	Mexico Obregon_Drought	2005-06	3.2 (2.3-4.0)	34 (24.8-43.7)	3405 (2463-4556)	-	-
MexObrD09	Mexico Obregon_Drought	2008-09	3.8 (2.4-5.1)	36.8 (31.7-44.1)	10457 (5820-14396)	72 (67-75)	96 (87-103)
MexObrH05	Mexico Obregon_Heat	2005	1.8 (1.0-2.8)	33.3 (28.1-38)	5530 (2872-9027)	-	-
MexObrH06	Mexico Obregon_Heat	2006	1.2 (0.9-1.5)	28.9 (23.9-35.2)	11220 (7969-14200)	-	-
MexObrH09	Mexico Obregon_Heat	2009	3.5 (1.8-4.4)	29.2 (24.2-41.6)	12019 (6712-15035)	53 (49-57)	78 (71-86)
MexObrHD09	Mexico Obregon_Heat + Drought	2009	2.7 (1.5-3.6)	26.6 (20.2-34.6)	10061 (6024-13990)	53 (48-57)	71 (63-82)
MexObrYP06	Mexico Obregon_Yield potential	2005-06	5.5 (4.7-6.0)	43.3 (36-50.6)	12778 (10855-14976)	-	-
MexObrYP09	Mexico Obregon_Yield potential	2008-09	6.6 (4.6-8.5)	41 (35.2-49.6)	16025 (10899-20819)	78 (73-81)	102 (91-114)
SudDon09	Sudan Dongola	2008-09	6.7 (4.6-8.8)	42.7 (36.5-52.1)	15703 (10387-21292)	64 (56-73)	88 (77-103)
SudWadM09	Sudan Wad Medani	2008-09	2.7 (1.6-3.9)	36 (25.6-45)	7667 (4098-11894)	55 (48-67)	76 (46-86)

471 **Table 2.** Genetic length, number of markers, average marker interval between two adjacent markers, and number of
472 QTLs identified for (GY, t ha^{-1}), thousand grain weight (TGW, g), grain number (GN), days to heading (DTH, d), and
473 plant height (PH, cm) on the 31 linkage groups of the Seri/Babax RIL population linkage map in the present study
474 compared with the previous genetic map (Lopes et al. 2013).

The updated map						The previous map					
LG	Chr/ Arm	Length (cM)	Marker number	Interval (cM)	QTL number	LG	Chr/ Arm	Length (cM)	Marker number	Interval (cM)	QTL number
1	1A	269.3	172	1.6	6	1	1A	119.9	21	5.7	1
2	1Ba	168.7	12	14.1		2	1B	85.0	45	1.9	
3	1Bb	249.8	59	4.2	6						
4	1D	350.6	84	4.2	9	3	1Da	132.2	30	4.4	1
						4	1Db	9.1	2	4.5	
5	2A	171.8	44	3.9	3	5	2Aa	37.0	2	18.5	
						6	2Ab	29.7	5	5.9	
						7	2Ac	0.4	3	0.1	
						8	2Ad	29.8	5	6.0	
6	2B	260.7	141	1.9	11	9	2B	99.0	43	2.3	3
7	2Da	100.6	28	3.6		10	2D	74.6	14	5.3	1
8	2Db	124.6	31	4.0	1						
9	3Aa	211.2	46	4.6	5	11	3Aa	49.9	15	3.3	1
10	3Ab	7.2	4	1.8		12	3Ab	13.6	9	1.5	
11	3Ba	52.4	14	3.7		13	3B	142.4	37	3.8	
12	3Bb	314.6	94	3.4	8						
13	3D	178.7	11	16.2	1						
14	4A	352.5	125	2.8	23	14	4A	110.9	43	2.6	5
15	4B	201.4	91	2.2	16	15	4B	70.1	18	3.9	3
16	4Da	43.2	6	7.2	1	16	4D	12.3	4	3.1	1
17	4Db	132.0	20	6.6	2						
18	5A	295.9	129	2.3	7	17	5A	76.0	22	3.5	2
19	5B	171.8	80	2.2	1	18	5B	14.5	17	0.9	1
20	5Da	139.9	20	7.0	5	19	5Da	25.5	2	12.7	1
21	5Db	26.9	14	1.9		20	5Db	13.0	3	4.3	1
22	6A	351.6	109	3.2	5	21	6Aa	76.2	25	3.0	2
						22	6Ab	44.6	11	4.1	
23	6B	299.9	114	2.6	14	23	6B	90.3	38	2.4	2
24	6Da	157.6	42	3.8	1	24	6Da	41.4	4	10.4	2
25	6Db	110.2	20	5.5	2	25	6Db	12.3	4	3.1	
26	7A	300.1	139	2.2	5	26	7A	115.2	35	3.3	
27	7Ba	173.0	28	6.2	12						
28	7Bb	69.7	15	4.7		27	7B	15.6	6	2.6	
29	7Da	166.7	30	5.6	22	28	7Da	19.0	6	3.2	
30	7Db	119.4	21	5.7		29	7Db	52.6	6	8.8	4
31	7Dc	4.7	5	0.9							
Total		5576.5	1748	3.2	166			1612	475	3.4	31

475

476 **Table 3.** Stable QTLs (identified in more than two environments) for grain yield (GY, t ha^{-1}), thousand-grain weight (TGW, g), grain number (GN), days to heading

477 (DTH, d), and plant height (PH, cm) in the Seri/Babax RIL population comprising 156 entries. PVE: phenotypic variance explained by QTL.

Trait	LG	Chr	Peak position (cM)	Marker interval (cM)	Left marker	Right marker	LOD	PVE (%)	Favorable parent
GY	9	3Aa	130	127-137	*aca-cta-13*	*1089533*	3.1	5.4	Seri
	12	3Bb	230	219-235	*wPt-1804*	*gwm301e*	6.5	10.7	Babax
	14	4A	268	265-269	*wmc048d*	*act-cag-3*	10	16.6	Babax
	14	4A	284	273-293	*gUa44*	*1089414*	6.3	14.8	Babax
	14	4A	308	307-311	*wmc048c*	*Ra_c7973_1185*	23.7	21.3	Babax
	23	6B	206	203-209	*agc-cta-4*	*agg-ctg-8*	5.4	8.4	Seri
	29	7Da	124	123-125	*D_contig12156_209*	*wsnp_Ex_c4637_8299644*	6.2	10.5	Seri
TGW	6	2B	44	37-47	*wPt-7320*	*acc-ctc-2*	4.7	9	Babax
	12	3Bb	44	43-53	*barc147*	*barc087*	4.1	8.1	Babax
	22	6A	218	217-219	*wsnp_Ku_c26784_36747712*	*998518*	2.9	6.2	Babax
	27	7Ba	28	23-33	*wsnp_Ex_c11658_18773086*	*wsnp_JD_c1285_1848292*	3.5	9.6	Babax
	29	7Da	114	99-123	*Excalibur_c22419_460*	*1068196*	7.7	20.1	Seri
	29	7Da	124	123-125	*D_contig12156_209*	*wsnp_Ex_c4637_8299644*	7.1	11.5	Seri
GN	3	1Bb	80	71-83	*act-ctc-7*	*aac-ctg-4*	8	13.9	Babax
	14	4A	308	307-311	*1072050*	*Ra_c7973_1185*	5.8	15.3	Babax
	23	6B	214	209-225	*wPt-4924*	*barc0178*	4.1	6.9	Seri
DTH	5	2A	160	153-167	*RFL_Contig5277_480*	*cfd0050*	4.2	4.1	Babax
	15	4B	70	69-71	*Tdurum_contig47552_957*	*Excalibur_c65023_62*	2.7	4.9	Seri
	15	4B	186	183-187	*gwm006a*	*aac-ctc-9*	5.6	8.9	Seri
	20	5Da	38	27-43	*Kukri_c444_833*	*Excalibur_c11929_1019*	7.8	9.4	Babax
	23	6B	206	201-209	*agc-cta-4*	*aac-ctc-3*	7.2	6.8	Babax
	27	7Ba	20	17-25	*IACX198*	*wsnp_Ex_c11658_18773086*	14.1	15	Seri
	29	7Da	112	107-117	*Excalibur_c22419_460*	*1068196*	7.6	15.2	Babax
	29	7Da	124	117-125	*Excalibur_c22419_460*	*wsnp_Ex_c4637_8299644*	14.4	16	Babax
PH	6	2B	84	81-87	*aca-ctg-1*	*wPt-5680*	4.2	8.6	Babax
	9	3Aa	166	161-181	*1086318*	*RAC875_c21944_117*	3	7.6-7.8	Seri
	14	4A	268	265-277	*wmc048d*	*wmc048c*	3.4-4.8	8.1-13	Seri

	15	4B	192	191-195	*aag-cta-5*	*wmc048a*	3.2-4.2	7.7-8.8	Seri

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Figure 1. Phenotyping the Seri/Babax RIL population comprising 156 entries was conducted in (a) several environments, (b) clusters of high-yielding and low-yielding environments, and (c) and phenotypic correlations of grain yield between the environments.

Figure 2. Genetic map and marker distribution of the Seri/Babax RIL population on 31 linkage groups.

Figure 3. Updated linkage map of the Seri/Babax population (left) projected to the physical map of IWGSC RefSeq genome v1.0 (right) showing a good collinearity between the genetic map and the physical map.

Figure 4. A stable QTL on chromosome 4A detected for grain yield (GY, t ha^{-1}) and grain number (GN) under yield potential (YP), drought (D), and head plus drought (HD) environments in Obregon, Mexico during 2004-2005, 2005-2006, and 2008-2009 growing seasons.

Figure 5. Venn diagram illustrating the common and specific QTLs for high-yielding and low-yielding environment clusters of the Seri/Babax RIL population. Subscript numbers indicate the *centi morgan* (cM) position on the linkage map, followed by traits. GY: grain yield (t ha^{-1}); TGW: thousand-grain weight (g); GN: grain number; DTH: days to heading (d); PH: plant height (cm).

Figure 6. Venn diagram illustrating the common genomic regions for grain yield (GY, t ha^{-1}), thousand-grain weight (TGW, g), and grain number (GN), days to heading (DTH, d) and plant height (PH, cm). Subscript numbers indicate the *centi morgan* (cM) position on the linkage map. For the GY, TGW, and GN QTLs independent from DTH and/or PH, the red up arrows indicates the favorable allele contributed by the same parent, and the black down arrows indicates the favorable allele contributed by different parents.

Supplementary Figure 1. Phenotypic correlations among grain yield (GY, t ha^{-1}), thousand grain weight (TGW, g), grain number (GN), days to heading (DTH, d), and plant height (PH, cm) of the Seri/Babax RIL population grown in different environments.

Supplementary Figure 2. Distribution of markers between two consecutive loci along genetic distance over all chromosomes.

Supplementary Figure 3. An updated genetic map of Seri/Babax RIL population with QTLs identified for grain yield (GY, t ha^{-1}), thousand-grain weight (TGW, g), grain number (GN), days to heading (DTH, d), and plant height

(PH, cm) in different environments. Markers in red and green color are distorted markers in favor of Babax and Seri alleles, respectively.

Supplementary Tables

Supplementary Table 1. Phenotypic data including grain yield (GY, t ha^{-1}), thousand-grain weight (TGW, g), grain number (GN), days to heading (DTH, d), and plant height (PH, cm) of the Seri/Babax RIL population grown in 16 environments from five countries.

Supplementary Table 2. Linkage map information of the Seri/Babax population. Seri is coded as "0", Babax is coded as "2", and missing data is coded as "-1".

Supplementary Table 3. Linkage map information of the Seri/Babax population using 90K SNPs. Seri is coded as "0", Babax is coded as "2", and missing data is coded as "-1".

Supplementary Table 4. QTLs detected for grain yield (GY, t ha^{-1}), thousand-grain weight (TGW, g), grain number (GN), days to heading (DTH, d), and plant height (PH, cm) of the the Seri/Babax RIL population in 16 individual environments.

Supplementary Table 5. QTLs detected for grain yield (GY, t ha^{-1}), thousand-grain weight (TGW, g), grain number (GN), days to heading (DTH, d), and plant height (PH, cm) of the Seri/Babax RIL population in high-yielding and low-yielding clusters.

Supplementary Table 6. Comparison of QTLs detected in the present study with the previous studies using the Seri/Babax RIL population.