

Genome-Wide Association Study for Adaptation to Agronomic Plant Density: A Component of High Yield Potential in Spring Wheat

Sivakumar Sukumaran, Matthew P. Reynolds,* Marta S. Lopes, and José Crossa

ABSTRACT

Previous research has shown that progress in genetic yield potential is associated with adaptation to agronomic planting density, though its genetic basis has not been addressed before. In the current study, a wheat (*Triticum aestivum* L.) association mapping initiative (WAMI) panel of 287 elite lines was assessed for the effects of plant density on grain yield (YLD), 1000-kernel weight (TKW), and grain number (GNO) in yield plots consisting of four evenly spaced rows. The YLD and GNO of inner (high plant density) rows compared with outer rows (low plant density) indicated a consistent pattern: genotypes that performed best under intense competition (inner rows) responded less to reduced competition (outer rows) while being generally the best performers on aggregate (inner plus outer rows). However, TKW was not affected by plant density. To identify the genetic loci, an adaptation to density index (ADi) was computed as the scaled difference in trait values between inner and outer rows. Results on biplot analysis indicated that ADi was correlated with YLD in high-yielding environments, suggesting that it is a component of high yield potential. Genotyping of the WAMI panel was done through 90K Illumina Bead single nucleotide polymorphism (SNP) array. Association mapping employed using 18,104 SNP markers for ADi identified a major locus in chromosome 3B at 71 cM that explained 11.4% variation in ADi for YLD and GNO. Functional marker for ADi will enable identification of the trait in early generations—not otherwise possible in spaced plants typical of pedigree breeding approach—and to select parents for hybrid development.

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Abbreviations: ADi, adaptation to density index; $G \times E$, genotype \times environment interaction; $G \times R$, genotype \times row interaction; GNO, grain number; GWAS, genome-wide association study; HYP, high-yield-potential; LYP, low-yield-potential; PC, principal component; Q-Q, quantile-quantile; TKW, 1000-kernel weight; WAMI, a wheat association mapping initiative; YLD, grain yield.

INCREASING YIELD POTENTIAL per se still remains a major objective of crop improvement programs worldwide (Braun et al., 2010; Reynolds et al., 2012). A significant proportion of the yield potential of CIMMYT's semidwarf spring wheat lines can be explained by genetic variability for adaptation to agronomic planting density (Reynolds et al., 1994a). Earlier-released lower yielding lines showed a higher yield response to reduction in interplant competition—treatments that increased light penetration to the lower canopy from boot stage onward, as well as treatments that combined increased light penetration with decreased below-ground competition—than modern higher yielding varieties. The results indicated the sensitivity of low-yielding genotypes to plant density and the potential of some high-yielding genotypes to perform well both under high interplant competition and reduced interplant competition. In other words, high-yield-potential (HYP) genotypes respond less when interplant competition was reduced than the earlier-released low-yield-potential (LYP) lines.

The results were consistent with genetic adaptation to agronomic planting density, an idea first proposed by Donald (1968), who suggested a specific set of traits associated with a communal

Published in *Crop Sci.* 55:1–11 (2015).

doi: 10.2135/cropsci2015.03.0139

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ideotype, that is, short, strong stem, sparsely tillering plant with few small erect leaves. In theory, by competing minimally with its neighbors, the ideotype will make fewer demands for limited resources (light, water, and nutrients) compared with plant types that express a more competitive growth habit associated with redundant tillers, for example (early vigor and canopy establishment). Other studies have also shown yield benefit of reduced plant density at low levels of available soil N (Reynolds et al., 1994b). The effect of plant density (ranging from 19 to 338 plants m⁻²) on canopy formation, radiation use efficiency, partitioning, and dry matter production was studied by Whaley et al. (2000). Results indicated that at the early stage of plant growth the green leaf area was low at lower plant densities, but in the later stages of plant growth, plants at lower plant density had higher green leaf area per plant and higher radiation use efficiency. As a result, grain number per ear increased from 32 to 48 at low plant density (Whaley et al., 2000). However, in the present study we are interested in genetic adaptation to high plant density rather than the agronomic response to seed rate. The role of direct selection in improving adaptation to plant density was proved by a study of unimproved base population of Iowa Stiff Stalk Synthetic compared with advanced recurrent selection populations in maize (*Zea mays* L.). Selection improved adaptation to high plant density for grain yield and stalk lodging traits but with little effect on root lodging, test weight, and grain moisture (Brekke et al., 2011).

Genome-wide association study (GWAS) is a complementary approach to biparental mapping where we have the leverage of using a genetic mapping population with large number of lines for higher mapping resolution and wider application of results. Several association studies—candidate gene as well as GWAS—have been conducted on this WAMI population for the genetic dissection of simple as well as complex traits (Edae et al., 2013, 2014; Lopes et al., 2015; Sukumaran et al., 2015). Little is known about the genetic basis of adaptation of wheat plants to agronomic density, which is interesting considering that it is not something that would have been selected for in nature, interplant competition of single plants being of clear survival value. Our hypothesis was that adaptation to high plant density is a component of yield potential and therefore amenable to genetic dissection. Plants under high density tend to reduce the number of grains set, but the genotypes better adapted to the density stress show less reduction in the number of grains per spike. Therefore, the objectives of the present study were twofold: (i) to quantify the effects of plant density on YLD, TKW, and GNO and (ii) to identify genomic regions for adaptation to plant density in the WAMI panel through GWAS.

MATERIALS AND METHODS

Germplasm

The genetic material was the WAMI panel of 287 lines assembled at CIMMYT for dissecting the genetic basis of complex traits through association mapping. The WAMI panel has been previously described in detail (Edae et al., 2013; Lopes et al., 2015; Sukumaran et al., 2015); its main characteristics are a restricted range of phenology in a genetically diverse panel of advanced lines all of which have been released at one time or another by the International Wheat Improvement Network coordinated by CIMMYT (Braun et al., 2010).

Adaptation to Plant Density Trials

The WAMI panel was evaluated under optimal management conditions at Norman E. Borlaug Experiment Station at Ciudad Obregon in northwestern Mexico (27.20° N, 109.54° W, 38 m asl) during the 2009 to 2010, 2010 to 2011, and 2011 to 2012 growing seasons, hereafter referred to 2010, 2011, and 2012, respectively. During these 3 yr, experiments were conducted under the raised bed system with four rows per bed. The site is a temperate high-radiation environment with full irrigation matching evaporative demand. The interrow spacing within each bed was 10 cm and the spacing between the beds was 80 cm. Therefore, the inner-row treatment represented two rows 10 cm apart with border rows on both sides at the same distance, while outer rows were bordered by rows at a 10 and 50 cm distance, respectively. The two inner rows represent high plant density, whereas the two outer rows represent low plant density. The rows were 2 m long (Fig. 1). Grain yield and TKW were measured on the two inner and two outer rows, and GNO was estimated from YLD and TKW.

In addition, WAMI was also evaluated, and grain yield was recorded at 33 international environments including Mexico, Iran, Sudan, Egypt, Nepal, India, Pakistan, and Bangladesh under two rows per bed system in 2010, 2011, and 2012 (Lopes et al., 2012). We used this data to evaluate the hypothesis that ADi is a component of high yield potential in wheat. When the average yield of the lines in an environment was higher than 5.0 Mg ha⁻¹, it was considered as a high-yielding environment.

Phenotypic Data Analysis

For simplicity, data for yield and yield components are also reported per 2 by 2 m linear rows, and this was used for the estimation of the ADi. Analysis of variance (ANOVA) and estimates of repeatability was done using the PROC MIXED and PROC GLM procedures in SAS version 9.2 (SAS Institute, 2008). ANOVA was done by accounting the experimental design (i.e., α lattice) with environments, replicates within environments, incomplete blocks within replications, replications, genotypes, and genotype \times environmental interactions ($G \times E$) considered as random effects using the MIXED procedure in SAS 9.2. Adjusted means were calculated for each trait.

Broad-sense heritability (h^2) (repeatability here because this is a collection of different lines) estimates were calculated as follows:

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{G \times E}^2}{l} + \frac{\sigma_e^2}{rl}}$$

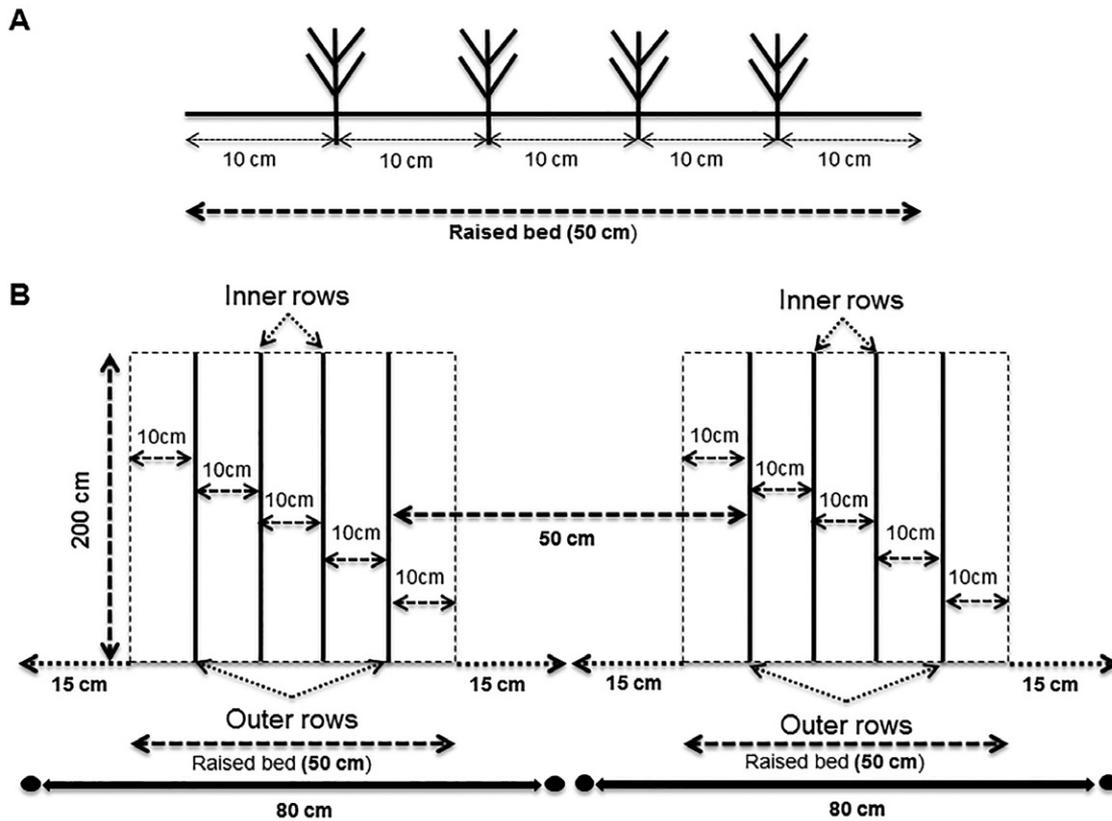


Figure 1. Diagram showing the planting of the experiment: (A) raised bed (50 cm) with four-row planting 10-cm spacing between the rows and (B) two raised beds with 30-cm spacing between the beds. Inner rows in each bed were under density stress and outer rows were at low plant density allowing more light penetration to the canopy.

where h^2 is the heritability estimates, σ_G^2 is the genetic variance, $\sigma_{G \times E}^2$ is the $G \times E$ variance, σ_e^2 is the residual variance, r is the number of replications, and l is the number of environments.

Genotype \times row ($G \times R$) interaction for each trait for each year and combined analysis were also estimated along with the heritability estimates. The $G \times R$ interaction was illustrated visually and apart from that it was also analyzed using PROC REG in SAS 9.2, where genotype, rows, and environment were the class variables. When each row was considered as a different environment, we had four environments in a year—one inner and one outer by two reps—and a total of 12 environments in 3 yr for the regression and factorial analyses. We also did factorial analysis using PROC GLM to estimate the significance of row position on YLD, TKW, and GNO.

Adaptation to Density Index

Grain yield and GNO were significantly affected by high density planting, but to rate cultivars for their adaptation to agronomic density, an ADi was estimated. The hypothesis that low-yielding plants tend to respond more to reduced competition was confirmed in an earlier study (Reynolds et al., 1994a). In the current study, the outer two rows experienced more light (and probably underground resources) on one side of the row—where the gap between rows was 50 cm and thus representing a low plant density environment—than the inner two rows that were at 10-cm spacing on both sides and a high plant density environment (Fig. 1). The outer rows, with low plant density, always yielded higher than the inner rows, so the difference in

yields between inner and outer rows was calculated as the ADi and scaled by multiplying with the grand mean of the trials and then divided by the trial specific mean.

We calculated ADi for YLD (ADi_{YLD}) as follows:

$$ADi_{YLD} = (YLD_O - YLD_I) \times \frac{\mu}{\mu l},$$

where YLD_O is the grain yield of outer rows, YLD_I is the grain yield of inner rows, μ is the grand mean of all 3 yr data, and μl is the mean of the specific trial. Similarly, the ADi for GNO (ADi_{GNO}) was also calculated. To test if ADi is a component of higher yield, correlation biplot was estimated by correlating the ADi with the yield of high- and low-yielding environments (Vargas Hernandez and Crossa, 2000).

Patterns of Genotype \times Environment Depicted in the Biplot

The biplot on the effects of the $G \times E$ plus the genotypic effect were obtained with the objective of examining the relationship of the ADi_{YLD} to the yield of different low- and high-yielding environments. Biplots use the principal component obtained from the singular value decomposition of the $G \times E$ matrix with the objective of describing the $G \times E$ patterns that will reflect the association (correlation) among environments and ADi measured at different years in Obregon.

Table 1. Mean, standard deviation (SD), and range of the traits (YLD, grain yield; GNO, grain number; TKW, 1000-kernel weight) studied in wheat association mapping initiative at Ciudad Obregon, Mexico, from 2009 and 2010 to 2011 and 2012 (3 yr) under four-row planting (I, inner two rows; O, outer two rows). Values for YLD and GNO are estimated per unit length (grams per meter) of the rows as well as in traditional unit (grams per square meters).

	YLD		YLD		GNO		GNO		TKW	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
	g m ⁻¹		g m ⁻²		m ⁻¹		m ⁻²		g	
I	104.03 ± 12.6	52.09–141.38	260.07 ± 31.5	130.24–353.46	2424 ± 358	1197–3312	6060 ± 896	2993–8280	43.48 ± 4.6	33.32–59.11
O	167.22 ± 16.7	117.76–227.10	418.06 ± 41.6	294.4–567.77	3923 ± 527	2558–5437	9808 ± 1318	6396–13,593	43.06 ± 4.6	33.19–56.70
I + O	271.25 ± 24.1	169.86–334.85	678.12 ± 60.3	424.66–837.13	6347 ± 796	3857–8644	15,868 ± 1990	9643–21,610	43.14 ± 4.5	33.30–57.50

Genotyping by 90K Illumina SNP Markers

DNA extraction of WAMI, genotyping, SNP calling are described in an earlier paper (Sukumaran et al., 2015). From 38,000 SNPs, 18,104 SNPs were resulted after screening for minor allele frequency (5%) and missing values cut off (10%). These SNPs had known position information (Wang et al., 2014).

Association Analysis

Population Structure, Kinship Matrix, Principal Component Analysis, and Trait Analysis

Population structure was assessed with 877 diversity array technology and 744 SNP markers in this panel using the STRUCTURE software (Pritchard and Rosenberg, 1999; Pritchard et al., 2000; Falush et al., 2003). A detailed analysis of the population structure, linkage disequilibrium, and minor allele frequency can be found in Lopes et al. (2015) and Sukumaran et al. (2015). We also looked at how the ADi varies across the subpopulations based on 1B.1R translocation in this WAMI panel.

Single Nucleotide Polymorphism Testing

Marker–trait association analysis was performed using mixed model (Yu et al., 2006; Zhang et al., 2010) and the simple generalized linear model (P3D) in TASSEL 5.0 (Bradbury et al., 2007; Zhang et al., 2010). Model testing for the best model to test marker–trait associations were done in SAS, comparing simple model, model with kinship matrix as random factor (K), population structure matrix (Q_{1-5}), $Q_{1-5} + K$, principal components (PC_{1-10}), and $PC_{1-10} + K$ according to previously described methods (Yu et al., 2006; Zhu et al., 2008; Sukumaran and Yu, 2014). Consistency of the results were also verified in R (R Development Core Team, 2011) using GAPIT codes (Lipka et al., 2012) by fitting kinship as the random term, PC10 as the population structure estimate with model selection. The quantile–quantile (Q–Q) plots showing the control of type I error in detecting marker–trait associations were also plotted to determine the best model. The threshold to call a marker–trait association significant was based on the Q–Q plot, where the observed distribution deviates from the expected distribution. We used the p -value threshold of 10^{-4} for the same set of markers in Sukumaran et al. (2015).

RESULTS

Phenotypic Analysis of Grain Yield, 1000-Kernel Weight, and Grain Number

Mean, standard deviation, and range of the 287 WAMI panel studies are shown in the Table 1. Phenotypic values were calculated as linear meter rows as well as per square meter. A comparison of inner and outer rows for YLD, TKW, and GNO showed that outer rows had higher YLD and GNO than inner rows. The TKW did not show any significant differences between the inner and outer rows. On average, inner rows yielded 37% less than outer rows, and the yield of inner rows ranged from 40 to 90% of the yield of outer rows. Inner rows had 37% less GNO, with a range of 41 to 88% of outer rows. For TKW, the percentage difference between inner and outer rows ranged from -9 to 5%, indicating that TKW was higher in some inner rows by 9%, and in some cases, outer rows had higher TKW by 5%. On average, the inner rows had an insignificantly 1% higher TKW than outer rows. To give a reference point for YLD and GNO in conventional units, values for inner plus outer ($I + O$) were calculated on an area basis as means averaged across years. The YLD per bed ($I + O$) had a range of 4.2 to 8.4 t ha⁻¹ with an average 6.8 t ha⁻¹.

Analysis of variance using PROC GLM and PROC MIXED indicated YLD, TKW, and GNO were significantly different among the 287 genotypes. Estimates of covariance parameters, and repeatability for $I + O$ rows showed that TKW (0.96) had the highest heritability, followed by GNO (0.83) and YLD (0.76). A separate analysis for $I + O$ rows showed variation in heritability estimates for $I + O$ rows for both YLD and GNO. Outer rows had higher repeatability values of 0.61 (YLD) and 0.74 (GNO), compared with inner rows 0.50 (YLD) and 0.62 (GNO). The TKW did not show differences in repeatability values between inner and outer rows (Table 2).

Interaction between Inner and Outer Rows

The $G \times R$ for YLD and GNO was highly significant and is visualized in Fig. 2. However, the interaction was not significant for TKW. Variation explained by rows was estimated by multiple regression analysis. Factorial analysis indicated that rows accounted for 49 and 46% variation in YLD and

Table 2. Variance parameter and repeatability estimates using mixed model for grain yield (YLD), 1000-kernel weight (TKW), and grain number (GNO) of the 287 lines wheat association mapping initiative panel grown in four-row plots in Mexico for 3 yr 2010, 2011, and 2012. Measurements and analysis were also done separately for inner (YLD_I, TKW_I, and GNO_I) and outer rows (YLD_O, TKW_O, and GNO_O).

Source [†]	Variance components								
	YLD	TKW	GNO	YLD_I	YLD_O	TKW_I	TKW_O	GNO_I	GNO_O
σ_G^2	7000.01**	80.822*	8,352,431*	1233.81*	2646.06*	20.426*	19.8727*	1,254,207*	3,228,007*
$\sigma_{G \times E}^2$	3085.32	4.753	2,862,171	1199.81*	1263.36*	1.502	1.4962	927,559	1,034,599
σ_e^2	6935.47	7.373	4,272,152	4958.14	7742.80	3.738	2.727	2,661,372	4,602,238
Repeatability [‡]	0.76	0.96	0.83	0.50	0.61	0.95	0.95	0.62	0.74

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

[†] σ_G^2 , genotypic variance; $\sigma_{G \times E}^2$, variance of genotype \times environment; σ_e^2 , residual variance.

[‡] Repeatability was calculated similar to heritability (h^2) from PROC MIXED analysis.

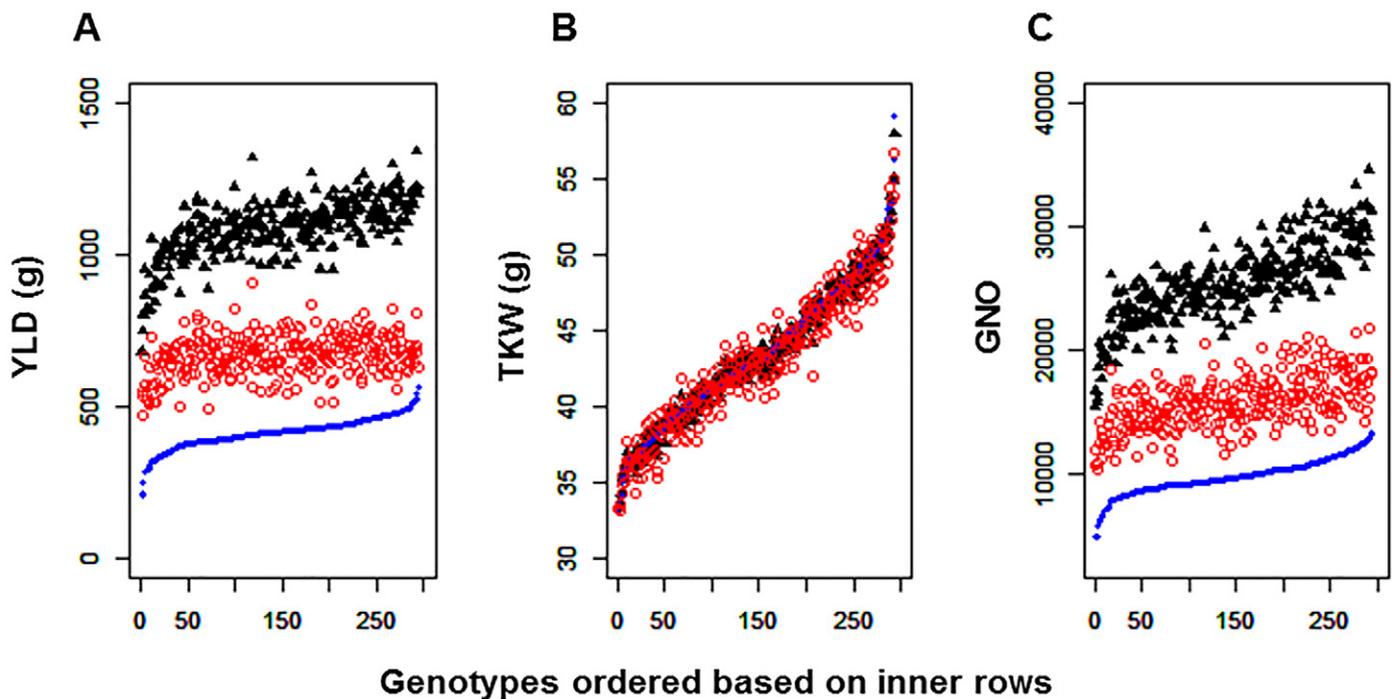


Figure 2. Comparison of (A) grain yield (YLD), (B) 1000-kernel weight (TKW), and (C) grain number (GNO) of inner rows (I, blue dots), outer rows (O, red circles), and the total (I+O, black triangle) of the rows of 287 lines of WAMI (wheat association mapping initiative) panel grown at Ciudad Obregon, Mexico from 2009 and 2010 to 2011 and 2012. The x-axis (genotypes) was sorted based on the value of respective traits at inner rows. For TKW, average of inner and outer was represented by black triangle. For YLD and GNO, outer rows were higher yielding than inner rows.

GNO, respectively, but rows explained little variation in TKW (0.001%). This indicates that TKW is not affected by plant density, but that YLD and GNO were affected by row position or the effect of plant density. Repeatability estimates considering inner and outer rows as separate environments within each year and combined repeatability estimates of inner and outer rows were estimated. Combined repeatability estimate of (I + O rows) across the years is lower than separate heritability estimates for I + O across years for YLD and GNO. Genotypic variance was lower than the $G \times R$ variance in some years. The combined repeatability estimates for inner and outer rows for 2010 and 2012 was close

to zero, indicating high $G \times R$. In 2011, repeatability was 0.4. The KW had high repeatability estimates across inner and outer rows each year indicating it is not affected by plant density (Supplemental Table S1).

Adaptation to Density Index

Our hypothesis, based on the earlier study (Reynolds et al., 1994a) in which low-yield potential lines responded more to reduce competition for light under low planting density than the high yield potential lines, was validated in this study. Our results indicated that plants that yielded low under normal spacing will respond more to low density planting. In other

words, the high yield potential lines showed less variation in YLD between high and low plant densities. The GNO also exhibited a similar trend, but TKW showed no response to plant density (Fig. 2). This indicates that interplant competition determines grain set early enough for the TKW target to be achieved. The genotypes adapted to high plant density do not show much reduction in YLD and GNO when comparing density treatment: ratio of inner to outer rows for yield will have higher value for adapted plants and lower value for not adapted plants. It also indicates that plants adapted to high plant density are “optimistic” plants that do not tend to reduce the number of grains per plant compared with nonadapted plants. The nonadapted plants are sensitive and show conservative response under high plant densities setting fewer grains.

Phenotypic Correlations of Adaptation to Density Index with the Yield

To understand if ADi is associated with the yield of high-yielding environments (Table 3), a biplot of ADi_{YLD} was made with the yield from high- and low-yielding environments. The biplot depicts the correlation patterns of the genotypes among environments. The interpretation of the biplot is as follows. The cosine of the angle between two lines in the biplot approximates the correlation between the ADi and the environments. Parallel vectors indicated a correlation close to unity, vectors in opposite directions indicate correlations close to -1 , and perpendicular vectors (orthogonal) indicate no correlations.

The ADi was positively correlated (correlation coefficient r) with yield of higher-yielding environments Me_YP_2010 (0.50), Me_YP_2012 (0.35), Pak_2011 (0.29), Eg_S_10, Me_YP_2011 (0.23), and In_I_10 (0.16). The ADi was not significantly correlated with the yield of low-yielding environments and even showed negative associations in environments Ban_11 (-0.09), In_V_11 (-0.06), In_L_11 (-0.05), Ban_10 (-0.21), Su_D_10 (-0.11), Nep (-0.10), and several other environments (Fig. 3, 4; Supplemental Table S2). The biplot of all the international environments with the ADi indicated that ADi_{YLD} indeed is positively correlated with the yield of high-yielding international environments (Fig. 4). The ADi_{YLD} and ADi_{GNO} were highly correlated in each site in Mexico (Supplemental Fig. S1).

Association Mapping

Linkage Disequilibrium, Population Structure, and Trait Analysis

Linkage disequilibrium decay was higher in the D genome than A and B genomes (Lopes et al., 2015; Sukumaran et al., 2015). The population structure of WAMI—two sub-populations based on the presence and absence of 1B.1R translocations and pedigree—was described in detail in earlier publications (Lopes et al., 2015; Sukumaran et al., 2015). A detailed analysis of the ADi_{YLD} and ADi_{GNO} indicated

Table 3. Average yield of wheat association mapping initiative under different environments in 2010 and 2011 and environments categorized based on yield. Average yield of the 33 environments was 5 t ha⁻¹ (Lopes et al. 2012).

Year	Abbreviation	Country	Location	Yield t ha ⁻¹	Site category
2010	Ban_10	Bangladesh	Joydebpur	2.22	Low yield
	Ir_A_10	Iran	Ahwaz	2.51	Low yield
	Nep_10	Nepal	Bhairahwa	2.70	Low yield
	Su_W_10	Sudan	Wad Madani	2.90	Low yield
	In_Dh_10	India	Dharwad	3.10	Low yield
	Pak_10	Pakistan	Islamabad	3.18	Low yield
	In_V_10	India	Varanasi	3.21	Low yield
	Me_HD_10	Mexico	Obregon	3.40	Low yield
	Me_D_10	Mexico	Obregon	3.71	Low yield
	Ind_D_10	India	Delhi	3.80	Low yield
	Me_H_10	Mexico	Obregon	4.01	Low yield
	Su_D_10	Sudan	Dongola	4.13	Low yield
	In_K_10	India	Karnal	4.21	Low yield
	Su_H_10	Sudan	Hudeiba	4.96	Low yield
	Ir_10	Iran	Darab	5.33	Average yield
	In_I_10	India	Indore	6.18	High yield
	In_L_10	India	Ludhiana	6.30	High yield
Eg_S_10	Egypt	Souhag	6.82	High yield	
Eg_E_10	Egypt	El mat	7.81	High yield	
Me_YP_10	Mexico	Obregon	7.02	High yield	
2011	Nep_11	Nepal	Bhairahwa	2.51	Low yield
	In_V_11	India	Varanasi	2.53	Low yield
	In_Dh_11	India	Dharwad	2.99	Low yield
	Ban_11	Bangladesh	Joydebpur	3.47	Low yield
	Me_D_11	Mexico	Obregon	3.76	Low yield
	In_L_11	India	Ludhiana	4.35	Low yield
	Me_H_12	Mexico	Obregon	3.12	Low yield
	In_I_11	India	Indore	5.71	Average yield
	Me_YP_11	Mexico	Obregon	5.86	High yield
	Pak_11	Pakistan	Islamabad	6.95	High yield
	In_D_11	India	Delhi	7.31	High yield
	In_K_11	India	Karnal	8.14	High yield
	Me_YP_12	Mexico	Obregon	7.97	High yield

that lines with 1B.1R translocation have lower ADi_{YLD} values (mean of $259 \pm$ standard error of 4.77) than the lines with 1B.1R (mean of $235.08 \pm$ standard error of 5.9) translocation (Supplemental Fig. S2). Student t -test indicated the two distributions are different at a p -value of 0.001.

Marker-Trait Associations

Association mapping was conducted in TASSEL using generalized linear model and mixed linear model testing for 18,104 SNPs markers from the 90K SNP data. The PC10 + K matrix fitted as the random factor in the mixed-model framework was the best model for ADi_{YLD} and for ADi_{GNO} . Manhattan plots of the marker positions and $-\log_{10}(p)$ values were also plotted (Fig. 5A,C). The Q-Q plots of the $-\log_{10}(p)$ values indicated goodness of fit of the selected model in controlling type I error (Fig. 5B,D).

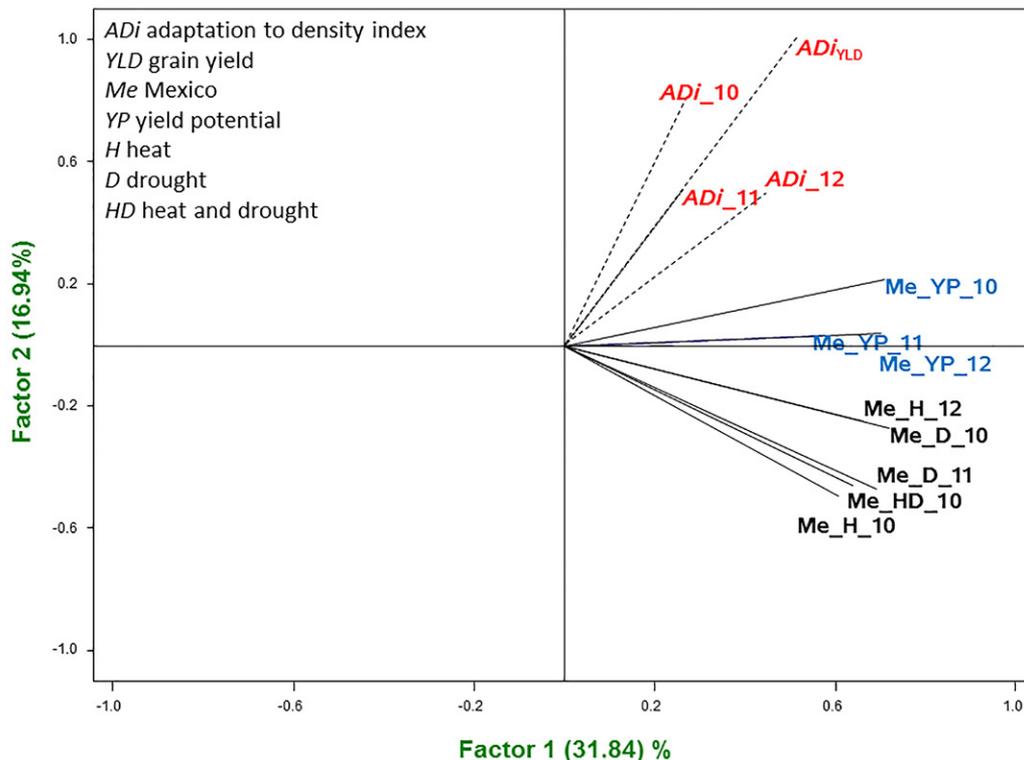


Figure 3. Biplot of adaptation to density index (ADi_{YLD}) with the yield of Mexican trials indicating ADi was highly correlated with yield of high-yielding environments ADi₁₀, ADi₁₁, and ADi₁₂ are adaptation to density index estimated for years 2010, 2011, and 2012, respectively. ADi_{YLD} is the combined estimate for 3 yr data. Environments labelled in blue and black colors are grain yield of each environment in Mexico (Me) under different conditions (YP, yield potential; H, heat; D, drought; HD, heat and drought) in years 2010, 2011, and 2012.

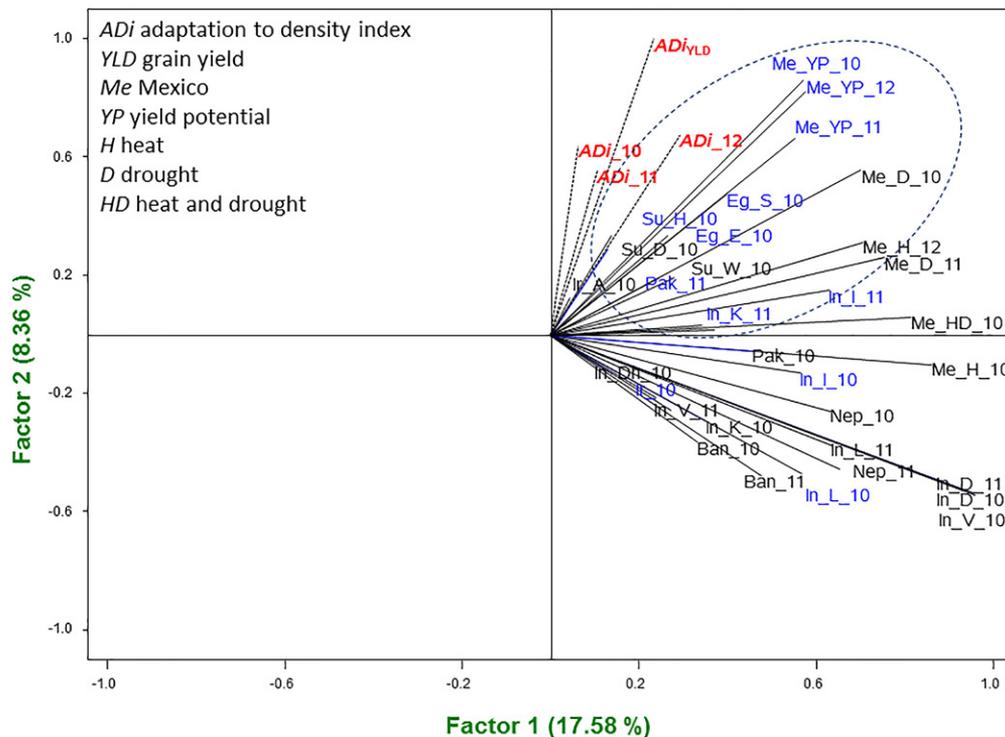


Figure 4. Biplot of adaptation to density index (ADi) with the yield of international environments in 2010, 2011, and 2012. Biplot indicates that adaptation to density index (ADi_{YLD}) is correlated with the grain yield in high-yielding environments. Principle component 1 (PC1) explains 17.58% of the variation and principle component 2 (PC2) explains 8.36% variation in the data. Locations with high yield are colored in blue and low-yielding locations in black. The ADi is shown in red. For abbreviations see Table 3. Dotted lines area indicates maximum number of locations with high yield.

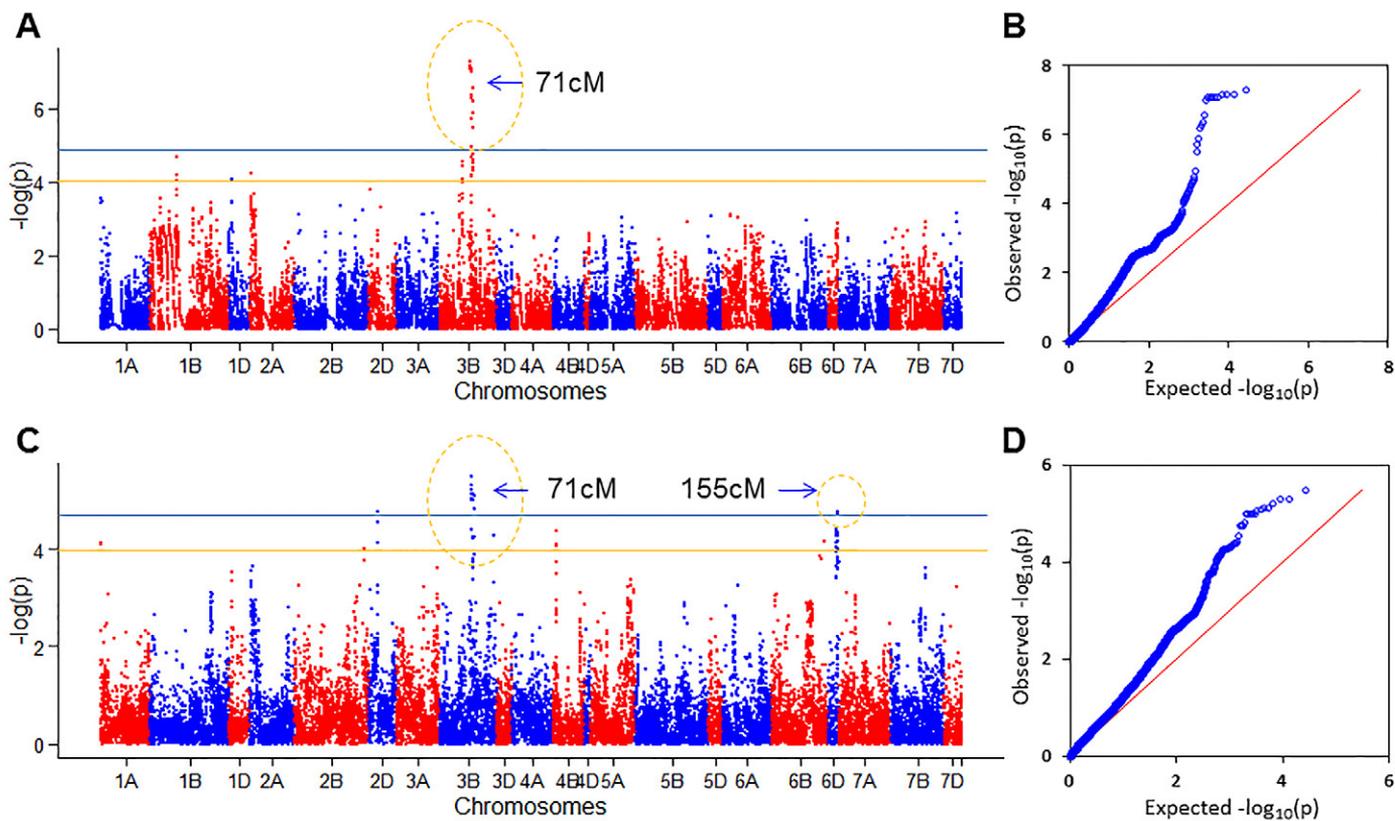


Figure 5. (A, C) Manhattan plots and (B, D) quantile–quantile (Q-Q) plots of the genome-wide association study results for adaptation to density index for yield (ADi_{YLD}) and for grain number (ADi_{GNO}), respectively, with the best model (PC10 + K). The threshold was taken as $-\log_{10}(p) < 10^{-4}$ and 5 . Significant loci above $-\log_{10}(p) < 10^{-5}$ are illustrated with circles and marker position with lowest p -value is noted.

Table 4. Genome-wide association study results for adaptation to density for yield (ADi_{YLD}) and grain number (ADi_{GNO}) in the wheat association mapping initiative population. Best model used to test marker–trait associations was PC10 + K Matrix.

Trait	Marker	Alleles	MAF [†]	Chromosome	Position cM	P-value, simple model	P-value, best model	Marker R^2
ADi_{YLD}	IAAV7721	A (35)/G (242)	0.13	3B	71.34	3.48×10^{-8}	6.98×10^{-8}	11.04
	<i>wsnp_BE443531B-Ta_1_1</i>	C (277)/T (33)	0.13	1B	64.31	1.04×10^{-4}	2.18×10^{-5}	7.12
	BS00022393_51	C (250)/T (32)	0.11	2A	25.97	1.88×10^{-4}	5.63×10^{-5}	5.89
ADi_{GNO}	IAAV7721	A (35)/G (242)	0.13	3B	71.34	2.54×10^{-6}	5.19×10^{-6}	7.63
	RAC875_c3156_630	C (194)/T (73)	0.27	6D	155.55	1.12×10^{-6}	1.81×10^{-8}	6.80
	D_GB5Y7FA01EHPZX_186	C (159)/T (105)	0.40	2D	50.82	9.10×10^{-9}	1.76×10^{-5}	7.12
	Tdurum_contig76213_958	C (145)/T (119)	0.45	4B	38.06	1.43×10^{-5}	4.30×10^{-5}	6.60
	Kukri_rep_c68091_971	C (202)/T (77)	0.28	6B	118.99	3.42×10^{-5}	6.85×10^{-5}	5.76

[†] MAF, minor allele frequency.

Significant and consistent marker–trait associations were detected for ADi_{YLD} and ADi_{GNO} in chromosome 3B (Fig. 5). In chromosome 3B, several markers at 71 cM that explained 11% variation in ADi_{YLD} were detected with a range of p -value from 10^{-5} to 10^{-8} . In addition, significant markers were detected for ADi_{YLD} in chromosomes 1B and 2A and for ADi_{GNO} on chromosomes 2D, 3B, 4B, 6B, and 6D that explained 7 to 11% variation for the trait (Table 4). Effects of significant markers are shown in Supplemental Fig. S3. The marker IAAV7721 in chromosome 3B was further analyzed through BLAST search in National Center for Biotechnology Information

(NCBI) and it resulted in a BLAST hit of SET domain protein gene with 99% identity.

DISCUSSION

Adaptation to High Plant Density

Most of the plant density studies in wheat consider the effect of planting density on weed populations or the effect of sowing date on planting densities in winter wheat (Bulson et al., 1997; Spink et al., 2000; Baccar et al., 2011). The effect of sowing date on optimum plant density was studied by Spink et al. (2000). They observed that YLD was reduced from 9.2 to 5.5 t ha⁻¹ when the plant number was changed

from 336 to 13 m⁻² at different sowing dates. But at low plant densities, the yield was maintained by increased shoot number per plant, increased grain number per ear, and, to a lesser extent, increased grain size (Spink et al., 2000).

In the 1960s (Stringfield, 1964) suggested that breeding for tolerance to high plant density is the greatest contribution that can be made in crop breeding, especially considering its use in hybrid development. Maize yield has increased over the years, not owing to increase in yield potential per plant, but by newer hybrids that outperform old hybrids because of their ability to withstand the pressure of high plant density. New hybrids at high plant densities make the same number of ears per plant as old hybrids do at low plant densities (Duvick, 2005). High planting density normally results in higher yield per hectare but reduced yield of individual plants. This has been demonstrated in several studies in maize. Adaptation to high-plant density is very important in hybrid maize production, and it was proven that selection for high yield in maize has resulted in lines adapted to higher plant density (Duvick, 1992; Brekke et al., 2011). While high density planting may reduce yield per plant as a result of aboveground competition for light, and possibly belowground competitive effects, developing plants that can better withstand high-density planting will have a tremendous impact on yield per hectare in wheat and other crops under intensive cultivation.

Adaptation to high plant density was estimated using an index that measures the scaled difference in the yield of inner and outer rows (ADi). The purpose of this index was to capture the lines that show a minimum difference in YLD under high and low plant densities. Even under high-plant density stress, an adapted plant maintains the number of grains in the plant and tries to fill them with available resources. However, under high-plant-density stress, the sensitive plants try to reduce the number of grains to conserve energy and produce viable seeds (Reynolds et al., 1994a; Whaley et al., 2000). High-yield-potential lines might use available light more efficiently than LYP lines under high plant density. The main evolutionary objective of a plant is to reproduce through its viable offspring. Previous studies indicate that there is an optimum balance in efficient use of the available energy in relation to the size and number of offspring (Smith and Fretwell, 1974). Our approach was to look for plants that are “optimistic” about filling grains under density stress and to maintain a maximum number of grains per plant, as opposed to those that responded conservatively, showing a reduced number of grains per plant and as a result, reduced grain yield per plant.

A number of hypotheses could be tested to explain genetic differences in adaptation to density. For example, a more optimal distribution of chlorophyll b (i.e., light antenna pigments) throughout the canopy, which permits

increased light penetration to lower leaves, may result in a greater proportion of leaves operating close to optimal light levels and, therefore, increased radiation-use efficiency (Melis, 2009; Ort et al., 2011). Another hypothesis, which is more difficult to test, is that high plant density—perhaps in response to red or far-red light receptors or other responses induced by a density stress—elicits a plant growth regulator response reducing grain set (Ugarte et al., 2010; Blum, 2013). Since spikes per square meter was higher in adapted lines than nonadapted lines (Reynolds et al., 1994a), a third hypothesis would be that lines adapted to density express a tiller dynamic whereby their production and abortion is minimized or optimized.

Molecular Markers for Adaptation to Plant Density

Considering the large range of responses shown by the genotypes in this study to different plant densities, this panel is ideal for association mapping to dissect the genetic architecture of this complex trait (Zhu et al., 2008; Sukumaran and Yu, 2014). This association mapping panel also serves as screening material for density studies aiming to determine the high and low density-adapted plants that could serve as parents for future hybridization in wheat in addition to parents of mapping populations. In this study, we used 18,104 SNP markers from 90K SNP assay with known genetic positions from consensus maps (Cavanagh et al., 2013).

We also analyzed how the adaptation to density traits vary among subpopulations and how 1B.1R lines adapt in comparison to absent lines. Grain number was not significantly different among the subgroups. A narrow phenology range for flowering time and plant height reduce confounding effects as a result of population structure in this panel for association mapping (Lopes et al., 2015). The markers in chromosome 1B, 2A, 2D, 3B, 4B, 6B, and 6D can differentiate lines adapted to density from nonadapted plants. The marker locus in 3B at 71 cM was also associated with YLD from a recent study (Sukumaran et al., 2015). BLAST analysis indicated the similarity of the marker to a SET domain protein gene. SET domain proteins are associated with pollen abortion in *Arabidopsis thaliana* (L.) Heynh. (Xu et al., 2010). This region may also harbor other genes and further validation is required to fully understand the biological function of this locus and the genes in the region associated with the trait. A previous meta-analysis using data from 21 Australian and Mexican environments detected a major quantitative trait loci associated with YLD, TKW, and early vigor in 3BL chromosome (Bonneau et al., 2013). We propose this region might be validated in different populations for use in molecular breeding.

No earlier reports on markers for adaptation to plant density or studies focused on molecular markers for adaptation to density in wheat and other crops exist. This novel study identified loci for adaptation to plant density

in wheat that may pave the way to select parents for hybrid wheat development. Although there are many longstanding studies related to cytoplasmic male sterility and hybrid wheat, it is still not developed and commercialized to its full potential (Driscoll, 1972; Mukai and Tsunewaki, 1979; Wilson and Driscoll, 1983). Ideally, hybrid wheat should not only produce high yields per hectare but also at the level of an individual plant.

Future Research

The present study is a first step toward elucidating the genetic mechanism of adaptation to plant density. Even though we used 287 lines and ~18,000 markers, the resolution could be improved with a larger mapping population and a higher number of markers. Among the above hypothesis related to adaptation to density, effect of density on tiller dynamics can be tested by looking at the number of productive and nonproductive tillers at cardinal growth stages. Testing whether adaptation to density is a function of antenna chlorophyll distribution would require verifying differences in light extinction down the canopy followed by direct measurement of chlorophyll a and b ratios at different canopy depths; others have proposed mutagenesis to induce variation in this trait (Melis, 2009). The most difficult hypothesis—related to red and far-red light—requires the use of phytochrome for light treatment and analysis of hormone levels in plants and different plant density.

CONCLUSIONS

Adaptation to plant density is a component of high yield potential in spring wheat. Wheat plants adapted to high plant density are “optimistic” and show reduced tendency toward producing fewer grains under density stress. Lower yield potential lines react more conservatively under high plant density, producing a reduced number of grains per plant, resulting in lower yields. Through GWAS we identified a novel locus for adaptation to plant density in spring wheat that might have an impact on hybrid wheat development and lead to higher yields. The identification of functional markers for this trait will have enormous value in pedigree breeding, as it is not possible to select for adaptation to high density in spaced plants typical of early-generation selection methods.

Author Contribution Statement

M.R. designed the research; M.R., S.S., M.L. performed the research; S.S., M.R. wrote the manuscript; M.R., S.S., J.C., performed the analysis.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

We would like to acknowledge Araceli Torres, Nayeli Quiche Yei, Mayra Jacqueline Barceló, and Perla Chavez for help in data collection in Cd. Obregon, Mexico. We also would like to thank M.R. Jalal Kamali, M. Moussa, Y. Feltaous, I.S.A. Tahir, N. Barma, M. Vargas, Y. Mannes, and M. Baum for phenotyping the WAMI panel trials at international environments. Funding support is acknowledged from ADAPTAWHEAT, SAGARPA, and USAID.

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