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Identification of QTLs for Drought Tolerance Traits on Wheat Chromosome 2A Using Association Mapping

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Abstract

Association mapping analysis was performed on a set of 108 diverse wheat accessions to dissect the genetic background of drought adaptive traits. These genotypes were characterized with 28 SSR loci on chromosome 2A. Fifteen agronomic traits were evaluated under well irrigated and drought conditions. Population structure and Kinship were inferred on the basis of 30 unlinked SSR loci covering all the 21 chromosomes, which enhanced the mapping strength by eliminating spurious associations. A total of 11 marker trait associations were detected by mixed linear model approach (MLM). The phenotypic variation explained by each marker ranged 4.26% to 13.6%. Out of 11, eight marker trait associations were found under drought and 3 marker trait associations were found under normal conditions. SSR locus *Xgwm312* showed association with relative water content (RWC) under normal conditions and detected in both years. Similarly, awn length showed association with *Xcfa2099* under drought conditions. SSR locus *Xwmc455* showed association with thousand grain weight (TGW) under well irrigated conditions. *Xbarc124* was associated with coleoptiles length, shoot length and extrusion length. This study has highlighted a novel set of loci which are associated with drought adaptive traits and can be used for marker assisted breeding to enhance the wheat performance under unfavorable conditions.

Keywords: Wheat; Drought; Marker trait association; Linkage disequilibrium; Mixed linear model; Association mapping

Introduction

Climate change consequences like drought, salinity and extreme temperature demands unprecedented efforts to sustain food production (Semenov and Halford, 2009). Understanding the genetic bases of morphological and physiological traits responsible for maintaining yield under stressful conditions is the key towards a more targeted breeding approach (Araus *et al.*, 2002; Salekdeh *et al.*, 2009). Recent developments in the field of genomics enable the selection and identification of the genomic regions harboring QTLs controlling important agronomic and yield traits in crops (Tuberosa and Salvi, 2006; Collins *et al.*, 2008; Cooper *et al.*, 2009). Family based QTL mapping has helped in identification of many genomic regions influencing complex traits in different plant species (Mauricio, 2001; Holland, 2007; Hall *et al.*, 2010). However, there are several limitations which lower the efficiency of linkage mapping. Firstly, as only two parents are used to establish mapping population, the allelic variation within mapping population is limited (Hall *et al.*, 2010). Secondly, only those experimental events can be exploited that have been taken place during the establishment of the mapping population and recombination do not have sufficient time to shuffle genome into small fragments (Myles *et al.*, 2009; Mir *et al.,* 2012).

Association mapping approach utilizes linkage disequilibrium (LD) in natural populations to identify markers with significant allelic differences (Ochieng *et al.*, 2007). Resolution of association mapping depends on LD levels, if LD decays rapidly, resolution of association mapping will be high and vice versa (Rafalski, 2002). There are two common ways to visualize the extent of LD between pairs of loci. LD decay plots are used to visualize the rate at which LD declines with genetic or physical distance. Scatter plots of r^2 values versus genetic/physical distance among all pairs of alleles within a gene, along a chromosome or across the genome are constructed. So a graphical view of LD can be presented either as a LD decay plot of D' or r^2 over physical or genetic distances or as a linear arrangement of LD between polymorphic sites within a gene or loci along a chromosome (Flint-Garcia *et al.*, 2003). Alternatively, disequilibrium matrices are also effective for visualizing the linear arrangement of LD between polymorphic sites within a gene or along chromosome.

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Association mapping can be classified into two categories, (i) candidate-gene association mapping, which dissect polymorphism in selected candidate genes that play role in controlling phenotypic variations for specific traits, and (ii) genome-wide association mapping, which trace genetic variation in the whole genome to find association for various complex traits (Risch and Merikangas, 1996). In the past, association mapping has been extensively used in medical genetics to dissect diseases, most notably Alzheimer's disease (Lander and Schork, 1994; Risch, 2000) and its application is gradually moving to other fields such as plant genetics (Ochieng *et al*., 2007).

Application of association mapping methods in plants has been limited by the fear of spurious associations that may result from population structure (Pritchard and Przeworski, 2001). Given the geographical origins, local adaptation, and breeding history of assembled genotypes in an association mapping panel, the non-independent samples usually contain both population structure and familial relatedness (Yu and Buckler, 2006). Association tests that don't attempt to account for the effect of population structure must be viewed with skepticism, however recent developments in statistical methodologies make it possible to properly interpret the results of association tests. To account for population structure many statistical methodologies have been designed such as structured association (SA) (Falush *et al.*, 2003) genomic control (GC) (Devlin *et al.*, 2004) mixed model approach (Yu and Buckler, 2006) and principal component approach (Price *et al.*, 2006). With these methods, the issues of false positives generated by population structure may be solved (Yu *et al.*, 2005; Price *et al.*, 2006).

Most of the association mapping studies so for, have been reported under favorable conditions (Breseghello and Sorrells, 2006; Ravel *et al.*, 2006; Crossa *et al.*, 2007; Yao *et al.*, 2009). In the previous studies, a number of QTLs for agronomic traits (spike length, spikelets spike^{-1}, grains spike^{-1}, plant height, spikelet density and thousand kernel weight) have been identified on different chromosomes (Yao *et al.*, 2009; Liu *et al.*, 2010; Mir *et al.*, 2012). For complex traits like drought tolerance and yield, the knowledge of interactions between marker/QTLs and environment are necessary for efficient utilization of marker assisted selection in plant breeding (Francia *et al.*, 2005; Gupta *et al.*, 2008; Zorić *et al.*, 2012). Comparing the performance of same genotypes across the environments, the best suited, environment for the expression of a particular QTL can be identified but the hundreds of markers are required for sufficient mapping resolution covering the whole genome of wheat, therefore, targeting an individual linkage group is a quite reasonable strategy as adopted by (Yao *et al.*, 2009) and (Liu *et al.*, 2010). So the following study was carried out. a) To identify the population structure in a collection of 108 germplasm lines. b) To identify the extent of LD decay on chromosome 2A. c) To identify the association of SSR markers with drought tolerance traits on chromosome 2A.

Materials and Methods

Plant Materials and Phenotyping

A core collection of 108 genotypes was selected after phenotyping a set of 200 genotypes on the basis of diversity found in 15 morphological traits. The germplasm was maintained at the department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (Latitude = 31°, 26' N, Longitude = 73°, 06' E, Altitude =184.4 m) during years 2009-2010 and 2010-2011 according to randomized complete block design with two replicates. One row of each germplasm accession was sown and evaluated and was kept 5m in length. Plants were kept 12cm apart and row to row distance was 30cm. Pre-anthesis water stress was created by withholding water after the 1st irrigation at tillering stage (Dhanda and Sethi, 2002) followed by a second irrigation after anthesis. The control treatment received four irrigations during the whole season. Ten plants were selected randomly and tagged for data recording.

All the genotypes were extensively phenotyped for two years like days to heading (DH), relative water content (RWC), proline content (µg/g), leaf rolling (LR), glaucousness/waxiness (Gla/wax), peduncle length (PL), peduncle extrusion length (EXL), awn length (AL), plant height (PH), thousand grain weight (TGW), grains per spike (GR/S) and yield/plant (Y). Software *STATISTICA* v7 was used to estimate the summary statistics like ANOVA and correlation analysis for all the traits. For seedling traits a separate experiment was conducted under laboratory conditions. For creating drought stress genotypes were grown on PEG-6000 solution and the traits like root length (RL), shoot length (SL) and coleoptiles length (CL) were recorded 10 days after sowing.

Genotypic Analysis

Fresh wheat leaf tissues were used to extract DNA according to the method described by (Rogowsky *et al.*, 1991). A set of 30 SSR markers covering whole genome were chosen from GrainGenes data base (http://wheat.pw.usda.gov) were used to detect population structure. For analysis of linkage disequilibrium (LD) and marker trait associations (MTA), 28 SSR markers flanking on chromosome 2A were scanned. High resolution agarose (2.5%) was used to analyze PCR products. The PCR profile for each SSR primer pair was the same as reported in Grain Genes (http://wheat.pw.usda.gov).

Polymorphism and Population Structure

The summary statistics of polymorphism and allelic information was determined using *Power-Marker* v3.25

(http://www.powermarker.net). Population structure was estimated with unlinked markers using *STRUCTURE* v2.3-a model based (Bayesian) cluster software (Pritchard *et al.*, 2000). The number of sub-populations (K) was set from 2-20 based on admixture and correlated allele frequencies models. For each K, 10 runs were performed separately. Each run was carried out with 30,000 iterations and 30,000 burn-in period. A value of K was selected where the graph of *InPr* (X/K) peaked in the range of 2-20 sub-populations. For selected K again 10 runs were performed each with 100,000 iterations and 100,000 burn-in period.

Linkage Disequilibrium

Linkage disequilibrium between all pairs of loci was estimated using LD parameter r^2 (the squared correlation coefficient between all bi-allelic combinations at two loci and summarizes both recombination and mutational history. Both unlinked and syntenic r^2 were evaluated using the software *TASSEL* v3 (http://www.maizegenetics.net) by setting 1000 permutation. The LD decay with genetic distance (cM) between markers on chromosome 2A was plotted by software *Power-Marker* v3.25. The 95% percentile of unlinked r^2 was used to derive a critical value for r^2, as evidence of linkage (Breseghello and Sorrells, 2006). The loci having $P<0.001$ were considered to be in significant LD. If all pairs of flanking loci within a chromosomal region were in significant LD, the region was designated as LD block (Stich *et al.*, 2005).

Marker-trait Association

Mixed linear model was used to calculate association between SSR marker alleles and traits by software *TASSEL* v3 (Yu *et al.*, 2005). The advantage of mixed linear model is that it reduces both type I and type II errors. The population structure Q matrix derived from *STRUCTURE* v2.2 and the relative kinship matrix K obtained from unlinked markers by *TASSEL* v3 was combined and co-variated in association test to minimize false positive rate. The $P < 0.01$ was used to declare the significance of SSR marker and trait associations and the magnitude of QTL effects were calculated by r^2-marker.

Results

Molecular Diversity

From the scoring of 58 SSR primers on a set of 108 genotypes, 361 alleles were detected with an average of 6.2 alleles per locus. The range of alleles varied from 2 to18. Polymorphic markers were used to identify molecular diversity, population structure, linkage disequilibrium and marker trait associations (MTAs). Major allele frequency ranged from 0.30-0.84. Average PIC values of primers ranged from 0.25-0.93 with an average of 0.65.

Population Structure

The admixture model-based analysis using software *STRUCTURE* was employed to investigate the nature of genetic relationships among genotypes. To select an optimal number of sub-populations, the *STRUCTURE* was run in the range of two to twenty sub-populations which revealed the peak of likelihood at 3 (Fig. 1). Therefore, K value was set to 3 to identify the sub-populations. The number of genotypes assigned to different sub-population was 33, 37, and 38 (Fig. 2). The F_{ST} values between sub-populations were significant ($P<0.001$) confirming the prevalence of genetic structure.

Linkage Disequilibrium

Background LD was determined by unlinked markers which were also used to detect population structure. This back ground LD caused by population structure was also used to set critical value of LD for markers on chromosome 2A. The unlinked r^2 value ranged from 0.000 to 0.06 for all unlinked loci pairs with an average of 0.002.

The 95th percentile of the distribution of unlinked r^2 (0.015) was used as population specific threshold for this parameter as an evidence of LD because of linkage. 28 SSRs on chromosome 2A were used to obtain syntenic r^2. The pairwise syntenic r^2 ranged from 0.0000 to 0.1194 with an average of 0.0189 which is higher than the average of unlinked r^2. The scatter plot showing LD decay of the syntenic LD values of r^2 in population is shown in Fig. 3. LD extent on chromosome 2A was ~ 8 cM with the critical value of 0.02. The chromosomal region having all the pairs of loci in LD was referred as LD block as shown in Fig. 4.

Marker-trait Associations

Mixed linear model approach was used to determine marker trait associations for 15 phenotypic traits and 28 SSRs loci on chromosome 2A of hexaploid wheat. Sub-populations were used as covariates for association mapping analysis. A total of 8 marker trait associations were identified for 6 traits under drought stress conditions at the probability level of 0.001 with the range of 6.56 to 14.4% of the phenotypic variation. On the other hand, well irrigated conditions generated only 3 marker trait associations with r^2 ranging from 10 to 17.8. Traits showed significant associations under drought include relative water contents (RWC), thousand grain weight (TGW) awn length (AL), plant height (PH), shoot length (SL), and coleoptile length (CL). The traits which showed significant marker trait associations under well irrigated conditions include relative water contents (RWC), peduncle extrusion length (EXL), and coleoptile length (CL). Of a total of 7 SSR markers showing significant marker traits association, four of them (wmc181, wmc407, gwm312, gwm558) were associated with only one trait and therefore can be considered as trait specific MTAs.

Table 1: Association (r^2) of the SSR markers with drought related traits in wheat

Traits	QTL	Marker	Position cM	1st year N	1st year D	2nd year N	2nd year D	R2	*P(Q+K)*
RWC	*QRWC.uaf.2A.2*	GWM 312	74	*		*		0.1526	0.0076
								0.1782	0.0009
RWC	*QRWC.uaf.2A.3*	WMC 181	103				*	0.0656	0.007
RWC	*QRWC.uaf.2A.1*	WMC 407	15				*	0.1225	0.0004
TGW	*QSZ.uaf.2A.1*	WMC 455	59				*	0.0763	0.0046
AL	*QAl.uaf.2A.1*	CFA 2099	66.4		*		*	0.0809	0.0073
								0.1463	0.0005
PH	*QPH.uaf.2A.1*	GWM 558	54		*			0.0946	0.0035
PH	*QPH.uaf.2A.2*	WMC 455	59				*	0.0734	0.0069
EXT	*QEXT.uaf.2A.1*	BARC 124	8			*		0.1121	0.0094
SL	*QSL.uaf.2A.1*	BARC 124	8		*			0.144	0.0026
CL	*QCL.uaf.2A.1*	BARC 124	8	*				0.1008	0.0079
CL	*QCL.uaf.2A.3*	CFA 2099	66.4		*			0.0863	0.0035

RWC=relative water content, TGW= thousand grain weight, Al= awn length, PH= plant height, EXT= extrusion length, SL= shoot length, CL= coleoptile length, 1st year N=First year Normal, 1st year D= First year drought

Two markers, cfa2099 and wmc455 were associated with two traits whearase, barc124 was found to be associated with three traits.

Under drought stress conditions wmc181 and wmc407 showed significant association with relative water contents. Awn length was found to be associated with cfa2099. This association for awn length was found across two years under drought environment. Marker trait associations for plant height were detected with primer wmc455 and gwm558 under drought conditions during 1st and 2nd year, respectively. Marker wmc455 showed overlapping association with two traits (thousand grain weight and plant height) during 2nd year under drought stress. Shoot length showed associations with barc124. Coleoptile length and awn length showed an overlapping association with cfa2099 under 2nd year drought. Only one MTA was found for thousand grain weight with wmc455 under drought conditions in the 2nd year. Under favorable conditions, only three marker trait associations were found. For relative water content, gwm312 was detected across two years under normal conditions. Barc124 showed significant association with coleoptile length and extrusion length under well irrigated condition. Barc124 showed association with three different traits under three different environments (Table 1).

Discussion

It was noted that 58 SSR loci were detected in this study on 2.5% high resolution agarose gel. More genetic diversity could have been observed if analysis performed using capillary electrophoresis and more diverse germplasm used. However, in previous study, (Sánchez-Pérez *et al.*, 2006) observed no significant difference in results obtained by capillary, agarose, polyacrylamide electrophoresis. The extent of observed genetic diversity in the current study 0.68 was higher than that of (Zorić *et al.*, 2012) having 0.64 genetic diversity, and by (Dreisigacker *et al.*, 2005) showing 0.57 gene diversity, and (Prasad *et al.*, 2009) showing 0.62 gene diversity, however, our extent of genetic diversity was very close to (Chao *et al.*, 2007) 0.66 genetic diversity. Huang *et al.* (2002) detected highest level of genetic diversity (0.77) from a set of 998 accessions collected from 68 countries of 5 continents across the globe.

Gene diversity values and average number of alleles per locus (6.2) is an evidence of sufficient genetic diversity present in the germplasm lines used in this study. Previously, Liu *et al.* (2010) found 6.1 numbers of alleles per locus in a core collection of Chinese wheat accessions. Yao *et al.* (2009) studied 108 Chinese wheat accessions and found 5.7 alleles per locus. 60 European wheat accessions showed 4.8 alleles per locus (Stachel *et al.*, 2000). Breseghello and Sorrells (2006) also found 4.8 alleles per locus in 95 soft winter cultivars of USA. High number of alleles per locus (18.1) was observed in IPK gene bank hexaploid wheat accessions (Huang *et al.*, 2002). Allelic diversity found in the present study is very close to the allelic diversity observed in the Chinese germplasm but higher than the European and USA wheat population as discussed above.

The admixture model based analysis grouped the accessions into 3 sub-populations. Presence of familial relationship and sub-population in cultivars and modern breeding materials is a common feature (Flint-Garcia *et al.*, 2003; Maccaferri *et al.*, 2005; Camus-Kulandaivelu *et al.*, 2006; Cockram *et al.*, 2008). Fig. 2 explains the nature of population structure obtained from structure analysis. First sub-population consisted of 33 genotypes and primarily contain genotypes originating from CIMMYT (CIMMYT =23, ICARDA=3, Pakistan=5, and India=2). The second sub-population consisted of accessions primarily from Pakistan (Pakistan=22, CIMMYT=9, ICARDA=5, and India=1). Similarly, third sub-population also contained genotypes mostly originating from Pakistan (Pakistan=26, CIMMYT=8 and Miscellaneous=2). Although a general division rule can be seen in the first sub-population containing mostly Mexican originated accession (also in the 2nd and 3rd with majority of Pakistani accessions), the sub-populations cannot be explained geographically due to overlapping of several accession from the same region in all groups. This is possibly due to regional breeding objectives and adaptation preferences. Presence of population structure results type I and type II errors in association mapping

studies and must be accounted during marker trait association studies (Pritchard and Rosenberg, 1999; Malosetti *et al.*, 2007). The identification of sub-populations within wheat genotypes to reduce spurious association for association mapping studies has been highlighted by many researchers (Breseghello and Sorrells, 2006; Crossa *et al.*, 2007; Yao *et al.*, 2009). Breseghello and Sorrells (2006) used the same statistical tool and identified four sub-populations in diverse group of soft winter wheat accessions. Yao *et al.* (2009) identified nine sub-populations within a set of 108 Chinese wheat accessions. To avoid spurious associations, matrix of population structure was co-variated with 2A data and the kinship matrix (Breseghello and Sorrells, 2006; Tommasini *et al.*, 2007; Yao *et al.*, 2009).

To establish the critical value of linkage disequilibrium, the 95th percentile of the distribution of unlinked r^2 was used (Yao *et al.*, 2009) instead of r^2 as used in most of the previous studies (Remington *et al.*, 2001; Tenaillon *et al.*, 2001; Palaisa *et al.*, 2003; Andersen *et al.*, 2007). The LD value defines the extent of LD attributable to linkage and the approach was first mentioned by (Breseghello and Sorrells, 2006). In this approach, unlinked LD distribution incorporates the effects of selection and population structure in the experiment (Breseghello and Sorrells, 2006; Yao *et al.*, 2009). LD decay in the targeted genomic region is essential for association mapping studies. Levels of LD or linkage disequilibrium are different, in different populations or species due to many genetic and breeding factors such as genetic drift, recombination's and mating system (Yao *et al.*, 2009; Al-Maskri *et al.*, 2012). Mating system is a major factor that affects LD extent. Self-pollinated crops showed higher levels of LD as compared to out-crossing crop species (Gupta *et al.*, 2005; Abdurakhmonov and Abdukarimov, 2008). Wheat being an auto-gamous has LD extent which is greater by three orders than the LD extent in maize which is an out-crossing species (Breseghello and Sorrells, 2006). Strong LD extending upto 20cM with r^2>0.6 was found by (Rhoné *et al.*, 2007). Crossa *et al.* (2007) found r^2 within ~40cM. In a study using a collection of 95 soft winter wheat accessions analyzed with 33 and 20 SSRs on chromosome 2D and 5A, respectively, Breseghello and Sorrells (2006) reported a strong LD within < 5cM and ~ 1cM for centromeric region of chromosome 5A and chromosome 2D, respectively. Higher level of LD in the centromeric region may be due to low frequency of recombination around centromere (Jones *et al.*, 2002) and loss of variability during domestication. Domestication related QTLs for plant height, yield plant^{-1} and days to heading have been detected in centromeric region of chromosome 2A by (Peng *et al.*, 2003). These levels observed in wheat were higher than the LD level observed in maize which decayed at about 1 kb distance (Remington *et al.*, 2001; Palaisa *et al.*, 2003). Remington *et al.* (2001) reported LD decay within 1500 bp in maize and (Jung *et al.*, 2004) surveyed *adhI* locus and found extent of LD within 500 kb. In a survey of 32 European maize lines, LD decay (3.7 kb) was persisted over the length of *PAL* gene locus (Andersen *et al.*, 2007). Mating system is the main cause for this difference of this deviation in LD in maize and wheat (Yao *et al.*, 2009).

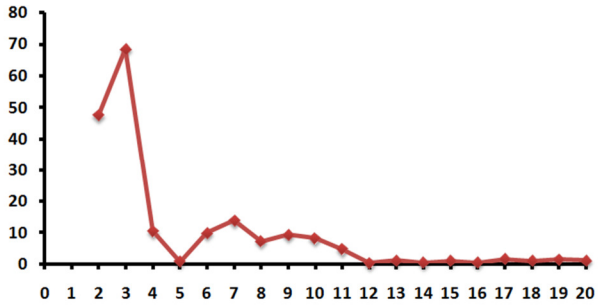

Fig. 1: The likelihood to identify optimal number of sub-populations when K ranged from 2 to 20

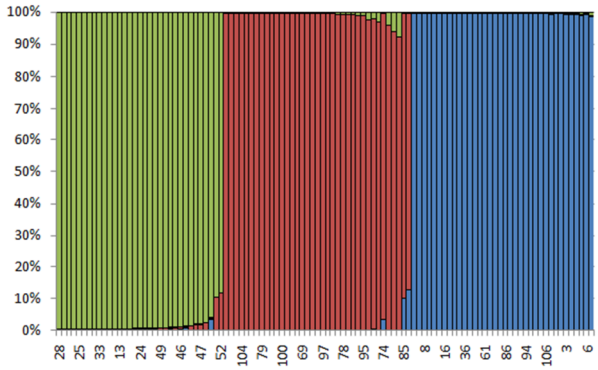

Fig. 2: Population structure of 108 wheat genotypes. The population was assigned to three color-coded sub-populations. Each bar represents single genotype and the colored portions in each bar reveal the proportional contribution of each sub-population to that genotype

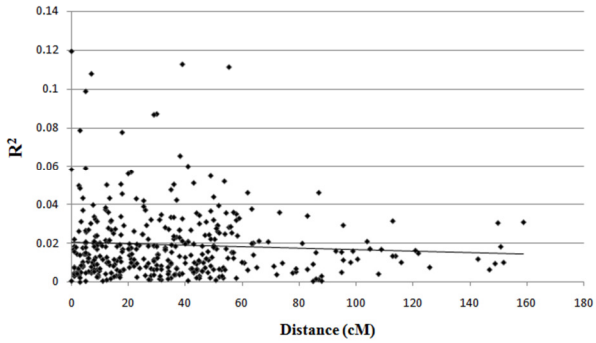

Fig. 3: Estimates of r^2 versus linkage distance on chromosome 2A. Horizontal line indicates the 95th percentile of the distribution of unlinked r^2

Note: Map position (cM) were based on the consensus map Ta-SSR-2004 (Somers *et al.*, 2004) R^2 indicates the percentage of the total variation explained at the loci significant at P<0.01 level

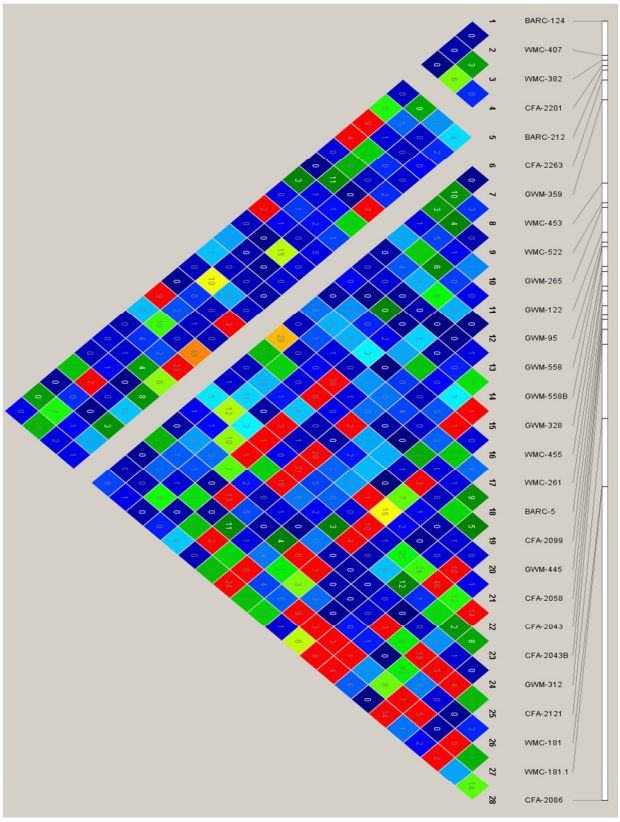

Fig. 4: Haplotype map showing the LD block on wheat chromosome 2A

Presence of rare alleles can also lead towards imprecise estimates of LD (Abdurakhmonov and Abdukarimov, 2008). Accordingly, rare alleles with less than 5% were pooled as missing data for linkage disequilibrium analysis in our study. Accurate estimates of LD are very important for marker trait association analysis. Criterion which establishes the critical values to test the significance syntenic LD as statistical factor may also affect the analysis. We observed LD extent upto ~ 8cM ($r^2 = 0.02$) on chromosome 2A in 108 spring wheat genotypes mostly Pakistani landraces, cultivars and elite lines surveyed with 28 polymorphic SSRs. Extent of LD varies in various wheat populations (Sajjad *et al.,* 2013).Various patterns of LD extent depict the selection pressure on respective genomic regions. Previously, Yao *et al.* (2009) observed very low LD extent (2.3cM) on chromosome 2A in a collection of 137 wheat accessions assayed with 37 SSR primers covering whole chromosome with an average marker interval of 3.75. They found two LD block on chromosome 2A, one in the centromeric region having eight loci within 6.3cM and other on the short arm within 3.3cM having three loci. It was also found that some markers which are very close in the map but was not as on LD (Yao *et al.,* 2009). It demonstrates the high resolution of LD mapping. Chao *et al.* (2010) observed higher level of LD extent in D-genome (20.8-27.1cM), followed by B-genome (20.4-21.5cM) and lowest level in A-genome (16.7-19.8cM) in a set of 478 winter and spring wheat accessions assayed by 394 SNPs. A set of 205 US winter lines genotyped with 254 SSRs showed a highly variable extent of LD throughout genome. The mean genome LD decay observed 10cM while the highest LD block was (>40cM) (Zhang *et al.*, 2011). Contrary, Liu *et al.* (2010) observed very low level of LD (3cM) in a set of 103 Chinese wheat accession assayed with 31 SSR markers. In a survey of 96 diverse wheat accessions assayed with 874 DArT, highly variable results were observed. Chromosome 7A showed very high values of LD about 45cM while few very closely linked markers showed no significant LD. Tenaillon *et al.* (2001) reported higher extent of LD in nine U.S. inbred lines than sixteen exotic lines. It is obvious that different populations of wheat selected from diverse germplasm exhibit different levels of resolution for association mapping studies. Therefore, LD patterns in wheat may vary with genomic regions, marker types and populations.

We used a mixed linear model (MLM) approach to determine marker-trait associations for fifteen traits and 28 2A specific SSRs markers. The mixed linear model (MLM) is a powerful approach as compared to general linear model (GLM) or any other model developed so far. Its power over GLM (that takes only population structure into account) lies in taking into accounts both population structure and kinship for accurate marker traits associations. Theoretically, kinship creates LD between genetically linked loci but it can also create LD between genetically unlinked loci when predominant parents are included in the population. In maize, kinship equally generated LD between genetically linked and unlinked loci (Stich *et al.*, 2005). In the present study QTLs detected under drought environment were higher in numbers than under normal conditions. Due to differences in structure in population, environmental conditions and methods of QTLs detection, it can be difficult to compare QTLs identified in this study with those of previously reported (Lakew *et al.*, 2013).

In our study, TGW was found associated with locus *Xwmc455* which is at 59cM distance on chromosome 2A. Yao *et al.* (2009) found marker trait association for TGW with wmc819 located at 60cM distance. QTLs controlling TGW on the same chromosome has also been reported previously in the marker interval gwm275 (56cM), gwm312 (79cM) and gwm372 (80cM) (Wang *et al.*, 2012) whereas, wmc455 is also located in this interval. Many researchers have also found genomic regions associated with TGW on chromosome 2A (Huang *et al.*, 2004; Gupta *et al.*, 2006; Snape *et al.*, 2007; Tsilo *et al.*, 2010). Peng *et al.* (2003) also found QTLs for TGW on chromosome 2A.

Peduncle extrusion length showed association with locus *Xbarc124* (8cM) in 2nd year under normal conditions. Peduncle extrusion and peduncle length facilitate the

emergence of spikes from boot. Inability of spikes to emerge out negatively effects seed setting and seed development due to reduced photosynthetic contribution (Rao *et al.*, 2007). In this study, it was also observed that genotypes having high peduncle length also have high peduncle extrusion length. Neumann *et al.* (2011) found six common QTLs for peduncle length and plant height across the genome in a study of 96 wheat core collections and showed that these traits are co-located at the same region. Yao *et al.* (2009) found significant marker trait association with plant height at 7.7cM and 10.4cM distance on chromosome 2A. In our study, *Xbarc124* is located at 8cM distance on chromosome 2A. So the results obtained by (Neumann *et al.*, 2011) and (Yao *et al.*, 2009) confirms the presence of QTLs for plant developmental trait like peduncle extrusion length as identified in this study. Many QTLs for peduncle length and peduncle extrusion has been identified in wheat and barley on different chromosomes. Börner *et al.* (2002) identified QTLs for peduncle length on chromosome 6A. Neumann *et al.* (2011) identified 13 QTLs in wheat for peduncle length across the genome. In barley, Rao *et al.* (2007) identified QTLs for peduncle length on chromosome 1H. Similarly, Tenaillon *et al.* (2001) identified QTLs for ear extrusion on chromosome 3H of barley.

Relative water content (RWC) showed 3 marker trait associations under normal and drought conditions. Chromosomal region *Xgwm312* showed association under normal conditions and detected under both years. Under drought condition, two distant markers showed association with RWC. Chen *et al.* (2010) reported QTLs for RWC on 2H barley chromosome under drought conditions. However, Teulat *et al.* (2003) also found QTLs for RWC on 6H chromosome of barley under dry environment. It shows influence of environment on this trait, that's why; different QTLs were detected under different environments.

In the present study markers cfa2099 showed association with awn length on chromosome 2A under drought stress conditions for two consecutive years. Awn length is controlled by 3 genes (*Hd*, *B1* and *B2*) which has been reported in previous studies (Sears, 1954, 1966). Sears (1954) also reported that some awn promoting genes are present on chromosome 2A. In a study of disomic addition lines of group 2 chromosome in Chinese spring (awnless) showed awned phenotype (Dvořák, 1980; Friebe *et al.*, 1999). So, awning seems to be a very complex trait, in addition to three major inhibitor genes, several minor genes also involved in awning which may be awning promoters or suppressors (Sood, 2008) as found in the present study.

Coleoptile length was found to be associated with two different genomic regions (*Xbarc124* and *Xcfa2099*) at 8 and 66.6cM under normal and drought, respectively. Landjeva *et al.* (2008) in a study of 114 recombinant inbred lines of ITMI population, found 5 genomic regions located 1 on chromosome 1A, 3 on chromosome 1B and 1 on 7D associated with coleoptile length. Rebetzke *et al.* (2001) found QTLs for coleoptile length on short and long arm of chromosome 4B near *Rht-B* locus. Qian *et al.* (2011) scanned 168 F2 and 57 DH populations with 323 SSR markers and found many associations between coleoptiles length and genomic regions on different chromosomes (4B, 3B and 6D).

Xbarc124 also showed association with shoot length under drought condition during first year. In a previous study, Landjeva *et al.* (2008) found four QTLs for shoot length on chromosome 2D under drought conditions and three QTLs on chromosome 2B under normal conditions, both of these chromosomal regions are homologous to 2A. Genomic region *Xbarc124* showed association with three height related traits as peduncle extrusion length, shoot length and coleoptile length. Positive significant association between extrusion length and plant height was also observed in this study. Similarly, positive significant association between shoot length and coleoptile length was observed during phenotyping under laboratory conditions. In previous studies, positive association between plant height and coleoptile length has been reported by many researchers (Allan, 1980; Rebetzke *et al.*, 1999) which indicates that QTLs affecting developmental traits might be clustered around this locus.

In conclusion, the study successfully identified many marker trait associations under normal and drought condition. Marker traits associations detected for RWC will help to improve our understanding about the water relations in plant. Marker wmc455 which is associated with thousand grain weight under drought conditions can be used to determine the yield potential of wheat genotypes under drought conditions using marker assisted selection. Similarly, marker trait associations for awn length, extrusion length and relative water content which are found to be associated with yield in this study or in previous studies could also improve our understanding about the performance of genotypes under drought. This study also identified some important QTLs for seedling traits under normal and drought stress conditions which improved our understanding of complex genetic nature of shoot length and coleoptile length.

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References

Abdurakhmonov, I.Y. and A. Abdukarimov, 2008. Application of association mapping to understanding the genetic diversity of plant germplasm resources. *Int. J. Plant Genomics,* 2008

Al-Maskri, A., M. Sajjad and S.H. Khan, 2012. Association mapping: a step forward to discovering new alleles for crop improvement. *Int. J. Agric. Biol.,* 14: 153–160

Allan, R., 1980. Influence of semidwarfism and genetic background on stand establishment of wheat. *Crop Sci.,* 20: 634–638

Andersen, J.R., I. Zein, G. Wenzel, B. Krützfeldt, J. Eder, M. Ouzunova and T. Lübberstedt, 2007. High levels of linkage disequilibrium and associations with forage quality at a Phenylalanine Ammonia–Lyase locus in European maize (*Zea mays* L.) inbreds. *Theor. Appl. Genet.,* 114: 307–319

Araus, J., G. Slafer, M. Reynolds and C. Royo, 2002. Plant breeding and drought in C3 cereals: what should we breed for? *Ann. Bot.,* 89: 925–940

Börner, A., E. Schumann, A. Fürste, H. Cöster, B. Leithold, M. Röder and W. Weber, 2002. Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.,* 105: 921–936

Breseghello, F. and M.E. Sorrells, 2006. Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. *Genetic.,* 172: 1165–1177

Camus-Kulandaivelu, L., J.–B. Veyrieras, D. Madur, V. Combes, M. Fourmann, S. Barraud, P. Dubreuil, B. Gouesnard, D. Manicacci and A. Charcosset, 2006. Maize adaptation to temperate climate: relationship between population structure and polymorphism in the *Dwarf8* gene. *Genetics,* 172: 2449–2463

Chao, S., W. Zhang, J. Dubcovsky and M. Sorrells, 2007. Evaluation of Genetic Diversity and Genome–wide Linkage Disequilibrium among US Wheat (L.) Germplasm Representing Different Market Classes. *Crop Sci.,* 47: 1018–1030

Chao, S., J. Dubcovsky, J. Dvorak, M.–C. Luo, S.P. Baenziger, R. Matnyazov, D.R. Clark, L.E. Talbert, J.A. Anderson and S. Dreisigacker, 2010. Population and genome specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (*Triticum aestivum* L.). *BMC Genomics,* 11: 727

Chen, G., T. Krugman, T. Fahima, K. Chen, Y. Hu, M. Röder, E. Nevo and A. Korol, 2010. Chromosomal regions controlling seedling drought resistance in Israeli wild barley, *Hordeum spontaneum* C. Koch. *Genet. Resour. Crop Ev.,* 57: 85–99

Cockram, J., J. White, F.J. Leigh, V.J. Lea, E. Chiapparino, D.A. Laurie, I.J. Mackay, W. Powell and D.M. O'Sullivan, 2008. Association mapping of partitioning loci in barley. *BMC Genetics,* 9: 16

Collins, N.C., F. Tardieu and R. Tuberosa, 2008. Quantitative trait loci and crop performance under abiotic stress: where do we stand? *Plant Physiol.,* 147: 469–486

Cooper, M., F.A. van Eeuwijk, G.L. Hammer, D.W. Podlich and C. Messina, 2009. Modeling QTL for complex traits: detection and context for plant breeding. *Curr. Opin. Plant Biol.,* 12: 231

Crossa, J., J. Burgueno, S. Dreisigacker, M. Vargas, S.A. Herrera–Foessel, M. Lillemo, R.P. Singh, R. Trethowan, M. Warburton and J. Franco, 2007. Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. *Genetics,* 177: 1889–1913

Devlin, B., S.A. Bacanu and K. Roeder, 2004. Genomic control to the extreme. *Nat. Genet.,* 36: 1129–1130

Dhanda, S. and G. Sethi, 2002. Tolerance to drought stress among selected Indian wheat cultivars. *J. Agric. Sci.,* 139: 319–326

Dreisigacker, S., P. Zhang, M. Warburton, B. Skovmand, D. Hoisington and A. Melchinger, 2005. Genetic diversity among and within CIMMYT wheat landrace accessions investigated with SSRs and implications for plant genetic resources management. *Crop Sci.,* 45: 653–661

Dvořák, J., 1980. Homoeology between *Agropyron elongatum* chromosomes and *Triticum aestivum* chromosomes. *Can. J. Genet. Cytol.,* 22: 237–259

Falush, D., M. Stephens and J.K. Pritchard, 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics,* 164: 1567–1587

Flint-Garcia, S.A., J.M. Thornsberry and B. IV, 2003. Structure of Linkage Disequilibrium in Plants. *Annu. Rev. Plant Biol.,* 54: 357–374

Francia, E., G. Tacconi, C. Crosatti, D. Barabaschi, D. Bulgarelli, E. Dall'Aglio and G. Vale, 2005. Marker assisted selection in crop plants. *Plant Cell Tiss. Org.,* 82: 317–342

Friebe, B.R., N.A. Tuleen and B.S. Gill, 1999. Development and identification of a complete set of *Triticum aestivum–Aegilops geniculata* chromosome addition lines. *Genome,* 42: 374–380

Gupta, P.K., H.S. Balyan, P.L. Kulwal, N. Kumar, A. Kumar, R.R. Mir, A. Mohan, J. Kumar. 2008. QTL analysis for some quantitative traits in bread wheat. *J Zhejiang Uni. Sci.,* 8: 807–814

Gupta, P.K., S. Rustgi and P.L. Kulwal, 2005. Linkage disequilibrium and association studies in higher plants: present status and future prospects. *Plant Mol. Biol.,* 57: 461–485

Gupta, P.K., S. Rustgi and N. Kumar, 2006. Genetic and molecular basis of grain size and grain number and its relevance to grain productivity in higher plants. *Genome,* 49: 565–571

Hall, D., C. Tegström and P.K. Ingvarsson, 2010. Using association mapping to dissect the genetic basis of complex traits in plants. *Brief Funct. Genomics,* 9: 157–165

Holland, J.B., 2007. Genetic architecture of complex traits in plants. *Curr. Opin. Plant Biol.,* 10: 156–161

Huang, X., A. Börner, M. Röder and M. Ganal, 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. *Theor. Appl. Genet.,* 105: 699–707

Huang, X., H. Kempf, M. Ganal and M. Röder, 2004. Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.,* 109: 933–943

Jones, L., K. Rybka and A. Lukaszewski, 2002. The effect of a deficiency and a deletion on recombination in chromosome 1BL in wheat. *Theor. Appl. Genet.,* 104: 1204–1208

Jung, M., A. Ching, D. Bhattramakki, M. Dolan, S. Tingey, M. Morgante and A. Rafalski, 2004. Linkage disequilibrium and sequence diversity in a 500–kbp region around the adh1 locus in elite maize germplasm. *Theor. Appl. Genet.,* 109: 681–689

Lakew, B., R.J. Henry, S. Ceccarelli, S. Grando, J. Eglinton and M. Baum, 2013. Genetic analysis and phenotypic associations for drought tolerance in *Hordeum spontaneum* introgression lines using SSR and SNP markers. *Euphytica,* 189: 9–29

Lander, E.S. and N.J. Schork, 1994. Genetic dissection of complex traits. *Sci. New York Washington,* 2037–2037

Landjeva, S., K. Neumann, U. Lohwasser and A. Börner, 2008. Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. *Biol. Plant.,* 52: 259–266

Liu, L., L. Wang, J. Yao, Y. Zheng and C. Zhao, 2010. Association mapping of six agronomic traits on chromosome 4A of wheat (*Triticum aestivum* L.). *Mol. Plant Breed.,* 2: 00426

Maccaferri, M., M.C. Sanguineti, E. Noli and R. Tuberosa, 2005. Population structure and long–range linkage disequilibrium in a durum wheat elite collection. *Mol. Breed.,* 15: 271–290

Malosetti, M., C.G. van der Linden, B. Vosman and F.A. van Eeuwijk, 2007. A mixed–model approach to association mapping using pedigree information with an illustration of resistance to Phytophthora infestans in potato. *Genetics,* 175: 879–889

Mauricio, R., 2001. Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. *Nat. Rev. Genet.,* 2: 370–381

Mir, R.R., N. Sreenivasulu, R.K. Varshney, M. Zaman–Allahand R. Trethowan, 2012. Integrated genomics, physiology and breeding approaches for improving drought tolerancein crops. *Theor. Appl. Genet.,* 125: 625–645

Myles, S., J. Peiffer, P.J. Brown, E.S. Ersoz, Z. Zhang, D.E. Costich and E.S. Buckler, 2009. Association mapping: critical considerations shift from genotyping to experimental design. *Plant Cell Online,* 21: 2194–2202

Neumann, K., B. Kobiljski, S. Denčić, R. Varshney and A. Börner, 2011. Genome–wide association mapping: a case study in bread wheat (*Triticum aestivum* L.). *Mol. Breed.,* 27: 37–58

Ochieng, J.W., A.W. Muigai and G.N. Ude, 2007. Localizing genes using linkage disequilibrium in plants: integrating lessons from the medical genetics. *Afr. J. Biotechnol.,* 6: 650–657

Palaisa, K.A., M. Morgante, M. Williams and A. Rafalski, 2003. Contrasting effects of selection on sequence diversity and linkage disequilibrium at two phytoene synthase loci. *Plant Cell Online,* 15: 1795–1806

Peng, J., Y. Ronin, T. Fahima, M.S. Röder, Y. Li, E. Nevo and A. Korol, 2003. Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. *Proc. Natl. Acad. Sci. USA,* 100: 2489–2494

Prasad, B., M. Babar, X. Xu, G. Bai and A. Klatt, 2009. Genetic diversity in the US hard red winter wheat cultivars as revealed by microsatellite markers. *Crop Past. Sci.,* 60: 16–24

Price, A.L., N.J. Patterson, R.M. Plenge, M.E. Weinblatt, N.A. Shadick and D. Reich, 2006. Principal components analysis corrects for stratification in genome–wide association studies. *Nat. Genet.,* 38: 904–909

Qian,Y., L. ZhuoKun, T. JiChun and H. ShuXiao. 2011. QTL mapping for coleoptile length and radicle length in wheat under different simulated moisture stresses. *Acta. Agron. Sin.,* 37: 294-301

Pritchard, J.K. and N.A. Rosenberg, 1999. Use of unlinked genetic markers to detect population stratification in association studies. *Amer. J. Human Genet.,* 65: 220–228

Pritchard, J.K. and M. Przeworski, 2001. Linkage disequilibrium in humans: models and data. *Amer. J. Human Genet.,* 69: 1–14

Pritchard, J.K.,M. Stephens and P. Donnelly, 2000. Inference of population structure using multilocus genotype data. *Genetics,* 155: 945–959

Rafalski, A., 2002. Applications of single nucleotide polymorphisms in crop genetics. *Curr. Opin. Plant Biol.,* 5: 94

Rao, H., O. Basha, N. Singh, K. Sato and H. Dhaliwal, 2007. Frequency distributions and composite interval mapping for QTL analysis in 'Steptoe'x 'Morex'barley mapping population. *Barley Genet. Newslett.,* 37: 5–20

Ravel, C., S. Praud, A. Murigneux, L. Linossier, M. Dardevet, F. Balfourier, P. Dufour, D. Brunel and G. Charmet, 2006. Identification of *Glu–B1–1* as a candidate gene for the quantity of high molecular weight glutenin in bread wheat (*Triticum aestivum* L.) by means of an association study. *Theor. Appl. Genet.,* 112: 738–743

Rebetzke, G., R. Richards, V. Fischer and B. Mickelson, 1999. Breeding long coleoptile, reduced height wheats. *Euphytica,* 106: 159–168

Rebetzke, G., R. Appels, A. Morrison, R. Richards, G. McDonald, M. Ellis, W. Spielmeyer and D. Bonnett, 2001. Quantitative trait loci on chromosome 4B for coleoptile length and early vigour in wheat (*Triticum aestivum* L.). *Crop Past. Sci.,* 52: 1221–1234

Remington, D.L., J.M. Thornsberry, Y. Matsuoka, L.M. Wilson, S.R. Whitt, J. Doebley, S. Kresovich, M.M. Goodman and E.S. Buckler, 2001. Structure of linkage disequilibrium and phenotypic associations in the maize genome. *Proc. Natl. Acad. Sci. USA,* 98: 11479–11484

Rhoné, B., A.L. Raquin and I. Goldringer, 2007. Strong linkage disequilibrium near the selected *Yr17* resistance gene in a wheat experimental population. *Theor. Appl. Genet.,* 114: 787–802

Risch, N. and K. Merikangas, 1996. The future of genetic studies of complex human diseases. *Sci. AAAS Weekly Paper Ed.,* 273: 1516–1517

Risch, N.J., 2000. Searching for genetic determinants in the new millennium. *Nature,* 405: 847–856

Rogowsky, P., F. Guidet, P. Langridge, K. Shepherd and R. Koebner, 1991. Isolation and characterization of wheat–rye recombinants involving chromosome arm 1DS of wheat. *Theor. Appl. Genet.,* 82: 537–544

Sajjad, M., S.H. khan, M.Q. Ahmad, A. Rasheed, A. Mujeeb–Kazi and I.A. Khan, 2013. Association Mapping Identifies QTLS on Wheat Chromosome 3A for Yield Related Traits.*CRC*.2013.0061

Salekdeh, G.H., M. Reynolds, J. Bennett and J. Boyer, 2009. Conceptual framework for drought phenotyping during molecular breeding. *Trends Plant sci.,* 14: 488–496

Sánchez-Pérez, R., J. Ballester, F. Dicenta, P. Arús and P. Martínez–Gómez, 2006. Comparison of SSR polymorphisms using automated capillary sequencers, and polyacrylamide and agarose gel electrophoresis: Implications for the assessment of genetic diversity and relatedness in almond. *Sci. Hortic.,* 108: 310–316

Sears, E., 1966. Chromosome mapping with the aid of telocentrics. *Proc 2nd Int Wheat Genet Symp. Hereditas Suppl,* 370–381

Sears, E.R., 1954: The aneuploids of common wheat. Uni of Missouri, College of Agric., Agric. Experiment Station, Missouri, USA

Semenov, M.A. and N.G. Halford, 2009. Identifying target traits and molecular mechanisms for wheat breeding under a changing climate. *J. Exp. Bot.,* 60: 2791–2804

Snape, J.W., M.J. Foulkes, J. Simmonds, M. Leverington, L.J. Fish, Y. Wang and M. Ciavarrella, 2007. Dissecting gene× environmental effects on wheat yields via QTL and physiological analysis. *Euphytica,* 154: 401–408

Somers, D.J., P. Isaac and K. Edwards, 2004. A high–density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.,* 109: 1105–1114

Sood, S., 2008: Molecular Characterization of Threshability Genes in Wheat ProQuest.

Stachel, M., T. Lelley, H. Grausgruber and J. Vollmann, 2000. Application of microsatellites in wheat (*Triticum aestivum* L.) for studying genetic differentiation caused by selection for adaptation and use. *Theor. Appl. Genet.,* 100: 242–248

Stich, B., A.E. Melchinger, M. Frisch, H.P. Maurer, M. Heckenberger and J.C. Reif, 2005. Linkage disequilibrium in European elite maize germplasm investigated with SSRs. *Theor. Appl. Genet.,* 111: 723–730

Tenaillon, M.I., M.C. Sawkins, A.D. Long, R.L. Gaut, J.F. Doebley and B.S. Gaut, 2001. Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* spp. mays L.). *Proc. Natl. Acad. Sci. USA,* 98: 9161–9166

Teulat, B., N. Zoumarou–Wallis, B. Rotter, M.B. Salem, H. Bahri and D. This, 2003. QTL for relative water content in field–grown barley and their stability across Mediterranean environments. *Theor. Appl. Genet.,* 108: 181–188

Tommasini, L., T. Schnurbusch, D. Fossati, F. Mascher and B. Keller, 2007. Association mapping of Stagonospora nodorum blotch resistance in modern European winter wheat varieties. *Theor. Appl. Genet.,* 115: 697–708

Tsilo, T.J., G.A. Hareland, S. Simsek, S. Chao and J.A. Anderson, 2010. Genome mapping of kernel characteristics in hard red spring wheat breeding lines. *Theor. Appl. Genet.,* 121: 717–730

Tuberosa, R. and S. Salvi, 2006. Genomics–based approaches to improve drought tolerance of crops. *Trends Plant Sci.,* 11: 405–412

Wang, M., J. Yan, J. Zhao, W. Song, X. Zhang, Y. Xiao and Y. Zheng, 2012. Genome–wide association study (GWAS) of resistance to head smut in maize. *Plant Sci.*, 196: 125–131

Yao, J., L. Wang, L. Liu, C. Zhao and Y. Zheng, 2009. Association mapping of agronomic traits on chromosome 2A of wheat. *Genetica,* 137: 67–75

Yu, J. and E.S. Buckler, 2006. Genetic association mapping and genome organization of maize. *Curr. Opin. Biotechnol.,* 17: 155–160

Yu, J., G. Pressoir, W.H. Briggs, I.V. Bi, M. Yamasaki, J.F. Doebley, M.D. McMullen, B.S. Gaut, D.M. Nielsen and J.B. Holland, 2005. A unified mixed–model method for association mapping that accounts for multiple levels of relatedness. *Nat. Genet.,* 38: 203–208

Zhang, J., C. Hao, Q. Ren, X. Chang, G. Liu and R. Jing, 2011. Association mapping of dynamic developmental plant height in common wheat. *Planta,* 234: 891–902

Zorić, M., D. Dodig, B. Kobiljski, S. Quarrie and J. Barnes, 2012. Population structure in a wheat core collection and genomic loci associated with yield under contrasting environments. *Genetica,* 140: 259–275

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