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MARKER-TRAIT ASSOCIATIONS FOR SPIKE-RELATED CHARACTERS IN A DOUBLED HAPLOID POPULATION OF WHEAT

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ABSTRACT

Phenotypic assessment of yield and its component traits needs to be performed in replicated trials under multiple environments. Mapping populations such as doubled haploids (DHs) are suitable material for studying marker-trait associations, because they can be evaluated repeatedly in different years or in variable environments, thus having considerable potential for the identification of reliable quantitative trait loci. In the present study a DH mapping population, generated from the crossing of two hexaploid high yield potential wheat varieties, Savannah (UK) and Renesansa (SRB), was used to identify associations between five microsatellite markers and seven spike-related traits. Statistically significant associations were observed between marker *GWM261* on 2DS chromosome and six spike-related traits: spike length, spikelet number per spike, sterile spikelet number per spike, grain weight, spike weight and spike index. The phenotypic variation explained by this marker was the highest for the trait spike length (from 8.3 to 24.7%).

Key words: breeding, microsatellites, QTL, *Triticum aestivum*, yield components.

INTRODUCTION

Grain yield is a complex trait that is usually controlled by a large number of quantitative trait loci (QTL) with minor effects. It is influenced by environmental factors, which make it difficult to be manipulated and improved in breeding programs. The grain yield of wheat is determined by three yield components, namely: productive spikes per unit area, kernels per spike and kernel weight, where the product of the first two components gives the total kernel number per unit area. Other traits, such as productive tillers per plant, spikelet number per spike, and number of fertile florets per spikelet, could all affect the total kernel number. Genetic and physiological dissection of these components and their relationships would facilitate achieving the maximum yield potential of the crop (Ma et al., 2007).

Over the last two decades wheat researchers have accumulated a range of genomic and genetic tools, particularly molecular markers, maps and genomic tools, which enable a precision of genetic analysis sufficient to locate genes controlling complex traits, such as yield (Snape et al., 2007). Microsatellites or SSR (simple sequence repeat) markers have gained considerable importance in wheat genetics and breeding owing to many desirable attributes including multiallelic nature, codominant inheritance, extensive genome coverage, chromosome specific location, amenability to automation and high throughput genotyping (Ganal and Röder, 2007). DH mapping populations are another very important tool for studying the complex wheat genome. They represent homozygous and true-breeding lines, which can be repeatedly phenotyped and are therefore ideal for studying complex traits with a quantitative inheritance, which may require replicated trials in several years and locations for accurate phenotyping and marker development (Tuvesson et al., 2007; Kobiljski et al., 2009). Seeds from DHs can be transferred between different laboratories for linkage mapping, to ensure that all collaborators examine identical material. Simulation studies demonstrate that DHs increase the efficiency of MAS (Marker Assisted Selection) and offer faster strategies

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for combining large numbers of genes with a minimum number of marker tests (Howes et al., 1998). In wheat, DH populations have been used to detect QTLs associated with plant height, flowering time, seed dormancy, weed competitiveness, and bread making quality parameters and to identify chromosomal regions involved in boron tolerance, tolerance to prevalent diseases, and traits associated with *in vitro* response, yield, and lodging resistance (see Landjeva et al., 2007, for detailed review).

In the present study a DH mapping population generated from crossing two hexaploid high-yielding wheat varieties was used to identify associations between five microsatellite markers and seven spike-related traits. The obtained results could contribute to the detection of markers that can be used in MAS for further improvement of wheat yield potential.

MATERIAL AND METHODS

A population of 177 DH lines was derived from crossing two hexaploid high-yielding wheat varieties, Savannah (UK) and Renesansa (SRB). These lines were evaluated in randomized block design field trials with three replications for seven spike-related characters of wheat. The plot size was 1.2 m^2 and each plot consisted of six rows separated from one another by 20 cm. Tests were conducted over six years, from 2003 to 2008, at the experiment field of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. Spike length (SL), in cm, was measured from the base of the rachis to the top of the uppermost spikelet excluding awns. Spikelet number per spike (SPN) included undeveloped or sterile spikelets at the basal part of the spike (SSPN). Spike weight (SW) and grain weight (GW) were expressed in grams. All these traits together with grain number (GN) were evaluated from five spikes sampled from five plants per plot. The spike index (SI) represents the ratio between GW and SW.

Total genomic DNA was extracted from fresh tissue of DH lines and parent varieties as described in Doyle and Doyle (1990). A set of five microsatellite markers (GWM18, GWM194, GWM261, PSP3071 and PSP3200), which, according to recent studies, are associated with some agronomic important traits (Quarrie et al., 2003; Suenaga et al., 2005; Kobiljski et al., 2007; Kumar et al., 2007; Chu et al., 2008), was applied. Primer sequences for markers were available from Röder et al. (1998) and at http://wheat.pw.usda.gov. Polymerase Chain Reactions (PCR) were performed in a volume of 20 µl in PCR System 9700 gold (Applied Biosystems). The reaction mixture contained 1xPCR buffer, 2 mM $MgCl_2$, 0.2 mM of each deoxynucleotide (dATP, dCTP, dGTP and dTTP), 0.5 µM of each primer (fluorescently labeled forward and unlabeled reverse primer), 2 units of Taq polymerase (Applied Biosystems) and 1.25 ng/µl of template DNA. After 5 min. at 94°, 45 cycles were performed with 30 sec. at 94°, 30 sec. at either 52° (for GWM18 and GWM194) or 62° (for GWM261, PSP3071 and PSP3200), 30 sec. at 72°, and the final extension step of 10 min. at 72°.

Fragment analysis was carried out on an automated laser fluorescence sequencer (ABI Genetic Analyzer 3130) using GeneMapper Software version 4.0.

Descriptive statistics and Pearson's correlation coefficient were estimated by Statistica 10.0 software. Marker-trait associations were analysed by the Single Marker Regression (SMR) method using QGene Software version 4.0 (Joehanes and Nelson, 2008). The following parameters were analysed: additive effect (Add effect), LOD (logarithm of odds), and regression (R^2). A LOD score of 3.0 was used for detecting putative QTLs.

RESULTS AND DISCUSSION

Trait analysis

The DH population Savannah/Renesansa offered fairly large phenotypic differences (Table 1) relevant for the detection of QTLs for traits of agronomic importance.

The distribution of the phenotypic data means for all spike-related traits (except for grain number) is given in Figure 1.

The polygenic nature of the traits was indicated by continuous frequency distributions, with transgressive segregants.

Table 1. Descriptive statistics for the seven spike-related traits

Statistics	SL (cm)	SPN	SSPN	SW (g)	GW (g)	GN	SI
Mean	10.35	20.83	1.21	2.59	2.00	53.06	0.76
Minimum	8.90	17.40	0.40	1.73	0.99	41.70	0.55
Maximum	12.70	24.50	2.40	3.26	2.68	67.60	0.84
Standard deviation	0.75	1.36	0.41	0.31	0.30	4.53	0.04
CV (%)	7.25	6.55	34.21	11.94	14.98	8.55	5.20

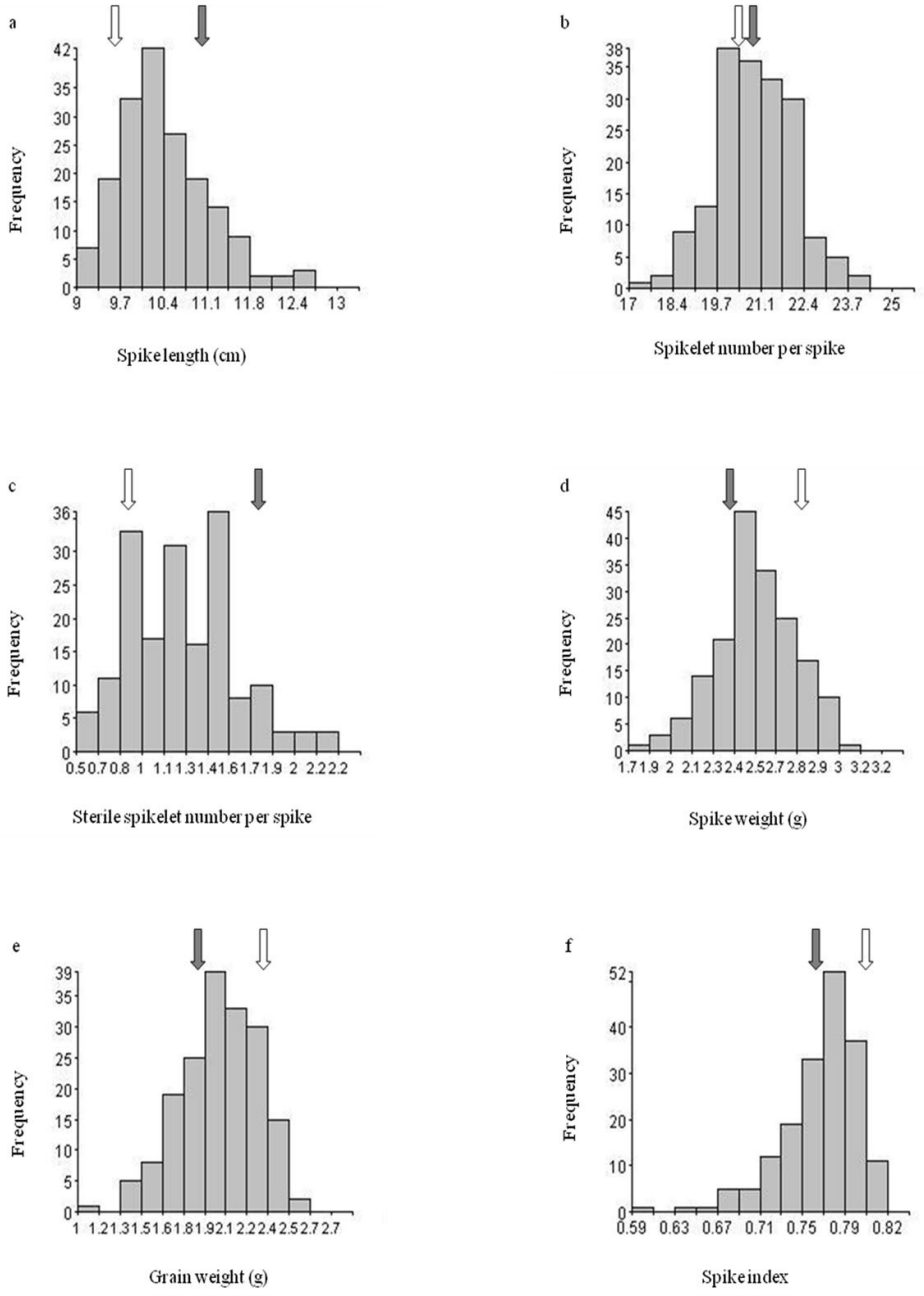

Figure 1. Phenotypic distributions for the trait means of the DHs:
a) SL; b) SPN; c) SSPN; d) SW; e) GW; f) SI.
Filled arrows = means of 'Savannah'; empty arrows = means of 'Renesansa'

The correlation coefficients were calculated to determine the degree of association between traits. According to Paterson et al. (1991), QTLs of correlated traits often have similar positions on the map. The correlation coefficients were generally significant for all trait combinations (Table 2). Spike length was positively correlated with spikelet number per spike and sterile spikelet number per spike and negatively correlated with spike weight, grain weight and spike index. Spikelet number per spike was negatively correlated with grain weight and spike index.

Table 2. Significant phenotypic trait correlations with mean values for each genotype

Traits	SL	SPN	SSPN	SW	GW	GN	SI
SL	1.000						
SPN	0.601**	1.000					
SSPN	0.514**	0.612**	1.000				
SW	-0.169*	-0.064	-0.398**	1.000			
GW	-0.295**	-0.156*	-0.421**	0.977**	1.000		
GN	-0.083	0.150*	-0.279**	0.268**	0.221**	1.000	
SI	-0.571**	-0.355**	-0.358**	0.607**	0.744**	0.060	1.000

Significance level indicated with asterisks as follows: * P< 0.05; ** P< 0.01.

Molecular analysis

A total of five polymorphic microsatellite markers were used to genotype the mapping population (Table 3).

Table 3. Alleles detected at analysed SSR loci in parent varieties

Locus	Alelle (bp)	
	Savannah	Renesansa
Xgwm18-1B	190	186
Xgwm261-2D	175	193
Xgwm194-4D	110	130
Xpsp3071-6A	161	153
Xpsp3200-6D	168	171

Of all the markers analysed in the present paper, marker GWM261 was the most interesting one for many authors, considering its association with reducing height gene *Rht8* (Korzun et al., 1998). Molecular screening of over 800 wheat varieties from 20 countries showed that 90% carried GWM261 alleles with 165, 174 or 192 base pairs (bp) (Worland et al., 2001). The 192 bp allele was the most frequent in southern and south-eastern European varieties, while the allele of 174 bp predominated in northern and western wheat material (Worland et al., 2001; Röder et al., 2002; Kobiljski et al., 2006). Varieties carrying the 192 bp allele, inherited from the variety Akakomugi or a Strampelli wheat ancestor (Ellis et al., 2007), showed a height reduction of 7-8 cm. Varieties carrying the 174 bp allele showed a height reduction of approximately 3 cm, and varieties carrying the 165 bp allele were of regular height (Korzun et al., 1998; Worland et al., 2001). In our study the allele of 193 bp was detected in the variety Renesansa, while the allele of 175 bp was found in the variety Savannah (Table 3). Those alleles would be equivalent to fragments of 192 bp and 174 bp detected in previous studies (Korzun et al., 1998; Worland et al., 2001; Kobiljski et al., 2006), and differences of 1 bp could be the consequence of using different systems for fragment analysis. For various fragment-analysis procedures, including analysis on automated sequencers, it was found that the absolute allele sizes varied by a few base pairs in different runs. It is therefore necessary to include a number of reference varieties in each run, which amplify representative alleles and can be used as direct comparisons for naming alleles (Röder et al., 2002).

QTL analysis

By SMR, consistent QTL for six traits (SL, SPN, SSPN, SW, GW and SI) were coincident with *Xgwm261* loci on 2DS chromosome. This QTL cluster was consistent

with the results of correlation analysis (Table 2) indicating that traits are controlled by closely linked QTLs or by the action of QTL with pleiotropic effects. Statistically significant associations between marker GWM261 and SL were detected in all analysed years, explaining 8.3-24.7% of the phenotypic variation. The QTL on 2DS explained 7.6-17.8% and 8.6-18.7% of the phenotypic variation for SPN and SSPN, respectively, in four out of the six years analysed. For SW, GW and SI, significant associations with *Xgwm261* loci were consistent in three of the analysed years, explaining 8.1-13.1%, 10.0-14.7% and 8.2-12.4% of the phenotypic variation, respectively (Table 4).

Table 4. Single Marker Regression analysis for seven spike-related traits (LOD > 3.0)

Trait	Chromosome	Loci	LOD	R^2 (%)	Add effect	Favorable allele
2003						
SL	2D	*Xgwm261*	**7.775**	**18.3**	-0.454	S
	6A	*Xpsp3071*	2.727	6.8	-0.283	
SSPN	2D	*Xgwm261*	**7.943**	**18.7**	-0.283	S
	4D	*Xgwm194*	2.929	7.3	-0.194	
SW	2D	*Xgwm261*	**3.786**	**9.4**	0.105	R
	4D	*Xgwm194*	2.511	6.3	0.095	
GW	2D	*Xgwm261*	**5.274**	**12.8**	0.111	R
	4D	*Xgwm194*	2.575	6.5	0.086	
SI	2D	*Xgwm261*	**5.096**	**12.4**	0.020	R
2004						
SL	2D	*Xgwm261*	**10.924**	**24.7**	-0.584	S
SPN	2D	*Xgwm261*	**7.529**	**17.8**	-0.817	S
2005						
SL	2D	*Xgwm261*	**10.134**	**23.2**	-0.432	S
SPN	2D	*Xgwm261*	**4.097**	**10.1**	-0.503	S
	6A	*Xpsp3071*	2.347	5.9	0.393	
2006						
SL	2D	*Xgwm261*	**7.425**	**17.6**	-0.267	S
SN	2D	*Xgwm261*	**3.036**	**7.6**	-0.410	S
SSPN	2D	*Xgwm261*	**3.632**	**9.0**	-0.187	S
SI	2D	*Xgwm261*	**4.155**	**10.2**	0.016	R
2007						
SL	2D	*Xgwm261*	**3.329**	**8.3**	-0.196	S
SPN	2D	*Xgwm261*	**3.233**	**8.1**	-0.472	S
SSPN	2D	*Xgwm261*	**4.851**	**11.9**	-0.216	S
SW	2D	*Xgwm261*	**5.376**	**13.1**	0.168	R
GW	2D	*Xgwm261*	**6.125**	**14.7**	0.167	R
2008						
SL	2D	*Xgwm261*	**7.490**	**17.7**	-0.459	S
SSPN	2D	*Xgwm261*	**3.462**	**8.6**	-0.192	S
SW	2D	*Xgwm261*	**3.260**	**8.1**	0.115	R
GW	2D	*Xgwm261*	**4.034**	**10.0**	0.118	R
SI	2D	*Xgwm261*	**3.273**	**8.2**	0.010	R

R^2 - indicates the total amount of variation explained by the markers
*The allele that increased the trait is either S for Savannah or R for Renesansa

The *Rht8* gene is located 20.9 cM proximal to the gene *Ppd-D1* for photoperiod insensitivity, while marker GWM261 was found to be 0.6 cM away from *Rht8* (Worland et al., 1998; Korzun et al., 1998). In Europe *Ppd-D1* typically accelerates flowering time by 6-14 days depending on the growing region, with significant pleiotropic effect on plant height, tiller number, spikelet number and spikelet fertility. The shortened life cycle resulted in a direct reduction in plant height and gave an average reduction of two spikelets in the ear. However, *Ppd-D1* increased spikelet fertility, which more than compensated for reductions in spikelet number and promoted increase in the number of grains per ear. In Southern and Eastern Europe, where summer conditions are usually hot and dry, genotypes with insensitivity to photoperiod produced larger grain than genotypes with the sensitive allele and consequently had significantly higher yields (Worland et al., 1996; Snape et al., 2001). The allele of 193 bp, detected in the variety Renesansa, had negative effects on SL, SPN and SSPN, but positive effects on GW, SW and SI (Table 4), which could be a consequence of linkage with the *Ppd-D1* gene.

Suenaga et al. (2005) determined one major QTL for spike length on chromosome 2DS that was stable to the greenhouse and field environments. The SSR locus *Xgwm261* was on or close to the peak of the likelihood ratio contour for this trait. The phenotype variation explained by this QTL varied from 19.3 to 33.3%, which is in accordance with our results (Table 4.). In the paper of Kumar et al. (2007), the marker interval *Xgwm261-Xcdo1379* was coincident with QTL for grain yield, harvest index, SL and SPN. Ma et al. (2007) also detected QTL for spike length in the marker interval on 2DS which included locus *Xgwm261*, but this locus was not associated with SPN and SSPN.

According to the results of Börner et al. (2002), the number of grains per ear and the trait grain weight per ear were determined by major QTL on chromosome arm 2DS. Dholakia et al. (2003) detected QTL on 2DS, which explained 3.5% of the phenotypic variation for grain weight. This QTL was associated with marker GWM261. Kumar et al. (2007) located QTL for GN (R2 = 12.55%) in the chromosome segment *Xgwm296-Xgwm261*. In this study, where we used SMR analysis, marker GWM261 explained between 10 and 14.7% of the phenotypic variation for GW in three of the six years studied (Table 4), while statistically significant association between marker GWM261 and GN were not detected (data not shown).

The importance of the SSR locus *Xgwm261* on 2DS chromosome for improving bread wheat yield potential could be also a resultant of its association with heading and flowering times (Xu et al., 2005; Narasimhamoorthy et al., 2006; Trkulja et al., 2011) and resistance to Fusarium head blight (Mao et al., 2010).

In the study of Chu et al. (2008) a major QTL on chromosome 4DL with peaking in marker interval *Xbarc48* - *Xgwm194* was associated with spikelet number per spike. This QTL was significant for both the greenhouse and field data, and explained 29 and 15% of the phenotypic variation, respectively. According to the results of the present paper, statistically significant associations between marker GWM194 and SPN were not detected. The LOD score for this SSR marker was around the threshold value for SSPN, SW and GW, but only in the year 2003 (Table 4).

SSR loci *Xpsp3071* on 6A chromosome had LOD scores near the threshold value - 2.727 for SL in 2003 and for 2.347 SPN in 2005 (Table 4). This marker showed significant associations with yield under drought in South-East Serbia, where yield components most related with this effect were thousand grain weight ($P<0.001$) and GN ($P<0.05$) (Quarrie et al., 2003).

The results of this study are based on the analysis of the biparental population Savannah/Renesansa. The identified marker-trait associations should be validated in genetic material relevant to Serbian breeding programs, so that they can be used in MAS for further improvement of wheat. Positive expectations could be based on the recent

study of Neumman et al. (2011). In that paper a genome-wide association study was reported, in which a large number of diversity array technology (DArT) markers was used to genotype a winter wheat core collection of 96 accessions from 21 countries across five continents. Significant marker-trait associations for GN, GW, SW, SL, SPN and SSPN were detected on chromosome 2DS, underscoring the importance of this region in the MAS targeting of these spike-related characters.

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CONCLUSIONS

In the present study, significant genetic variability for the spike-related traits was found in the mapping population Savannah/Renesansa, which makes it suitable for the detection of important QTLs. In this genetic background, significant associations were found between marker *GWM261* on 2DS chromosome and six traits: spike length, spikelet number per spike, sterile spikelet number per spike, grain weight, spike weight and spike index. The obtained marker-trait associations should be validated in a relevant breeding material in order to check their applicability in MAS.

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