

Marker traits association of flag and second leaf traits in bread wheat (*Triticum aestivum* L.)

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Abstract – Leaf traits (leaf length, width, and area) are closely associated with photosynthetic ability and grain yield in bread wheat (*Triticum aestivum* L.). Identifying QTLs that control leaf related traits under stressed environment is very useful for marker assisted selection (MAS). QTL studies on flag and second leaf traits were rarely reported. In this study, marker traits associations of leaf traits using a collection of bread wheat accessions were performed. Using MLM and GLM approaches, at $-\log_{10} P \geq 3$, a total 64 SNPs markers associated with flag and second leaf traits were identified on all chromosomes except for 3A, 4D, 5A, 6B and 7D. QTLs identified on chromosomes 7A and 7B were found to have a pleiotropic effect on almost leaf traits controlling FLA, FLL, FLW, SLA, and SLL. This region could serve as a target for fine mapping and marker assisted breeding in bread wheat (*Triticum aestivum* L.).

Keywords: Leaf traits, Bread wheat (*Triticum aestivum* L.), SNP Markers, QTL mapping, semi arid climate

1. Introduction

Bread wheat (*Triticum aestivum* L.), one of the most important food crop along with rice and maize, is grown under rain fed climate in semi-arid and arid region. Grain yield is a complex trait controlled by several genetic and environmental factors. It is also associated with the carbohydrate accumulation and the photosynthetic activity attributed to the top two leaves organ. Flag leaf area contributed to more than 50 % to the total photosynthetic activity (Xu et al.1995) and about 41–43 % to carbohydrates needed for grain filling (Sharma et al. 2003). Leaf area and size is an indicator of potential grain yield (Monyo et al.1973). Yaopeng (2015) reported a negative correlation between leaf related traits and grain yield suggesting that a small leaves would contribute to high yield. In wheat (*Triticum aestivum* L.), a large number of morphological and physiological traits are linked to drought tolerance (Del Pozo et al. 2012). Under drought conditions, rolled leaves and reduction in leaf area is considered as a positive adaptation to avoid excessive transpiration loss. Understanding the genetic basis of leaf traits is of importance in the breeding programs of bread wheat. Several studies have reported about the QTLs controlling flag leaf morphological traits such as flag leaf area (FLA), flag leaf length (FLL), flag leaf width (FLW). Little is known about QTL controlling second leaf morphological traits. In a RIL population, 38 QTLs were found to control FLW, FLL and FLA on 12 chromosomes. Of these QTLs, five on chromosomes 4B (three QTLs) and 6B (two QTLs) were major QTLs controlling FLW (Fan et al. 2015). Two QTLs for leaf width were mapped to chromosomes 2A and 6A, with a phenotypic variance of 6% and 14% respectively (Spielmeyer et al. 2007). On chromosome 5A, a QTL controlling flag leaf width was found in Nanda2419/Wangshuibai recombinant inbred line population (Ma et al. 2008) and (Jia et al. 2013).

In this report we investigated (i) the correlation between flag leaf and second leaf related traits including; flag leaf area (FLA), flag leaf length (FLL), flag leaf width (FLW), second leaf area (SLA), second leaf length (SLL), and second leaf width (SLW), and (ii) a marker trait association using 134 bread wheat accessions was also carried out to identify SNPs linked to leaf traits in bread wheat (*Triticum aestivum* L.).



2. Materials and Methods

2.1. Genetic materials Genotyping

The genetic material evaluated in this study including 134 bread wheat genotypes (*Triticum aestivum* L.) was selected from the U.S National Plant Germplasm System (NPGS). All accessions were typed with 1744 SNP markers selected from the iSelect 90K array containing 90,000 wheat SNP markers (Cavanagh et al. 2013) and (Wang et al. 2014). Genotypic data of the 134 selected accessions are publicly available on <https://triticeaetoolbox.org/wheat>. The positions of SNP markers along chromosomes in terms of genetic distance (cM) were based on the wheat 2014 consensus genetic map (Wang et al. 2014). Markers were removed if they were either monomorphic or exhibited allele frequencies of less than 5% (minor alleles).

2.2. Field trial and leaf traits measurement

Field trials were conducted under rain fed condition in the research station, EIKEf, Tunisia, characterized by a semi arid climate with an annual rainfall below 380 mm. All accessions were planted in two rows, 2.5 m long and a row spacing of 25 cm. The measurement of flag leaf and second leaf related traits was performed using Image J (Abramoff MD 2004), a Java-based image processing program developed at the National Institutes of Health. Leaf length was measured at 10 days after heading, from the beginning of the ligule to the top of the leaf and leaf width was taken at the widest part of the leaf.

2.3. Statistical analysis

For each of the leaf traits, descriptive statistical measures were obtained based on the average data of the 134 bread wheat accessions. The data on all traits were subjected to variance. Correlation matrix between all leaf traits was performed using the R package: Performance Analytics (Brian GP 2014).

2.4. Association Mapping (AM)

The software TASSEL v.5.0 (Bradbury PJ 2007) was used to perform association mapping of leaf traits in bread wheat. For best linear unbiased estimates, a general linear model (GLM) and the mixed linear model (MLM) procedure taking into account estimated population structure (Q), and kinship matrix (K) were used. At a threshold of $-\log_{10} P \geq 3.0$, a significant marker trait association is declared.

3. Results

3.1. Phenotypic analysis

The results from the descriptive statistics and the correlation matrix of the investigated characteristics are presented in Table 1 and in figure 1. The flag leaf area (FLA) ranged from 6.5 cm² to 34 cm² with a mean value of 14.53 cm. In all accessions, the second leaf shows a larger area than the flag leaf with a mean of 22.79 cm². The second leaf was longer than the flag leaf with 20.39 cm and 13.07 cm respectively. In both traits, the flag leaf represents 65% of the second leaf. All skewness and kurtosis values were less than 1.0 except for FLA, indicative of continuous variation and a quantitative genetic basis controlled by multiple genes. All leaf traits are positively correlated with each other ($P < 0.001$).

3.2. Association mapping of leaf traits

Using GLM approach, without taking into account the kinship matrix (Q) and the estimated population structure (K), 50 associated SNPs to the studied traits were identified on all chromosomes except for 2D, 3A, 3D, 4D, 5A, and 6B. Using MLM approach, 14 significant markers associated to leaf related traits were found except for the second leaf length (SLL). A summary of the results of MTAs detected using MLM and GLM are given in the table 2 and 3.

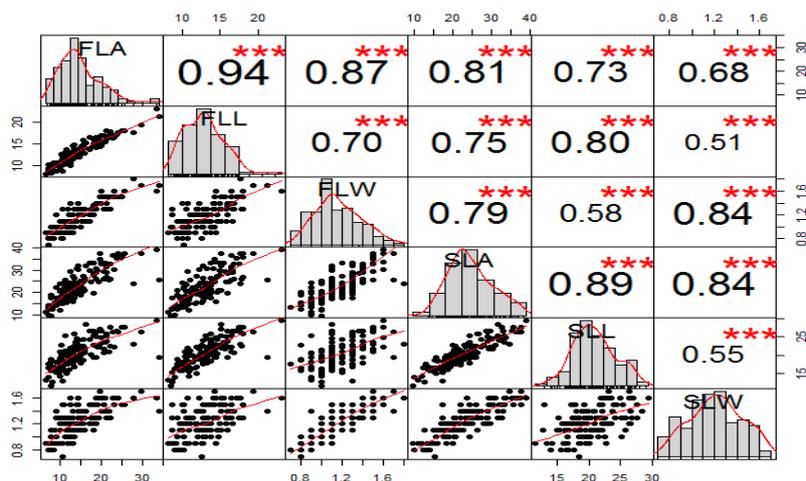


Figure 1. Correlation matrix of all leaf related traits using R package: PerformanceAnalytics. All traits show a high positive correlation. The variation of leaf traits approximates a statistical normal distribution suggesting its complex genetic inheritance. *** Correlation is significant at the $P < 0.001$.

Table 1. Descriptive statistics of leaf related traits

	Range	Minimum	Maximum	Sum	Mean	Std. Deviation	Variance	CV%	Skewness	Kurtosis
FLA	27.5	6.5	34	1946.4	14.53	5.28	27.9	36	1.15	1.73
FLL	15	8.1	23.1	1750.8	13.07	2.79	7.76	21	0.62	0.53
FLW	1.1	0.7	1.8	156.86	1.17	0.22	0.05	19	0.4	-0.22
SLA	29.5	9.6	39.1	3053.87	22.79	6.74	45.36	30	0.43	-0.4
SLL	17.8	11.6	29.4	2732.9	20.39	3.67	13.5	18	0.23	-0.35
SLW	1	0.7	1.7	163.7	1.22	0.23	0.05	19	-0.01	-0.7

Table 2. Results of the association mapping using MLM approach with $-\log_{10}(p) \geq 3$

Traits	Markers	Chr.	Pos. (Kb)	$-\log_{10}(p)$	R^2
FLA	IWA7233	7B	58172	5.77E-4	0.13
	IWA5802	5B	188578	9E-4	0.12
FLL	IWA8059	3D	148409	1.92E-04	0.16
	IWA5381	4D	80677	1.68E-04	0.16
FLW	IWA7233	7B	58172	1.72E-4	0.15
	IWA4746	2D	8520	2.4E-4	0.15
SLA	IWA3536	1A	71096	8E-4	0.12
	IWA4746	2D	8520	3.84E-4	0.14
	IWA7816	6D	87174	5.12E-4	0.13
SLL	IWA7711	4B	69779	6.8E-4	0.12
	IWA3623	5A	98901	9.4E-4	0.12
	IWA3114	7B	68835	4E-4	0.14
	IWA3608	4B	73860	4.38E-4	0.14
SLW	IWA4746	2D	8520	3.8E-4	0.14

FLA, flag leaf area; FLL, flag leaf length; FLW, flag leaf width; SLA, second leaf area; SLL, second leaf length; SLW, second leaf width

3.3. Flag leaf area (FLA)

MLM approach identified two SNP markers associated to FLA were located on chromosome 5B and chromosome 7B respectively. The phenotypic variation ranged from 12% and 13%. GLM approach identified 11 SNP markers located on chromosome 1A, 1B, 2B, 3B (2), 4A, 4B, 5B, 5D, 7A, 7B. The phenotypic variation ranged from 8% and 10%.

3.4. Flag leaf length (FLL)

Using MLM approach, two SNPs markers associated with FLL explaining 16% each of the total phenotypic variation were identified on chromosome 3D and 4D. Only 3 QTLs were identified using GLM approach on chromosome 1A, 7A and 7B.

3.5. Flag leaf width (FLW)

Using MLM approach, three SNP markers associated to FLW were located on chromosome 1A, 2D, 7B, with a phenotypic variation ranging from 12% to 15%. The QTL located on chromosome 7B has pleiotropic effect on FLA. Thirteen SNPs were identified using GLM approach, with a phenotypic variation ranging from 8% to 14%. Both approaches shared the SNP marker IWA7233 located on chromosome 7B, associated with FLW and FLA.

3.6. Second leaf area (SLA)

Four SNP markers were found using MLM with a phenotypic variation ranging from 12% and 14%. Those located on chromosome 2D and 6D were detected to control FLW and FLL respectively. Ten SNP markers were identified using GLM approach. Markers found on chromosome 1A, 1B, 2B, 3B, 4A were also associated with FLA. Those located on chromosome 1D, 2A have pleiotropic effect on FLW. Using GLM, only the SNP markers located on chr.6D was specific to SLA.

3.7. Second leaf length (SLL)

No marker trait association was found for SLL using MLM. However, 4 SNP located on chromosomes 1A, 4B, 6A, and 7B were associated with SLL. SNP located on chromosome 7B had a pleiotropic effect on SLA, FLL and FLA.

3.8. Second leaf width (SLW)

MLM approach identified 3 SNP markers located on chromosomes 2D, 4B, and 7B with 14% phenotypic variation. GLM approach identified 9 SNP markers with a phenotypic variation ranging from 8% to 12%.

4. Discussion

Many studies indicated the importance of leaf characteristics such as shape, size, and width of the cereal leaf in relation to yield. A positive correlation between wheat flag leaf and yield was noted by Simon et al. (1999) and Quarrie et al. (2006). Identifying QTLs that control leaf related traits under stressed environment is very useful for marker assisted selection (MAS). However a few studies reported the genetic control of flag leaf and second leaf characteristics. A total of sixty-four QTLs were identified including eleven QTLs for FLA, five for FLL, fourteen for FLW, thirteen for SLA, four for SLL and eleven for SLW. These QTLs were distributed in the wheat genome. Among them, 22 were on genome A (34.3%), 32 on genome B (50%) and 10 on D genome (15.6%). The phenotypic variance explained by each QTL ranged from 8% to 13% for FLA, 8% to 16% for FLL, 8% to 15% for FLW, 8% to 14% for SLA, 8% to 11% for SLL, and 8% to 14% for SLW. QTL co-localization was found for several markers suggesting their pleiotropic effect. The QTL detected on chr.7B controlling FLA has also a pleiotropic effect on FLL, FLW, SLA and SLL. The detected marker IWA7816 on chr. 6D control both FLL and SLA. The QTL located on chr.2D has a significant effect on FLW, SLA and SLW. Previously, several QTLs controlling leaf characteristics were found. The QTL located on the long arm of chr.1A controlling FLL coincide with the *QFll.cz-1A.3* (Yaopeng 2015). The co-localization of both QTLs controlling FLA and FLW on chr.1B was also reported by (Qihong Wu 2015) by the identification of *QFla.cau-1B* and *QFlw.cau-1B.2*. QTL detected on chromosome 2D controlling FLW coincides with *QFlw.cau-2* and *QFlw.tam-2D* (Qihong Wu et al. 2015) and (Mason et al. 2011). In this study, QTLs located on chr. 7A and 7B were found to have a pleiotropic on almost leaf traits effect that control FLA, FLL, FLW, SLA, and SLL. This 7B region was only reported by (Qihong Wu 2015) by the identification of *QFla.cau-7B.2* controlling FLA.

Table 3. Results of the association mapping using GLM approach with $-\log_{10}(p) \geq 3$

Traits	Markers	Chr.	Pos. (Kb)	$-\log_{10}(p)$	R^2	
FLA	IWA6655	3B	65554	1.78E-4	0.10	
	IWA4594	7A	208714	2.35E-4	0.09	
	IWA1692	4A	66279	2.37E-4	0.09	
	IWA7422	1B	114576	3.4E-4	0.09	
	IWA4121	1A	155800	3.4E-4	0.09	
	IWA3780	4B	104788	3.8E-4	0.09	
	IWA7233	7B	58172	4.1E-4	0.09	
	IWA8053	3B	85517	5.7E-4	0.09	
	IWA4135	2B	84691	6.8E-4	0.08	
	IWA5802	5B	188578	7.8E-4	0.08	
	IWA302	5D	76951	7.9E-4	0.08	
	FLL	IWA2997	7B	69391	2.5E-4	0.10
		IWA4594	7A	208714	7E-4	0.08
FLW	IWA4121	1A	155800	9.9E-4	0.08	
	IWA6655	3B	65554	8.15E-6	0.14	
	IWA624	3B	80129	9.45E-6	0.14	
	IWA1692	4A	66279	1.75E-5	0.13	
	IWA7233	7B	58172	4.9E-5	0.11	
	IWA7422	1B	114576	5.3E-5	0.11	
	IWA2585	4A	48981	7E-5	0.11	
	IWA4135	2B	84691	3.3E-4	0.09	
	IWA2755	4B	73840	3.4E-4	0.09	
	IWA4594	7A	208714	4.7E-4	0.09	
	IWA8040	2A	142355	4.7E-4	0.09	
	IWA7276	1D	87358	7E-4	0.08	
	IWA4757	5B	40546	8.5E-4	0.08	
	IWA2273	7D	139280	9.5E-4	0.08	
	SLA	IWA7422	1B	114576	2E-4	0.09
		IWA8040	2A	142355	2.3E-4	0.09
		IWA2979	7B	69391	2.8E-4	0.09
IWA4594		7A	208714	5.26E-4	0.09	
IWA7276		1D	87358	5.7E-4	0.09	
IWA4135		2B	84691	5.8E-4	0.09	
IWA4121		1A	155800	5.9E-4	0.09	
IWA7816		6D	87174	5.9E-4	0.09	
IWA493		4A	48524	6.7E-4	0.08	
IWA4685		3B	63962	8E-4	0.08	
SLL	IWA2997	7B	69391	3.64E-4	0.11	
	IWA1081	1A	102319	7E-4	0.08	
	IWA4115	4B	86990	8.6E-4	0.08	
	IWA2295	6A	78502	9.33E-4	0.08	
SLW	IWA7422	1B	114576	3.95E-5	0.12	
	IWA2585	4A	48981	1.22E-4	0.10	
	IWA1692	4A	66279	1.4E-4	0.10	
	IWA624	3B	80129	1.6E-4	0.10	
	IWA2755	4B	73840	2E-4	0.10	
	IWA4594	7A	208714	3.95E-4	0.09	
	IWA4757	5B	40546	5.26E-4	0.09	
	IWA3536	1A	40546	8.45E-4	0.08	
IWA8040	2A	71096	9.4E-4	0.08		
			142355			

FLA, flag leaf area; FLL, flag leaf length; FLW, flag leaf width; SLA, second leaf area; SLL, second leaf length; SLW, second leaf width

5. Conclusion

In this study, we succeeded to identify several SNP associated markers to flag and second leaf characteristics in bread wheat (*Triticum aestivum* L.). These findings will be useful for markers assisted breeding of wheat under semi arid climate under drought conditions.

6. Acknowledgments

We thank the direction of the PRRDANOSA-EIKeF and the Technical Support Section of INRAT research station for field management and soil preparation.

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