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MARKER TRAIT ASSOCIATION STUDY IN WHEAT GENOTYPES UNDER NORMAL AND DROUGHT CONDITIONS USING SSR MARKERS

SONIA MUNIR[1*], MUHAMMAD AZMAT[2], FAROOQ AHMAD KHAN[1], USMAN SALEEM[2], SALMAN SALEEM[3] AND MUHAMMAD SALMAN[4]

[1]Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan
[2]Department of Plant Breeding and Genetics, college of Agriculture, University of Sargodha, Sargodhe, Pakistan
[3]MSM&F Program, National Agricultural Research Center, Islamabad, Pakistan
[4]Institute of plant breeding and genetics, MNS-University of agriculture, Multan, Pakistan
**Corresponding author's email: Soniapbg23@gmail.com*

Abstract

The study was conducted in the department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. In total 226 genotypes were grown in polythene bags and drought stress was imposed using PEG-6000 (20%) and data were collected for shoot and root length, root shoot ratio, fresh weight and dry weight. The genotypes which showed good performance for all the parameters were selected (100 genotypes) and evaluated during the next two years 2013-14 and 2014-15 under both the normal and meiotic stage (pre-anthesis) drought stress conditions. The data for the following 14 traits were recorded, numbers of days to heading, numbers of days to maturity, plants height, peduncle length, spike length, leaf length, leaf width, leaf area, spikelets / spike, grains number / spike, grains weight / spike, tillers / plant, 1000 grain weight and with yield per plant. The significant results were shown by the genotypes, environment and (G x E) their interaction for almost all the parameters studied. The DNA of these 100 genotypes was extracted and 25 SSR markers were applied. It was concluded that about nine of the markers were linked with nine parameters studied and the level of probable was taken as one percent and phenotypic changes ranged between 14 to 32% under the water stress condition. And under the normal, irrigated conditions only 6 markers were found to be linked and the phenotypic variability ranged from 14 to 22%. The SSR marker CFA2086 showed significant association with 1000 grain weight under normal as well as under drought conditions. The 5 markers such as WMC382, CFA2086, CFA2121, CFA2263 and WMC2610 were associated with spikelets per spike which could be considered as trait specific MTA.

Key words: Marker trait association, Breeding wheat, Irrigated and drought conditions, Diversity, Yield parameters.

Introduction

Human population depends directly on plants to get their food. Common wheat is a major crop which is cultivated not only in Pakistan but also in all over the world as a major food crop. Pakistan is far behind to meet the increasing demand of population and we must work efficiently through proficient source management such as the breeders who try to produce new varieties that can also give more production in a wide range of environments. Wheat has the best capability to adopt the cool growing conditions (Modhej *et al.*, 2008). It is now being grown in areas which are too hot and are not good for its cultivation and growth.

Because of the continuous increase in global warming it is necessary to develop the genetically modified crops which are resistant to drought. There are different morphologic, physiologic, biochemically and at molecular responses shown by crop plants. The most critical stage for drought stress is reproductive stage of plants. Drought is a very complex parameter that is being polygenically controlled whose expression is affected by different environmental factors. Due to multiple genes the breeding for drought tolerance is very difficult. There is a dire need to apply novel molecular techniques, mapping of QTL and patterns of expression of genes to develop such genotypes which can tolerate the drought stress. The wheat has various genes which are involved in drought stress tolerance. These genes synthesize many kinds of chemicals such in the form of proteins and enzyme (Nezhad Ahmadi *et al.* 2013).

Association mapping is a good technique which provides reliable information regarding the genetic structure of different lines also called as genotypes which are very helpful to cross the genotypes for the desired characters as reported by Maccaferri *et al*., (2011). It is the demand of the modern era to produce the varieties which can gave more production even in the harsh environment so that the increasing population can be fed. As the yield in cereal crops such as wheat is a very complex parameter and its nature of inheritance is polygenic that is controlled by many genes and increase one thing reduces the other therefore a multidisciplinary technique should be adopted to know the problem (Tubrosa *et al.* 2007, Flury *et al*., 2010 and Uga 2013).

Materials and Methods

Two hundred and twenty-six genotypes were grown in polythene bags and PEG-6000 (20%) solution was applied to create drought and 100 high yielding were selected for further analysis. The selected 100 genotypes were grown during the years 2013-2014 and 2014-2015 at experimental site of department of PBG, Agriculture University, and Faisalabad. Randomized and completely blocked (RCBD) design was followed (2) replications in a form of 5 meters plot length with 15 centimeters distance was taken between plants and about 30 centimeters in rows. The crop was sown using the dibbling procedure in which the holes were formed using the dibbler and then two or three grains were dropped in each hole and then gently covered with moist soil.

The randomization among the hundred (100) genotypes was done using Crops Stat-v-7.2-software. At meiotic stage the irrigation was stopped. Under normal conditions 4 irrigations were applied but in case of drought irrigation at anthesis or at flowering was discontinued. All agronomical and plants protections measured were undertaken to get healthy crop. The data were collected for 10 guarded plants and mean value was used for further analysis The data were recorded for 14 traits which were as follows, numbers of days to heading, numbers of days to maturity, plants height, peduncle length, spike length, leaf length, leaf width, leaf area, spikelets number per spike, grains number per spike, grain weight per spike, tillers / plant, 1000 grain weight and yield / plant.

Isolation of deoxyribonucleic acid (DNA): The deoxyribonucleic acid (DNA) was isolated following the procedure of Paterson and Kresovich 1993 in which C tab was applied**.**

Preparing the Gel: The gel was prepared for quality determination of the isolated deoxyribonucleic acid (DNA) through C tab procedure. 2% concentration of Gel was applied for quality determination of deoxyribonucleic acid. The flask of 200 ml volume was taken and got ready for gel preparation by washing it with moderately warm distilled water. Now it was filled with double distilled water up to the mark of 200 ml volume. Then 4 gram of good quality agarose was weighed in an electric balance. The measured agarose was gently lifted and poured in the flask containing 196 ml water. Now it was made up to 200 ml mark. The flask was stirred gently to mix them uniformly. Then put the flask in oven till it was seeing clear. If it was not, then again put in the oven for further few moments till it made clear. Now put the flask in cold water so that it may cool down and took pipette and drop 2 ml of ethidium bromide in the gel. Put the gel tank in the fume hood and pour the gel in the tank. The combs were inserted in the gel to make the holes in the gel for loading the samples of deoxyribonucleic acid.

Preparing the master mixture: About 4 microliters of double distilled water was taken for every sample of deoxyribonucleic acid (DNA) in the tubes, 0.2 microlitre of taq-polymerase, 0.2 microlitre volume of forwarded and reversed primers, 0.2 microlitre volume of salt of magnesium, 0.2 microlitre volume of buffer of enzyme in the form of ammonium chloride, 1 microlitre of freshly prepared dNTPs were put in the tube.

The PCR tubes were fixed in the stand that was already placed in the ice. The PCR tubes were labeled. Good quality and freshly isolated deoxyribonucleic acid in the labeled tubes was added. An already prepared master mixture was poured in the tubes in equal quantity.

Running the PCR: In the meanwhile, the PCR tubes containing the master mixture was placed in ice. In the display, the PCR machine was set taking the temperature of lid at 105ºC, 94-96ºC was set for incubation and time was set for 6 minutes. The denaturing temperature was set for 1-2 minutes at 94-96ºC, the deoxyribonucleic acid was annealed at 50-52ºC. The temperature for final extension was set at 95ºC for 8 minutes. The temperature of 5ºC was set for holding (forever).

Markers analysis: For the analysis of marker association software named TASSEL three was used, and model of mixed linearity was taken as this model can minimize both the type one and two errors. The model of mixed linearity at the same time calculated the variations of kinship along with the trait association. The matrix of kinship was calculated using the same software as above and the population structure was obtained using the software namely Structure 2.2. The significance of the results was declared at probability level of one percent.

Results and Discussions

The genotypes showed highly significant results for all the parameters studied. The environment also exhibited highly significant results for most of the characters except for plant height which showed non-significant results. The genotypes into treatment interaction showed highly significant results for most of the parameters and significant results for days to heading except for peduncle length, spike length, grain weight per spike and tillers per plant and yield per plant as shown in the Table 1.

The model of mixed linearity was applied to know the link between the 14 parameters and 25 SSR markers which were linked to the chromosome number 2A of wheat, a 6X (hexaploid) crop. The association study was completed using the covariance of sub populations. The eight characters were identified which were linked with 9 SSR markers at probability level of 1% and the phenotypic value was in range of 14 to 32% under water stress conditions. But under the irrigated conditions the same genotypes showed that 6 parameters were linked with six SSR markers with the same probability level and phenotypic variability was ranged between 14% to 22%. The parameters which showed prominent association under water stress environment are the number of days taken to heading, numbers of days to maturity, length of spikes, area of leaves, spikelet's numbers in spikes, grains number per spike, thousand grains weight and the yield per plant. The parameters which gave significant association under the irrigated conditions were the numbers of days to heading, days taken to maturity, length of peduncle leaf, area of leaves, grains number per spike and thousand grains weight. Among the 10 linked SSR markers which showed the prominent trait association, only 5 of them (WMC382, CFA2086, CFA2121, CFA2263, WMC2610) were linked to only one trait which could be the trait specific MTAs. And the markers WMC382, CFA2043, CFA2086, were linked with numbers of days to maturity.

Table 1. The effects due to the genotypes, environment and their interaction for different yield parameters in wheat.

Effect	DF	DH	DM	PH	PL	SL	Lw	LL	LA	SP/S	G/S	GWT/S	T/PL	1000GWT	Y/PL
GEN	99	10.28**	8.91**	243.8**	59.89**	15.08**	0.19**	47.63**	389.12**	5.8**	294.23**	0.9**	18.09**	717.14**	65.4**
E	1	8019**	8019**	99	25**	891**	4**	81**	3749.04**	900**	3564**	25**	63.36**	3600**	1600*
G x E	99	4.5*	5.0**	110**	10.01	2.5	0.07**	20.01**	110.62**	8.01**	22.0**	0.001	3.5	210.01**	20.02

DH; days to heading, DM; days to maturity, PH; Plant height, PL; Peduncle length, SL; Spike length, LW; Leaf width, LL; Leaf length, LA; Leaf area, SP/S; Spikelet's per spike, G/S; Grains per spike, GWT/S; Grain weight per spike, T/PL; Tillers per plant, 1000GWT; Thousands grain weight, Y/p; Yield per plant

Table 2. Association (r2) of the SSR markers with different traits in wheat.

Traits	Marker	1st Year	1st Year	2nd Year	2nd Year	R2	P(Q+K)
		N	D	N	D		
DH	WMC382	*				0.19	0.000054
DH	WMC382		*			0.19	0.000054
DH	CFA2263	*				0.17	0.0013
DH	WMC382				*	0.18	0.000051
DH	CFA2263				*	0.17	0.0014
DM	CFA2043	*				0.16	0.0012
DM	CFA2043		*			0.15	0.00043
DM	WMC382				*	0.18	0.00006
DM	CFA2086				*	0.16	0.0014
PL	WMC2614			*		0.14	0.0015
SL	CFA2086				*	0.29	0.00012
LA	CFA2201	*				0.14	0.00146
LA	CFA2201		*			0.16	0.0013
SP/S	WMC382		*			0.19	0.00005
SP/S	CFA2263		*			0.17	0.0013
SP/S	CFA2086				*	0.25	0.000005
SP/S	CFA2121				*	0.32	6E-07
SP/S	WMC2610				*	0.21	0.000033
G/S	CFA2086	*				0.17	0.0012
G/S	CFA2263				*	0.18	0.00007
1000GWT	CFA2086	*				0.22	0.000015
1000GWT	CFA2086		*			0.22	0.000015
1000GWT	CFA2086			*		0.18	0.000062
1000GWT	BARC21				*	0.18	0.000085
Y/PL	WMC261				*	0.15	0.00006

* Significant at 0.01, N; irrigated conditions D; drought conditions

DH; days to heading, DM; days to maturity, PH; Plant height, PL; Peduncle length, SL; Spike length, LW; Leaf width, LL; Leaf length, LA; Leaf area, SP/S; Spikelets per spike, G/S; Grains per spike, GWT/S; Grain weight per spike, T/PL; Tillers per plant, 1000GWT; Thousands grain weight, Y/p; Yield per plant

Under meiotic stage drought stress conditions WMC382 and CFA2263 exhibited significant association with days to heading. Days to maturity was found to be associated with WMC382, CFA2043 and CFA2086. Marker trait associations for peduncle length were detected with primer WMC2614 under meiotic stage drought conditions during 2nd year. The marker CFA2201 was associated with leaf area under normal as well as under drought conditions. The markers which showed significant results under normal as well as under drought conditions were WMC382, CFA2201, CFA2263 and WMC2614. The markers WMC382 and WMC407 showed significant results for days to heading under normal as well as under meiotic stage drought conditions during 1st year. The marker CFA2086 showed significant association with 1000 grain weight under normal as well as under meiotic stage in drought conditions. The 5 markers such as WMC382, CFA2086, CFA2121, CFA2263 and WMC2610 were associated with spikelets per spike which could be considered as trait specific MTA (Table 2).

The association procedure used in this study called as population dependant association rectified the mistakes made in family based linkage study (Spielman *et al.*, 1993; Long *et al.*, 1997; Rossl barra *et al.*, 2007). As the crossing over occurring naturally to create new combinations there is very small distance between linkages. So the more numbers of molecular markers are required to determine the link of parameters with markers (Nordborg and Tavare, 2002 and Yu & Buckler, 2006).

There is direct link between association mapping and linkage disequilibrium and provided the more accurate and precise results for marker assisted selection and association mapping (Remington, *et al.*, 2001and Flint-Garcia *et al.*, 2003; Zhang *et al.*, 2010).

Table 3. List of SSR primers used for markers trait association.

1.	WMC181	TCCTTGACCCCTTGCACTAACT ATGGTTGGGAGCACTAGCTTGG	14.	CFA2263	GGCCATGTAATTAAGGCACA CTCCCAGGAGTACAGAAGAGGA
2.	WMC261	GATGTGCATGTGAATCTCAAAAGTA AAAGAGGGTCACAGAATAACCTAAA	15.	BARC5	GCGCCTGGACCGGTTTTCTATTTT GCGTTGGGAATTCCTGAACATTTT
3.	WMC382	CATGAATGGAGGCACTGAAACA CCTTCCGGTCGACGCAAC	16.	BARC12	TGCACCCCTTCCAAATCT TGCGAGTCGTGTGGTTGT
4.	WMC407	GGTAATTCTAGGCTGACATATGCTC CATATTTCCAAATCCCCAACTC	17.	BARC21	GGCAACTGGAGTGATATAAATACCG CAGGAAGGGAGGAGAACAGAGG
5.	WMC453	ACTTGTGTCCATAACCGACCTT ATCTTTTGAGGTTACAACCCGA	18.	GWM95	GATCAAACACACACCCCTCC AATGCAAAGTGAAAAACCCG
6.	WMC455	GCGTCATTTCCTCAAACACATC AGAAGGAGAAGTGCCTCACCAA	19.	GWM265	TGTTGCGGATGGTCACTATT GAGTACACATTTGGCCTCTGC
7.	WMC522	AAAAATCTCACGAGTCGGGC CCCGAGCAGGAGCTACAAAT	20.	GWM312	ATCGCATGATGCACGTAGAG ACATGCATGCCTACCTAATGG
8.	CFA2043	CAGCCGAAGAAGGATTTCTG GAGGCAGGAACTTAGGGGAG	21.	GWM328	GCAATCCACGAGAAGAGAGG CACAAACTCTTGACATGTGCG
9.	CFA2058	CCCATTGCCATCTCAGTCTT ATAGTAGGCCCAAAGCGATG	22.	GMW359	CTAATTGCAACAGGTCATGGG TACTTGTGTTCTGGGACAATGG
10.	CFA2086	TCTACTTTCAGGGCACCTCG TCTCTCCAAACCTCCCTGTAA	23.	GMW122	GGGTGGGAGAAAGGAGATG AAACCATCCTCCATCCTGG
11.	CFA2099	TGCGAAGTATTCAGTGCGTC TCAAGACCATCAGCACTCAGA	24.	GMW445	TTTGTTGGGGGTTAGGATTAG CCTTAACACTTGCTGGTAGTGA
12.	CFA2121	TAAATGGCCATCAAGCAATG GCTTGTGAACTAATGCCTCCC	25.	GMW558	GGGATTGCATATGAGACAACG TGCCATGGTTGTAGTAGCCA
13.	CFA2201	CAAACCAACCTCATTGACCC CCACCAGAACTTCAACCTGG			

Marker trait association was calculated using 46 simple sequence repeats scattered in the whole genome of wheat with yield and other agronomic characters (Dodig *et al.* 2012) (Table 3). A genome contains higher number of SSR alleles (Breseghello & Sorrells, 2006). The SSR loci were scattered in the three genomes of wheat (Peng *et al.* 2009). It was found out that Dreb 1 gene was located on chromosome 3A in all genotypes, including drought-tolerant and drought-sensitive ones, except in semi-tolerant genotype (Tale-38) (Huseynove & Rustamova, 2010). Two approaches, general and mixed linear models were used to perform association analysis. In both models 385 associations between markers and traits were detected (Neumann *et al.* 2011). Association mapping also provide greater resolution as more meiotic events occur throughout the development of germplasm (Al-Maskari *et al.*, 2012).

Conclusion

It was concluded that 8 characters were identified which were linked with 9 SSR markers at probability level of 1% and the phenotypic value was in range of 14 to 32% under water stress conditions. But under the irrigated conditions the same genotypes showed that six parameters were linked with six SSR markers with the same probability level and phenotypic variability ranged from 14 to 22%. The five SSR primers were identified such as WMC382, CFA2086, CFA2121, CFA2263 and WMC2610 which were linked with spikelets per spike which could be consisted as trait specific MTA.

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