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MARKER – ASSISTED SELECTION (MAS) FOR DROUGHT TOLERANCE IN WHEAT USING MARKERS ASSOCIATED WITH MEMBRANE STABILITY

SELECȚIA ASISTATĂ DE MARKERI ASOCIAȚI STABILITĂȚII MEMBRANELOR PENTRU TOLERANȚA GRÂULUI LA SECETĂ

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Abstract

Abiotic stresses such as drought, high or low temperatures, salinity and others reduce wheat productivity. From these, the drought stress is the major constraint to wheat.

To improve yield in water limited environments, breeders have been trying to identify secondary traits contributing to drought resistance and selecting for those traits in a breeding program.

C i u c ă and P e t c u (2009) found SSR molecular markers weakly, but significantly, associated with cell membrane stability after water stress in wheat, and concluded that they can be used for increasing the frequency of progenies with better performance under drought.

Eighty nine winter wheat lines and three cultivars from Romania and thirteen winter wheat lines and cultivars from Bulgaria were analyzed with two SSR markers (wmc603 and barc108). The analysis distinguished 31 homozygous and two heterozygous wheat lines with electrophoresis profile similar with the profile of the drought resistant cultivar Izvor. DNA amplification with barc108 showed only 28 homozygous and two heterozygote (but not the same as those obtained with wmc603) wheat lines with electrophoresis profile similar with Izvor. Profiles similar with Izvor for both wmc603 and barc108 markers were found in 25 Romanian common wheat lines selected from 13 hybrid combinations involving Izvor as one of the parents and in one Bulgarian durum line

Different alleles at the marker loci might be associated with improved drought resistance in different wheat germplasm.

Key words: wheat, drought, SSR markers, membrane stability.

INTRODUCTION

Abiotic stresses such as drought, high or low temperatures, salinity and others reduce wheat productivity. From these, the drought stress is the major constraint to wheat.

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Using the conventional approach, breeders achieved significant genetic improvement of adaptation to drought by selecting for yield stability. But such selection programs are slow in attaining progress. One alternative for yield improvements in water limited environments could be to identify secondary traits contributing to drought resistance and to select for those traits in a breeding program. Among the secondary traits that contribute to drought resistance in wheat are osmotic adjustment (OA), membrane stability, reduced cuticular transpiration, improved stomatal conductance, stem reserve mobilisation etc (B l u m, 2005), but phenotypic selection for these traits is difficult and labor intensive. For these reasons acceleration of genetic progress for drought resistance by using selection for contributing secondary traits is limited. These limitations could be overtaken using molecular markers technology, leading to the possibility of indirect selection for these secondary traits by marker-assisted selection (MAS) strategy.

C i u c ă and P e t c u (2009) found SSR molecular markers weakly but significantly associated with cell membrane stability after water stress, in wheat, and concluded that these markers can be used for increasing the frequency of progenies with better performance under drought conditions.

This paper presents results about marker - assisted selection (MAS) strategy using SSR markers associated with membrane stability in Romanian and Bulgarian wheat genotypes.

MATERIAL AND METHODS

Eighty nine winter wheat lines and three cultivars from Romania and thirteen winter wheat lines and cultivars from Bulgaria were analyzed. These included the Romanian cultivar Izvor, described as having superior yield stability due to its drought resistance (M u s t ă ț e a et al., 2009) and characterized for membrane stability and osmotic adjustment through grain pollen test by immersion in PEG solution (B a n i c ă et al., 2008).

PCR was performed into 0,2 ml tubes containing 25 µl final volume of a reaction mixture consisting of 1X buffer,1,5 mM $MgCl_2$, 0,25 µM primer, 1U of Taq polymerase (Go Taq Flexi-Promega), 0,2 mM each dNTP and 50-100 ng of genomic DNA. The Applied Biosystem 9600 thermal cycler, was programmed for 38 cycles, each consisting of: 1 min at 94° C, 1 min, 61°C (for barc108 we used 53°C), 1 min., 2 min. at 72º C, and 10 min. at 72°C. PCR products were evaluated by electrophoresis, on 2% agarose gels in 0,5x TBE buffer, stained with ethidium bromide and BioPrint images record.

Primers for SSR markers wmc603 and barc108 located on chromosome 7A, previously found as associated with membrane stability after severe drought exposure (C i u c ă and P e t c u, 2009) were used.

RESULTS AND DISCUSSIONS

The results obtained by analysis on Romanian and Bulgarian wheat with markers wmc603 and barc108 are presented in table 1.

Table 1

Winter wheat breeding lines and cultivars tested with markers wmc603 and barc108 associated with membrane stability

Nr.	Country and Name	Country	Wheat species	Wmc603	Barc108	Observations
1	2	3	4	5	6	7
1	F02065G5-21	Ro	*Triticum aestivum*	+	+	
2	F02065G5-22	Ro	*T. aestivum*			
3	F02065G5-23	Ro	*T. aestivum*			
4	F02065G8-12	Ro	*T. aestivum*			
5	F06389GP1	Ro	*T. aestivum*			
6	F06389GP2	Ro	*T. aestivum*	+	+	
7	F06389GP5	Ro	*T. aestivum*			
8	F06389GP6	Ro	*T. aestivum*	+	+	
9	F06389GP7	Ro	*T. aestivum*	+	+	
10	F06389GP8	Ro	*T. aestivum*			
11	F06387GP1	Ro	*T. aestivum*			
12	F06387GP2	Ro	*T. aestivum*			
13	F06387GP3	Ro	*T. aestivum*	+	+	
14	F06387GP4	Ro	*T. aestivum*	+	+	
15	F06387GP6	Ro	*T. aestivum*			
16	F02065G5-22	Ro	*T. aestivum*			
17	F05036G3-1	Ro	*T. aestivum*	+	+	
18	F05036G3-2	Ro	*T. aestivum*	+	+	
19	F05036G3-3	Ro	*T. aestivum*	+	+	
20	F05037G1-1	Ro	*T. aestivum*	+	+	
21	F05037G2-1	Ro	*T. aestivum*	+	+	
22	F05075G1-1	Ro	*T. aestivum*			
23	F05075G2-1	Ro	*T. aestivum*			
24	F05105G1-1	Ro	*T. aestivum*			
25	F05174G1-2	Ro	*T. aestivum*			
26	F05174G2-1	Ro	*T. aestivum*			
27	F05174G2-2	Ro	*T. aestivum*			
28	F05228G4-1	Ro	*T. aestivum*			
29	F05228G5-1	Ro	*T. aestivum*			
30	F05248G1-1	Ro	*T. aestivum*			
31	F05248G1-2	Ro	*T. aestivum*			
32	F05248G2-1	Ro	*T. aestivum*	+	+	
33	F05248G2-2	Ro	*T. aestivum*			
34	F05248G2-3	Ro	*T. aestivum*	+	+	
35	F05248G3-1	Ro	*T. aestivum*	+	+	
36	F05248G3-2	Ro	*T. aestivum*	+	+	
37	F05248G4-1	Ro	*T. aestivum*	+	+	
38	F05256G3-1	Ro	*T. aestivum*	+	+	
39	F05418G1-1	Ro	*T. aestivum*	+	+	
40	F05443G1-1	Ro	*T. aestivum*	+	+	
41	F05498G2-1	Ro	*T. aestivum*			
42	F05503G1-1	Ro	*T. aestivum*	+	H	
43	F05503G1-2	Ro	*T. aestivum*	+	+	
44	F05503G2-1	Ro	*T. aestivum*	+	+	
45	F05503G3-1	Ro	*T. aestivum*			
46	F05505G2-1	Ro	*T. aestivum*			

Table 1 (continuation)

1	2	3	4	5	6	7
47	F05508G2-1	Ro	*T. aestivum*			
48	F05512G1-1	Ro	*T. aestivum*			
49	F05512G1-2	Ro	*T. aestivum*			
50	F05512G1-3	Ro	*T. aestivum*			
51	F05512G1-4	Ro	*T. aestivum*			
52	F05512G1-5	Ro	*T. aestivum*	+	H	
53	F05517G3-1	Ro	*T. aestivum*	H		
54	F05517G3-2	Ro	*T. aestivum*	H		
55	F05537G2-1	Ro	*T. aestivum*			
56	F05921G1-1	Ro	*T. aestivum*			
57	F05921G1-2	Ro	*T. aestivum*			
58	F05921G2-1	Ro	*T. aestivum*			
59	**F05921G3-1**	Ro	***T. aestivum***	**+**		
60	**F05921G3-2**	Ro	***T. aestivum***	**+**		
61	F05921G3-3	Ro	*T. aestivum*			
62	F05922G3-1	Ro	*T. aestivum*			
63	F05922G3-2	Ro	*T. aestivum*			
64	F05922G4-1	Ro	*T. aestivum*			
65	F05503GP2	Ro	*T. aestivum*			
66	F05511GP2	Ro	*T. aestivum*	+	+	
67	F05511GP3	Ro	*T. aestivum*	+	+	
68	F05511GP4	Ro	*T. aestivum*			
69	F05511GP5	Ro	*T. aestivum*			
70	F02065G5-201	Ro	*T. aestivum*			
71	F02065G5-202	Ro	*T. aestivum*			
72	F02065G5-203	Ro	*T. aestivum*			
73	F02065G5-204	Ro	*T. aestivum*			
74	F02065G8-102	Ro	*T. aestivum*			
75	F04162G1-1	Ro	*T. aestivum*			
76	F04825G2-1	Ro	*T. aestivum*	+	+	
77	F04826G1-1	Ro	*T. aestivum*	+	+	
78	**F04826G2-1**	Ro	***T. aestivum***	**+**		
79	F04826G3-1	Ro	*T. aestivum*			
80	F03378G1-M1	Ro	*T. aestivum*			
81	F03378G1-2M1	Ro	*T. aestivum*			
82	F05802G1	Ro	*T. aestivum*			
83	F05921G1	Ro	*T. aestivum*			
84	F05921G2	Ro	*T. aestivum*			
85	F05922G2	Ro	*T. aestivum*			
86	F05922G3	Ro	*T. aestivum*			
87	F05922G4	Ro	*T. aestivum*			
88	F05922G5	Ro	*T. aestivum*			
89	F05922G6`	Ro	*T. aestivum*			
90	BOEMA	Ro	*T. aestivum*			
91	LIMAN	Ro	*T. aestivum*			
92	IZVOR	Ro	*T. aestivum*	+	+	
93	GERGANA	BG	Triticum durum			
94	APULLICUM	BG	Triticum durum			
95	BG-7048	BG	Triticum durum			

Table 1 (continuation)

1	2	3	4	5	6	7
96	BG-Trd3	BG	Triticum durum	+	+	
97	**BG-6189**	**BG**	**Triticum durum**		**+**	
98	BG-6467	BG	Triticum durum			
99	BG-6911	BG	Triticum durum			
100	BG-6953	BG	Triticum durum			
101	**GALATEA**	**BG**	***T. aestivum***		**+**	
102	VUZHOD	BG	Triticum durum			
103	ZAGORKA	BG	Triticum durum			
104	TODORA	BG	*T. aestivum*			
105	SATURN	BG	Triticum durum			
106	CHINESE SPRING	BG	*T. aestivum*			

Note: Ro - genotypes from Romania; BG - genotypes from Bulgaria;
"+" electrophoresis profile similar with IZVOR;
Bold letters indicate genotypes showing differences between results obtained with wmc603 and barc108;
H – heterozygote.

Our analysis with marker wmc603 distinguished 31 homozygous and two heterozygous wheat lines with electrophoresis profile similar with Izvor. DNA amplification with barc108 showed only 28 homozygous and two heterozygote (but not the same as those obtained with wmc603) wheat lines with electrophoresis profile similar with Izvor. Profiles similar with Izvor for both wmc603 and barc108 markers were found in 25 Romanian common wheat lines selected from 13 hybrid combinations involving Izvor as one of the parents (Table 2) and in one Bulgarian durum line.

Table 2

Genealogy of wheat lines with electrophoresis profiles similar with Izvor

Nr.	Name of the hybrid combination	Genealogy	Number of lines with electrophoresis profiles similar with Izvor
1	F02065G	IZVOR/95272G1-11	1
2	F04825G	IZVOR7/LIMAN	1
3	F04826G	IZVOR7/00099GP2	1
4	F05036G	IZVOR/00099GP2	3
5	F05037G	IZVOR/ 00143GP1	2
6	F05248G	GLOSA/IZVOR	5
7	F05256G	GRUIA/IZVOR	1
8	F05418G	99654GP2/IZA//IZVOR	1
9	F05443G	BOEMA/2*IZVOR	1
10	F05503G	IZVOR/LIMAN//IZVOR	2
11	F05511G	IZVOR/99654GP2//IZVOR	2
12	F06387G	LIMAN/00099GP2//LIMAN/IZVOR	2
13	F06389G	LIMAN/2*IZVOR	3

Visual field observations on drought response of the Romanian wheat lines during the relatively mild drought of 2009, confirmed a relatively good behavior of the lines carrying both markers.

Two Bulgarian durum wheat cultivars (*Triticum durum*): Gergana and Apullicum were previously characterized as drought susceptible and drought resistant respectively. When tested with markers wmc603 and barc108, none of these cultivars had electrophoresis profiles similar to Izvor. However, the electrophoresis profiles of these genotypes were different. This suggests that other alleles at the marker loci might also be associated with a better response to drought, or that other mechanisms for resistance to drought are present in Apullicum. Y u n e s (2009) also found that none of the studied Algerian wheat cultivars, characterized as having good levels of drought resistance, had an electrophoretic profile similar with Izvor, but identified a large polymorphism for markers located on chromosome 7A.

CONCLUSIONS

- In lines selected from crosses having Izvor as one of the parents, markers wmc603 and barc108 co-segregated, but 3 lines were detected where only one marker was inherited from Izvor.
- Selection assisted by SSR markers wmc603 and barc108 could be useful in identifying segregants that inherited from the drought resistant cultivar Izvor a region of the 7A chromosome carrying gene(s) which improve the response to water stress.
- Different alleles at the marker loci might be associated with improved drought resistance in different wheat germplasm.

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