DETECTION OF QTLs LINKED TO FUSARIUM HEAD BLIGHT RESISTANCE IN ROMANIAN WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) is one of the main diseases of wheat in Romania. Breeding for resistance to FHB is the most reliable method to control the field contamination and to reduce the detrimental effects of disease on yield, quality and contamination of kernels. Marker-assisted selection (MAS) is considered as a valuable tool in order to accelerate and increase genetic progress for resistance to FHB in wheat.

Our research was focused on detection of QTLs previously described as associated with resistance to FHB, based on evaluation of polymorphisms identified with specific SSR primers, in winter wheat breeding lines obtained at NARDI Fundulea.

Investigation of sixty lines using the primers for seven SSR markers (Xgwm and Xbarc), located on chromosomes *6B, 3B* and *3A,* revealed polymorphism possibly associated to FHB resistance in several F201R derivatives, but also in lines, with no known relation with F201R. Further investigations for confirmation of FHB resistance levels in these lines are necessary.

Primers for markers Xgwm *493* and Xgwm 533, located on chromosome *3BS*, identified the same electrophoresis pattern with Sumai 3, in only one of 13 winter wheat lines, which had Sumai 3 among their parents.

The information about the presence of markers associated with FHB resistance in lines from the Romanian winter wheat breeding program can be used for pyramiding QTLs to further increase the level of resistance.

Key words: Fusarium head blight, FHB, QTLs, marker-assisted selection-MAS, winter wheat

INTRODUCTION

Fusarium head blight (FHB) is a wheat disease that causes serious yield losses and deteriorated grain quality in Romania. FHB is caused by several fungi from the genus *Fusarium*, among which *Fusarium graminearum* is predominant. As a result of natural occurrence, significant reduction of yield and quality, often associated with mycotoxin (secondary metabolites) contamination of kernels, are registered. Among the mycotoxins produced by pathogen strains of *Fusarium*, the most widespread is deoxynivalenol (DON, vomitoxin), with a harmful effect on humans and animals. DON is a significant factor affecting the food safety on the entire food chain.

Breeding for resistance to FHB is the most reliable method to control the field contamination and to reduce the detrimental effects of disease on yield, quality and contamination of kernels.

Breeding progress for resistance to FHB in wheat is much more difficult, as compared with other pathosystems, because of both the complexity of host resistance and the strong interference with environment. That's why, the marker-assisted selection (MAS) is considered as a valuable perspective tool in order to acelerate and to increase genetic progress in breeding for resistance to FHB in wheat.

Resistance to FHB is complex, many genes controlling different aspects of the disease, such as resistance to initial infection (type I), resistance to the spread of the disease in the spike (type II) and resistance to the accumulation of mycotoxins such as deoxynivalenol (type III) (Gilbert and Tekauz, 2000; Somers et al., 2005).

Only few cultivars have been identified to have a relatively high level of FHB resistance (Buerstmayr et al., 2002; Saur, 1991; Shen et al., 2003; Snijders, 1990; Yang et al., 2005). These include the Chinese cultivar Sumai 3, the Brasilian Frontana, the Romanian F201R, and the Korean Chokwang.

Several research groups have identified a common set of FHB resistance QTLs in different crosses and genetic backgrounds (Anderson et al., 2001; Buerstmayr et al., 2002; Somers et al., 2003). Although, each identified QTL appears to explain only small amounts of the variation in infection, the effects appear to be additive. As a result, it seems essential to select for multiple genes in order to provide a sufficient level of resistance to FHB in wheat (Somers et al., 2003; Yang et al., 2003).

Our research was focused on detection of QTLs, previously described as associated with FHB resistance, in several winter wheat breeding lines obtained at NARDI Fundulea, through evaluation of polymorphism evidenced with specific SSR primers.

MATERIAL AND METHODS

Genotypes: 60 winter bread-wheat Romanian breeding lines, obtained at the National Agricultural Research & Development Institute Fundulea, were analyzed. The lines were selected from crosses involving F201R or Sumai 3, or showed various levels of FHB resistance in tests performed by M. Ittu in the Wheat Breeding Laboratory field.

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DNA isolation

Total DNA was isolated from leaves following the protocol proposed by Doyle & Doyle (1991).

SSR analysis

PCR reaction was performed in 384 plates, containing 10 µl of a reaction mixture consisting of 1X buffer, 1,5 mM $MgCl_2$, 0,2 µM primer (SSR primer pairs developed by Röder et al. (1998) and Xbarc primer pairs developed by P. Cregan, Q. Song and associates - http://wheat.pw.usda.gov/cgi-bin/graingenes), 0,4U of Taq polymerase, 0,2 mM each dNTP and 20 ng of genomic DNA. The reaction mixture was denaturized at 94ºC for 3 min.; followed by 35 cycles, each consisting of: 1 min. at 94°C, 1 min. at different annealing temperature (50ºC, 55ºC, 60ºC), 2 min. at 72ºC and a final extension step of 72ºC for 10 min.

The polymorphism was revealed with LI-COR system on 6% polyacrilamide gels, during our stay in IFA –Tulln, Austria.

RESULTS AND DISCUSSION

The lines and cultivars were screened with 30 microsatellites distributed across the genome, but only a few gave polymorphism and are presented here (Table 1).

The first group of microsatellites targeted the Sumai 3 resistance genes. A major QTL for Fusarium head blight (FHB) resistance, *Qfhs.ndsu-3BS*, derived from 'Sumai 3', which explained a large portion (25–60%) of the variation in FHB resistance, has been identified and verified by several research groups via molecular marker analysis (Liu et al., 2003).

Primer pairs for two microsatellite primers (Xgwm 493 and Xgwm 533), previously described as linked with the FHB resistance QTL on *3BS* (Anderson et al., 2001; Buerstmayr et al., 2002), were used to screen for polymorphism in lines derived from crosses with Sumai 3. Out of 13 lines derived from Sumai 3 (Table 1), only one line F 488 T1-01 presented the polymorphism linked to FHB resistance QTL, for both microsatellite markers (Figure 1).

This suggests that selecting for other traits followed in the winter wheat breeding program, without simultaneous selection for the presence of Sumai 3 FHB resistance gene *Qfhs.ndsu-3BS*, preferentially eliminated this gene.

The Romanian winter wheat line, Fundulea 201R (F 201R), was reported as having FHB resistance genes cumulated from cultivars NS732, Amigo and possibly other parents involved in its complex genealogy. It has no relation to any of previously described sources of resistance (Ittu et al., 2001). Shen et al. (2003) found four interval regions, located on chromosomes *1B, 3A, 3D,* and *5A* that conferred resistance to FHB in F 201R. The QTLs located on chromosomes *1B* and *3A,* contributed by F 201R, had large effects and were consistently expressed in three environments. The QTL that was located on the short arm of chromosome *3A,* near the centromere explained 13% of the phenotypic variation and was tightly linked to markers Xgwm 674 and Xbarc 67, which are 2.8 cM apart (Shen et al., 2003).

We evaluated F 201R and 15 lines obtained from crosses between F 201R and other Romanian winter wheat genotypes (Table 1), for polymorphism, using one pair of SSR primers previously described by Shen et al. (2003), for the marker Xbarc 67 on chromosome *3AS.* Our analyses with Xbarc 67 showed polymorphism in 10 lines, out of which 7 lines were derived from crosses with F 201R, while in three lines the origin of resistance to FHB could not be traced back to this line. This suggests that the FHB resistance QTL linked with Xbarc 67 might be widespread in the Romanian breeding germplasm.

Similar results were obtained in most lines with primers for SSR marker Xbarc19 located on *3A* chromosome, close to Xbarc 67 marker. We found polymorphism for this marker both in lines derived and not derived from F 201R (Figures 2 and 3). Further research should compare the reliability of markers Xbarc 19 and Xbarc 67 for the FHB resistance QTL on chromosome *3A*.

The marker Xgwm 508, located on chromosome *6B* identified polymorphism in Sumai3 and in 2 lines for which the source of resistance is not known. Further research should investigate the usefulness of this marker for FHB resistance.

The information about the presence of markers associated with FHB resistance in lines from the Romanian winter wheat breeding program can be used for pyramiding QTLs to further increase the level of resistance.

Table 1. Screening with molecular markers for resistance to Fusarium head blight in 60 winter wheat lines

No.	GENOTYPE	RESISTANT PARENT	POLYMORPHIC SSR MARKERS						
			Xgwm 508	Xgwm 285	Xgwm 493	Xgwm 533	Barc 133	Barc 19	Barc 67
			Location on chromosome:						
			6B	*3B*	*3BS Qfhs.ndsu-3BS*	*3BS Qfhs.ndsu-3BS*	*3B*	*3A*	*3A*
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1	Boema	No	-	-	-	-	-	-	-
2	Dropia	No	-	-	-	-	-	-	-
3	Delabrad	No	-	-	-	-	-	-	-
4	Dor	No	-	-	-	-	-	-	-
5	Crina	No	-	-	-	-	-	-	-
6	Faur	No	-	-	-	-	-	-	-
7	Glosa	No	-	-	-	-	-	-	-
8	**F 201R**	**source**	**-**	**-**	**-**	**-**	**-**	+	+
9	**F 201R**	**source**	**-**	**-**	**-**	**-**	**-**	+	+
10	F 201R sel. 1(7)	F 201R	-	-	-	-	-	+	+
11	F 201R sel. 1(8)	F 201R	-	-	-	-	-	+	+
12	F 201R sel. 1(9)	F 201R	-	-	-	-	-	+	+
13	F 92080 G 01102	F 201R	-	-	-	-	-	-	-
14	F 95171 G 013-13	F 201R	-	-	-	-	-	+	-
15	Izvor	F 201R	-	-	-	-	-	+	-
16	F 01370 G1-1	F 201R	-	-	-	-	-	-	-
17	F 96035 G1-10	F 201R	-	-	-	-	-	+	+
18	F 96035 G-10102	F 201R	-	-	-	-	-	+	+
19	F 96035 G1-10	F 201R	-	-	-	-	-	+	+
20	F 96035 G11-2	F 201R	-	-	-	-	-	+ H	+ H
21	F 96035 G1-10	F201R	-	-	-	-	-	+	+
22	F 96035 G1-10	F201R	-	-	-	-	-	-	+
23	F 00143 G3-1	F 201R	-	-	-	-	-	-	-
24	F 02102 G3-1	F 201R							
25	F 02104G2-1	F 201R	-	-	-	-	-	-	-
26	F 02379 G2-1	F 201R	-	-	-	-	-	+H	+
27	F 02379G3-1	F 201R	-	-	-	-	-	+	-
28	**Sumai 3**	**source**	+	+	+	+	+	**-**	**-**
29	F02002 G1-1	Sumai 3	-	-	-	-	-	-	-
30	F01873 G2-1	Sumai 3	-	-	-	-	-	-	-
31	F01127 G2	Sumai 3	-	-	-	-	-	-	-
32	F01127 G3	Sumai 3	-	+	-	-	-	-	-
33	F01127 G3-1	Sumai 3	-	-	-	-	-	-	-
34	F01128 G 1-1	Sumai 3	-	+	-	-	-	-	-
35	F01127 G3-3	Sumai 3	-	-	-	-	-	-	-
36	F01872 G1-2	Sumai 3	-	-	-	-	-	-	-
37	F01876 G1	Sumai 3	-	-	-	-	-	-	-
38	F01876G5-2	Sumai 3	-	-	-	-	-	-	-
39	F01127 G3	Sumai 3	-	+	-	-	-	-	-
40	F488T1-01	Sumai 3	-	-	+	+	+	-	-
41	F257S2-3	Sumai 3	-	-	+?	+?	-	-	-
42	F00240G3-2	**Unknown**	-/+	-	-	-	-	-	-
43	F00240G3-2	**Unknown**	-/+	-	-	-	-	-	-
44	F00329 G1-1	**Unknown**	-	-	-	-	-	-	-

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
45	F00356 G8-104	**Unknown**	-	-	-	-	-	-	-
46	F00399G2-11	**Unknown**	-	-	-	-	-	+H	-
47	F00628 G34-1	**Unknown**	+	-	-	-	-	+!	+!
48	F01156 G3-1	**Unknown**	-	-	-	-	-	-	-
49	F01400 G1-2	**Unknown**	-	-	-	-	-	+!	+!
50	F01429 G4-1	**Unknown**	-	-	-	-	-	+!	+!
51	F01461 G3-2	**Unknown**	-	-	-	-	-	+!	+!
52	F01459 G4-1	**Unknown**	-	-	-	-	-	+!	+!
53	F02083 GP-3	**Unknown**	-	-	-	-	-	-	-
54	F02122 GP1	**Unknown**	-	-	-	-	-	-	-
55	F02211 G2-1	**Unknown**	-	-	-	+!	-	-	-
56	F96831 G6-1001	**Unknown**	-	-	-	-	-	-	-
57	F98039 G5-10INC-1	**Unknown**	-	-	-	-	-	-	-
58	F99051 G3-3INC2	**Unknown**	-	-	-	-	-	-	-
59	F99146 G1-101	**Unknown**	-	-	-	-	-	-	-
60	F99419 G4-1Al 1-1	**Unknown**	-	-	-	-	-	-	-

Note:

Polymorphism linked to FHB resistance:	Symbol:
- present;	+
- absent	-
- present, genotype heterozygote	+H
- polymorphism, other position	+ ?
- present, resistance source not Sumai 3 or F 201R	+!
- present, polymorphism different from Sumai 3	-/+

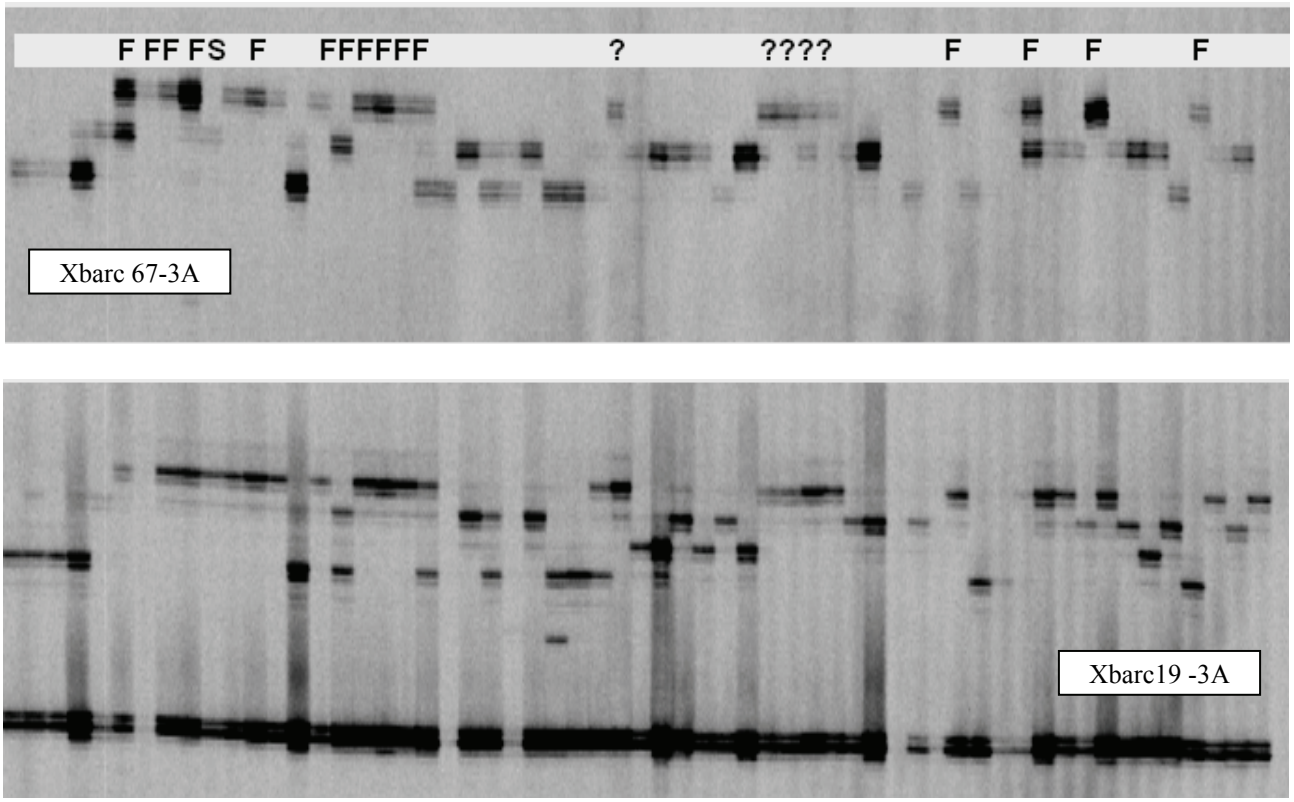

Figure 1. Electrophoresis pattern obtained with primers for microsatellite markers Xbarc 67 and Xbarc 19: F-F 201R and progenies; S- Sumai 3; ?- source unknown.

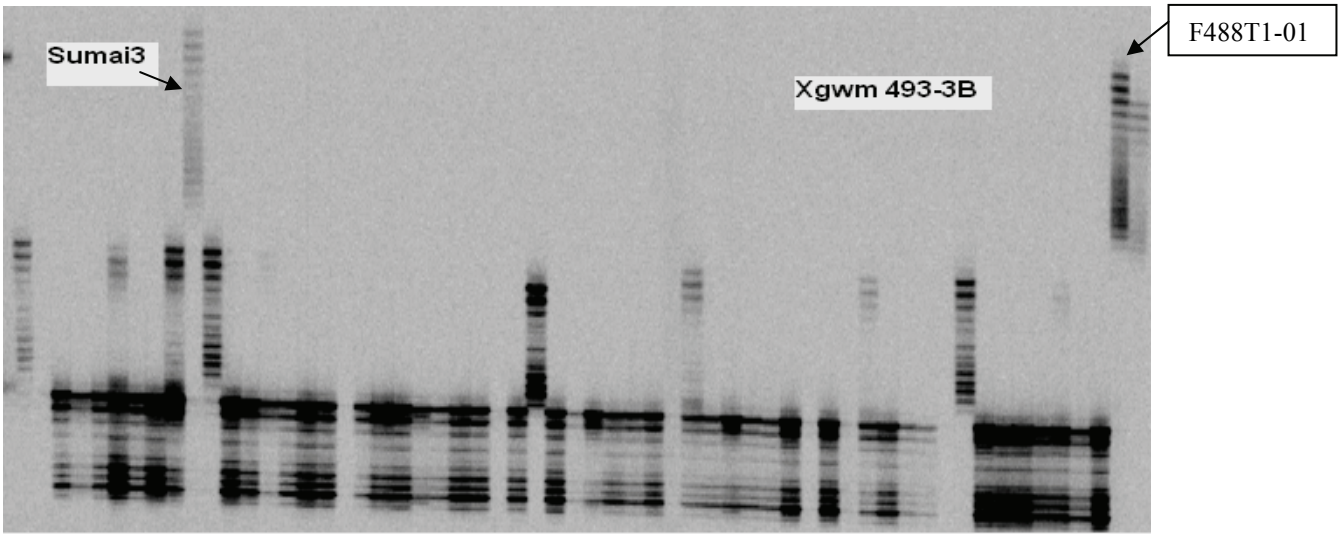

Figure 2. Electrophoresis pattern obtained with SSR primers for Xgwm 493

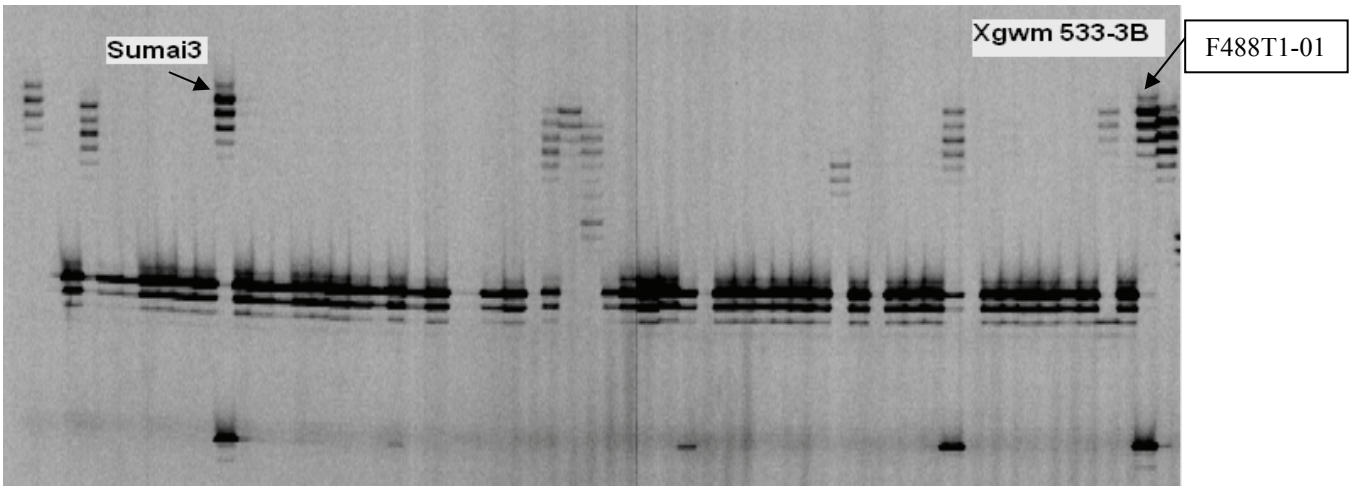

Figure 3. Electrophoresis pattern obtained with SSR primers for Xgwm 533

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