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Marker Assisted Selection for *Septoria tritici* Resistance in Wheat Dihaploid Lines

Constantin BOTEZ[1)], Doru PAMFIL[1)], Dana CURTICIU[1)], Laura COTA[1)], Nicolae N. SAULESCU[2)]

[1)] *University of Agricultural Sciences and Veterinary Medicine, 3-5 Manastur St., 400372, Cluj-Napoca, Romania; constantinbotez@yahoo.com*

[2)] *Res. Inst. for Cereals and Industrial Crops, Fundulea, 8264 Calarasi County, Romania*

Abstract

The selection with co-dominant SSR markers existent on the market, accomplished in dihaploid wheat lines, offers the opportunity to rapidly stabilize the selected resistant individuals into homozygous estate. As biological material there were used 21 resistant and two sensitive dihaploid lines. All dihaploid lines tested, with field resistance to *Septoria tritici* leaf blotch, expressed at least once such a resistant gene. The most frequently there were encountered in the tested material the *Stb* 7 and *Stb* 8 leaf blotch resistant genes. The size of amplification products specific for SSR markers linked with *Stb* 7 and *Stb* 8 resistant genes were similar to those obtained by Adhikari *et al*. (2004) but the size of amplification products linked with *Stb* 2 resistant genes were quite different, probably due to the polymorphism that characterizes microsatellite markers at the allelic level. The *Stb*3, *Stb* 4 and *Stb* 6 resistant genes were totally absent. The results obtained for *Stb* 1 and *Stb* 5 were inconclusive.

Keywords: SSR markers, wheat dihaploid lines, resistant genes to *Septoria tritici*

Introduction

Septoria tritici leaf blotch (*Stb*), caused by fungus *Mycosphaerella graminicola*, is one of the most serious foliar pathogens of wheat in Romania. Many loci for resistance to *Stb* have been identified, loci designed from *Stb*1 to *Stb*8 (Adhikari *et al*., 22003, Adhikari *et al*., 2004) and *Stb* 9 to *Stb* 12 (Chartrain *et al*., 2005; Goodwin 2007). The conventional selection methods for *Stb* resistance can be very much improved by using genetic markers tightly linked with *Stb* resistance genes. Attempts to find RAPD markers linked with resistance genes to *Septoria* and *Tilletia* have been done and several candidate RAPD markers, possibly linked with *Septoria* resistance genes, were identified relying on co-segregation (Botez *et al*., 2006). The better is to attribute such molecular markers to resistant genes by other methods like Bulked Segregant Analysis (BSA) or by utilization of Near Isogenic Lines (NIL) (Micelemore *et al*., 1991; Mohan *et al*., 1997; Chague *et al*., 1999). SSR markers, based on PCR amplification of microsatellites sequences (Simple Sequence Repeats) offer a much higher monolocus polymorphism than any other marker system (Röder *et al*., 1998). Such kind of marker should be also attributed to resistant genes by a genetic analysis of linkage between markers and resistant genes (Ganal and Röder, 2007). The selection accomplished in dihaploid wheat lines, with co-dominant SSR markers existent on the market, offers the opportunity to rapidly stabilize the selected resistant individuals into homozygous estate.

Materials and Methods

As biological material there were used 21 resistant dihaplod lines (marked from 1 to 21) and 2 sensitive dihaploid lines (marked from 22 to 23), lines generated and tested for field resistance at the NARDI Fundulea-Romania.

For DNA amplification there was used 15 SSR pair of primers that marked different *Stb* loci (Tab. 1).

Results and disscusion

The P5 pair of primers, specific for Xgwm493 marker linked with *Stb* 2 locus, gave straightforward results (Fig. 1). Most of the field resistant dihaplod lines had amplification products similar to those of the sensitive lines (22 and 23), being considered deprived of *Stb*2 allele for resistance to *Septoria tritici* leaf blotch. These lines could harbour other *Stb* genes for resistance. Only two lines (9 and 11) had amplification products of different size from that of sensitive ones, being considered as harbouring *Stb* 2 allele for resistance. The molecular dimension of amplification products for sensitive dihaploid lines and for lines deprived of *Stb* 2 allele for resistance varies between 149 and 155 bp, while for lines harbouring *Stb* 2 allele for resistance is 171 bp, quite different from molecular dimensions obtained by Adhikari *et al*. (2004).

The amplification products obtained for P6, specific for Xgwdm 132 marker linked with *Stb* 3 locus, are monomorphic for all dihaploid lines, sensitive or with field

Tab. 1. Markers linked with different *Stb* resistance genes to *Septoria tritici* leaf blotch

Markers	Pairs of primers used	*Stb* genes linked with markers	Markers	Pairs of primers used	*Stb* genes linked with markers
Xgwm213	P1 (201-202)	*Stb*1	Xgwm44	P8(215-216)	*Stb*5
Xgwm335	P2 (203-204)	*Stb*1	Xgwm369	P9(217-218)	*Stb*6
Xgwm389	P3(205-206)	*Stb*2	Xwmc313	P13(97-98)	*Stb*7
Xgwm533	P4(207-208)	*Stb*2	Xgwm146	P10(219-220)	*Stb*8
Xgwm493	P5(209-210)	*Stb*2	Xgwm577	P11(221-222)	*Stb*8
Xgwdm132	P6(211-2129)	*Stb*3	Xwmc611	P12(223-224)	*Stb*.8
Xgwm111	P7(213-214)	*Stb*4			

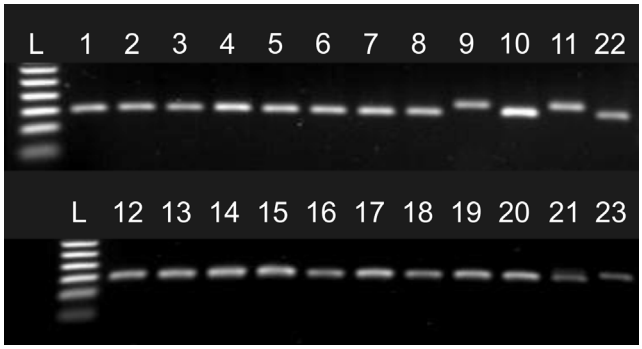

Fig. 1. Amplification products obtained from different dihaploid wheat lines with P5 pair of primers, specific primer for Xgwm 493 marker linked with *Stb* 2 locus. L - 50 bp ladder. Columns 1 to 21- there are lines with field resistance to *Septoria tritici* leaf blotch, 22 and 23 columns- sensitive lines

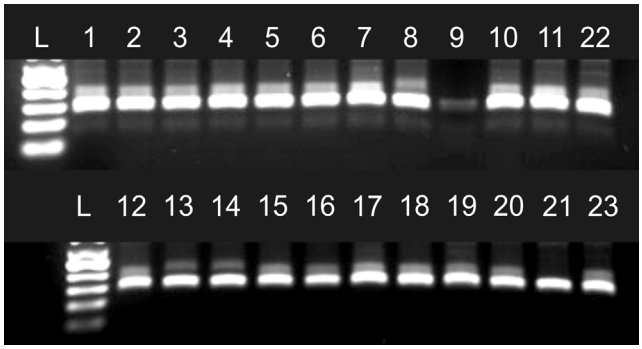

Fig. 2. Amplification products obtained from different dihaploid wheat lines with P7 pair of primer, specific primer for Xgwm 111 marker linked with *Stb* 4 locus. L- 50 bp ladder. Columns 1 to 21 - there are lines with field resistance to *Septoria tritici* leaf blotch, 22 and 23 columns- sensitive lines.

resistance to *Septoria tritici* leaf blotch. So we can consider our dihaploide lines deprived of *Stb* 3 resistance gene.

Similar results have been obtained for *Stb* 4, with P7 pair of primers, specific for Xgwm 111 marker (Fig. 2), and *Stb* 6 with P9 pair of primers, specific for Xgwm 369 marker (Fig. 3).

The results obtained for *Stb*1 and *Stb*5 were inconclusive.

The amplification products obtained for P13, specific for Xgwmc 313 marker linked with *Stb* 7 locus, are monomorphic for one amplification product (300 bp) and polymorphic for the other (202-241 bp), (fig 4). For some dihaploid lines (1, 3, 6, 8, 9, 11, 13, 14, 21) with field resistance to *Septoria tritici* leaf blotch, the amplification products around 200 bp are similar to those from sensitive dihaploid lines. These lines are considered deprived of *Stb* 7 resistant gene. The others (4,5,7,10,12,15,16,17,18,19,20 dihaploi lines), with amplification products around 240 bp, are considered carrier of *Stb* 7 resistant gene.

The amplification products obtained for P11, specific for Xgwmc 577 marker linked with *Stb* 8 locus, are polymorphic (Fig. 5).

Most of the dihaploid lines with field resistance to *Septoria tritici* leaf blotch have amplification products among 160 and 200 bp, specific for resistant alleles, being considered carriers of *Stb* 8 genes. The amplification products around 146 bp, of the sensitive dihaplid line (22) and two dihaplid lines with field resistance to *Septoria tritici* leaf blotch (18 and 19), are specific for the sensitive alleles (Adhikari *et al*., 2003) and as consequence these lines are deprived of *Stb* 8 gene but the two dihaploid lines with field resitance to *Septoria tritici* could carry other resistant genes.

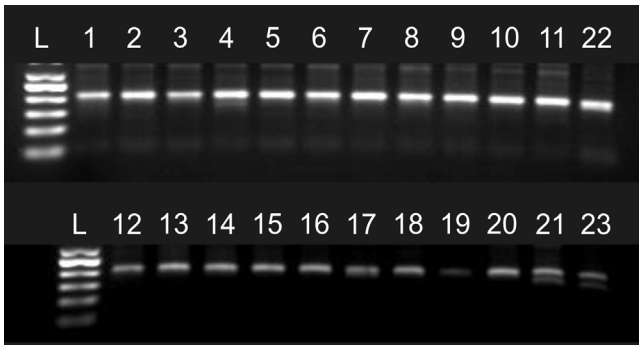

Fig. 3. Amplification products obtained from different dihaploid wheat lines with P9 pair of primers, specific primer for Xgwm 369 marker linked with *Stb* 6 locus. L - 50 bp ladder. Columns 1 to 21 - there are lines with field resistance to *Septoria tritici* leaf blotch, 22 and 23 columns- sensitive lines.

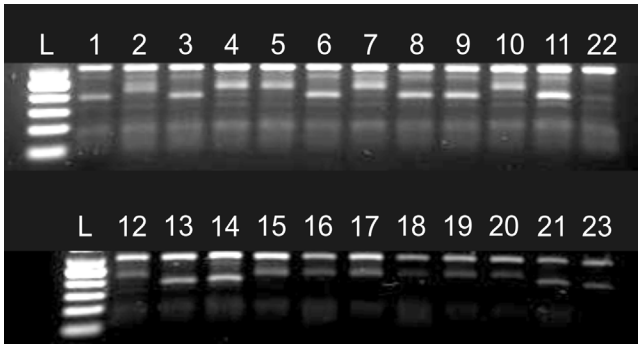

Fig. 4. Amplification products obtained from different dihaploid wheat lines with P13 pair of primers, specific primer for Xgwmc 313 marker linked with *Stb* 7 locus. L - 50 bp ladder. Columns 1 to 21 - there are lines with field resistance to *Septoria tritici* leaf blotch, 22 and 23 columns- sensitive lines.

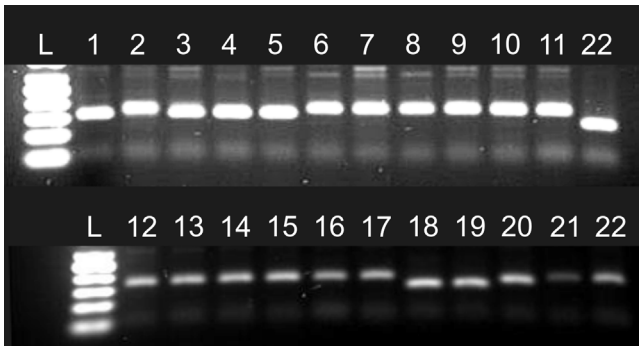

Fig. 5. Amplification products obtained from different dihaploid wheat lines with P11 pair of primers, specific primer for Xgwmc 577 marker linked with *Stb* 8 locus. L- 50 bp ladder. Columns 1 to 21- there are lines with field resistance to *Septoria tritici* leaf blotch, 22 and 23 columns- sensitive lines.

Conclusions

SSR marker could be useful in the wheat-breeding program in order to identify genes for wheat resistance to *Septoria tritici* leaf blotch and to facilitate the selection of individuals harbouring these genes.

All our dihaploid lines with field resistance to *Septoria tritici* leaf blotch carry at least one such a gene for resistance. Most frequently, in the tested material, *Stb* 7 and *Stb* 8 leaf blotch resistant genes were encountered.

The size of amplification products specific for SSR markers linked with *Stb* 7 and *Stb* 8 resistant genes was similar to those obtained by Adhikari *et al*., (2004) but the size of amplification products linked with *Stb* 2 resistant genes were quite different. The *Stb*3, *Stb* 4 and *Stb* 6 resistant genes were totally absent.

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