Testing of RAPD and SSR markers for wheat resistance to *Tilletia spp.* in *F*₂ segregating populations

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Abstract

The purpose of our study was testing some markers for the resistance genes to *Tilletia spp.* and using these markers in the selection for resistance. In order to do this, we have tested, at the *F*₂ generation, RAPD markers and microsatellite markers (SSR) known as being linked with some resistance genes. 

*F*₂ generation was obtained from crosses between resistant parental lines (99419G4-1A/1-1, 00274G2-31) and sensitive varieties (Glosa, Dropia). The DNA extracted from each individual *F*₂ plant was used to apply the Bulk Segregant Analysis method. The RAPD primers which identified polymorphism were for the markers UBC196 and Mic13, and the polymorphic fragments identified are possibly linked to some resistance genes. We have also tested 11 microsatellite markers (SSR), out of which Xgwm633, Xgwm114 have generated polymorphic fragments among resistant and sensitive plants. Analyses with microsatellite markers have shown the presence of resistance genes in the biological material tested (*Bt10, Bt11* and QTLs) and there is the possibility of using them in the selection for wheat resistance to *Tilletia spp.*

Key words: RAPD marker, SSR marker, wheat resistance, *Tilletia spp.*

Introduction

The species of *Tilletia* which cause common bunt of wheat (*Tilletia caries* and *Tilletia foetida*) are known from ancient times as a fungal pathogen of common wheat (*Triticum aestivum* L.). Both the wheat grain quality and the yield are reduced dramatically in all common wheat growing regions by the attack of *Tilletia* species (Nagy and Moldovan, 2006). By breeding for resistance and by obtaining resistant cultivars, the spread of *Tilletia* species can be reduced in time and space. Different wheat cultivars and lines presently cultivated were identified carrying some resistance gene to *Tilletia* (Dumalasová and Bartoš, 2006; Oncică and Săulescu, 2007). At least 15 resistance genes have been identified, some of them being localized on the wheat chromosomes. In the last years, for some of the these genes linked molecular markers were identified (Laroche et al., 2000, Fofana et al., 2008, Wang et al., 2009).

The microsatellites markers (SSR) are codominant, highly polymorphic and very informative markers and, once identified, they can be used to identify major genes or QTLs conferring wheat resistance to *Tilletia*; if tightly linked markers are identified, they can be used in selection programs for resistance by Marker Assisted Selection (MAS). The aim of our study was to test some already known and new RAPD and microsatellites (SSR) markers for resistance to *Tilletia spp.* (*Tilletia caries* and *Tilletia foetida*) in our local wheat cultivars and lines. For RAPD analysis the BSA (Bulk Segregant Analysis) method was applied (Michelmore et al., 1991).
Material and methods

Plant material. To obtain F₂ population, hybridization between resistant wheat parental lines and susceptible cultivars and lines was done during 2006-2007. The parental wheat cultivars and lines were obtained from NARDI Fundulea, Romania, being characterized by breeders as having resistance and susceptibility to *Tilletia*. The parental line 999419G4-1A/1-1 is known to be very resistant to *Tilletia*, but we do not know which resistance gene is carrying (the resistance comes from their parents: Colonias – a brazilian cultivar, or from Bucur – a breeding Romanian line). The line 999419G4-1A/1-1 was used in the crosses as a parental form. The line 00274G2-31 is a parental resistant line carrying Bt5 resistance gene and the cultivar Glosa is known as a very susceptible cultivar.

After the F₂ populations were obtained, the F₂ plants were tested for resistance to *Tilletia* by artificial inoculation with spores of *Tilletia tritici* and *Tilletia foedida*; tests were carried out in the field. The two populations 999419G4-1A/1-1xGLOSA and 00274G2-31xGLOSA, consisting of 50 individual plants, were tested for presence of the markers.

DNA isolation and Bulk Segregant Analysis - Wheat genomic DNA was isolated from leaves following the protocol described by Lodhi et al., (1994) based on CTAB (cetyltrimethylammonium bromide) protocol. Resistant and susceptible plants were selected to apply Bulk Segregant Analysis method. The DNA from 5-10 resistant and respectively susceptible plants was mixed (150 ng) to form the bulks. For RAPD analysis the primers Mic13 and UBC196 were tested. The primer UBC196 (CTCTCCCTCCC) was previously identified by Demeke et al., (1996), linked with Bt10 resistance gene and amplifying a polymorphic fragment of 590 pb for resistance.

Microsatellite primers - Eleven microsatellite primer pairs for eleven markers localized on different wheat chromosomes were tested. Some of these markers were previously identified linked with the resistance gene: Xgwm818, Xgwm63 and Xgwm633 (Fofana et al., 2008); Xbarc128, Xgwm264 and Xgwm374 (Wang et al., 2009); Xgwm114 (Cichy and Goates, 2009); Xgwm469, Xgwm749, Xgwm259 (Menzies et al., 2006) and Xgwm403. These markers were localized on the wheat chromosomes 1B, 2B, 3B, 7A and their sequence was obtained form the GrainGenes database at http://wheat.pw.usda.gov. The primers were sintetized by Microsynth, Switzerland.

PCR amplification and gel electrophoresis - The PCR amplification reaction was performed in a volume of 25 μl of reaction mixture containing: Dream PCR Buffer (10x) (Fermentas), 20mM MgCl₂, 200 μM of each nucleotide (dATP, dTTP, dCTP, dGTP), 1 unit DreamTaq DNA Polymerase (Fermentas), 4μM primer, 60 ng genomic DNA. The parameters of temperature were: at 94ºC for 3 min (1 cycle); 94ºC for 1 min, 32-34 ºC – depending on the primer – for 1 min, 72 ºC for 2 min (45 cycles); 72 ºC C for 7 min (1 cycle). For SSR amplification the parameters of temperature for PCR were: 94ºC for 3 min (1 cycle); 94 ºC for 1 min, 50-60 ºC (depending on the primers) for 1 min, 72 ºC for 1 min (35 cycles) and at 72ºC for 7 min (1 cycle). The amplification products was separated by electrophoresis in agarose gel 1,2% with TBE buffer (Tris-borate-EDTA). For SSR markers analysis polyacrylamide gel electrophoresis was carried out (6 % polyacrylamide denaturing gel in 0.5 x TBE running buffer). The PCR products were visualized by silver staining. As a molecular weight marker in the gel was loaded 3μl of 20-pb ladder (Fermentas).

Results and discussions

Testing of RAPD markers - The primer UBC196 was tested on the parental lines and cultivars and identified a polymorphic fragment of 790 bp in the line 999419G4-1A/1-1 which was absent in all susceptible lines and cultivars (Figure 1, A). The primer Mic 13 (TTCCCCCCAG) identified also a polymorphic fragment of approximatley 500 bp in the resistant line 999419G4-1A/1-1, which was absent in all susceptibles lines and cultivars (Figure 1, B). This primer was also tested at the F₂ population.
Microsatellite markers analysis - Initially, the microsatellite primers we tested for polymorphism between parents (susceptibles and respectively resistant to Tilletia). Two SSR primer pairs for two markers revealed polymorphisms between the two parents: Xgwm633 and Xgwm114, eight were monomorphic. The marker Xgwm633 localized on the wheat chromosome 7A was reported previously close linked with the QTL for resistance to Tilletia (Fofana et al., 2008). In our analysis the primers for the marker Xgwm633 identified a polymorphic fragment of 220 bp in the resistant line 999419G4-1A/1 and a fragment of 240 bp in the susceptible parental lines (including cultivar Glosa), (Figure 2, B). The polymorphism was tested at the entire F2 population. The marker was also polymorphic in the 999419G4-1A/1xGlosa F2 population. The primers for the marker Xgwm114 identified also a polymorphic fragment in the resistant wheat line 00274G2-31 of 150 bp and the fragment of susceptibility was present in the susceptible cultivar Glosa at 190 bp.

The F2 plants from 999419G4-1A/1xGLOSA population was genotyped for the marker Xgwm633. Preliminary analysis in the agarose gel electrophoresis shows that plants are carrying either the marker resistant, or the marker for the susceptible marker or they are heterozygous (Figure 3, up). However, some unspecific PCR products are visible in the polyacrylamide gel (Figure 3, down). The data were used for linkage analysis between the marker and the resistance gene. Chi square test was applied to test for linkage between marker and the resistance gene. The value of chi square for the linkage was \( \chi^2=1.97 \) at a probability of \( P=20\% \). Although the marker Xgwm633 is loosely linked with the resistance gene, future analysis in the region of the wheat chromosome 7A will identify new markers for the resistance to Tilletia.
Figure 2. Microsatellite DNA products amplified by the primers for the marker Xgwm114 (A) and Xgwm633 (B) at the parental wheat cultivars and lines. R1-999419G4-1A/1-1, R2-00274G2-31, R3-00281G2-11, R4-00399G2-11, R5-00450G1-1, S1-Farmec, S2-Delabrad, S3-F96869G1-108, S4-Glosa, S5-Boema, S6-Jiana, S7-Crina, S8-Dropia, L-Molecular weight marker, 100 bp.

Figure 3. Genotyping of the F2 population 999419G4-1A/1-1xGLOSA with the marker Xgwm633. P1-resistant parent 999419G4-1A/1-1, P2-susceptible parent Glosa, 28 of the 50 F2 plants, M-Molecular weight marker, 100 bp.

Conclusions

When testing the RAPD markers for Bt10 resistance gene, a polymorphic fragment of 790 bp was identified absent in all susceptible lines and cultivars, possibly linked with the resistance gene. The RAPD primer Mic13 identified also a fragment of approximately 500 pb in the resistant bulk, which was absent in the susceptible bulk. Screening of eleven microsatellite marker, two of them identified polymorphism for resistance to Tilletia. The primers for the markers Xgwm633 identified a fragment of 220 bp for the resistant lines 999419G4-1A/1-1 and a fragment of 240 bp for the susceptibility marker in cultivar Glosa. The distance between the marker and the resistance gene was estimated to be 23cM by linkage analysis. The primers for the marker Xgwm114 (for Bt11 resistance gene) also identified a fragment of 150 bp for the resistance in the line 00274G2-31 and a fragment of 190 bp for susceptibility in the cultivar Glosa. A further analysis is necessary to test this marker in polyacrylamide gel electrophoresis.
References


