SCREENING ROMANIAN WINTER WHEAT GERMPLASM FOR PRESENCE OF *Bt10* BUNT RESISTANCE GENE, USING MOLECULAR MARKERS

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ABSTRACT

Common bunt and dwarf bunt caused by *Tilletia caries* (DC) Tul., *T. foetida* (Wallr) Liro. and *T. controversa*, respectively, can cause considerable yield and quality losses in wheat. Growing resistant cultivars is the most economic and environmentally friendly option in bunt control, especially important in organic agriculture systems. *Bt10* is one of the most effective bunt resistance genes, widely used in wheat breeding programs worldwide, including programs in Romania. We used the PCR marker developed by Laroche et al. (2000), to screen Romanian semidwarf winter lines for presence of *Bt10* gene. The marker detected the presence of a specific 275 bp amplification product in PI 554118 and in one semidwarf line which has PI 554118 in its pedigree, but not in any of the lines obtained from crosses involving PI 178383, carrier of *Bt8 + Bt9 + Bt10*. Presumably, *Bt10* was eliminated during selection, either through genetic drift or because it might be associated with some unfavourable traits. Selection for bunt resistance only retained one or both other resistance genes present in PI 178383. Screening three lines in which bunt resistance genes could not be identified from genealogies, showed that they do not carry *Bt10*. Further studies should demonstrate if they carry other known *Bt* genes, not obvious from their parentage, or they are indeed carriers of new resistance genes.

Key words: Bunt resistance, molecular markers, wheat.

INTRODUCTION

Common bunt and dwarf bunt caused by *Tilletia caries* (DC) Tul., *T. foetida* (Wallr.) Liro. and *T. controversa*, respectively, can cause yield and quality losses in Romania and many other countries. The problem caused by these diseases seemed to be solved once with introduction of effective chemical treatments, but common bunt and dwarf bunt still occur for different reasons, such as: organic farming, small-scale farming or incorrect chemical treatment. There are not very effective organic compounds for seed treatment approved at farm level and the probability of high infection exists (Borgen & Davanlou, 2000, cited by Liatukas and Ruzgas, 2006). This suggests that there is considerable need for bunt resistant cultivars.

There are more than 15 specific resistance genes, named *Bt*, which control the resistance to common and dwarf bunt (Goates, 1996).

Introduction of *Bt*-resistance genes into adapted varieties has been the most widely used breeding approach for reducing the risk associated with these pathogens, but breeding high-yielding bunt resistant wheat cultivars faces many difficulties related to the poor agronomic performance of most known sources of resistance and the incomplete expressivity of susceptibility alleles. Even after several cycles of crossing with high-yielding cultivars, the association of bunt resistance with some unfavourable traits remains difficult to break. Molecular markers associated with bunt resistance genes could accelerate introduction of these genes in good agronomic type genotypes (Ciucă et al., 2007).

The *Bt10* gene proved to be efficient in controlling common bunt in many parts of the world. For example, the *Bt10* resistance gene was reported as effective against all the known races of common bunt caused by *Tilletia tritici* and *T. laevis* in western Canada, and in Germany all analyzed races were avirulent against *Bt10* (Wächter et al., 2007). A RAPD markers for *Bt10* was identified by Demeke et al. (1996), and later Laroche et al. (2000) developed a highly specific PCR marker. *Bt10* was located on the short arm of chromosome 6D (Menzies et al., 2006).

In Romania, studies at the National Agricultural Research and Development Institute (NARDI) Fundulea, regarding efficiency of race-specific *Bt* genes revealed a good level of resistance in genotypes carrying *Bt10* gene (Ittu et al., 2000; Ciucă et al., 2007).

This paper presents results of using the PCR marker for bunt resistance gene *Bt10*, published by Laroche et al. (2000), to screen wheat breeding lines for the presence of this gene.

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MATERIAL AND METHODS

Plant material

Four groups of winter wheat lines, originating from the breeding program of the National Agricultural Research and Development Institute Fundulea, Romania and of the Agricultural Research and Development Station Şimnic, were studied (Table 1).

These included:

- PI 554118 (the original *Bt10* source) and one line selected from a cross involving this carrier of bunt resistance gene *Bt10*;
- 13 lines obtained by crossing susceptible wheat cultivars with the Turkish local population PI 178383, as a source of resistance to bunt. PI 178383 was described as carrying three bunt resistance genes, namely *Bt8*, *Bt9* and *Bt10*;
- 12 lines selected from crosses with resistance sources, carrying other known genes;
- 3 bunt resistant lines, for which the resistance gene(s) could not be established based on genealogy.

All lines had been previously tested under artificial inoculation with common bunt, both at NARDI Fundulea and Agricultural Research and Development Station Simnic, and found to be resistant (Ittu et al., 2001; Oncică and Săulescu, 2007).

Table 1. Winter wheat breeding lines tested for the presence of bunt resistance gene *Bt10*

Name	Genealogy	Number of diseased spikes under artificial inoculation
Original *Bt10* source and germplasm obtained from crosses with a *Bt10* carrier parent		
PI 554118	PI 178383 / Elgin	0
F94975GM1-11	Dropia/ *Bt10*	0
Germplasm obtained from crosses with PI178383 (a *Bt8+Bt9+Bt10* carrier)		
Greti	PI 178383/Iulia//Aura/3/ Flamura/4/Dropia/5/ F30R2-1	0
F99146G1-101	F7022W/5/PI 178383/Iulia//Aura/3/ Flamura/4/ Dropia/6/ F577U1-106	0
F99146G4-122	F7022W/5/PI 178383/Iulia//Aura/3/ Flamura/4/ Dropia/6/ F577U1-106	0
F00263G2-11	PI 178383/Iulia//Aura/3/ Flamura/4/ Dropia/5/ Boema	0
F00399G2-11	F7022W/5/PI 178383/Iulia//Aura/3/ Flamura/4/ Dropia/6/ F577U1-106/7/ Delabrad	0
F00399G2-112	F7022W/5/PI 178383/Iulia//Aura/3/ Flamura/4/ Dropia/6/ F577U1-106/7/ Delabrad	0
F00399G2-113	F7022W/5/PI 178383/Iulia//Aura/3/ Flamura/4/ Dropia/6/ F577U1-106/7/ Delabrad	0
F01438G1-11	PI 178383/Iulia//Aura/3/ Flamura/4/ Dropia/5/ F93122G4-1/6/ Crina	0
F02065G4-12	Izvor/6/ PI 178383/Iulia//Aura/3/ Flamura/4/ Dropia/5/ F30R2-1	0
F02065G5-1	Izvor/6/ PI 178383/Iulia//Aura/3/ Flamura/4/ Dropia/5/ F30R2-1	0
F02065G5-22	Izvor/6/ PI 178383/Iulia//Aura/3/ Flamura/4/ Dropia/5/ F30R2-1	0
F02065G8-1	Izvor/6/ PI 178383/Iulia//Aura/3/ Flamura/4/ Dropia/5/ F30R2-1	0
LRM 16	Diana / PI 178383//2*Aurora	0
Germplasm obtained from crosses with other bunt resistance genes		
F00274G2-31	Dropia/ Hohenheim // Delabrad	0
F02030G1-11	Dropia/ Hohenheim // F96831G2-1	0
F02030G1-12	Dropia/ Hohenheim // F96831G2-1	0
F02030G1-21	Dropia/ Hohenheim // F96831G2-1	0
F02030G1-22	Dropia/ Hohenheim // F96831G2-1	0
F03282G1	Dropia/ Hohenheim // *Eruthrospermum*26221/3/ Horea	0
F03282G1-11	Dropia/ Hohenheim // *Erythrospermum*26221/3/ Horea	0
F00281G2-11	Dropia/PI 166910 // Delabrad	0
F01450G1-1	Dropia/ PI 166910 // Delabrad/3/ F93122G6-209	0
F01450G1-11	Dropia/ PI 166910 // Delabrad/3/ F93122G6-209	0
F00287G1-11	R93-233/2*Dropia// Delabrad	0
F02062G1-11	R93-233/2*Dropia// F96831G7-2	0
Bunt resistant germplasm carrying not identified resistance genes		
F96915G1-1	WGRC 23/ Dropia	0
F99419G4-1	Colonias / Bucur	0
F00628G34-1	Triticale191TR1-1221 / 2* wheat	0

Methods

DNA isolation

Total DNA were isolated from leaves following the protocol proposed by Saghai-Maroof et al. (1984).

PCR was performed according to Laroche et al. (2000), with small modification, in 0.2 ml tubes containing 25 µl final volume of a reaction mixture consisting of 1X buffer, 1.5 mM $MgCl_2$, 0.4 µM primer, 0.65U of Taq polymerase (Go Taq Flexi-Promega), 0.2 mM each dNTP and 18-20 ng of genomic DNA. The used primers were FSD-GTT TTA TCT TTT TAT TTC; and RSA-CTC CTC CCC CCA. The small modifications included the type of Taq polymerase enzyme and its concentration, a little higher DNA concentration and not using gelatine.

The amplification was performed using Applied Biosystem 9600 thermal cycler, programmed for 35 cycles, each consisting of: 1 min. at 94°C, 1 min. at 42°C, 2 min. at 72ºC, and 7 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.

RESULTS AND DISCUSSION

PCR reaction using FSD/RSA primers produces a 275 bp fragment associated to *Bt10* resistance gene (Laroche et al., 2000).

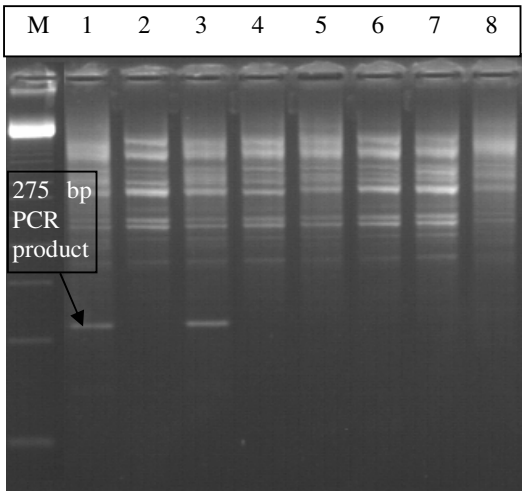

Figure 1. Amplification products obtained using the FSD/RSA primers (M. Ladder 123 bp; 1. *Bt10* source; 2. Dropia (susceptible); 3. F94975GM1-11; 4. LRM 16; 5. F96915G1-1; 6. Delabrad (susceptible); 7. F00263G2-11; 8. F00274G2-31)

Figure 1 shows that this fragment is clearly visible in our BioPrint images in the original *Bt10* source PI 554118 and in line F94975GM1-11 (obtained from the cross between the susceptible cultivar Dropia and this *Bt10* source), while being absent in Dropia (Table 2).

Table 2. Presence of the 275 bp PCR product after amplification with FSD/RSA primers

Name	Bunt resistance source	Presence of 275 bp PCR product
Original *Bt10* source and germplasm obtained from crosses with a *Bt10* carrier parent		
PI 554118	*Bt10* form PI 178383	+
F94975GM1-11	*Bt10*	+
Germplasm obtained from crosses with PI178383 (a *Bt8* + *Bt9* + *Bt10* carrier)		
Greti	PI 178383	-
F99146G1-101	PI 178383	-
F99146G4-122	PI 178383	-
F00263G2-11	PI 178383	-
F00399G2-11	PI 178383	-
F00399G2-112	PI 178383	-
F00399G2-113	PI 178383	-
F01438G1-11	PI 178383	-
F02065G4-12	PI 178383	-
F02065G5-1	PI 178383	-
F02065G5-22	PI 178383	-
F02065G8-1	PI 178383	-
LRM 16	PI 178383	-
Germplasm obtained from crosses with other bunt resistance genes		
F00274G2-31	Hohenheim (carrier of *Bt5*)	-
F02030G1-11	Hohenheim (carrier of *Bt5*)	-
F02030G1-12	Hohenheim (carrier of *Bt5*)	-
F02030G1-21	Hohenheim (carrier of *Bt5*)	-
F02030G1-22	Hohenheim (carrier of *Bt5*)	-
F03282G1	Hohenheim (carrier of *Bt5*)	-
F03282G1-11	Hohenheim (carrier of *Bt5*)	-
F00281G2-11	PI 166910 (carrier of *Bt11*)	-
F01450G1-1	PI 166910 (carrier of *Bt11*)	-
F01450G1-11	PI 166910 (carrier of *Bt11*)	-
F00287G1-11	R93-233 (carrier of *Bt8*)	-
F02062G1-11	R93-233 (carrier of *Bt8*)	-
Bunt resistant germplasm carrying not identified resistance genes		
F96915G1-1	WGRC23 (gene not identified)	-
F99419G4-1	Colonias (gene not identified)	-
F00628G34-1	Triticale (gene not identified)	-

The Turkish local population PI 178383 was intensively used in breeding programs worldwide, as it carries three very efficient bunt resistance genes (*Bt8, Bt9* and *Bt10*). It was also the first source of bunt resistance used in the breeding program at NARDI Fundulea (Ittu et al., 2000), as well as at ARDS Şimnic (Oncică, 2007).

When we tested several bunt resistant wheat lines produced by crosses between PI 178383 and various susceptible parents (Iulia, Flamura 80, Aura, Dropia, Boema, Aurora, Diana etc.), the amplification product of 275 bp was not detected. Presumably, selection for adaptation and plant type among bunt resistant progenies led to discarding the *Bt10* carriers and retained *Bt9* or *Bt8* carriers.

Further studies should confirm if *Bt10* gene, itself or genes linked with it, has indeed unfavourable effects in our environment, or its loss was the result of genetic drift.

As expected, none of the lines known to carry other bunt resistance genes showed the 275 bp amplification product. This is a confirmation that this amplification product is specific only to *Bt10*.

Screening three lines in which bunt resistance genes could not be identified from genealogies, showed that they do not carry *Bt10*. Further studies should demonstrate if they carry other known *Bt* genes, not obvious from their parentage, or they are indeed carriers of new resistance genes, as suggested by Oncică and Săulescu (2008).

CONCLUSIONS

The PCR marker developed by Laroche et al. (2000) proved to be efficient for screening breeding germplasm for the presence of bunt resistance gene *Bt10.* Presence of *Bt10* gene was confirmed in one semidwarf line of good agronomic type, bred at NARDI Fundulea.

In our study, several resistant lines derived from crosses with PI 178383 did not inherit *Bt10* and are probably *Bt8* or *Bt9* resistance gene carriers. We suppose that the gene *Bt10*, itself or genes linked with it, might have unfavourable effects on plant type and adaptation to our environment, and therefore was not retained following concomitant selection for bunt resistance and plant type.

Presence of *Bt10* gene was not detected in bunt resistant lines, for which none of the known *Bt* genes could be detected in the parentage. Further studies are necessary to prove if they carry new bunt resistance genes.

Acknowledgements

The authors are very grateful to Dr. Mariana Ittu for providing plant material and informations about resistance to bunt in artificial inoculation.

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