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A Preliminary Report on the Identification of SSR Markers for Bunt (*Tilletia* sp.) Resistance in Wheat

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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 still cause yield and quality losses, despite the availability of effective chemical treatments. Growing resistant cultivars remains the best option for economical and environmental reasons, and is the only effective alternative in organic farming. As the durability of bunt resistance has proved to be rather poor, the pyramiding of resistance genes has been envisaged as a method of extending the life of resistance genes. Molecular markers can considerably increase the efficiency of gene pyramiding, but, because incomplete expression of both susceptibility and resistance genes makes accurate phenotyping difficult, very few markers associated with bunt resistance genes have been identified to date. This is why, at the National Agricultural Research & Development Institute Fundulea-Romania, along with the breeding program for bunt resistance, research on the possible use of molecular markers for Marker Assisted Selection (MAS) was developed. Random F_5 or F_4 lines from crosses between a *Bt11* line or a bunt resistant line derived from a Triticale/2 × wheat, and susceptible parents, were phenotyped under artificial inoculation conditions, and were genotyped using primers for several markers. Preliminary results suggest that the *Bt11* gene is located on chromosome 3B, and may be associated with marker loci *Xbarc180*, *Xwmc623*, *Xwmc808* and *Xgwm285*. The gene for bunt resistance transferred from Triticale (line F00628G34-1 – possessing a 1A/1R translocation) can make MAS possible by using 1R specific markers. Although these results are preliminary, they already prove to be useful for the diversification and pyramiding of bunt resistance genes in breeding for durability of bunt resistance.

Keywords: *Bt11*; durability of resistance; MAS; rye introgression

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. Despite the availability of effective chemical treatments, growing resistant cultivars is best option for economic and environmental reasons, and is the only effective alternative in organic farming.

There are more than 15 specific resistance genes, named *Bt* genes, which can be used to breed resistant cultivars (Goates 1996), and other, possibly different, sources have been identified (Oncică & Săulescu 2008). However, resistance may be overcome by the selective increase of virulent races or by the development of new combinations of virulence genes in the bunt population (Goates 1996). Because virulence in *Tilletia* has proved to evolve rapidly, the usefulness of individual resistance genes has been short lived.

The pyramiding of resistance genes can increase the durability of resistance, and this can be best achieved by using molecular markers. Unfortunately, because incomplete expression of both susceptibility and resistance genes makes accurate phenotyping difficult, very few markers associated with bunt resistance genes have been identified.

The results of Laroche *et al.* (2000), Galaev *et al.* (2006) and Wang *et al.* (2009) are among the few exceptions.

This is why, at the National Agricultural Research & Development Institute Fundulea-Romania, along with the breeding program for bunt resistance, research on the possible use of molecular markers for Marker Assisted Selection (MAS) is being carried out. Besides using already described markers (Ciucă & Săulescu 2008), we are attempting the identification of other markers for use in breeding for durable bunt resistance. This paper summarizes some of the preliminary results obtained so far on SSR markers associated with bunt resistance genes.

MATERIALS AND METHODS

We present here results obtained in two experiments:

Experiment 1 aimed at the identification of SSR markers associated with *Bt11*, a not much used gene, but found to be effective against the bunt races present in Romania (Ittu *et al.* 2001; Oncică & Săulescu 2007);

Experiment 2 was directed towards identification of SSR markers for gene(s) controlling bunt resistance detected in a potentially new resistance source F00628G34-1, derived from a Triticale/2 × wheat cross. This resistance has proved to be effective in Romania (Ittu *et al.* 2006; Oncică & Săulescu 2008), as well as in the European *Tilletia* Ring test, coordinated by Fabio Mascher-Frutschi, ACW, Switzerland.

Plant material

Since doubled haploid lines, which would have been more appropriate for phenotyping, were not available, we attempted to obtain as much information as possible using segregating populations already available in the breeding program.

For experiment 1, 117 F_5 lines, randomly selected from the cross between F94976GM28 (a *Bt11* gene carrier selected from a cross between DROPIA and PI 554119 – the source of *Bt11*) and a Romanian bunt susceptible breeding line Liman, were artificially inoculated with bunt in 2009, and classified as resistant, susceptible or segregating.

For experiment 2, 70 random F_4 lines from a cross between F00628G34-1 and a susceptible Romanian cultivar Litera were artificially inoculated with bunt, and one individual plant from each row was used for collecting leaves for molecular analysis and for characterizing the presence/absence of bunt at harvest.

Molecular analysis

Total DNA was isolated from leaves and purified following the protocol proposed by Sagai-Maroof *et al.* (1984), using 2% CTAB. Amplification was performed in a 25 µl final volume using Applied Biosystem 9600 thermal cycler, with Go Taq Polymerase – Promega.

In experiment 1, for identification of markers associated with the *Bt11* gene we tested about 100 SSR primer pairs with different chromosome localizations. Out of these, only 6 showed polymorphism between the parents of the analyzed cross, and were used for Bulk Segregant Analysis (BSA) of F_5 lines (Table 1). We bulked DNA from leaves collected earlier from 20 homogenous resistant and 20 susceptible lines, respectively.

In experiment 2, to trace rye chromatin in the parent F00628G34-1 and in segregating lines, we used:

- the set of F3/R3 primers, described by Katto *et al.* (2004) as a universal marker for rye chromatin, following the protocol of these authors;
- primers for SCM9, specific to the 1RS rye chromosome (Saal & Wricke 1999), used according to the protocols described at http://maswheat.ucdavis.edu.protocols/Drought/index.htm;
- primers for IAG95-STS (using the protocol described at http://wheat.pw.usda.gov/GG2/index.shtml;
- primers for Sec-1 (o-sec-5'/a and o-sec-3'/r) as described by Shimizu *et al.* (1997);
- primers for *Xtsm106* and *Xtsm123*, located on chromosome 1R by Kofler *et al.* (2008).

Additionally, primers for *Xbarc1048*, *Xwmc818*, *Xgwm136*, *Xgdm33*, and *Xbarc263*, all located on wheat chromosome 1A, were used to identify heterozygous segregating individuals (according to the protocol indicated on http://wheat.pw.usda.gov/GG2/index.shtml).

RESULTS AND DISCUSSION

In experiment 1, all markers showing polymorphism between the *Bt11* carrier and non carrier parent were located on chromosome 3B, or had multiple locations

Table 1. The primers that showed polymorphism between parents F94976GM28 (a *Bt11* carrier) and Liman

Primers name	Annealing temperature used in this study (°C)	Chromosome localization
barc180	52	3B, 5A, 6B,7A
gwm285	60	3B
wmc808	61	3B
gwm566	60	3B
wmc231	65	3B
wmc623	62	3B

which included chromosome 3B. This information is useful in view of further genetic studies.

Primers for markers *Xbarc180*, *Xwmc623*, *Xwmc808*, and *Xgwm285* showed clear differences between the bulks of resistant and susceptible lines selected from the cross F94976GM28/Liman. This is a good indication of the association of these markers with the *Bt11* gene, even before the validation of the results by inspection of the resistance or susceptibility of individual lines. We shall continue our study to establish which marker is closest to the *Bt11* resistance gene and is the most suitable for MAS. However, on the basis of results obtained to date, we already consider that the above mentioned markers could be used in MAS for introducing the *Bt11* gene in wheat breeding programmes.

In experiment 2, molecular analysis using Katto's universal marker for the detection of rye chromatin showed that F00628G34-1 carries rye chromatin, and analysis with SCM9 proved that rye chromosome 1R was present. Parallel analysis using markers located on the wheat homoeologous group 1 chromosomes, as well as FISH and GISH analysis, performed with the assistance of the wheat genetics team of the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary, showed that this line carries a 1R/1A translocation.

DNA from individual F_4 plants was analyzed using primers for the universal marker for rye chromatin, and 5 markers specific for rye chromosome 1R. In most cases all these markers co-segregated, suggesting that, as rule, the translocation was inherited as such, without deletions or recombination with wheat chromosomes. The plants where we detected 1 or 2 missing 1R specific markers are being further investigated to confirm whether, in these cases, the translocated 1R chromosome arm was indeed modified.

Parallel characterization of all plants for the presence of wheat 1A specific chromosomes allowed identification of heterozygous individuals, carrying both the 1A/1R translocation and the 1A chromosome.

The percentage of bunt infected plants was more than two-times higher in plants without the 1A/1R translocation than in plants carrying the translocation (Table 2). The percentage of bunt infected individuals was similar in the heterozygous plants and in plants homozygous for the 1A chromosome, suggesting that the resistance gene is recessive. Although there is a considerable overlap of bunt infection levels in the distributions of 1A/1R carriers and non-carriers, a χ^2 test showed that the observed distribution was significantly different from one expected assuming that the translocation had no effect on bunt attack ($P < 1\%$). Therefore, markers specific for the 1R chromosome could be used for MAS in crosses between line F00628G34-1 and bunt susceptible parents, with the condition that the susceptible parents are not carriers of 1B/1R or 1A/1R translocations, and 1R comes from other sources.

As expected, results from phenotyping bunt resistance in the segregating populations studied were not clear, for many different reasons. As Goates (1996)

Table 2. Percentage of bunt infected or un-infected F_4 plants in relation to the presence or absence of rye chromatin

	Percentage of plants		
	with rye chromatin	heterozygous	without rye chromatin
Attacked	18.9	38.4	40.0
Healthy	81.1	61.6	60.0

pointed out, even under severe artificial inoculation not all susceptible plants are infected. In our environment the percentage of diseased individuals in a susceptible cultivar can sometimes be as low as 60%. On the other hand, resistant cultivars are rarely completely immune to infection, and they are often considered as resistant to this disease, even with up to 5% diseased plants. Modifying genes can also influence infection data.

More precise studies of the association between bunt resistance and bunt resistance genes, mainly using doubled haploid lines, are necessary, and these are under way.

CONCLUSIONS

Molecular analysis of hybrid populations segregating for the *Bt11* gene or an unnamed gene for bunt resistance transferred from Triticale suggest that:

– the *Bt11* gene is located on chromosome 3B and may be associated with markers *Xbarc180*, *Xwmc623*, *Xwmc808,* and *Xgwm285*;
– a gene for bunt resistance detected in our study might be located on the 1A/1R translocation of line F00628G34-1, making MAS possible by using 1R specific markers.

Although these results are preliminary, they are already proving useful for diversification and pyramiding of bunt resistance genes in breeding for durability of bunt resistance.

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