

Research Article

Molecular Characterization of Bunt Resistance in Romanian Wheat Line F00628G34-M, Selected From a Triticale (*Triticosecale*) X Winter Bread Wheat (*Triticumaestivum*) Cross

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Abstract

Bunts are diseases produced by *Tilletiacaries*, *T. laevis*, *T. controversa* and *T. secalis* with severe effects on wheat yield and quality. Their importance has increased, especially in organic agriculture, where chemical treatments are forbidden. Rye is highly resistant to *Tilletia sp.* Breeders at NARDI Fundulea obtained a bunt resistant line F00628G34-M derived from a Triticale/2' wheat cross. Sixty eight randomly extracted F4 lines from a cross between F00628G34-M and susceptible wheat cultivar Literawere phenotyped (using local populations of *Tilletia sp.*) and genotyped with 20 specific markers for 1 RS and 15 SSR markers located on 1AS. Chi square test showed significant deviation ($P < 5\%$) from the expected Mendelian monogenic segregation, suggesting that the resistance gene is recessive or partially dominant and/or the resistance is affected by suppressing factors from wheat genome. Molecular markers assay proved a significant association of the bunt resistance inherited from the F00628G34-M line, with the 1A/1R translocation, suggesting that the bunt resistance gene originated from rye could be located in the region homeologous with the Glu-A3 locus and close to Xgwm1223 microsatellite locus. To our knowledge, this is the first time when it is proven that a bunt resistance gene is associated with the rye chromatin transfer to wheat.

Keywords: Wheat; Rye; Triticale; Bunt; Molecular markers; *Tilletia sp*

Introduction

Agriculture in Europe has been moving toward organic farming and with “low-inputs”, including reduced chemicals use. Reduction or lack of chemical seed treatments have led to resurgence of many seed-borne diseases including bunts, which were previously controlled with chemicals [1]. In Romania, if untreated seeds are used, the incidence of bunt can reach up to 70-80% and the yield losses can reach up to 40% [2].

Wheat bunts are produced by *Tilletia caries* (DC) Tul, *T. laevis* (Wallr) Liro. (Common bunt) and *T. controversa* (dwarf bunt), but *T. secalis* (on rye) can also affect wheat. Although chemical treatments have been successfully used to control bunt, genetic resistance is the most economical and ecological way of control, and is the only acceptable approach in organic agriculture. This is why bunt resistance has become an important objective in wheat breeding. At present, have been identified more than 15 resistant genes to common bunt (*Bt1* to *Bt15*) and in addition, the gene *Bt-Z* in *Agropyroninter medium*, the gene *Bt-P* in wheat variety PI 173438, a new gene in Blizzard and also, a QTL on 7B chromosome in McKenzie cv designated *QCbt.spa-7B.1* [3-6]. The efficiency of resistance genes is different, but not very different from one area to another. In Romania the following genes have been identified with a good efficiency: *Bt5*, *Bt8*, *Bt10*, *Bt11*, *Bt12*, *Bt13* [7].

Rye (*Secalecereale* L.) has already provided genes that proved useful in wheat breeding [8,9] and many cultivars that enjoyed a great success over time are carriers of translocations from rye, especially of some translocations in which the short arm of 1R chromosome is involved. There are still many genes of interest for wheat improvement, not yet transferred, that are available in the rye genome, and that includes bunt resistance genes.

Phenotype observations conducted in the Czech Republic on 17 triticale cultivars showed presence of *T. caries* pathogen in a very low percent (2.4%) only on one triticale cultivar (Triamant) [10]. This result suggested that, due to the presence of rye chromatin, triticales are very resistant to common bunt attack. However, both rye and triticale were attacked by *T. controversa* (dwarf bunt). Similar results were reported by other researchers [4].

Triticale can be successfully used as a bridge for transferring useful rye genes to wheat [9]. The line F00628G34-M, created at NARDI Fundulea by crosses between triticale (*Triticosecale*) and wheat (*Triticumaestivum*), showed good resistance to bunt in artificial infections, both in tests from Romania, done at Fundulea [7] and Simnic [11] and in most locations of international tests from the European project “*Tilletia* Ring Test”. In the frame of this project, the F00628G34-M line was affected by *Tilletia spp.* at different levels, but considerably less than the susceptible entries (Austria -11.5%, Germany -15.5%, Croatia -race T7- 7.4%; race T9- 4.2%; race T17-

Table 1: Molecular markers used in this study and their specificity.

No.crt.	Name	Primerssequences	Location on chromosome
Markers for rye – specific localization on 1RS			
1	F3 R3	F: GATCGCCTCTTTGCCAAGA R: TCACTGATCACAAAGAGCTTG	Universal marker for rye
2	SCM9	F: TGACAACCCCTTTCCCTCGT R: TCATCGACGCTAAGGAGGACCC	1R, 1B
3	Bmac213	F: ATGGATGCAAGACCAAAC R: CTATGAGAGGTAGAGCAGCC	1RS, 1H
4	Sec-1	F: CTATTAGTTGAAAAGCTTATGA R: GCATATGACTCAAATATTTTT	1R
5	IAG95	F: CTCTGTGGATAGTTACTTGATCGA R: CCTAGAACATGCATGGCTGTTACA	1R
6	REMS1280	F: CAACGGCATGGAGTACCCT R: GAAGTTAACCTGCGGGAACA	1R
7	REMS1135	F: GCGTCCGTGTAGAGAGAGA R: ACCTGATGCACCTCCAAAAG	1R, 3R, 7R
8	NOR	F: GCA TGT AGC GAC TAA CTC ATC R: CCC AGT TTT CCA TGT CGC	1R
9	TSM12	F: CTGCGCACACATGAGTCAAT R: ATGGAAGGCGAGAGTCCCTT	1R
10	TSM94	F: GGAGGCACCGAGATATTGAA R: ACTTCCTCTAGGGCCGAAAC	1R
11	TSM106	F: AACGAACGGCAAGAACCTAA R: GTCGGCTGCATCATCTCC	1R
12	TSM123	F: CCATCTCCCTCCTCTGCTA R: TGAACGACATGCTGTTAATATG	1R
13	TSM303	F: CAACACCGATAGGGTAGAGAGG R: GATCATCGCCATCGTCATC	1R
14	TSM322	F: TGCCACACACAACTTGACA R: GCGAGATCGATGAAGAGAGC	1R
15	TSM325	F: CTGCACATGTCGCACCTC R: AGGAGCCAAAGAAGCATCAA	1R
16	TSM422	F: GTCCTGCTGCTACTGTGCTG R: CACTCTCGCATCCTTTGCTA	1R
17	TSM556	F: GGGTAGGCAGAGGCCTAACTA R: TACCCCTCTCCCTCCCTCT	1R
18	TSM608	F: AGGACGGGAAATAGGATGG R: AACACATCCCCACTCTTGTTG	1R
19	TSM625	F: GTGTGAGAGAAGCGAGAGAGAG R: ATTTGTGATGCCGCTTATCC	1R
20	TSM690	F: CTGAATTGCTTTGCGGTTTT R: GTAGTTTGGCCAGGCTGAAG	1R
Markers for 1AS chromosome			
21	Xbarc1	F: GCGATGCTTTTGCCCTGTTTCAG R: GCGGCCCTTTGACTCTTCATAG	1A, 5A
22	Xbarc25	F: GCGGTGCATCAAGGACGACAT R: GCGTAGTTCATCCATCCGTAAT	1A, 3A, 4B
23	Xbarc148	F: GCGCAACCACAATGTATGCT R: GGGGTGTTTTCTTCTTTCTT	1A, 3A, 1D, 5B
24	Xbarc119	F: CACCCGATGATGAAAAT R: GATGGCACAAGAAATGAT	1A, 1B, 1D
25	Xbarc263	F: GGAAGCGCGTCAGCACTAGGCAAC R: GGCTTCTAGGTGCTGCGGCTTTTGTG	1A
26	Xbarc1048	F: ACG TGG TAA TTA GTT GGG AGT CTG TA R: GCG AAG TCA AGA AGT GGG CTT TTC AAG AG	1A
27	Xgwm136	F: GACAGCACCTTGCCCTTTG R: CATCGGCAACATGCTCATC	1A
28	Xgwm33	F: GGAGTCACACTTGTGTTGTCG R: CACTGCACACCTAACTACCTGC	1A, 1B, 1D
29	Xgdm33	F: GGCTCAATTC AACGGTCTT R: TACGTTCTGGTGGCTGCTC	1A, 1B, 1D
30	Xwmc278	F: AAACGATAGTAAAATTACCTCGGAT R: TCAAAAAATAGCAACTTGAAGACAT	1A
31	Xwmc336	F: GTCTTACCCCGCATCTGC R: GCGGCCTGAGCTTCTTGAG	1A, 1D
32	Xwmc818	F: TGAAGGGTGCCTGTGGTC R: GCGTCGATTTAATTTGATGATGG	1A, 1B
33	Xgwm1223	Material Transfer Agreement (Dr. Martin Ganal)	1R, 1A, 1D
34	Xgwm750	Material Transfer Agreement (Dr. Marion Roder)	1R, 1A
35	PSP2999 (Glu-3)A	F: TCCCGCATGAGTCAATC R: TTGGGAGACACATTGGCC	1A

Table 2: Chi-squared test for bunt attack in 68 progenies of the cross F00628G34-M/Literal.

	Non-bunted lines	Bunted lines	Total
Observed	18	50	68
Expected (segregation ratio 1,75:0,5:1,75)	29,75	8,5+29,75	68
O-A	-11,75	+11,75	
	$\chi^2 = 8,25$	$P = 0.00407468$	Degrees of freedom = 1

9.3%; race T02 – 4.7%) [12]. This line was previously characterized as carrying rye chromatin as a 1RS:1AL translocation, using hybridization techniques (GISH and FISH) at Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary (Marta Molnar-Lang's Department) by Constantina Banica (personal communication).

Marker Assisted Selection (MAS) has proved to be a very efficient method for increasing genetic progress and efficiency of breeding programs [13]. Starting from the necessity to obtain bunt resistant cultivars, taking into account the complexity of wheat genome, the difficulties of testing and selection of bunt resistant genotypes and the utility of molecular markers, this work proposes to look at the wheat genome with the help of molecular markers and to determine if the resistance to bunt in F00628G34-M line is associated with rye chromatin.

Biological material

The study was done on 68 F4 lines randomly extracted from the cross of the cultivar Litera, as susceptible parent, with the line F00628G34-M (Triticale/2' Wheat), as resistant parent.

Methods

Inoculation-teliospors

The teliospores were mixed with seed in a paper envelope, next was shaken by hand until the seed was evenly and visibly darkened with teliospores (performed under the guidance of Dr. Mariana Ittu).

Field tests

Inoculated seeds were planted on one meter long rows, using as susceptible cultivars Dropia and Literal. At maturity, the lines were classified in bunted and non-bunted (0 % common bunt). Infected spikes (where at least one grain was replaced by bunt balls) were counted and expressed as percentage from total number of spikes.

DNA isolation was made from leaves, using CTAB method [14]. PCR- All amplification reactions were carried out in a 25 µl volume. We used 20 markers specific for rye and for the 1R short arm chromosome [15-21] and 15 SSR markers localized on the 1AS chromosome [22]. The sequences of REMS markers were kindly offered by Dr. Victor Korzun -KWS LOCHOW, Germany, and for marker Xgwm752 the sequence was obtained by MTA (Material Transfer Agreement) from Dr. Marion Roder. For the marker Xgwm1223 the sequence was obtained by MTA from "TraitGenetics" company, Germany -Dr. Martin Ganal (Table 1). The PCR products were separated on 1.2-1.5-2-2.5% agarose for routine use, in 0.5X TBE buffer, stained with ethidium bromide and photographed under ultraviolet light with Vilber Lourmat system.

As positive control for rye chromatin, we used DNA from *Secale cereale* "Harkowskaya", from wheat cultivar Liman (which

carries rye chromatin) and from another wheat genotype which carries the 1RS:1BL translocation Ludogoria (seeds were kindly provided by Dr. Elena Todorovska, ABI, Sofia, Bulgaria).

For nested PCR (F3/R3 - Xgwm1223), the final reaction was done in a 25 µl final volume using 3 µl of PCR product obtained with "universal marker for rye" F3/R3 [14]. We used 1U Taq DNA polymerase (Promega) and 0,2 µM primers. Association between markers and level of bunt attack was estimated using Chi square test, interactive software [23].

Results

Phenotype observations

Phenotype observations made in 2010 for the F4 generation on 68 progenies lines, showed 18 progenies lines non-bunted and 50 progenies lines bunted, the percentage of bunted ears varying between 9 and 80 percent, whilst the susceptible parent, Literal showed 65 percent of bunted ears. We also observed partially bunted spikes and seeds. The phenotype segregation suggests that the bunt resistance gene transmitted from F00628G34-M manifested as recessive or partially dominant gene. However, Chi square test suggests significant deviation ($P < 5\%$) from the expected Mendelian monogenic segregation (Table 2). This might be explained by repression of resistance by certain factors from wheat genome.

The characterization of rye translocation present in F00628G34-M line

Molecular analysis with specific markers for rye chromatin offered some information about 1RS:1AL translocation present in F00628G34-M line. The marker SCM9 [19] that make difference between 1RS:1AS translocation (220bp PCR product) and 1RS:1BS translocation (200bp PCR product), confirmed the presence of 1RS:1AS translocation in F00628G34-M (Figure 1).

The molecular marker STS-IAG95 [15] showed a PCR product of 1150bp (Figure 2) in the line F00628G34-M. Based on previously reported results [15], with this marker one can obtain either 1050bp or 1150bp PCR product, the first being associated with "Petkus" rye type and the second PCR product (1150bp) with "Insave" rye type.

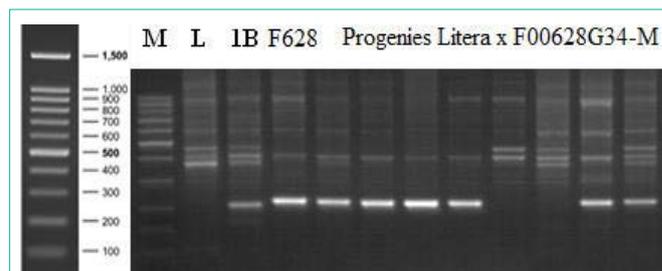


Figure 1: Electrophoresis pattern of SCM9 marker. M-DNA Ladder (100bp); L-cv Literal; 1B-Ludogoria (1R/1B translocation); F628 - F00628G34-M line.

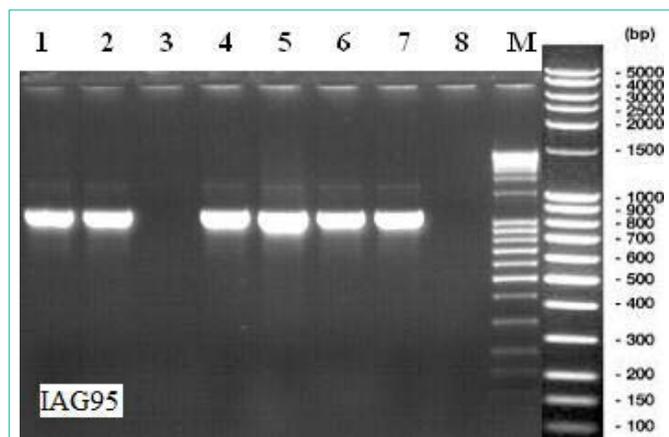


Figure 2: Electrophoresis pattern of IAG95 marker: 1-cv Liman (1R translocation); 2-F00628G34-M; 3-cv Litala; 4-8 – progenies (Litala x F00628G34-M); M- DNA ladder 100bp extended (Roth).

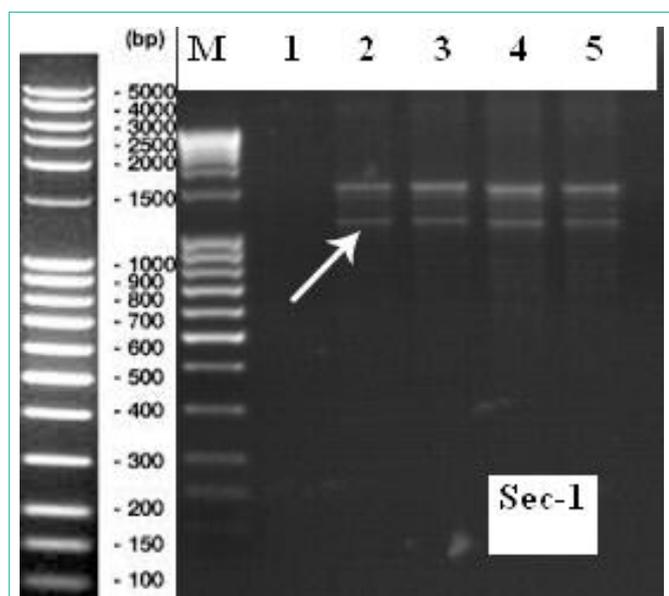


Figure 3: Electrophoresis pattern for Sec-1 locus. M-DNA ladder 100bp extended (Roth); 1-cv Litala; 2-F00628G34-M; 3,4,5-progenies. The arrow mark the 1,2 kb PCR product.

This locus is associated with a gene for resistance to *Blumeriagraminis* (*Pm8* for “Petkus” type and *Pm17* for “Insave” type). Because we obtained an 1150bp product, the rye chromatin from our line is INSAVE type, and probably contains the *Pm17* powdery mildew resistance gene.

The 1RS arm chromosome carries a locus for secaline (ω secaline and γ secaline). Using the PCR marker for Sec-1 locus (ω secaline) [20] results in 2 PCR products (1.5kb and 0.7kb), but in our case we obtained the 1.5kb product and another 1.2kb product (Figure 3). Apparently, the Sec-1 locus showed polymorphism, presented by a PCR product of about 1200bp in our line. Maybe, this polymorphism is determined by the specificity of *Insave* rye chromatin present in the line F000628G34-M.

The association of bunt resistance with rye chromatin inline F000628G34-M based on molecular markers assays. Of all 35 markers

tested in this study, only 12 markers showed polymorphism between parents. The results of chi-square test for these markers are presented in (Table 3). The results for the IAG95 were not included because its sequence has a transposable nature [24].

Combined analysis of results obtained with molecular markers for *Sec-1* locus and for Glu-A3 locus (PSP2999) permitted to distinguish heterozygous genotypes [25].

Both the presence of the universal marker for the rye chromatin and of four other specific markers for the 1R chromosome, and the absence of 6 specific markers for the 1A chromosome proved a significant association of the bunt resistance inherited from the F00628G34-M line, with the 1A/1R translocation. This proves that bunt resistance identified in the F00628G34-M line is associated with the presence of rye chromatin.

To our knowledge, this is the first time when it is proven that a bunt resistance gene is associated with the rye chromatin transfer to wheat. Molecular assays suggest a possible location of the bunt resistance gene in F00628G34-M line, on the 1RS chromosome, in the homeologous region with the Glu-A3 locus and close to Xgwm1223 microsatellite locus. Therefore the bunt resistance gene present in F00628G34-M is different from other known resistance genes. The markers association with the bunt resistance gene was more significant on homozygous genotypes.

For the transfer of this gene in other genotypes, we proved that the marker assisted selection can be used, the most indicated markers being Xbarc1048, Xgwm136 or PSP2999, whose absence notifies the presence of rye chromatin where the gene is localized.

Also, with the help of molecular markers (by nested - PCR with markers (F3/R3 / Xgwm1223), we showed that the 1RS:1AL translocation present in the line F000628G34-M has some chromosome rearrangements, by wheat chromatin insertion. This result was suggested by the polymorphism obtained using the PCR product (F3/R3) as DNA template for Xgwm1223 marker amplification, which was similar with PCR products by Xgwm1223 when DNA template came from genotypes without rye chromatin (Figure 4). This region could be heterogenic and can influence the molecular marker assay for association with resistance gene to bunt.

Discussion

The lower than expected number of non-bunted genotypes could

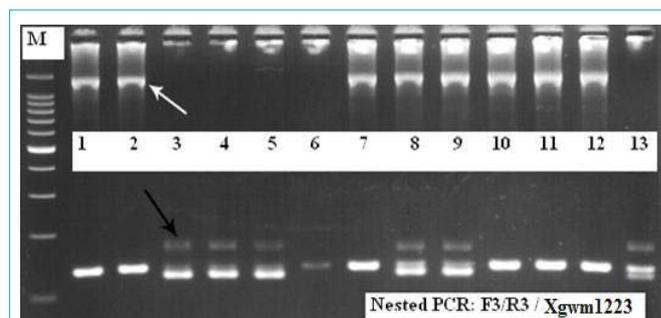


Figure 4: Electrophoresis pattern of nested PCR (F3R3/wms1223). M- DNA ladder 100bp; 1. F00628G34-M; 2-12 progenies; cv Litala. The white arrow mark the rye chromatin (F3R3 PCR product). The black arrow mark the Xgwm1223 PCR product.

Table 3: Association of molecular markers to bunt resistance.

Marker Name	Location on chromosome	Chi-squared test- Probability (for 68 F4 lines)	Chi-squared test- Probability (for only the homozygous lines)
F3 /R3	Rye-marker	0.00094212	0.00004733
SCM9	1R, 1B	0.00958200	0.00028462
Sec-1	1R	0.00222864	0.00002873
TSM106	1R	0.00958200	0.00028462
TSM123	1R	0.00958200	0.00028462
Xbarc263	1A	0.00012177	0.00010338
Xbarc1048	1A	0.00001093	0.00000832
Xgwm136	1A	0.00002097	0.00004733
Xgwm1223	1R,1A,1D	0.00036605	0.00004733
PSP2999 (Glu A3)	1A	0.00006456	0.00004733
Xwmc818	1A, 1B	0.00012177	0.00010338

be explained by incomplete gene expression or by suppression of the resistance gene from rye by modified/inhibitor factors from wheat genome. Similar results for disease resistance genes transferred from rye genome have already been reported [26].

The slow progress in learning about the genetic resistance to bunt is explained by a series of diseases characteristics [4]. In most of the cases, not all the plants with the susceptible genotypes are attacked, even if a good artificial infection is obtained. The resistant genotypes are rarely completely immune. Different authors define as resistant the genotypes that are attacked at a level less than 5 to 10%.

The existence of modifying genes can influence the infection level. Many of the resistance genes present a partial dominance. Additionally, the level of bunt infection is strongly influenced by the autumn environmental conditions, which makes the heritability relatively low.

All these aspects, but especially the incomplete expression of susceptibility or resistance alleles, distort the phenotypic data of resistance to bunt in mapping populations, and this is an obstacle in the determination of genetic distance between resistance gene and molecular markers. This problem could be partially solved if the study is realized on homozygous recombinant lines, preferably doubled haploid lines, which permit the phenotyping of the same genotype for more years to establish more clearly the resistance classes. When these kinds of populations are not available, the solution is a qualitative determination of the associations between resistance genes and markers using Chi-square test, without estimating the genetic distance.

The difficulties which remain for the study of genetic resistance to bunt can also be found out in breeding bunt resistant cultivars. Moreover, the low agronomic performances of most of the known resistance sources make the association of the resistance to bunt with some unfavorable agronomical traits difficult to deal with, even after several crossing cycles. Compared to other wheat diseases, all these problems make the wheat selection for bunt resistance to be considered one of the most difficult. The determination, even only at the qualitative level, of some molecular markers association with bunt resistance genes could accelerate the introduction of these genes in wheat genotypes with good agronomic traits. That is why,

for obtaining wheat cultivars with high-performance and resistance to bunt, the use of molecular markers associated to bunt resistance genes is of great importance.

Conclusion

The bunt resistance identified in the F00628G34-M line is associated with the presence of rye chromatin.

For the transfer of this gene in other genotypes we proved that the marker assisted selection can be used, the most indicated markers loci being Xbarc1048, Xgwm136 or PSP2999 (GluA3), whose absence notifies the presence of rye chromatin in the region where the gene is located. The Xgwm1223 marker locus, which produces specific products for the rye translocation, can also be used.

The bunt resistance gene present in F00628G34-M is different from other known resistance genes.

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