# PRELIMINARY RESULTS ON USE OF MOLECULAR MARKERS TO IDENTIFY RYE INTROGRESSION INTO THE WHEAT GENOME

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## ABSTRACT

**Rye *(Secale cereale)* has already provided many valuable genes for wheat breeding, but many other valuable genes which might be useful for improving wheat, such as genes for disease (bunt, *Septoria*, Barley Yellow Dwarf Virus etc.) resistance, for rapid growth, for allelopathic response and other, are still available in the rye genome. Molecular markers could be very efficient in identifying new gene transfers from the rye genome to wheat, especially small rye chromosomal segments, which are most desirable in wheat breeding.**

**Our research was aimed at using already reported markers and identifying new PCR-based markers for detecting rye introgressions in wheat cultivars and breeding lines selected from triticale x wheat crosses. We analyzed 13 wheat cultivars or lines, some known as 1RS/1BL translocation carriers, one rye cultivar, one triticale cultivar and 26 breeding lines selected from triticale / wheat crosses, some previously noticed in the field for their superior resistance to bunt and *Septoria tritici.***

**The F3/R3 „universal marker" for rye chromatin described by Katto et al. (2004) identified a single-band PCR product at about 1400 bp in 15 out of 26 lines derived from triticale x wheat crosses. The decamer primer OP H20, which according to Ko et al. (2002) produced rye genome specific RAPD fragments, identified in our studies a 1035 bp band in the same entries that produced the 1400 bp band with the F3/R3 set of primers. Among the 75 UCB primers tested, only the primer UCB 318 identified polymorphism among the analyzed genotypes, producing a 390 bp band in most but not all of the entries identified with the F3/R3 primers and the OP H20 primer as carrying rye chromatin and is potentially useful as a specific marker for some segments of rye chromatin. Our results suggest that triticale x wheat crosses can be efficient for transferring useful rye genes to wheat.**

**Key words**: Introgression, rye genome, wheat genome, specific PCR markers

## INTRODUCTION

Rye (*Secale cereale*) has already provided many valuable genes for wheat breeding. Many wheat cultivars carry the 1RS/1BL or the 1RS/1AL translocation, containing useful genes, mainly for disease resistance (Singh and al., 1990; Lukaszewski, 2000). Other rye chromosome segments, such as 2RL, 5RL, 6RS, 7RL, have also been introgressed into the wheat genome (Friebe et al., 1990, 1994, 1995, 1999; Ribeiro-Carvalho et al., 2001; Lukaszewski et al., 2004).

However, many other valuable genes which might be useful for improving wheat are available in the rye genome. These include genes for disease resistance (including bunt, *Septoria*, Barley Yellow Dwarf Virus etc.), for rapid growth, for allelopathic response and others. This is why many wheat breeders are interested in obtaining and identifying new gene transfers from the rye genome to wheat.

Small rye chromosomal segments, which are most desirable in wheat breeding, are difficult to identify by cytological methods. This explains the recent interest in using molecular markers to identify rye introgressions into the wheat genome.

Koebner (1995) developed PCR-based markers that detect rye chromatin in wheat. Shimizu (1997) developed a PCR-based marker for the locus *Sec-1* that controls the synthesis of a rye specific grain protein. Lee et al. (1993, 1995) used a PCR technique for detecting the rye chromosome *2R* in wheat, while Brunell et al. (1999) described a RAPD arm specific marker for the rye chromosome *2R* transferred to wheat. However, these markers were only chromosome specific and were not useful for detecting other possible rye introgressions.

The rye genome, like many other *Triticeae,* contains 92% repetitive DNA sequences, and it is known that repetitive DNA sequences are often highly conserved at the taxon level. This opens the possibility of identifying markers associated with many chromosome segments, dispersed throughout all chromosomes. Katto et al. (2004) indicates that McIntyre et al. were the first to report a molecular marker specific to and widely dispersed throughout rye chromosomes. Ko et al. (2002) identified two rye genome-specific RAPD markers that detected all tested rye introgressions into wheat. They also developed two large cloned markers, related to retrotransposons, one of which (pSC20H) was shown to be dispersed throughout the rye genome, except at telomeric and nucleolar organizing regions. The authors claim that these markers should be useful to track all wheat-rye translocation lines derived from whole arms of rye chromosomes.

More recently, Katto et al. (2004), using primers designed based on the nucleotide sequence of the clone pSc20H, developed a PCR-based marker which detected the presence of rye DNA in all tested wheat plants, carrying different rye chromosomes or small segments of

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chromosomes, including the *B* chromosome, as well as in various wild species and cultivars of rye. They indicated that the set of primers designated as F3/R3 can be regarded as a universal marker for introgression of rye chromosome segments in the wheat genome.

Our research was aimed at using already reported markers and identifying new PCR-based markers for detecting rye introgressions in wheat cultivars and breeding lines selected from triticale x wheat crosses.

## MATERIAL AND METHODS

We analyzed 13 wheat cultivars (CE 15-22 and W 1-3) or lines (CE 1 and W 4-5), some known as 1RS/1BL translocation carriers, one rye cultivar (W 28), one triticale cultivar (W 29) and 26 breeding lines selected from triticale / wheat crosses (CE 11-14 and W6-27) (Table 1). Several of these lines were previously noticed in the field for their superior resistance to bunt and *Septoria tritici.* We used for amplification the following primers:

- the set of primers F3 (5'-GATCG CCTCT TTTGC CAAGA-3') / R3 (5'-TCACT GATCA CAAGA GCTTG-3'), described by Katto et al. (2004) as „universal marker" for rye chromatin;
- the primer OP H20 (5'-GGGAGACATC-3'), as described by Ko et al. (2002);
- 75 UBC random primers.

The DNA izolation was performed according to the technique based on CTAB. The DNA amplification following the protocol of 94°- 55°- 72°C in a termocycler Applied Biosystem has been used to screen the polymorphic bands.

The evaluation of electrophoresis bands was performed on 1.5 % agarose gel, BET staining and Imatech Image analyses.

*Table 1*. List of analyzed wheat, rye and triticale genotypes

| Lab. code | Name | Genealogy | Type of material |
|---|---|---|---|
| CE 1 | 01172G1-1 | IBWSN252-31/96869G1-1 | Wheat line |
| CE 11 | F00599G17-2 | Tcl 191TR1-1221/BUCUR//ECOU | Triticale x wheat |
| CE 12 | F00599G19-1 | Tcl 191TR1-1221/BUCUR//ECOU | Triticale x wheat |
| CE 13 | F00628G43-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| CE 14 | F00628G34-2 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| CE 15 | FARMEC | | Wheat cultivar |
| CE 16 | DELABRAD | | Wheat cultivar |
| CE 17 | F96869G1-108 | | Wheat cultivar |
| CE 18 | GLOSA | | Wheat cultivar |
| CE 19 | BOEMA | | Wheat cultivar |
| CE 20 | JIANA | | Wheat cultivar |
| CE 21 | CRINA | | Wheat cultivar |
| W1 | DROPIA | | Wheat cultivar |
| W 2 | FUNDULEA 4 | | Wheat cultivar |
| W 3 | IZVOR | | Wheat cultivar |
| W 4 | F91419G4-1A1 | | Wheat line |
| W 5 | F91418G4-1A2 | | Wheat line |
| W 6 | F01476G2-1 | Tcl 191TR1-1221/2*EXPRES | Triticale x wheat |
| W 7 | F01531G2-1 | Tcl 92438T1-102/DBD//? | Triticale x wheat |
| W 8 | F00310G1-1 | Tcl 191TR1-1221/BOEMA | Triticale x wheat |
| W 9 | F00628G4-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 10 | F00628G12-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 11 | F00628G12-2 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 12 | F00628G13-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 13 | F00628G18-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 14 | F00628G20-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 15 | F00628GG22-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 16 | F00628G22-2 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 17 | F00628G23-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 18 | F00628G24-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 19 | F00628G24-2 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 20 | F00628G34-1-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 21 | F00628G34-2-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 22 | F00628G34-3-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 23 | F00628G35-1 | Tcl 191TR-1-1221Fu/ BUCUR//? | Triticale x wheat |
| W 24 | F01474G1-1 | Tcl 191TR1-12/F236U1//CRINA | Triticale x wheat |
| W 25 | F00599G17-2-1 | Tcl 191TR1-1221/BUCUR//ECOU | Triticale x wheat |
| W 26 | F00599G19-1-1 | Tcl 191TR1-1221/BUCUR//ECOU | Triticale x wheat |
| W 27 | F00903G3-2 | DBD/Tcl 191TR1-1221//DBD | Triticale x wheat |
| W 28 | GLORIA | | Rye cultivar |
| W 29 | TITAN | | Triticale cultivar |

## RESULTS AND DISCUSSION

The „universal marker" developed by Katto et al. (2004) identified a single-band PCR product at about 1400 bp in many of the analyzed entries. These included the wheat cultivar Fundulea 4, known as carrier of the secaline locus and of disease resistance genes *Yr9/Sr31/Lr26*, and therefore a carrier of the 1RS/1BL translocation, the wheat line F96869G1-108 and one of its derivatives (F01172G1-1), the rye and triticale cultivars, and 15 out of 26 lines derived from triticale/wheat crosses (Figure 1).

The fainter band present in genotypes W 4, 15, 19 and 21 might be due to heterogeneity of the sample.

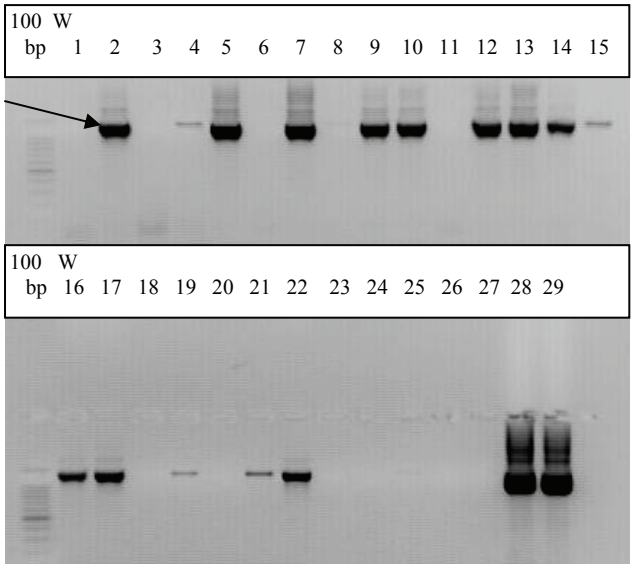


*Figure 1.* The rye specific PCR product at 1400 bp identified with the F3/R3 set of primers in genotypes W 1-29

The same band was identified in only some plants of entries CE 11, 13 and 14, indicating segregation for the rye introgression (Figure 2).

The decamer primer OP H20, which according to Ko et al. (2002) produced rye genome specific RAPD fragments, identified in our studies a 1035 bp band in the same entries that produced the 1400 bp band with the F3/R3 set of primers.

These included the wheat cultivar Fundulea 4 (W 2), the rye and triticale cultivars (W 28 and 29), as well as the same lines derived from triticale/wheat crosses noticed with the F3/R3 primers (Figure 3).

However, the band amplified with the F3/R3 primers was more clearly distinguishable and the polymorphism more obvious.

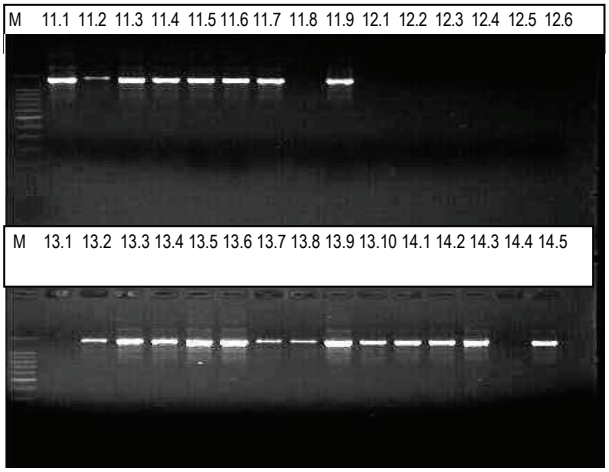


*Figure 2.* Segregation for the „universal rye genome specific marker" in entries CE 11-14.

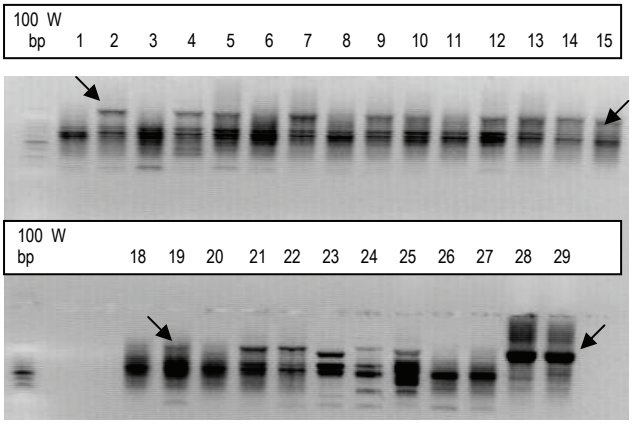


*Figure 3.* Polymorphism found in entries W 1-W 29, as identified by the 10-mer primer OP H20

Similarly with the primers F3/R3, the primer OP H20 also identified segregation for the marker at 1035 bp in individual plants from the entries CE 11, CE 13 and CE 14, and failed to produce the rye specific band in entry CE 12 and in bulks of CE 15 - CE 21 (Figure 4).

The only difference from the results obtained with the primers F3/R3 is for the entry CE17. We suppose that this difference might be due to the heterogeneity of the line.

Among the 75 UBC primers tested, only the primer UBC 318 identified polymorphism among the analyzed genotypes, producing a 390 bp band in most of the entries identified with the F3/R3 primers and the OP H20 primer

as carrying rye chromatin (Figure 5). The 390 bp band was clearly visible in the 1RS/1BL wheat cultivar Fundulea 4. It was also present in all lines derived from triticale/wheat crosses, previously identified as having rye introgressions, except the entries W 10 and W 13. It is probable that the primer UBC 318 only identifies some specific and not all rye introgressions. Further studies should establish with what rye chromatin segments this primer is associated.

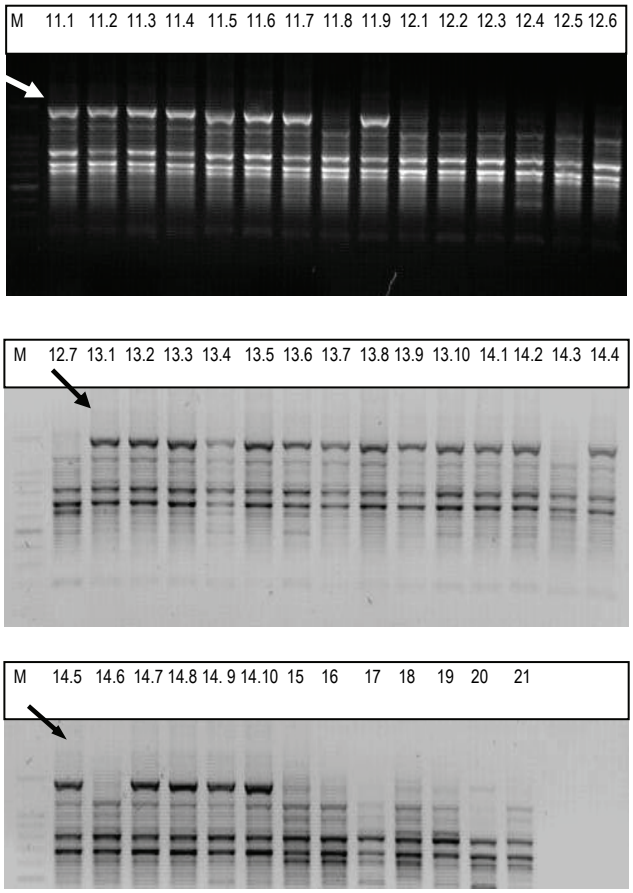


*Figure 4*. Polymorphism identified with the primer OP H20 among individual plants of entries CE 11-14 and among the bulks of entries CE 15-21

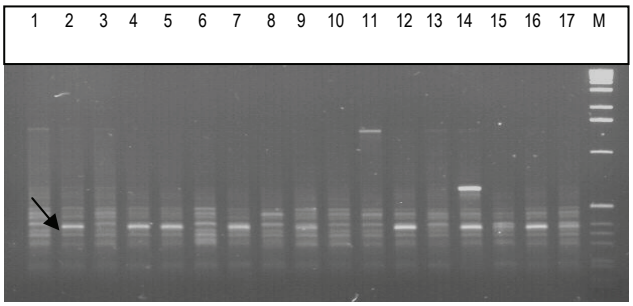


*Figure 5*. Polymorphism identified with the primer UBC 318 in entries W 1 - W 17 (M = 1Kb)

## CONCLUSIONS

The „universal marker" for rye chromatin described by Katto et al. (2004) detected presence of introgressions from rye in 15 out of 26 lines derived from triticale x wheat crosses.

The decamer primer OP H20 gave similar results with the „universal marker".

The primer UBC 318 identified many, but not all the entries carrying rye chromatin, and is potentially useful as a specific marker for some segments of rye chromatin.

Triticale x wheat crosses can be efficient for transferring useful rye genes to wheat.

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