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### GENETIC MAPPING AND TAGGING OF WHEAT GENES USING RAPD, STS AND SSR MARKERS

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**Abstract:** We applied SSR markers for mapping genes determining red coleoptile colour in wheat (*Rc1*, *Rc2*, *Rc3*) using F<sub>2</sub> populations. All three genes map at about 15 to 20 cM distally from the centromere of chromosomes 7AS, 7BS and 7DS, respectively. The locations of the glume colour (*Bg*, *Rg1*) and glume hairiness (*Hg*) genes relative to the SSR markers of the homoeologous chromosomes group 1 were determined using molecular analysis of near-isogenic lines (NILs). One RAPD marker for the vernalisation response gene *Vrn-A1* was identified by screening 95 random primers against two pairs of NILs. New PCR (STS) markers were developed based on RFLP-markers PSR426 (5A, 5B, 5D) and PSR1201 (1A, 5A, 5B). Analysis of nulli-tetrasomic and near-isogenic lines of wheat using the STS markers developed gave an indication that these new STS markers have the same chromosomal and intrachromosomal positions as the correspondent RFLP markers. Therefore, they could be used for mapping and/or tagging the vernalisation response (*Vrn-A1*, *Vrn-B1*, *Vrn-D1*) and homoeologous pairing (*Ph1*) genes.

**Key Words:** Glume Colour, Glume Hairiness, Gene Mapping, Homoeologous Pairing, Molecular Markers, Red Coleoptile Colour, *Triticum aestivum*, Vernalisation Response.

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## INTRODUCTION

Since saturated molecular maps became available, it has been possible to localize genes within genomes more precisely. For mapping or tagging the genes of interest, different approaches such as RFLP, RAPD, STS or SSR analysis can be applied. During the last decade, various genes were mapped in wheat using molecular markers. Updated reviews are frequently summarised in the 'Catalogue of gene symbols for wheat' [1] and its supplements [2, 3, 4]. In this study, we present data on the mapping and/or tagging of a selection of *T. aestivum* genes determining red coleoptile colour (*Rc1* on 7AS), (*Rc2* on 7BS), (*Rc3* on 7DS), glume colour (*Bg* on 1AS), (*Rg1* on 1BS), glume hairiness (*Hg* on 1AS), vernalisation response (*Vrn-A1*, *Vrn-B* and *Vrn-D1* on 5AL, 5BL and 5DL, respectively) and homoeologous pairing (*Ph1* on 5BL). Homologous and homoeologous relationships with comparable major genes or QTLs already described in wheat or other *Triticeae* members are discussed.

## MATERIALS AND METHODS

Three F<sub>2</sub> populations (Chinese Spring/Hope 7A x TRI15010 (103 plants), Chinese Spring/Hope 7B x TRI2732 (153 plants) and Mironovskaya 808 x Aibian 1 (74 plants)) were used for mapping the red coleoptile colour genes *Rc1*, *Rc2* and *Rc3*. The markers applied were SSR markers [5] known to map on chromosomes 7A, 7B and 7D.

Another approach (SSR analysis of NILs) was used to determine the location of the glume colour (*Bg*, *Rg1*) and glume hairiness (*Hg*) genes in relation to SSR markers mapped on chromosomes 1AS and 1BS [5]. The use of molecular markers to compare the genetic profiles of pairs of NILs in order to search for DNA markers near the target gene was proposed by Muehlbauer *et al.* [6]. A set of near-isogenic lines in the background of the common wheat varieties 'Novosibirskaya 67' and/or 'Saratovskaya 29' (BC<sub>8,9</sub>) were investigated. Different varieties and accessions of common and durum wheat carrying dominant alleles of the *Bg*, *Rg1* and *Hg* genes were used as donor parents [7].

Two pairs of NILs in the background of 'Novosibirskaya 67' (BC<sub>8,9</sub>) [8], both carrying the recessive allele of the vernalisation response locus of chromosome 5AL (*Vrn-A1*) were used to search for RAPD and STS markers near this gene. For the chromosomal locations of STS markers developed in this study, nulli-tetrasomic lines of 'Chinese Spring' were used as described by Sharp *et al.* [9] for the RFLP markers, and by Plaschke *et al.* [10] for the SSR markers.

DNA was isolated according to a modified procedure of Plaschke *et al.* [11]. Wheat microsatellite (or SSR) markers known to map on chromosomes 1AS (4), 1BS (5), 7A (31), 7B (34) and 7D (26) were selected and used as described by Röder *et al.* [5]. Linkage maps were constructed with the 'MAPMARKER 2.0' computer program [12]. To convert RFLP markers PSR426 and PSR1201 [13] into STS markers, they were sequenced according to Sanger *et al.* [14]. Specific

primers for PCR were constructed using the 'OLIGO' computer program (W. Rychlik, USA). A set of 95 random primers, 10-11 bp in length, with 45-72% G-C content, were used in the RAPD analysis. The PCR products were analysed on polyacrylamide (STSs) or agarose (RAPDs) gels (for details see [8]).

## RESULTS AND DISCUSSION

### Molecular mapping of *Rc* genes

The phenotypic segregation data, obtained from scoring F<sub>2</sub> populations (in case of the third population, the phenotype was determined using F<sub>3</sub> plants) gave a clear indication for a monogenic inheritance of the target trait as proved by the  $\chi^2$ -test. From the wheat microsatellites tested, 20 out of 31 (chromosome 7A; 65%), 23 out of 34 (chromosome 7B; 68%), and 11 out of 26 (chromosome 7D; 42%) were found to be polymorphic between the parents. Polymorphic markers of the centromere region were chosen for analysis of the DNA of the F<sub>2</sub> individuals. The three coleoptile colour genes were mapped about 15 to 20 cM distally from the centromere on the short arms of the homoeologous group 7 chromosomes (Fig. 1). The mapping data confirm the previous results on gene localisation presented in [15] or [16]. For further details, see Khlestkina *et al.* [17].

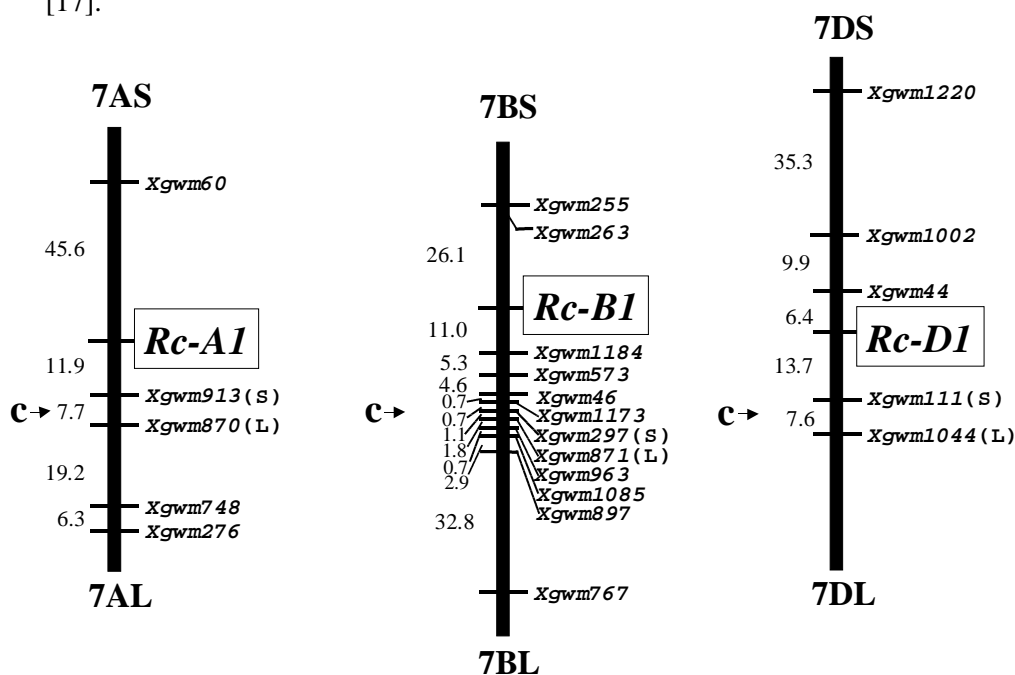


Fig. 1. Comparative molecular mapping of *Rc* genes on homoeologous group 7 chromosomes.

Because the map positions of all three genes are highly comparable, it may be concluded that they are members of a homoeologous series. Therefore, the loci were designated *Rc-A1*, *Rc-B1* and *Rc-D1* (Fig. 1). Further homoeologous loci may exist on chromosome 7R in *S. cereale* (*an1*; [18]) and 7H in *H. vulgare* (*ant1*; [19, 20]).

#### Localisation of *Bg*, *Hg* and *Rg1* on the molecular map using NILs

SSR analysis of NILs was used to find markers related to the *Bg*, *Hg* and *Rg1* genes located on chromosomes 1AS and 1BS. *Bg* and *Hg* are known to be closely linked to each other, so that the same set of NILs was used for *Bg* and *Hg* gene tagging. One (*Xgwm136* – proximal) out of the 4 tested SSR markers known to be mapped on chromosome 1AS [5] was located near genes *Bg* and *Hg* (Fig. 2a). Two (*Xgwm33b* – proximal, *Xgwm550* – distal) of the 5 SSR markers of chromosome 1BS tested were shown to be associated with *Rg1* (Fig. 2b). Interestingly, markers related to genes *Bg* and *Rg1* are at comparable distances to the centromeres of chromosomes 1A and 1B [5]. Furthermore, *Rg2*, mapped as a QTL on chromosome 1D [21], was found to be again in a highly comparable position. It may be suggested that the glume colour genes of the group 1 chromosomes are members of a homoeologous series.

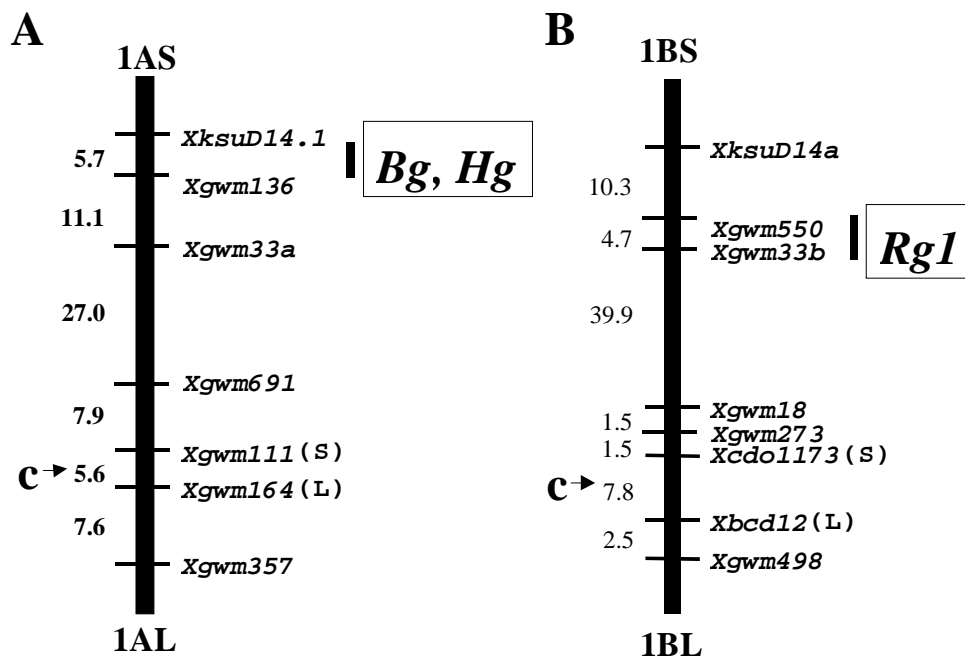


Fig. 2. Molecular maps of chromosomes 1AS (A) and 1BS (B) of *T. aestivum* [5; Röder, unpubl. data]. The locations of *Bg*, *Hg* and *Rg1* are indicated by vertical bars (c = centromere, centi Morgans are given on the left).

### Development of PCR-markers for the genes *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Ph1* located on the homoeologous group 5 chromosomes

One RAPD marker was identified as the result of screening 95 random primers across two pairs of NILs differing for the vernalisation response gene, *Vrn-A1*. The low level of intervarietal polymorphism observed did not allow us to identify more RAPD markers for these genes. Another approach applied to develop PCR markers for all three *Vrn-1* genes (*Vrn-A1*, *Vrn-B1*, *Vrn-D1*) and *Ph1*, was STS analysis. STS markers could be created based on RFLP markers via their primary structure analysis followed by PCR primer construction [22, 23]. Two RFLPs were selected to be converted into STS markers, which are much easier to use: 1) PSR1201, known to have loci on chromosomes 5A, 5B and 1A, one of which, on chromosome 5B, was shown to locate near *Ph1* [24, 13]; 2) PSR426, known to have homoeologous loci on chromosomes 5A, 5B and 5D. The 5A locus is known to be closely linked (0 cM) to *Vrn-A1* [25]. Both RFLP markers were successfully converted to STS markers (the results of the sequencing are given in Fig. 3, with the regions correspondent to specific primers sequences underlined), amplifying different loci, designated *Xsts1201a* (1A), *Xsts1201b,d* (5A), *Xsts1201c* (5B), *Xsts426a* (5A), *Xsts426b,d* (5B), *Xsts426c* (5D). The chromosomal locations (indicated in brackets) of amplified STS loci were determined using nulli-tetrasomic lines of 'Chinese Spring'.

#### PSR1201 476 bp

5' AGGATCCGCCTCAATATTTGTTGGATACGGTCAGAAAGAGTTGGCCTGCAA  
ATTCTGAAGTTCATAATAATGGTGTGTCAGTTATTTTTTCATTGGTGGTAAGGGGA  
TGCGTTTCACTCTAGAGGGAATAAAGCGTTACCTACGTCGACCTGCTCATAA  
GAAAAAGAAAGGATGGATGTCATATACGCGGCAAACCTTCTGAAACAGTGG  
CGTACATTCGTTAATACTATAGTTTTTGTGTTTGCTACCTGACCAATCTGACA  
GACCTGCCTAAAGGGAAAGTCATTAAGAGAAAAAGGACAGCATGCGGGTAA  
TAGCGAATCGGCCACTCCAAATCCCAGAGCCAAGGTTTGCACGTCGCCAGG  
AAACTGTGCGTGTGCGCATCCTTGATGGCACTTCAATTACATGAGTGATGTA  
TAAGCAAAAACAGAGGGAGTACCAGTAAAGTGCACAATGCAGTATGCATATA  
ATGTAGAGA-3'

#### PSR426 497 bp

5' AATTATACGTGCTGGTGGGAAATCGAAATCGGGTTATCTTTCTCATTTCGA  
CCGATCGTTCTCGTGTTCGCTGAATCGTGGTTCATTGCTTATCAGGAATCAC  
TTGGCTTTAGTAGATGAAAGGCAAACGGCTGCTAGTTGCACTTGCATCATCG  
GTCGTGGTGTGGCTCTTTATCCGGTTGTCGACCTCTTGTGCGGAGCTCTCGGGG  
GAAAAGGGGAAATTCTGAACTAGTGTCGTCTCGCTTTCTCTGTGTGATC  
CACTCACTGGTAAAGAAAGTGTCTTATGAGAAGTCTAGGTCGTACCATGAC  
CTAATCCAGCACCAAGTTATGAGTTATCCTGCACACTAGTGACACCTGAGAT  
GCGACGATCAGCTAACAATGTCTTTGTAACGAGAACACGTTCCAAAAGCTA  
CCAAATGTCTTTGTAACCAGGAGGTGACTAGTGGTACGTCCCATGCGACAGA  
CTTTGTGAGGATCAGTGATATCGAATTC-3'

Fig. 3. The primary structure of the RFLPs used.

It is demonstrated that the STS markers developed have the same chromosome locations as the correspondent RFLP markers. In addition *Xsts426a* was used for analysing NILs differing for *Vrn-A1*. The amplified fragment, about 450 bp in length, was observed in NIL and the donor parent, but not in the recurrent parent 'Novosibirskaya 67', meaning that marker *Xsts426a* is located near *Vrn-A1* as a correspondent RFLP marker PSR426-5A [22]. This indicates that the STS markers developed seem to have not only the same chromosomal but also the same intrachromosomal location as their correspondent RFLP markers. STS markers *Xsts426a*, *Xsts426b,d*, *Xsts426c* and *Xsts1201c* are recommended to be used for mapping and/or tagging the genes *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Ph1*, respectively.

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