

- Van Dekken, H., Pinke, I.D., Mullikin, J., and Gray, J.W. 1988. Enzymatic production of single-stranded DNA as a target for fluorescence in situ hybridization. *Chromosoma*, **97**: 1–5.
- Vogt, P. 1990. Potential genetic functions of tandem repeated DNA sequence blocks in the human genome are based on a

- highly conserved “chromatin folding code”. *Hum. Genet.* **84**: 301–336.
- Wu, C. 1984. Two protein-binding sites in chromatin implicated in the inactivation of heat-shock genes. *Nature (London)*, **309**: 229–234.

Chromosomal location of three wheat sequences with homology to pollen allergen encoding, DNA replication regulating, and DNA (cytosine-5)-methyltransferase genes in wheat and rye

V. Korzun, H.-J. Balzer, A. Balzer, H. Bäumlein, and A. Börner

Abstract: Three wheat sequences, shown to be homologous to pollen allergen encoding, DNA replication regulating, and DNA (cytosine-5)-methyltransferase genes were localized on chromosomes using nullisomic–tetrasomic wheat (‘Chinese Spring’) and wheat–rye (‘Chinese Spring’/‘Imperial’) addition lines. Whereas the loci for the pollen allergen encoding sequence (*Tri a III*) were shown to be located on homoeologous group 4, the DNA replication regulating (*Rep*) and DNA (cytosine-5)-methyltransferase (*Mtase*) genes were located to homoeologous groups 1 and 7, respectively, of Triticeae. Chromosomal rearrangements in wheat and rye relative to each other are discussed.

Key words: chromosomal location, known function probes, RFLP, rye, wheat.

Résumé : Trois séquences nucléotidiques du blé montrant de l’homologie avec des gènes codant pour l’allergène du pollen, régulant la réplication de l’ADN et codant pour l’ADN (cytosine-5)-méthyltransférase ont été localisés au moyen de lignées nullisomique–tétrasomiques du blé (‘Chinese Spring’) et des lignées d’addition blé–seigle (‘Chinese Spring’/‘Imperial’). Tandis que la séquence codant pour l’allergène du pollen (*Tri a III*) était située sur le groupe homéologue 4, les gènes régulant la réplication de l’ADN (*Rep*) et codant pour l’ADN (cytosine-5)-méthyltransférase (*Mtase*) étaient assignés aux groupes homéologues 1 et 7, respectivement, de Triticeae. Les réarrangements chromosomiques chez le blé et le seigle, l’un par rapport à l’autre, sont discutés.

Mots clés : emplacement chromosomique, sondes de fonction connue, RFLP, seigle, blé.

[Traduit par la Rédaction]

One of the necessities for the construction of high density RFLP (restriction fragment length polymorphism) maps is the availability of as many low copy clones as possible. Besides anonymous probes (cDNAs, gDNAs), known function probes have also been used for genome mapping.

Millan et al. (1992) found a gene with homology to a 7S storage-globulin sequence of wheat (*Glo*) to be located on chromosomes 4A, 4B, 4D, 4H, and 4R. Other examples are sequences (genes) for sedoheptulose-1,7-bisphosphatase (*Sbp*) located on chromosomes 2BS, 2BL, 3A, 3B, 3D, and 7B (Devos et al. 1992) and for ferredoxin (*Fed*) localized on the chromosomes of homoeologous group 7 of wheat, barley, and rye (Chinoy et al. 1991). Plaschke et al. (1993, 1995) used the *EmBP-1* gene, encoding a bZIP protein (Devos et al. 1991), for mapping the GA insensitive dwarfing genes *ct2* and *ct1* on chromosomes 5R and 7R, respectively. In both cases a linkage of less than 6 cM between *XEmbp* and the *ct* genes was found. The present

Corresponding Editor: R. Kemble.

Received February 27, 1996. Accepted July 11, 1996.

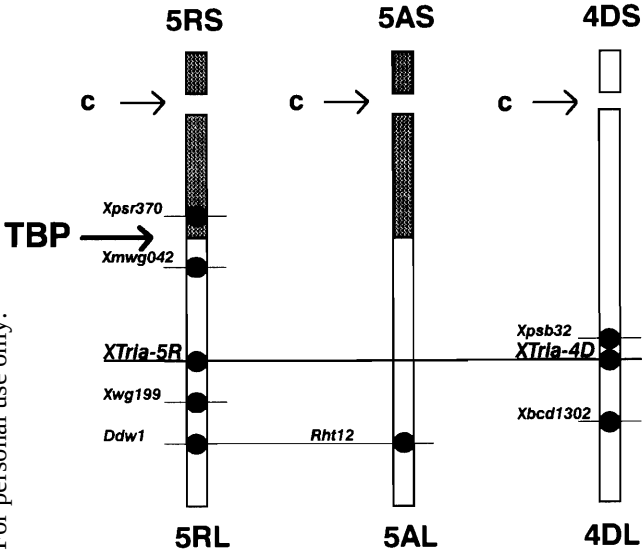
V. Korzun, H.-J. Balzer, A. Balzer, H. Bäumlein, and A. Börner.¹ Institut für Pflanzengenetik und Kulturpflanzenforschung, D-06466 Gatersleben, Germany.

¹ Author to whom all correspondence should be addressed.

Table 1. Locus symbols and availability of the known function probes studied.

Gene	Locus symbol	Availability
Pollen allergen encoding gene	<i>XTria</i> (<i>XTria</i> -5A, -4B, -4D, -5R)	Contact H.-J. Balzer
DNA replication regulating gene	<i>XRep</i> (<i>XRep</i> -1A, -1B, -1D, -1R)	Contact H.-J. Balzer
DNA (cytosine-5)-methyltransferase gene	<i>XMtase</i> (<i>XMtase</i> -7A, -7B, -7D, -6R)	Contact A. Balzer

Fig. 1. Comparative genetic maps of homoeologous parts of chromosomes 5RL (Korzun et al. 1996), 5AL, and 4DL of rye and wheat, including the probes *XTria*-5R and *XTria*-4D. c, centromere; TBP, 5L/4L translocation break point.



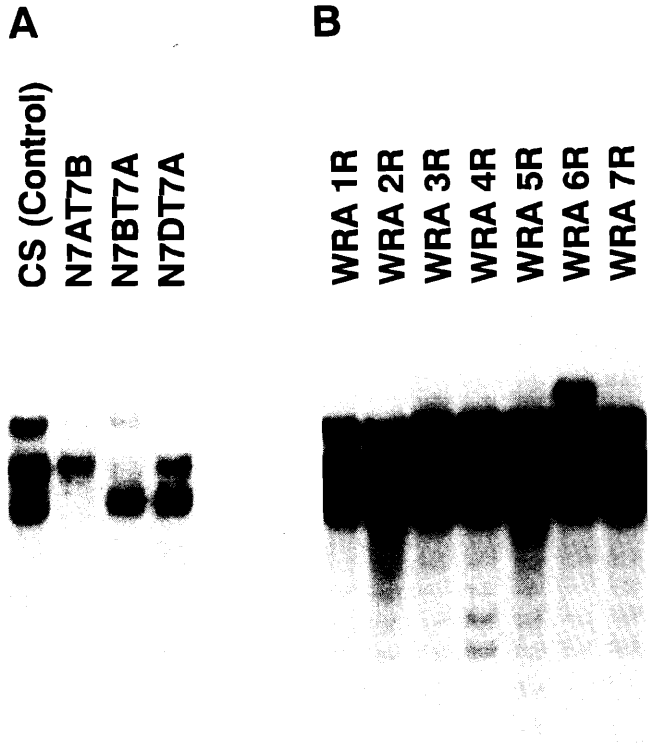
paper describes the chromosomal location of three wheat sequences, shown to be homologous to pollen allergen encoding, DNA replication regulating, and DNA (cytosine-5)-methyltransferase genes. For the pollen allergen encoding gene additional mapping data is presented.

Pollen allergen encoding gene (*Tri a* III)

A cDNA library in λZAP (Stratagene) was constructed from poly(A)⁺ RNA extracted from gynoecia of the wheat ‘Salmon’. The library was screened using a cDNA fragment (SA7X1) from a collection of differentially expressed clones from a subtractive hybridization assay (Balzer and Bäumlein 1994) including ‘Salmon’ and an alloplasmatic line of ‘Salmon’ having the cytoplasm of *Aegilops caudata* (Matzk et al. 1995). The resulting full length cDNA clone shows strong sequence homology (68–75%) to pollen allergens of type II/III from *Lolium perenne* (Ansari et al. 1989a, 1989b) and *Phleum pratense* (Dolecek et al. 1993). In accordance with the rules of nomenclature for pollen allergens (Marsh et al. 1987), the cloned gene was designated *Tri a* III. A detailed description of *Tri a* III is given by Balzer et al. (1996).

For chromosomal localization, *Tri a* III was hybridized to genomic DNA from 21 nullisomic–tetrasomic lines of ‘Chinese Spring’ digested with *Eco*RI, *Eco*RV, *Dra*I, and *Hind*III. It was shown that the genes were present in a

Fig. 2. Hybridization of *Mtase* to *Eco*RI-restricted DNA from homoeologous group 7 nullisomic–tetrasomic lines of ‘Chinese Spring’ (A) and ‘Chinese Spring’ wheat – ‘Imperial’ rye addition lines (B).



single locus per genome on chromosomes 5A, 4B, and 4D. Chromosome location in rye was obtained using the seven wheat–rye (‘Chinese Spring’/‘Imperial’) addition lines. Again only one locus, located on chromosome 5R, was identified. The data demonstrate that *Tri a* III is located on the long arms of the particular chromosomes, distal to the translocation break points on chromosomes 5AL and 5RL described by Liu et al. (1992) and Devos et al. (1993). The locus symbols together with the information about the availability of the probes are listed in Table 1.

Based on the knowledge about the location of *Tri a* III, the clone was used as a probe for mapping the GA sensitive dwarfing genes *Ddw1* of rye (Korzun et al. 1996) and *Rht12* of wheat. These genes are located on the segments of chromosomes 5RL and 5AL that were ancestrally translocated and are homoeologous to Triticeae 4L (Börner et al. 1996). Whereas in rye the locus *XTria*-5R was mapped on chromosome 5RL distal to the translocation break point in wheat, no polymorphism was detected with the enzymes

used for the 5A locus. *Tri a* III was, however, mapped on chromosome 4DL (Fig. 1).

DNA replication regulating gene (*Rep*)

A 606 base pair cDNA fragment was selected from a collection of clones derived from the subtractive hybridization assay described above for *Tri a* III and differentially expressed in the gynoecea of the alloplasmic wheat line '(caudata)-Salmon'. Database analysis revealed a high level of homology to yeast (56–65%) and mammalian (56–59%) DNA replication regulating genes (H.-J. Balzer, unpublished data). Therefore, the clone was designated *Rep*. The hybridization patterns obtained with *Rep* in three restriction digests (*Eco*RI, *Eco*RV, and *Dra*I) consist of the three bands in the wheat 'Chinese Spring'. This indicates that *Rep* is present in a single copy per genome. Nullisomic-tetrasomic and wheat-rye addition line analysis showed these copies to be located on chromosomes 1A, 1B, 1D, and 1R. The proposed symbols for the loci are listed in Table 1.

DNA (cytosine-5)-methyltransferase gene (*Mtase*)

Synthetic oligomers corresponding to conserved regions of DNA (cytosine-5)-methyltransferase were used to screen a cDNA library (kindly provided by Dr. M. Röder, Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany) developed from young plants of the wheat 'Chinese Spring'. An initial partial cDNA fragment (*Wc13*) was used to screen a genomic DNA library (LλSep6-CNN) derived from mature embryos of the wheat 'Cheyenne'. The library was kindly provided by O. Anderson, United States Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Albany, U.S.A. One of the identified positive clones (*Wg8*) was analysed further. Sequence alignments showed convincing homology to the 5-methylcytosine DNA methyltransferase genes from *Arabidopsis thaliana*, mouse, and human (A. Balzer and H. Bäumlein, unpublished data).

A 1.6-kilobase *Eco*RI fragment of the clone was subcloned in pBS vector (Stratagene), designated *Mtase*, and used as a probe. The hybridization against *Eco*RI-, *Eco*RV-, and *Dra*I-restricted genomic DNA of nullisomic-tetrasomic and wheat-rye addition lines showed that the gene copies were located on chromosomes 7A, 7B, 7D, and 6R (Fig. 2). Three hybridization fragments were detected with all three restriction enzymes, indicating the presence of one single copy of the *Mtase* gene in each of the three wheat and rye genomes (for availability and probe symbols see Table 1). For chromosome 6R it is known that the long arm comprises a distal region with homoeology to the long arms of the wheat group 7 chromosomes (Devos et al. 1993). Therefore, the most probable location of *Mtase* in wheat is on the long arms of chromosomes 7A, 7B, and 7D.

References

Ansari, A.A., Shenbagamurthi, P., and Marsh, D.G. 1989a. Complete amino acid sequence of a *Lolium perenne* (Perennial Rye Grass) pollen allergen, *Lol p* II. J. Biol. Chem. **264**: 11 181 – 11 185.

- Ansari, A.A., Shenbagamurthi, P., and Marsh, D.G. 1989b. Complete primary structure of a *Lolium perenne* (Perennial Rye Grass) pollen allergen, *Lol p* III: comparison with known *Lol p* I and II sequences. Biochemistry, **28**: 8665–8670.
- Balzer, H.-J., and Bäumlein, H. 1994. An improved gene expression screen. Nucleic Acids Res. **22**: 2853–2854.
- Balzer, H.-J., Borysiuk, L., Meyer, H.-M., Matzk, F., and Bäumlein, H. 1996. A pollen allergen encoding gene is expressed in wheat ovaries. Plant. Mol. Biol. In press.
- Börner, A., Plaschke, J., Korzun, V., and Worland, A.J. 1996. The relationships between dwarfing genes of wheat and rye. Euphytica, **89**: 69–75.
- Chinoy, C.N., Devos, K.M., Bringle, D., Gray, J.C., Gale, M.D., and Dyer, T.A. 1991. Chromosomal location of the genes for ferredoxin in wheat, barley and rye. Theor. Appl. Genet. **82**: 1–2.
- Devos, K.M., Atkinson, M.D., Chinoy, C.N., Guiltinan, M.J., Quatrano, R.S., and Gale, M.D. 1991. Chromosomal location and variability in wheat, barley and rye of a wheat gene encoding a bZIP protein (EmBP-1). Theor. Appl. Genet. **82**: 665–667.
- Devos, K.M., Atkinson, M.D., Chinoy, C.N., Lloyd, J.C., Raines, C.A., Dyer, T.A., and Gale, M.D. 1992. The coding sequence for sedoheptulose-1,7-bisphosphatase detects multiple homologues in wheat genomic DNA. Theor. Appl. Genet. **85**: 133–135.
- Devos, K.M., Atkinson, M.D., Chinoy, C.N., Francis, H.A., Harcourt, R.L., Koebner, R.M.D., Liu, C.J., Masojc, P., Xie, D.X., and Gale, M.D. 1993. Chromosomal rearrangements in the rye genome relative to that of wheat. Theor. Appl. Genet. **85**: 673–680.
- Dolecek, C., Vrtala, S., Laffer, S., Steinberger, P., Kraft, D., Scheiner, O., and Valenta, R. 1993. Molecular characterization of *Phl p* II, a major timothy grass (*Phleum pratense*) pollen allergen. FEBS Lett. **335**: 299–304.
- Korzun, V., Melz, G., and Börner, A. 1996. RFLP mapping of the dwarfing (*Ddw1*) and hairy peduncle (*Hp*) genes on chromosome 5 of rye (*Secale cereale* L.). Theor. Appl. Genet. **92**: 1073–1077.
- Liu, C.J., Atkinson, M.D., Chinoy, C.N., Devos, K.M., and Gale M.D. 1992. Nonhomoeologous translocations between group 4, 5 and 7 chromosomes within wheat and rye. Theor. Appl. Genet. **83**: 305–312.
- Marsh, D.G., Goodfriend, L., King, T.P., Lowenstein, H., and Platts-Mills, T.A.E. 1987. Allergen nomenclature. J. Allergy Clin. Immunol. **80**: 639–645.
- Matzk, F., Meyer, H.-M., Bäumlein, H., Balzer, H.-J., and Schubert, I. 1995. A novel approach to the analysis of the initiation of embryo development in Gramineae. Sex. Plant Reprod. **8**: 266–272.
- Millan, T., Devos, K.M., Chinoy, C.N., Litts, J.L., Quatrano, R.S., and Gale M.D. 1992. Chromosomal location and RFLP utility in wheat and barley of a wheat gene with homology to a 7S storage-globulin sequence. Theor. Appl. Genet. **85**: 387–388.
- Plaschke, J., Börner, A., Xie, D.X., Koebner, R.M.D., Schlegel, R., and Gale M.D. 1993. RFLP-mapping of genes affecting plant height and growth habit in rye. Theor. Appl. Genet. **85**: 1049–1054.
- Plaschke, J., Korzun, V., Koebner, R.M.D., and Börner, A. 1995. Mapping of the GA₃-insensitive dwarfing gene *ctl* on chromosome 7R in rye. Plant Breed. **114**: 113–116.