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Chromosome painting of Amigo wheat

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Abstract Chromosome painting using multicolor fluorescence *in situ* hybridization showed that, in addition to the T1AL·1RS translocation derived from rye, a segment from chromosome 3Ae#1 of *Agropyron elongatum* (2n=10x =70), is present in Amigo wheat. The *Agropyron* chromosome segment is located on the satellite of chromosome 1B and the translocation chromosome is designated as T1BL·1BS-3Ae#1L. T1BL·1BS-3Ae#1L was inherited from Teewon wheat and carries resistance genes to stem rust (*Sr24*) and leaf rust (*Lr24*). The *Agropyron* chromosome segments in different *Sr24/Lr24* carrier wheat lines, including Agent, TAP 48, TAP 67, Teewon, and Amigo, showed a diagnostic C-band, and were derived from the same chromosome, 3Ae#1.

Key words *In situ* hybridization · C-banding
Rust resistance · *Agropyron elongatum*

Introduction

'Amigo', a cultivar of hexaploid wheat (*Triticum aestivum* L. em. Thell, 2n=6x=42), is well known for its greenbug, *Schizaphis graminum* (Rondani), resistance derived from 'Insave F.A.' rye (*Secale cereale* L.) (Sebesta and Wood 1978). Cytogenetic analysis showed that Amigo has a wheat-rye T1AL·1RS translocation chromosome (Zeller and Fuchs 1983; Lapitan et al. 1986; Heun et al. 1990). The greenbug resistance gene (*Gb2*) is located on the rye arm 1RS (Hollenhorst and Joppa 1981). Amigo also carries a powdery mildew resistance gene, *Pm17*, on 1RS (Lowry et al. 1984; Heun et al. 1990).

Recently, The et al. (1992) reported that Amigo also carries the stem rust resistance gene *Sr24* and the leaf rust resistance gene *Lr24*. Both *Sr24* and *Lr24* were previously located on the long arm of chromosome 3Ae#1 from *Agropyron elongatum* [syn. *Lophopyrum ponticum* (Podp.) Löve, 2n=10x=70] (McIntosh 1988). *Agropyron* chromatin was detected in Amigo, and in backcrossed derivatives carrying *Sr24/Lr24*, using a repetitive DNA sequence probe pAcc2p (The et al. 1992). However, the origin and location of *Sr24/Lr24* in Amigo were not investigated. In this paper, we describe the molecular cytogenetic characterization of an *Agropyron* chromosome segment on the short arm of chromosome 1B in Amigo.

Materials and methods

Amigo seeds were provided by R. A. McIntosh, Plant Breeding Institute, University of Sydney, Australia. Seeds of TAP 48 and 'Teewon' were from D. R. Porter, USDA-ARS, Stillwater, Oklahomo, USA. Seeds of TAP 67 were provided by K. Ross, USDA-ARS, Columbia, Missouri, USA. TAP 67 is a wheat-*Ag. elongatum* 3Ae#1 (3D) substitution line and chromosome 3Ae#1 carries *Sr24* and *Lr24* (see Sears 1972; McIntosh 1988).

C-banding was performed using the technique described by Gill et al. (1991). The multicolor FISH (fluorescence *in situ* hybridization) technique was used for painting the rye and *Agropyron* chromosomes. Genomic DNA from *Ag. elongatum* and rye was labelled with biotin-11-dUTP and digoxigenin-11-dUTP, respectively. Sheared wheat DNA was included in the hybridization mixture to block the cross hybridization of the probes to wheat chromosomes (see Le et al. 1989). For painting TAP 48 and Teewon, avidin-FITC (Boehringer Mannheim) solution (1:500) was applied after post-hybridization washing, and the slides were counterstained with propidium iodide. For painting Amigo, both avidin-FITC and anti-digoxigenin-rhodamine (Boehringer Mannheim) (1:10) solutions were applied, and the slides were counterstained with DAPI. Photographs were taken using a Kodak EKTAR film ASA 1000.

Results and discussion

One of the parents of Amigo, Teewon, was derived from a cross, TAP 48/Wichita (F_1 pollen X-rayed)//Wichita/3/Triumph 64 (Sebesta et al. 1994 a, b). TAP 48 is a 44-chro-

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mosome line carrying a pair of *Ag. elongatum* chromosomes and is derived from a wheat *Ag. elongatum* hybrid made by W.J. Sando (USDA-ARS) (Sebesta et al. 1994 a). The *Agropyron* chromosome in TAP 48 has *Sr24/Lr24* and is closely related to the *Agropyron* chromosome in TAP 67 that also has *Sr24/Lr24* (The et al. 1992). The et al. (1992) also reported that several sib lines of Teewon have a stem rust and leaf rust resistance reaction similar to Agent, a T3DS·3DL-3Ae#1L translocation line (Jiang et al. 1994) known for carrying *Sr24/Lr24*. Therefore, it is most likely that the *Sr24/Lr24* in Amigo was inherited from Teewon and the *Sr24/Lr24* in Teewon was from TAP 48.

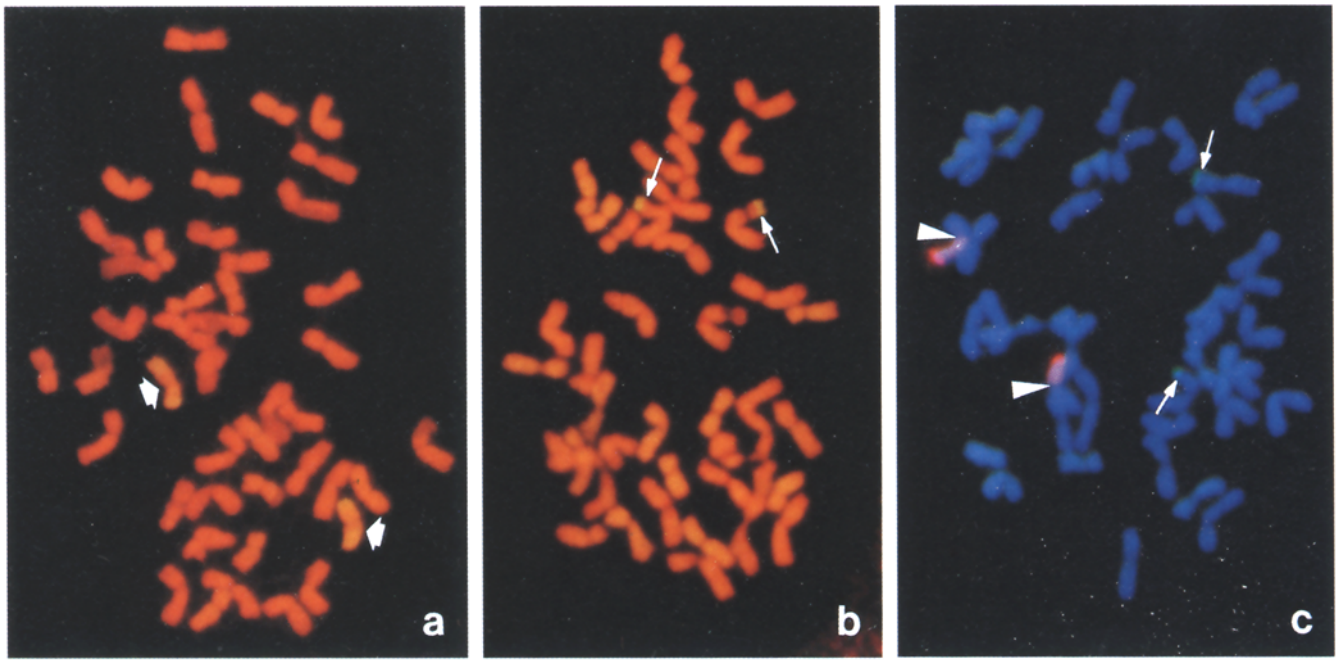
To verify this hypothesis, we analyzed TAP 48, Teewon, and Amigo by GISH (genomic *in situ* hybridization). TAP 48 (2n=44) has a pair of *Ag. elongatum* chromosomes (Fig. 1 a), confirming that it is a disomic wheat-*Ag. elongatum* chromosome addition line (Sebesta et al. 1994 a). GISH analysis on Teewon showed that a pair of satellited chromosomes were involved in a wheat-*Agropyron* translocation (Fig. 1 b). Based on the measurements from ten chromosomes after GISH, about 50% of the satellite was derived from *Ag. elongatum* (Fig. 2 c). This translocation chromosome can be identified as 1B based on its arm ratio. An identical GISH pattern for chromosome 1B was found in Amigo, indicating that chromosome 1B in Amigo was inherited from Teewon. This translocation chromosome is designated as T1BL·1BS-3Ae#1L.

Fig. 1a–c Chromosome painting of: **a** TAP 48, *arrows* point to chromosome 3Ae#1 from *Ag. elongatum*; **b** Teewon, *arrows* point to the breakpoint of wheat-*Agropyron* translocation chromosome T1BL·1BS-3Ae#1L; **c** Amigo, *arrows* points to the breakpoint of wheat-*Agropyron* translocation chromosomes T1BL·1BS-3Ae#1L; *arrowheads* point to the breakpoint of wheat-rye translocations chromosomes T1AL·1RS

Multicolor FISH was applied to confirm the presence of both *Agropyron* and rye chromatin in Amigo. Biotin-labelled rye DNA and digoxigenin-labelled *Ag. elongatum* DNA were included in the hybridization mixture. Figure 1 c shows the simultaneous detection of the *Agropyron* segment on 1BS (with a turquoise color) and the rye arm 1RS on the translocation chromosome T1AL·1RS (with a red color).

The *Agropyron* chromosome in TAP 48 has a major telomeric and a faint subtelomeric C-band on the short arm, and a minor subtelomeric C-band on the long arm (Fig. 2 b). Chromosome 3Ae#1 in TAP 67 has an identical C-banding pattern to the *Agropyron* chromosome in TAP 48. Thus, these two chromosomes may be derived from the same wheat *Ag. elongatum* cross. The *Agropyron* chromosome segment on chromosome T3DS·3DL-3Ae#1L in Agent also has a minor subtelomeric C-band (Jiang et al. 1994), indicating that this *Agropyron* segment was derived from the same 3Ae#1 chromosome as that in TAP 67 and TAP 48. Therefore, the rust resistance genes *Sr24/Lr24* in different wheat lines were originally derived from the same *Ag. elongatum* chromosome. So far, we have found that the 3Ae#1L segments in all wheat lines carrying *Sr24/Lr24*, including the 3D/3Ae#1 recombinant lines derived from TAP 67 (Sears 1972), have the diagnostic subtelomeric C-band (our unpublished data). Thus, both *Sr24* and *Lr24* are probably located proximal, or very close, to this C-band.

The identification of T1BL·1BS-3Ae#1L was also confirmed by C-banding analysis. The distal end of the satellite on 1B in Teewon and Amigo has no C-bands (Fig. 2 d). The minor subtelomeric band on the satellite (Fig. 2 d) is most likely the diagnostic subtelomeric C-band for the long arm of 3Ae#1. All the wheat bands on the satellite of 1BS are present on the satellite of T1BL·1BS-3Ae#1L. Thus, the breakpoint on 1B is very close to the distal tip of the satellite, and only very little wheat chromatin



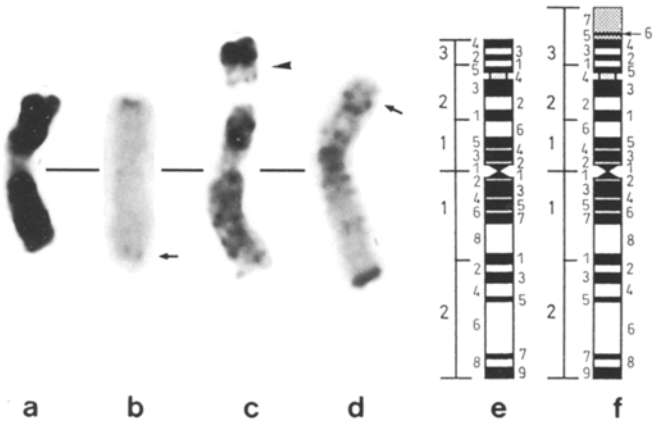


Fig. 2a–f **a** Genomic *in situ* hybridization (GISH) pattern of 3Ae#1 in TAP 48 and TAP 67; the centromeric region is less stained, indicating that the *Agropyron*-specific repetitive DNA sequences are mainly distributed at the distal half of the chromosome (see also Fig. 1 a); **b** C-banding pattern of 3Ae#1 in TAP 48 and TAP 67, the *arrow* points to the diagnostic subtelomeric C-band for the long arm; **c** GISH pattern of translocation chromosome T1BL·1BS-3Ae#1L in Teewon and Amigo, the *arrowhead* points to the breakpoint. The *Agropyron* segment is detected by a peroxidase-DAB method (Rayburn and Gill 1985); **d** C-banding pattern of T1BL·1BS-3Ae#1L in Teewon and Amigo, the *arrow* points to the diagnostic subtelomeric C-band derived from 3Ae#1L; **e** Idiogram of normal chromosome 1B (from Gill et al. 1991); **f** Idiogram of T1BL·1BS-3Ae#1L, the *arrow* points to the diagnostic subtelomeric C-band derived from 3Ae#1L, the chromosome segment (from band 3.5 to 3.7) derived from 3Ae#1L is *shaded*

was lost in the translocation event. This is also confirmed by the presence of the gliadin locus, *Gli-B1*, on T1BL·1BS-3Ae#1L (R. B. Gupta and R. A. McIntosh, personal communication). *Gli-B1* is physically located at the very tip of the short arm of 1B (Mukai and Endo 1992).

T1BL·1BS-3Ae#1L was most likely induced by X-rays during the production of Teewon, and it is a genetically non-compensating translocation. This translocation involves a small deficiency of the distal tip of 1BS, and a duplication (addition) of the distal tip of a homoeologous group-3 chromosome (3Ae#1L). The effect of this deficiency/duplication on yield is not known. The source of *Sr24/Lr24* in the compensating 3D/3Ae#1 translocations, such as Agent or various 3D/3Ae#1 recombinant lines produced by Sears (1972), may be more suitable than from T1BL·1BS-3Ae#1L for breeding applications.

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References

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum* L.). Genome 34: 830–839

Heun M, Friebe B, Bushuk W (1990) Chromosomal location of the powdery mildew resistance gene of Amigo wheat. Phytopathology 80:1129–1133

Hollenhorst MM, Joppa LR (1981) Chromosomal location of genes for resistance to greenbug in Largo and Amigo wheats. Agron Abstr, p 63

Jiang J, Friebe, B, Gill BS (1994) Recent advances in alien gene transfer in wheat. Euphytica 73:199-212

Lapitan NLV, Sears RG, Rayburn AL, Gill BS (1986) Wheat-rye translocations. J Hered 77:415–419

Le HT, Armstrong KC, Miki B (1989) Detection of rye DNA in wheat-rye hybrids and wheat translocation stocks using total genomic DNA as a probe. Plant Mol Biol Rep 7:150–158

Lowry JR, Sammons DJ, Baenziger PS, Moseman JG (1984) Identification and characterization of the gene conditioning powdery mildew resistance in 'Amigo' wheat. Crop Sci 24:129–132

McIntosh RA (1988) Catalogue of gene symbols for wheat. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp. Cambridge, England, pp 1225–1323

Mukai Y, Endo TR (1992) Physical mapping of a fertility-restoring gene against *Aegilops kotschyi* cytoplasm in wheat. Jpn J Genet 67:199–207

Rayburn AL, Gill BS (1985) Use of biotin-labeled probes to map specific DNA sequences on wheat chromosomes. J Hered 76:78–81

Sears EA (1972) Chromosome engineering in wheat. Stadler Genet Symp 4:23–38

Sebesta EE, Wood EA (1978) Transfer of greenbug resistance from rye to wheat with X-rays. Agron Abstr 61–62

Sebesta EE, Smith EL, Young HC, Porter DR, Webster JA (1994 a) Registration of Teewon wheat germplasm. Crop Sci (in press)

Sebesta EE, Wood EA, Porter DR, Webster JA, Smith EL (1994 b) Registration of Amigo greenbug-resistant wheat germplasm. Crop Sci (in press)

The TT, Gupta RB, Dyck PL, Appels R, Hohmann U, McIntosh RA (1992) Characterization of stem rust-resistant derivatives of wheat cultivar Amigo. Euphytica 58:245–252

Zeller FJ, Fuchs E (1983) Cytologie und Krankheitsresistenz einer 1A/1R und mehrerer 1B/1R-Weizen-Roggen-Translokationssorten. Z Pflanzenzüchtg 90:285–296