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Molecular cytogenetic characterization of *Thinopyrum* and wheat–*Thinopyrum* translocated chromosomes in a wheat–*Thinopyrum* amphiploid

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The wheat–*Thinopyrum* amphiploid 'Agrotriticum # 3425' (AT 3425), which is highly resistant to Cephalosporium stripe, was identified to carry seven pairs of *Thinopyrum* chromosomes, three pairs of wheat–*Thinopyrum* translocated chromosomes and 18 pairs of wheat chromosomes. Fluorescence genomic *in situ* hybridization (FGISH), C-banding, sequential C-banding and FGISH, and denaturing polyacrylamide gel electrophoresis (SDS-PAGE) were used to characterize and identify the chromosomes. The *Thinopyrum* chromosomes in AT 3425 were designated as T_1 through T_7 based on their C-banding patterns. The FGISH and C-banding patterns of mitotic chromosomes in AT 3425 and meiotic chromosomes in the hybrid between AT 3425 and wheat cultivar 'Chinese Spring' (CS) revealed that wheat chromosomes 1D, 2B and 3D were involved in the three wheat-*Thinopyrum* chromosome translocations designated as $(W\text{-}T)_1$, $(W\text{-}T)_2$, and $(W\text{-}T)_3$ respectively. The analysis of high-molecular-weight glutenin subunits in single seeds of AT 3425 confirmed the involvement of wheat chromosome 1D in the translocation $(W\text{-}T)_1$. The designations 1DS·1DL-1TL, 2BS·2BL-2TL and 3DS·3DL-3TL were suggested for the wheat–*Thinopyrum* translocated chromosomes $(W\text{-}T)_1$, $(W\text{-}T)_2$ and $(W\text{-}T)_3$ in AT 3425 respectively.

Key words: C-banding, chromosome translocation, fluorescence genomic *in situ* hybridization, SDS–PAGE

Introduction

Thinopyrum species, including *Thinopyrum ponticum* (Podp.) Barkworth & D. R. Dewey [syn. *Lophopyrum ponticum* (Podp.) A. Love, *Agropyron elongatum* (Host) Beauv, $2n = 10x = 70$], *Th. elongatum* (Host) D. R. Dewey [syn. *L. elongatum* (Host) Love, *A. elongatum* (Host) Beauv., $2n = 2x = 14$] and *Th. intermedium* (Host) Barknorth & D. R. Dewey [syn. *A. glaucum* Roem. et Schlt., *A. intermedium* (Host) Beauv., *Elytrigia intermedia* (Host) Nevski, $2n = 6x = 42$] have been extensively hybridized to wheat and have played an important role in wheat improvement (Larson & Atkinson 1970, Shepherd & Islam 1988, Jiang *et al*. 1993, Friebe *et al*. 1994, Xu *et al*. 1994, Zhong G. *et al*. 1994, Chen *et al*. 1995, Cai *et al*. 1996, Cai & Jones 1997). Many wheat–*Thinopyrum* amphiploids with varied chromosome constitutions have been produced from crosses between wheat and *Thinopyrum* species (Dvorak 1976, Schulz-Schaeffer & Friebe 1992, Xu *et al*. 1994, Chen *et al*. 1995, Zhang *et al*. 1996). Wheat–*Thinopyrum* amphiploids carrying desired genes can be used as efficient 'bridges' to transfer genes from *Thinopyrum* species to wheat (Jiang *et al*. 1994, Jones *et al*. 1995).

Cephalosporium stripe, caused by *Cephalosporium gramineum* Nis. and Ika., is an important disease of winter wheat (*T. aestivum* L. em. Thell.) in the USA Pacific Northwest and other winter wheat-growing areas of the world (Mathre *et al*. 1985, Martin *et al*. 1989). No resistance genes for Cephalosporium stripe have been identified within wheat genomes, however one *Th. ponticum* chromosome, 6Ae#2, was determined to confer resistance to Cephalosporium stripe in a wheat background (Cai *et al*. 1996). More effective resistance to Cephalosporium stripe than that conferred by 6Ae#2 was identified in the wheat–*Thinopyrum* amphiploid AT 3425 ($2n = 56$) (Mathre *et al*. 1985, Jones & Murray, upublished data) and, therefore, AT 3425 should be a useful intermediate for transferring the resistance to Cephalosporium stripe of the *Thinopyrum* species into wheat.

The characterization of *Thinopyrum* chromosomes or chromosome segments in AT 3425 will allow a further determination of the *Thinopyrum* chromosome(s) or chromosome segment(s) conferring the resistance to Cephalosporium stripe, and thus aid in the efficient transfer of the resistance gene(s) to wheat. In the present study, the *Thinopyrum* and wheat–*Thinopyrum* translocated chromosomes in AT 3425 were characterized using FGISH, C-banding, sequential C-banding and FGISH, and SDS–PAGE.

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Materials and methods

Seed of wheat-*Thinopyrum* amphiploid AT 3425 was provided by Dr D. E. Mathre, Department of Plant Pathology, Montana State University, USA. AT 3425 was used as a female to hybridize with *T. aestivum* cv. 'Chinese Spring' (CS) in the greenhouse. The F_1 hybrid was obtained by conventional hybridization. Seven F_1 hybrid plants were planted in the greenhouse for the analysis of meiotic chromosomes. Mitotic and meiotic chromosomes were prepared using the methods described earlier (Cai *et al*. 1996). FGISH was performed on the mitotic chromosomes of AT 3425 and the meiotic chromosomes at metaphase I (MI) in pollen mother cells (PMCs) of the F_1 hybrid between AT 3425 and CS. Total genomic DNA of *Th. ponticum* was used as the probe for FGISH and labeled with biotin-16-dUTP by nick translation (Enzo Diagnostics Nick Translation). Total genomic DNA of CS was sheared by boiling in 0.4 M NaOH for 40–50 min and used as blocking DNA for FGISH. The hybridization and signal detection of FGISH were carried out as described by Mukai *et al*. (1993). Fluorescein isothiocyanate-conjugated avidin (FITC-avidin) (Boehringer Mannheim) was used to detect hybridization of the biotinylated probes with chromosomal DNA. Slides were mounted in Vectashield (Vector Laboratories) antifading medium containing 1 μg/ml propidium iodide for counterstaining. FITC and propidium iodide were excited at 450–490 nm. Photographs were taken with Kodak Royal Gold 400 color print film through a Zeiss Axiophot microscope. To identify individual *Thinopyrum* chromosomes and the chromosomes involved in the wheat–*Thinopyrum* chromosome translocations, the mitotic chromosomes of AT 3425 were analyzed by sequential C-banding and FGISH. Standard C-banding procedures of Gill *et al*. (1991) and the FISH procedure of Mukai *et al*. (1993) were used for the sequential analysis. C-banding was also performed on the meiotic chromosomes at MI in PMCs of the F_1 hybrid between AT 3425 and CS (Gill *et al*. 1991).

The high-molecular-weight (HMW) glutenin subunits in single seed of AT 3425 were analysed using the SDS–PAGE procedures of Jones *et al*. (1990). Wheat cultivar 'Federation' (*T. aestivum* L.) and *Th. ponticum* accession 'PI 206624' were used as controls.

Results

FGISH patterns of mitotic chromosomes in AT 3425

The *Thinopyrum* chromatin in AT 3425 was distinguished from wheat chromatin by FGISH with *Th. ponticum* genomic DNA used as the probe. Seven pairs of *Thinopyrum* chromosomes, three pairs of wheat–*Thinopyrum* translocated chromosomes and 18 pairs of wheat chromosomes were identified among the 56 chromosomes at AT 3425 (Figure 1a). Each of the three pairs of translocated chromosomes consisted of one arm of a wheat chromosome, the centromere of the wheat chromosome, a portion of the other arm of the wheat chromosome proximal to the centromere and a terminal segment of a *Thinopyrum* chromosome (Figure 1a). The three wheat–*Thinopyrum* translocated chromosomes in AT 3425 were designated as $(W\text{-}T)_1$, $(W\text{-}T)_2$ and $(W\text{-}T)_3$. Among the three translocated chromosomes, $(W\text{-}T)_1$ carried the largest *Thinopyrum* chromosome segment that constituted over two-thirds of the translocated arm, and $(W\text{-}T)_3$ carried the smallest *Thinopyrum* chromosome fragment that constituted less than one-tenth of the translocated arm. The *Thinopyrum* chromosome segment in $(W\text{-}T)_2$ constituted less than one-third of the translocated arm (Figure 1a).

Sequential C-banding and FGISH

C-banding patterns of *Thinopyrum* and wheat–*Thinopyrum* translocated chromosomes in AT 3425 were determined by sequential C-banding and FGISH analysis (Figure 1b & c). Each of the *Thinopyrum* chromosomes in AT 3425 showed a characteristic C-banding pattern. Based on C-banding patterns, seven pairs of *Thinopyrum* chromosomes in AT 3425 were designated as T_1 through T_7 (Figure 1b). C-banding patterns of the translocated chromosomes showed that wheat chromosomes 1D, 2B and 3D were involved in the translocated chromosomes $(W\text{-}T)_1$, $(W\text{-}T)_2$ and $(W\text{-}T)_3$ respectively (Figures 1b & 2). The *Thinopyrum* chromosome segments involved in the translocated chromosomes also showed different C-banding patterns (Figures 1b & 2). The breakpoints of the translocated chromosomes were determined by FGISH (Figure 1a & c) and defined by C-bands (Figures 1b & 2).

FGISH and C-banidng patterns of meiotic chromosomes in AT 3425 X CS

Forty-eight instead of the expected 49 chromosomes were observed in the hybrid plants between AT 3425 and CS. Eighteen wheat chromosomes of AT 3425 paired with the corresponding chromosomes of CS as bivalents in the hybrid (Figure 3a–c). Six *Thinopyrum* chromosomes and three wheat–*Thinopyrum* translocated chromosomes were visualized among the meiotic chromosomes at MI of the hybrid by FGISH (Figure 3a–c). *Thinopyrum* chromosome T_5 of AT 3425 was not observed in the hybrid (Figure 4). In 66 out of 68 PMCs studied, the translocated chromosome $(W\text{-}T)_3$ paired with a corresponding wheat chromosome of CS as a rod bivalent or ring bivalent, and $(W\text{-}T)_1$ paired with a corresponding wheat chromosome of CS as a rod bivalent (Figure 3a–c). However, the translocated chromosome $(W\text{-}T)_2$ appeared as a univalent in all 68 PMCs observed. In all of the PMCs studied, six *Thinopyrum*' chromosomes appeared as univalents (Figure 3a–c).

To confirm the identification of the wheat chromosomes involved in the wheat–*Thinopyrum* translocated chromosomes by sequential C-banding and FGISH, C-banding was performed on the meiotic chromosomes at MI in the hybrid between AT 3425 and CS. Wheat, *Thinopyrum* and wheat–*Thinopyrum* translocated chromosomes were identified based on their C-banding patterns determined by sequential C-banding and FGISH analysis (Figure 4). The translocated chromosomes $(W\text{-}T)_1$ and $(W\text{-}T)_3$ paired with chromosomes 1D and 3D of CS in the hybrid respectively. However, translocated chromosome $(W\text{-}T)_2$ and chromosome 2B of CS appeared as univalents in all of 74 PMCs observed (Figure 4).

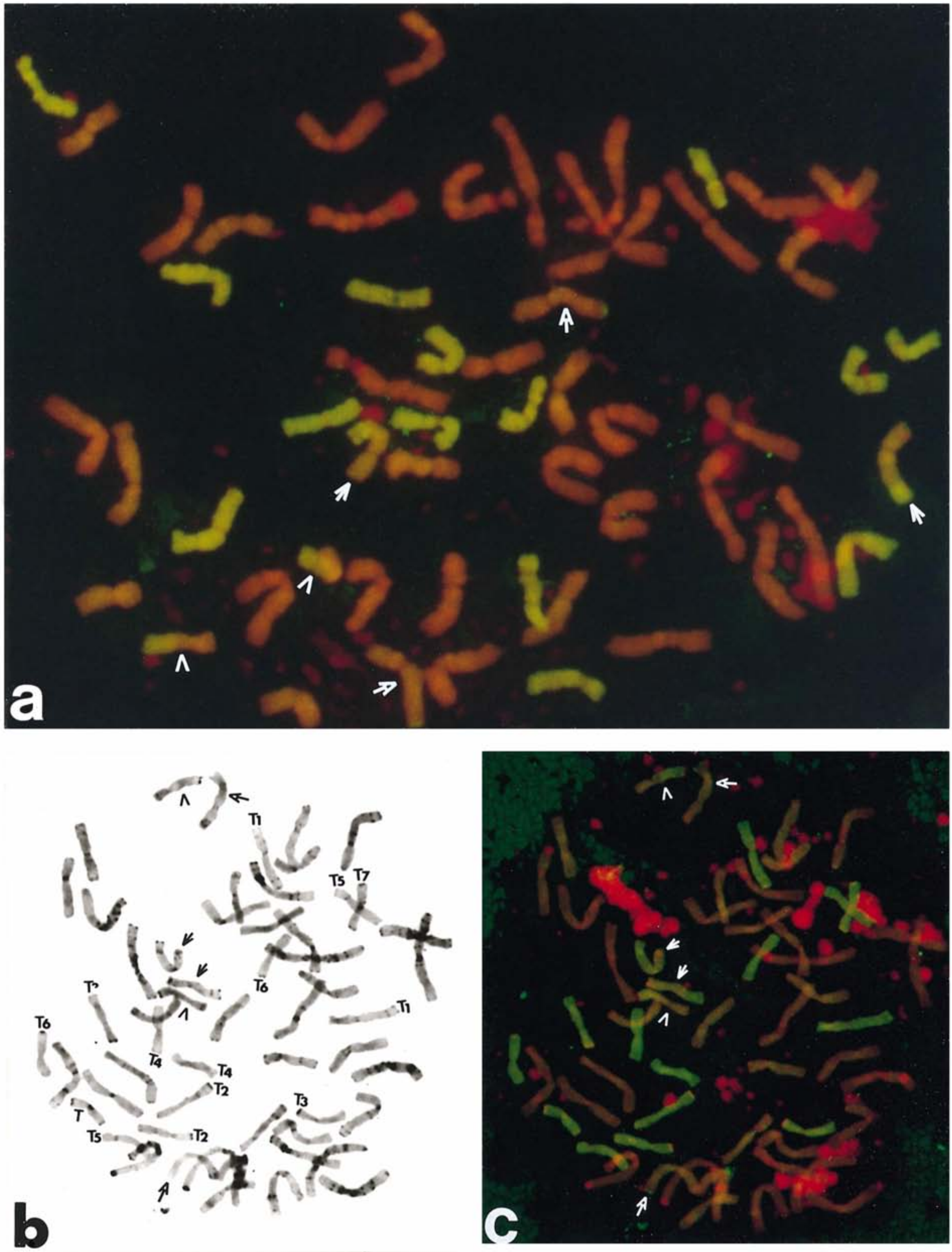

Figure 1. a–c FGISH and C-banding patterns of mitotic chromosomes in AT 3425. **a** Direct FGISH patterns; **b** C-banding patterns of sequential C-banding and FGISH analysis; **c** FGISH patterns of sequential C-banding and FGISH analysis. ∧, ↑, and ⇧ point to (W-T)$_1$ (1DS·1DL-1TL), (W-T)$_2$ (2BS·2BL-2TL) and (W-T)$_3$ (3DS·3DL-3TL) respectively.

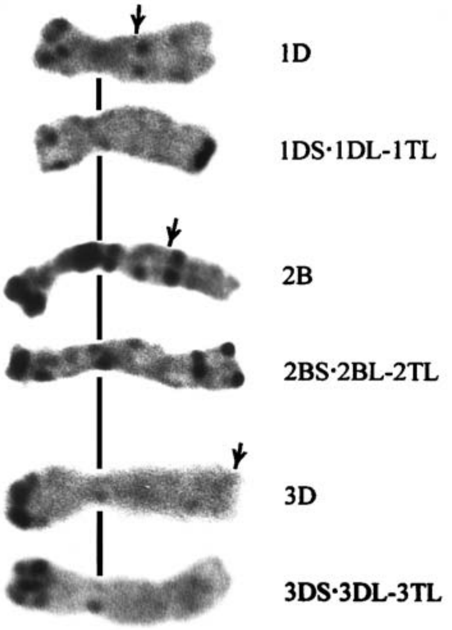

Figure 2. C-banded wheat-*Tinopyrum* translocated chromosomes in AT 3425 and 1D, 2B and 3D chromosomes of CS. Arrows indicate translocation points.

HMW glutenin subunits

The HMW glutenin subunit electrophoretic profiles showed that AT 3425 does not carry the subunits conferred by *Glu-D1* located on the long arm of wheat chromosome 1D (Figure 5). The subunits coded by *Glu-A1a* (1) and *Glu-B1e* (20), however, were present in AT 3425 (Figure 5).

Discussion

Genomic *in situ* hybridization (GISH) has proven to be a very powerful technique to detect alien chromosomes or wheat-alien translocated chromosomes in a wheat genetic background (Heslop-Harrison 1991, Schwarzacher *et al*. 1992, Jiang *et al*. 1993, Mukai *et al*. 1993, Friebe *et al*. 1994, Zhong *et al*. 1994, Cai *et al*. 1996). In the present study, FGISH was successfully used to distinguish *Thinopyrum* chromosomes and chromosome segments from wheat chromosomes and chromosome segments in the wheat–*Thinopyrum* amphiploid AT 3425. Through this approach, AT 3425 was identified to carry seven pairs of *Thinopyrum* chromosomes, three pairs of wheat–*Thinopyrum* translocated chromosomes and 18 pairs of wheat chromosomes (Figure 1a). However, FGISH cannot be used to distinguish one *Thinopyrum* chromosome from another, or to determine the specific chromosomes involved in the translocations. C-banding can be an effective technique to identify individual chromosomes (Gill *et al*. 1991), however a lack of diagnostic bands on chromosomes or chromosome segments limits the application of C-banding to identify these chromosomes or chromosome segments (Fernandez-Calvin *et al*. 1995, Cai *et al*. 1996). Sequential C-banding and FGISH analysis uses the advantages of C-banding and FGISH and can be a powerful approach to analyze chromosome constitutions of derivatives from crosses between wheat and related alien species (Jiang & Gill 1993, S. B. Zhong *et al*. 1996). In the sequential C-banding and FGISH analysis of the present study, FGISH allowed a precise distinction of *Thinopyrum* chromosomes and chromosome segments from wheat chromosomes and chromosome segments in AT 3425, and C-banding allowed an identification of individual *Thinopyrum* chromosomes and determination of the chromosomes involved in the translocations (Figure 1b & c).

Seven pairs of *Thinopyrum* chromosomes identified by sequential C-banding and FGISH analysis in AT 3425 were designated as T_1 through T_7 instead of 1T through 7T as the homoeologous relationships of *Thinopyrum* chromosomes in AT 3425 with wheat chromosomes were unknown. Sequential C-banding and FGISH analysis of the translocated chromosomes in AT 3425 revealed that wheat chromosomes 1D, 2B and 3D were involved in the translocated chromosomes $(W\text{-}T)_1$, $(W\text{-}T)_2$ and $(W\text{-}T)_3$ respectively. As radiation or other artificial factors were not used to induce chromosomal breakage in the production of AT 3425, it can be suggested that the wheat–*Thinopyrum* chromosomal translocations in AT 3425 resulted from recombination events between wheat chromosomes 1D, 2B, 3D and their homoeologous chromosomes in the *Thinopyrum* species. Therefore, the *Thinopyrum* chromosomes involved in the translocations were designated as 1T, 2T and 3T, and their homoeology was inferred to wheat chromosomes in groups 1, 2 and 3. Thus, the wheat–*Thinopyrum* translocated chromosomes $(W\text{-}T)_1$, $(W\text{-}T)_2$ and $(W\text{-}T)_3$ in AT 3425 can be designated as 1DS·1DL-1TL, 2BS·2BL-2TL and 3DS·3DL-3TL respectively (Figure 2).

The meiotic chromosomes and chromosome segments of wheat were distinguished from the meiotic *Thinopyrum* chromosomes and chromosome segments in the hybrid between AT 3425 and CS by FGISH and C-banding (Figures 3a–c & 4). Chromosome pairing of the translocated chromosomes with corresponding wheat chromosomes in the hybrid revealed that wheat chromosomes 1D, 2B and 3D were involved in the wheat–*Thinopyrum* chromosome translocations of AT 3425, which was consistent with the conclusion based on sequential C-banding and FGISH analysis of mitotic chromosomes. FGISH patterns of meiotic chromosomes in the hybrid of AT 3425 with CS showed that both arms of the translocated chromosome 3DS·3DL-3TL paired with corresponding arms of wheat chromosome 3D (Figure 3a–c). This indicated that the *Thinopyrum* chromosome fragment 3TL involved in the translocation did not affect pairing between the translocated chromosome and wheat chromosome 3D as much as *Thinopyrum* chromosome segments 1TL and 2TL involved in

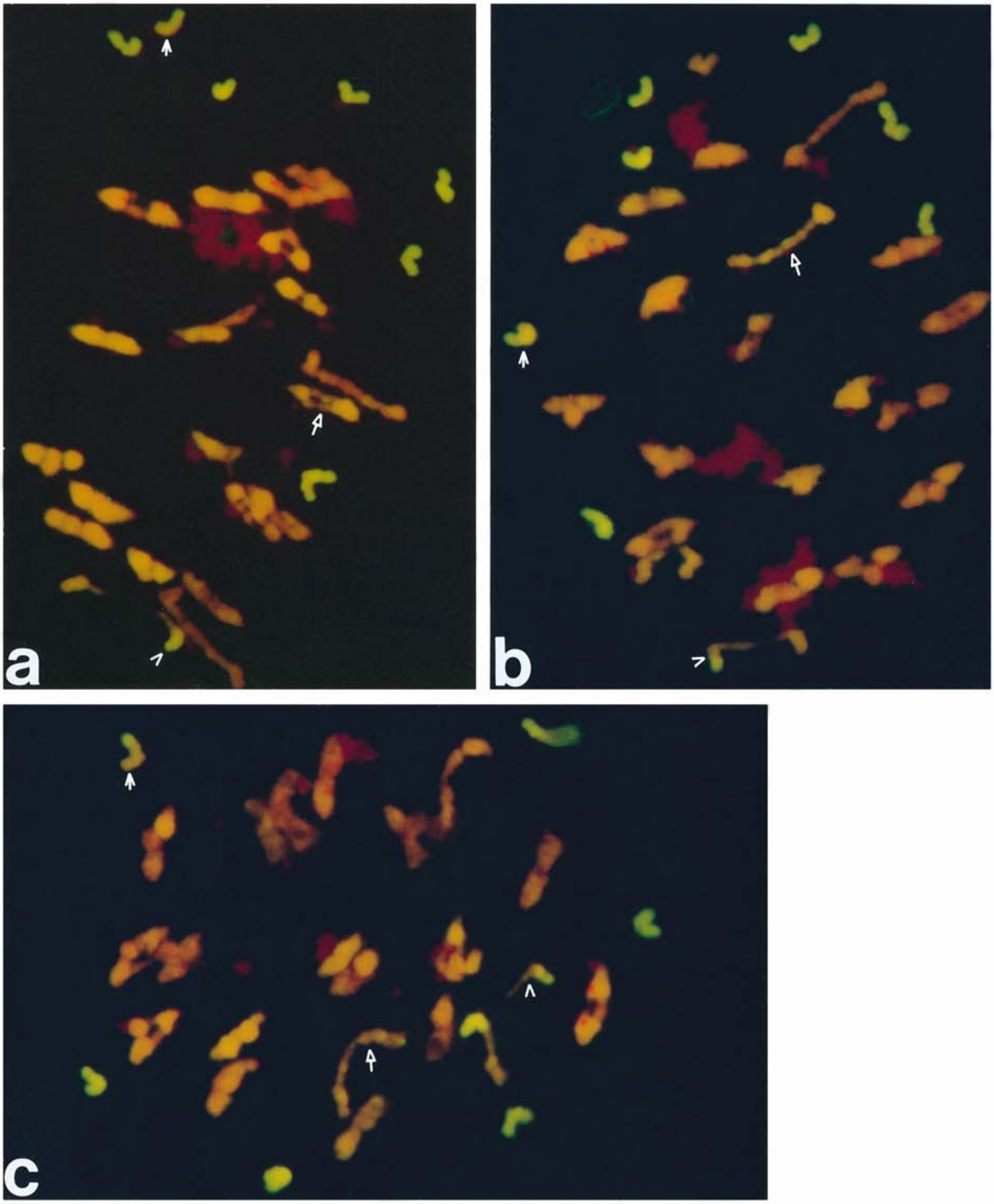

Figure 3. **a**–**c** FGISH patterns of meiotic chromosomes in the hybrid between AT 3425 and CS. **a** A ring bivalent formed by 3DS·3DL-3TL and 3D; **b** a rod bivalent configuration 3DS·3DL↔3TL-3DL·3DS; **c** a rod bivalent configuration 3DL·3DS↔3DS·3DL-3TL. ∧ points to a rod bivalent formed by 1DS·1DL-1TL and 1D; ↑ points to 2BS·2BL-2TL; ⇧ points to a rod or ring bivalent formed by 3DS·3DL-3TL and 3D.

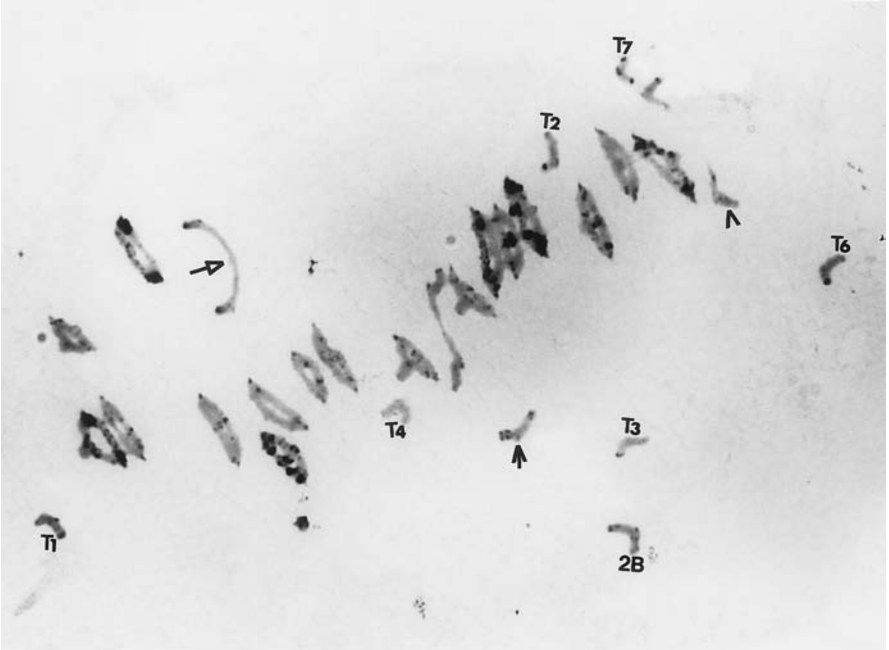

Figure 4. C-banding patterns of meiotic chromosomes in the hybrid between AT 3425 and CS. ∧ points to a rod bivalent formed by 1DS·1DL-1TL and 1D; ↑ points to 2BS·2BL-2TL; ⇧ points to a rod bivalent formed by 3DS·3DL-3TL and 3D.

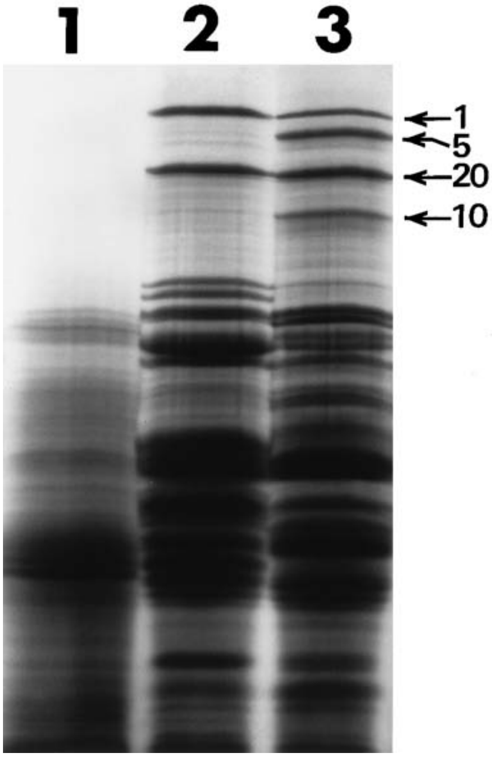

Figure 5. Analysis of HMW glutenin subunits fractionated by SDS–PAGE of *Th. ponticum* (lane 1), AT 3425 (lane 2) and Federation (lane 3).

the translocations 1DS·1DL-1TL and 2BS·2BL-2TL did for pairing of these two translocated chromosomes with wheat chromosomes 1D and 2B respectively (Figure 3a–c).

Six instead of seven *Thinopyrum* chromosomes were observed in the hybrid plants studied between AT 3425 and CS (Figures 3a–c & 4). *Thinopyrum* chromosome T_5 of AT 3425 was not transmitted to the hybrid plants. This may have resulted from a failure in pairing between two T_5 homologous chromosomes during meiosis. Plants carrying 54 and 55 instead of 56 chromosomes have been found in the selfed progenies of AT 3425 on several occasions (X. Cai, unpublished data). This indicates that AT 3425 produces gametes with less than 28 chromosomes and is the probable cause of the F_1 plants carrying 48 chromosomes in the cross between AT 3425 and CS.

Absence of the HMW glutenin subunit coded by *Glu-D1* located on the long arm of wheat chromosome 1D (1DL) revealed that the chromosome segment carrying *Glu-D1* of 1DL was not present in AT 3425, which confirmed the presence of wheat–*Thinopyrum* chromosome translocation 1DS·1DL-1TL in AT 3425. Genetic mapping of *Glu-D1* indicated that *Glu-D1* is 16.5 cM from the centromere of 1D (Jones *et al*. 1991). Therefore, the genetic distance between centromere and translocation point of the translocated chromosome 1DS·1DL-1TL was estimated to be about 16.5 cM or less.

Based on the present results, we concluded that AT 3425 is a wheat–*Thinopyrum* amphiploid with seven pairs of *Thinopyrum* chromosomes, three pairs of wheat–*Thinopyrum* translocated chromosomes and 18 pairs of wheat chromosomes. AT 3425 shows high resistance to Cephalosporium stripe and will be an important intermediate to transfer the resistance of *Thinopyrum* species to wheat.

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