

A Set of Cytogenetic Markers Allows the Precise Identification of All A-Genome Chromosomes in Diploid and Polyploid Wheat

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Key Words

A-genome evolution · Chromosome classification · *Triticum boeoticum* · *Triticum monococcum* · *Triticum urartu*

Abstract

Karyotypes of 3 diploid wheat species containing different variants of the A-genome, *Triticum boeoticum* (A^b), *T. monococcum* (A^b), and *T. urartu* (A^u), were examined using C-banding and FISH with DNA probes representing 5S and 45S rDNA families, the microsatellite sequences GAA_n and GTT_n, the already known satellite sequences pSc119.2, Spelt52, *Fat*, pAs1, and pTa535, and a newly identified repeat called Aesp_SAT86. The C-banding patterns of the 3 species in general were similar; differences were observed in chromosomes 4A and 6A. Besides 2 major 45S rDNA loci on chromosomes 1A and 5A, 2 minor polymorphic NORs were observed in the terminal part of 5AL and in the distal part of 6AS in all species. An additional minor locus was found in the distal part of 7A^bL of *T. boeoticum* and *T. monococcum*, but not in *T. urartu*. Two 5S rDNA loci were observed in 1AS and 5AS.

The pTa535 probe displayed species- and chromosome-specific hybridization patterns, allowing complete chromosome identification and species discrimination. The distribution of pTa535 on the A^u-genome chromosomes was more similar to that on the A-genome chromosomes of *T. dicoccoides* and *T. araraticum*, thus confirming the origin of these genomes from *T. urartu*. The probe pAs1 allowed the identification of 4 chromosomes of *T. urartu* and 2 of *T. boeoticum* or *T. monococcum*. The Aesp_SAT86-derived patterns were polymorphic; main clusters were observed on chromosomes 1A^u and 3A^u of *T. urartu* and chromosomes 3A^b and 6A^b of *T. boeoticum*. Thus, a set of probes, pTa535, pAs1, GAA_n and GTT_n, pTa71, pTa794, and Aesp_SAT86, proved to be most informative for the analysis of A-genomes in diploid and polyploid wheat species.

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The cultivated diploid wheat *Triticum monococcum* L. is one of the oldest domesticated crop species. Together with emmer and barley, einkorn constituted the principal

grain stock that founded Neolithic agriculture and was the main element responsible for its successive spread [Feldman, 2001; Zohary and Hopf, 2001]. It has been assumed that *T. monococcum* was domesticated from its wild progenitor *T. boeoticum* Boiss in southeast Turkey, the Karacadağ mountain range [Heun et al., 1997]. Another wild diploid wheat species, *T. urartu* Tumanian ex Gandilyan, was the A-genome donor for polyploid durum and bread wheat as well as for the species of the Timopheevi group [Dvořák et al., 1993; Ling et al., 2013]. Natural hybridization of *T. timopheevii* (Zhuk.) Zhuk. with cultivated einkorn *T. monococcum* gave rise to *T. zhukovskyi* Menabde et Ericzjan, a new hexaploid wheat species with a unique genome constitution [Bowden, 1959; Tavrín, 1964; Upadhia and Swaminathan, 1969]. Although *T. monococcum* is not cultivated anymore, it is used in wheat breeding as a source of disease resistance genes, high carotenoid content, and other agronomically important characters [Damania et al., 1998; Singh et al., 2008].

Wild einkorns *T. urartu* (A^u) and *T. boeoticum* (A^b) share similar morphological characters [Dorofeev et al., 1979]. They carry closely related genomes that probably diverged ~0.5–1 mya [Huang et al., 2002]. Genetically, *T. monococcum* is similar to *T. boeoticum* [Konarev, 1980], and many taxonomists treat it as a subspecies of *T. monococcum* [Van Slageren, 1994]. Genomes of these species can be discriminated using immunological, biochemical, and molecular markers [Jaaska, 1980; Brandolini et al., 2006; Badaeva et al., 2010a; Konovalov et al., 2010]. However, they are karyotypically similar and have similar Giemsa C-banding patterns [Kuz'menko et al., 1987; Friebe and Gill, 1996]. No reliable markers permitting unequivocal species identification can be provided using C-banding.

An alternative method of chromosome analysis is FISH, which allows direct localization of DNA sequences on chromosomes. FISH in plants often employs probes containing conservative high-copy sequences, like 5S and 45S ribosomal RNA genes [Gerlach and Bedbrook, 1979; Gerlach and Dyer, 1980]. The number and relative position of 5S and 45S rDNA loci in the Triticeae species was shown to be a conservative feature of genomes or genome groups. Therefore, these markers are often used in phylogenetic studies, in the analysis of intra- and interspecific divergence, and in the elucidation of the origin of polyploid species [Badaeva et al., 1996a, 2002, 2004, 2010b; Taketa et al., 1999, 2001, 2005]. Karyotype evolution is also associated with alterations and reorganization of other repetitive sequences [Kubis et al., 1998]. Localization of some families of tandem repeats in plant chromosomes was found to coincide with the position of C-bands

[Bedbrook et al., 1980; Pedersen and Langridge, 1997; Cuadrado et al., 2000].

Chromosomes of diploid wheats possess only few small C-bands [Kuz'menko et al., 1987; Friebe et al., 1990; Friebe and Gill, 1996]. Therefore, one may expect that the 'classical' marker for a Giemsa-positive region, the GAA_n microsatellite [Pedersen and Langridge, 1997; Cuadrado et al., 2000; Cuadrado et al., 2008], will be non-informative for their analysis. Indeed, application of microsatellite probes to *T. monococcum* revealed small signals on only chromosomes 2A^m and 6A^m [Megyeri et al., 2012]. Although clear GAA_n signals have been detected on several A-genome chromosomes of polyploid wheat [Pedersen and Langridge, 1997; Kubaláková et al., 2005], they could have emerged as a result of species-specific translocations with B-genome chromosomes [Liu et al., 1992]. Probe pSc119.2 [Bedbrook et al., 1980], which is frequently employed for examination of polyploid wheat, also proved to be little informative for the analysis of A-genomes [Schneider et al., 2003; Megyeri et al., 2012].

The *Afa* DNA probe could be useful for chromosome identification of diploid wheats and discrimination between A^b- and A^u-genome types. The *Afa*-type probes are also used for chromosome identification of polyploid wheat [Mukai et al., 1993; Pedersen and Langridge, 1997; Schneider et al., 2003; Kubaláková et al., 2005] and *Aegilops* species [Rayburn and Gill, 1986a, 1987; Badaeva et al., 1996b; Bardsley et al., 1999] as well as in phylogenetic studies of other cereals [Taketa et al., 2000]. *Afa* allowed the identification of most chromosomes of cultivated einkorn [Megyeri et al., 2012] and some A-genome chromosomes of durum and bread wheat [Schneider et al., 2003; Sepsi et al., 2008]. The karyotype of *T. urartu*, however, has not been studied using this probe.

More recently, several novel repeat families were isolated from the bread wheat genome [Komuro et al., 2013]. The most informative labelling patterns were generated by pTa535, pTa-713, and pT-86 (homolog of pSc119 sequence). Among them, the probe pTa535 produced the highest number of signals on the A-genome chromosomes, and labelling patterns were chromosome-specific. This clone is a 342-bp tandemly repeated DNA sequence, showing ~80% homology with clone pTa173, a member of the *Afa*-family [Komuro et al., 2013].

The aim of our study was the analysis of 3 diploid wheat species using C-banding and FISH with different families of tandemly repeated sequences to develop a set of informative probes for precise chromosome identification, allowing the discrimination between species and the analysis of chromosome evolution in the genus *Triticum* L.

Materials and Methods

Plant Material

The list of accessions of *T. boeoticum*, *T. monococcum*, and *T. urartu* used in our study is shown in online supplementary table 1 (see www.karger.com/doi/10.1159/000433458 for all online suppl. material). The materials were provided by Dr. O.P. Mitrofanova (VIR, St. Petersburg, Russia), Dr. H. Bockelman (USDA-ARS, Aberdeen, Idaho, USA); Dr. J. Valkoun (ICARDA, Aleppo, Syria), Dr. T. Kawahara (Kyoto University, Kyoto, Japan), Dr. M. Feldman (Weizmann Institute of Science, Rehovot, Israel), Drs. E.A. Nazarova and A.G. Gukasyan (Institute of Botany, National Academy of Sciences of Armenia, Erevan, Armenia), Dr. B. Kilian (IPK, Gatersleben, Germany), and Dr. N.K. Aminov (Institute of Genetic Resources of Azerbaijan, Baku, Azerbaijan).

DNA Probes

The following probes were used for FISH:

- (1) pTa71 – a 9-kb-long sequence of common wheat encoding 18S, 5.8S, and 26S rRNA genes including spacers [Gerlach and Bedbrook, 1979]
- (2) pTa794 – a 420-bp-long sequence of wheat containing the 5S rRNA gene and intergenic spacer [Gerlach and Dyer, 1980]
- (3) pAs1 – a 1-kb fragment derived from *Ae. tauschii* and belonging to the *Afa* family [Rayburn and Gill, 1986b]
- (4) pSc119.2 – a 120-bp-long sequence isolated from rye [Bedbrook et al., 1980]

These probes were labeled with FITC (fluorescein-12-dUTP, Roche, Germany) or biotin (biotin-16-dUTP, Roche, Germany) by nick translation according to the manufacturer's instruction.

(5) Spelt-52 (homolog of pAesKB52), a 276-bp-long sequence was labeled with fluorescein-12-dUTP by PCR with the primer pair (5'-TGA AAT ACT TGC ACA CAA ACC-3'; 5'-GTT AGT TCT GAG TGA ATT GCC-3') using *Ae. speltoides* genomic DNA as template, as described in Salina et al. [2004].

(6) *Fat*, a 460-bp-long sequence, was labeled with fluorescein-12-dUTP by PCR with the primer pair (5'-GGG GAG CTT CTC ACA ACA AGC-3'; 5'-TAT TTA CCA CGG CAT GTC GGG-3') using genomic DNA of bread wheat as template, as described in Badaeva et al. [2010c].

(7) Aesp_SAT86 is a new satellite family with a monomer length of 86 bp identified in *Ae. speltoides* genomic DNA via in silico cluster analysis [Novak et al., 2010]. It shows 91–94% similarity to the wheat repeat pTa-713 described in Komuro et al. [2013]. The probe was labeled with fluorescein-12-dUTP or biotin-16-dUTP by PCR with the primer pair (5'-AGC ACG TGA CAC CAT TGC-3'; 5'-GAC TCG CGT TAC GCT AAG ACA-3') using genomic DNA of *Ae. speltoides* as template.

(8) pTa535-1 was used as a 5' 6-carboxyfluorescein (6-FAM) or 6-carboxytetramethylrhodamine (TAMRA) end-labelled (MWG, Germany) oligo probe (5'-AAA AAC TTG ACG CAC GTC ACG TAC AAA TTG GAC AAA CTC TTT CGG AGT ATC AGG GTT TC-3') [Komuro et al., 2013; Tang et al., 2014].

(9) Microsatellite (CTT)₁₀ was directly labelled with 5/6 sulforhodamine 101PEG3_azine by click chemistry (Baseclick, Germany); the complementary (GAA)₉ oligo probe, labelled at the 3' end with fluorescein-12-dUTP, was synthesized in the laboratory of biological microchips at the Institute of Molecular Biology, Moscow, Russia.

(10) The oligo-(GTT)₉ probe, labelled at the 3' end with fluorescein-12-dUTP, was synthesized in the laboratory of biological microchips at the Institute of Molecular Biology, Moscow, Russia.

Giemsa C-Banding Method

The C-banding method described in Badaeva et al. [1994] was used for analysis. Chromosomes were classified according to the nomenclature suggested in Friebe and Gill [1996] and Friebe et al. [1990].

Fluorescence in situ Hybridization

FISH was carried out according to a standard protocol [Badaeva et al., 1996b] with minor modifications [Zoshchuk et al., 2007]. The probes labelled with fluorescein were detected using anti-fluorescein/Oregon green, rabbit IgG fraction, Alexa Fluor 488 conjugate (Molecular Probes, USA). Biotin was detected with streptavidin-Cy3 (Amersham Pharmacia Biotech, USA). The slides were counter-stained with DAPI (4',6-diamidino-2-phenylindole) in Vectashield mounting media (Vector laboratories, Peterborough, UK) and analyzed using a Zeiss Imager D-1 microscope. Selected metaphase cells were captured with an AxioCam HRm digital camera using software AxioVision, version 4.6. Images were processed in Adobe Photoshop, version CS5 (Adobe Systems, Edinburgh, UK). The FISH painted chromosomes were classified according to Komuro et al. [2013] and Megyeri et al. [2012].

Results and Discussion

Giemsa C-Banding Analysis of *T. boeoticum*, *T. monococcum*, and *T. urartu* Karyotypes

The karyotypes of 8 accessions of *T. boeoticum*, 2 of *T. monococcum*, and 7 of *T. urartu* were analyzed by C-banding (online suppl. table S1). All species were characterized by few Giemsa-positive bands in accordance with previous observations [Kuz'menko et al., 1987; Friebe et al., 1990; Friebe and Gill, 1996]. Most C-bands were small and localized in interstitial chromosome regions (online suppl. figs. S1, S2). Bands on chromosomes 1A and 5A adjacent to NORs were usually the largest. C-banding patterns of *T. boeoticum* chromosomes were virtually identical to those of *T. monococcum* (online suppl. fig. S2) but differed from *T. urartu* (online suppl. fig. S1) and were similar to those described earlier [Kuz'menko et al., 1987; Friebe et al., 1990; Friebe and Gill, 1996]. Most obvious differences between the A^u and A^b genomes were observed in the C-banding patterns of chromosomes 4A and 6A. In particular, chromosome 4A^u of *T. urartu* carried one weak interstitial band in the proximal part of the short arm (online suppl. fig. S1), whereas chromosome 4A^b of *T. boeoticum*/*T. monococcum* contained 2 weak C-bands in the short arm and 1 distinct band adjacent to the pericentromeric region in the long arm (fig. 1e, online suppl. fig. S2). We failed to find any constant C-bands on

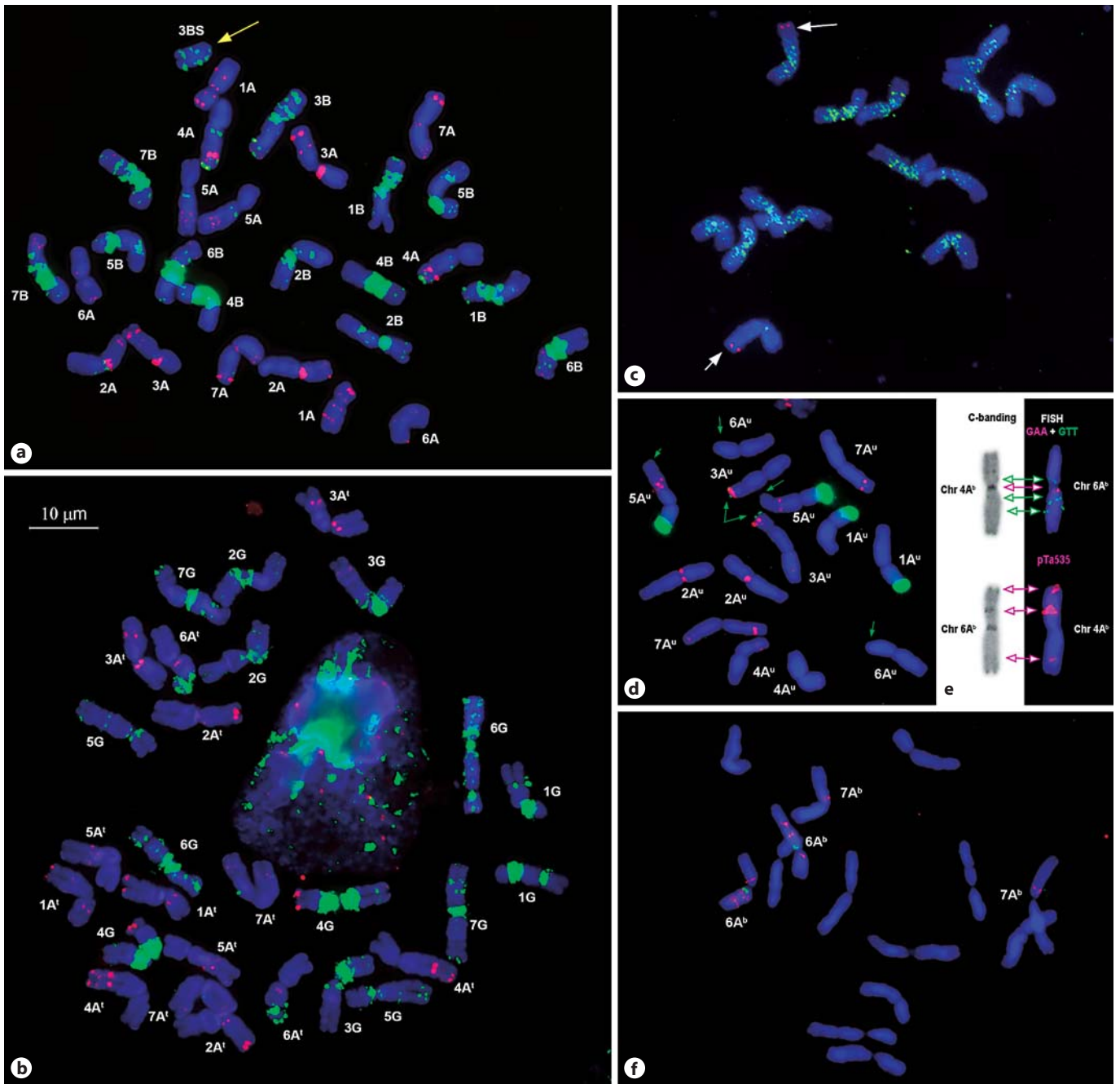


Fig. 1. Distribution of different families of repetitive DNA on selected metaphase chromosomes of diploid and tetraploid wheat species (chromosomes are designated according to the genetic nomenclature). **a, b** *T. dicoccoides* (KU 8836A, Iraq; **a**) and *T. araraticum* (KU 8926, Turkey; **b**) hybridized with pTa535 (red) and GAA_n (green) probes. The yellow arrow points to a spontaneous telocentric 3BS chromosome. **c** *T. boeoticum* (K-25811) hybridized with pSc119.2 (red, indicated with white arrows) and *Fat* (green) probes. **d** *T. urartu* (TA 831) hybridized with pTa71 (green) and pTa535 (red) probes. Green arrows show minor NORs detected in

the subtelomeric region of 3A^{uL}, 5A^{uL}, and 6A^{uS}. **e** Comparison of C-banding (left) and FISH (right) patterns of chromosomes 4A^b and 6A^b of *T. monococcum*. C-banded chromosomes are classified according to Friebe and Gill [1996]; FISH-labelled chromosomes are classified according to Megyeri et al. [2012] and with consideration of hybridization patterns of the 45S rDNA probe. Positions of C-bands and corresponding GAA_n and GTT_n or pAs1 sites are indicated with arrows. **f** *T. monococcum* (MG 4278) hybridized with GAA_n (green) and GTT_n (red) probes.

chromosome 6A^u of *T. urartu*, while 6A^b of *T. boeoticum* always carried clear C-bands in the middle and sometimes in a distal region of the short arm.

C-banding patterns of *T. urartu* satellite chromosomes were polymorphic. Chromosome 1A^u often possessed C-bands in the proximal region of the short arm, and C-bands located in the middle of the long arm were characteristic for chromosome 5A^u. These bands, however, were present in some but not all accessions studied. Chromosome 5A^b can be discriminated from 1A^b by the presence of a pair of weak C-bands in the distal region of the long arm.

Chromosomes assigned to homoeologous groups 2, 3, and 7 in all einkorn species possessed only small C-bands located in similar positions. Thus, C-banding does not permit the precise identification of all chromosomes of diploid wheat species, though it allowed the discrimination of *T. urartu* from *T. boeoticum* based on the presence or absence of marker C-bands on chromosomes 4A and 6A.

Distribution of Highly Repetitive DNA Sequence on Chromosomes of Diploid Wheats

All DNA probes tested in our study generated signals on chromosomes of diploid wheat; however, not all probes in diagnostic patterns. Thus, only small signals of pSc119.2 (fig. 1c) and Spelt-52 probes were rarely observed in subtelomeric regions of one chromosome pair. These chromosomes probably belong to homoeologous group 2 as the same probes were mapped to the short (Spelt-52) and long (pSc119.2) arms of chromosome 2A of tetraploid wheat species [Salina et al., 2006a; Zoshchuk et al., 2007]. Although the *Fat* probe produced many signals on all chromosomes of einkorn species, their distribution was not specific for a particular chromosome or genome type (fig. 1c). Owing to these features, these 3 sequences were considered less informative for the identification of A-genome chromosomes.

Satellite Sequence pTa535. Hybridization of common wheat chromosomes with pTa535 generated signals mainly on the A- and D-genome chromosomes and, in combination with other probes, allowed the identification of all common wheat chromosomes [Komuro et al., 2013]. Hybridization of oligo-pTa535-1 probe to chromosomes of all 3 einkorn species revealed clear and highly specific labelling patterns (fig. 2). All chromosomes except 6A^b carried 1–4 hybridization sites in chromosome-specific positions, although some intraspecific variation in signal localization and intensity has been observed (fig. 2, polymorphic sites are indicated by an asterisk).

Chromosomes belonging to homoeologous groups 1, 3, and 7 in different species had similar labelling patterns, and intraspecific differences were found for chromosomes of groups 2, 4, 5, and 6. Noteworthy, distribution of pTa535 on the A^u genome chromosomes was very similar with that on *T. dicoccoides* (fig. 1a), *T. aestivum* [Komuro et al., 2013], and *T. araraticum* (fig. 1b) chromosomes, thus confirming the origin of their A-genomes from *T. urartu*. This similarity allowed us to apply the chromosome nomenclature developed for common wheat A-genome chromosomes [Komuro et al., 2013] for the classification of the A-genome chromosomes of diploid wheats. Intraspecific differences in the distribution of pTa535 on chromosomes 3A^u-3A^t, 4A^u-4A-4A^t or 6A^u-6A^t can be caused by species-specific translocations identified in 2 allopolyploid wheat species [Jiang and Gill, 1994a; Liu et al., 1992; Salina et al., 2006b].

Hybridization patterns of pTa535 on *T. boeoticum* and *T. monococcum* chromosomes were similar. Some differences between these species observed in the probe distribution on chromosome 2A^b can be due to intraspecific polymorphisms and the small number of accessions studied.

Afa-Family. According to Megyeri et al. [2012], the wheat-derived *Afa* repeat probe allowed the identification of 5 out of 7 chromosomes of cultivated einkorn. The pAs1 clone isolated from *Ae. tauschii* [Rayburn and Gill, 1986b] belongs to the same *Afa* family [Nagaki et al., 1995]. Hybridization patterns of pAs1 on *T. monococcum* chromosomes obtained in our work (fig. 2) only partially corresponded to those published in Megyeri et al. [2012]. Differences of hybridization patterns produced by closely related probes isolated from different species can be due to the divergence of their nucleotide sequences. In *T. monococcum*, 2 weak pAs1 signals were found in the opposite arms of 6A^b, which were not labelled with pTa535. The strongest pAs1 signals were detected on chromosomes 4A^b and 7A^b, and they did not overlap with pTa535 hybridization sites (fig. 2).

Up to 6 pAs1 hybridization sites have been detected in *T. urartu*; among them the largest ones (2A^{uS} and 5A^{uL}) were polymorphic (fig. 2). Localization of pAs1 on chromosomes 7A^{uL} and 4A^{uS} was similar to that in *T. boeoticum*, while chromosome 6A^u carried 2 clear pAs1 sites in the long arm.

Microsatellites GAA_n and GTT_n. Hybridization of the GAA_n probe revealed only very weak signals on chromosomes 1A^u and 5A^u of *T. urartu* (fig. 2). *T. monococcum* contained only 1 strong signal in the pericentromeric region of 6A^b (figs. 1f, 2), and the additional hybridization

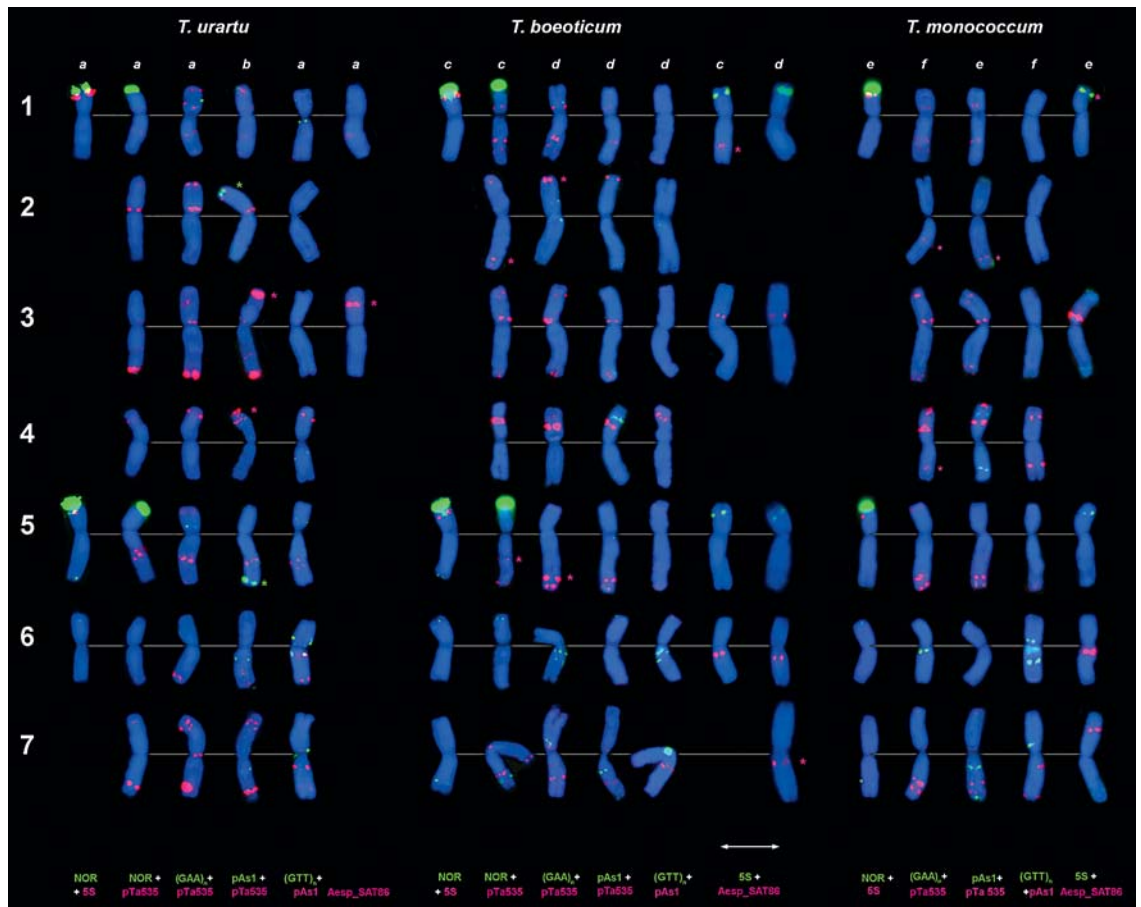


Fig. 2. Comparison of the distribution of different types of repetitive DNA on chromosomes of 3 diploid wheat species: *T. urartu* (a = TA 787, b = TA 831), *T. boeoticum* (c = 11–60; d = PI 554546), and *T. monococcum* (e = MG 4278, f = PI 428282). Chromosomes are assigned to homoeologous groups 1–7. Probe combinations are shown at the bottom of the figure. Hybridization sites showing intraspecific polymorphisms are indicated with an asterisk in the color corresponding to the respective probe.

site was found on chromosome 7A^b of both studied accessions of *T. boeoticum*. In contrast to Megyeri et al. [2012], we have not revealed a telomeric GAA_n signal on chromosome 2A^b, which can be due to an intraspecific polymorphism.

Chromosomal distribution of GTT_n and GAA_n signals differed between the chromosomes of diploid wheat species. In *T. urartu*, 2 very small GTT_n signals were detected on the short and long arms of chromosome 1A^u, in the proximal third of 5A^{uS} and proximal part of 4A^{uL} (fig. 2). Several signals with higher intensities were observed in both arms of 6A^u and in the pericentromeric region of 7A^u. A^b-genome species showed a similar signal distribution on chromosomes 6A^b and 7A^b, but all smaller sites found in the *T. urartu* genome were absent.

Comparison of the hybridization patterns of GAA_n and GTT_n microsatellite probes and of the pAs1 sequences on *T. monococcum* chromosomes with their C-banding patterns raised the question, whether the classification of 4A^b and 6A^b chromosomes should be reconsidered. The identity of 4A^b of *T. boeoticum* was established in a 29-chromosome wheat form, in which chromosome 4A^b spontaneously substituted 4A of *T. durum* [Friebe et al., 1990]. At the same time, the C-banded chromosome 4A^b is more similar with the FISH-labelled chromosome 6A^b and vice versa (fig. 1e). As demonstrated by Cuadrado et al. [2000], the positions of C-bands on wheat chromosomes coincide with the location of GAA_n or GTT_n microsatellite sequences. The distribution of C-bands on the D-genome chromosomes of *Triticum* and *Aegilops* often

overlaps with the distribution of pAs1 hybridization sites [Friebe et al., 1992]. In addition, *T. monococcum* carries a minor pTa71 site (45S rDNA locus) on 6A^{bS}, which could be the remnant of an ancient, inactive NOR locus derived from a common progenitor of the Triticeae. Based on these facts, we suggest that the classification of C-banded chromosomes should be verified.

Satellite Sequence Aesp_SAT86. Hybridization of the Aesp_SAT86 probe on *T. urartu* chromosomes revealed 1–2 large signals located in the middle of 1A^{uL} and in 3A^{uS}, the latter one being polymorphic between the accessions. Three large clusters have been detected in *T. monococcum* chromosomes: in 3A^{bS}, 6A^{bL} close to the centromere, and in 7A^{bL} (fig. 2). Two very small signals were observed in pericentromeric regions of chromosome 7A^b and in the short arm of chromosome 1A^b, close to the 5S rDNA site. The labelling pattern of *T. boeoticum* chromosomes was similar to that of *T. monococcum*, although we found a difference between the accessions in signal distribution. Only 2 loci on 3A^{bS} and 6A^{bL} were constant. Accession PI 554546 contained an additional site in the middle of 1A^{bL}, similar to chromosome 1A^u of *T. urartu*. Small pericentromeric signals were found on chromosome 7A^b of accession 11–60. Based on these results, we suggest that Aesp_SAT86 can be used in the analysis of einkorn for species discrimination and identification of particular chromosomes.

Localization of 5S and 18S-5.8S-26S rDNA Loci on Chromosomes of Diploid Wheats. Mapping of the ribosomal RNA gene probes pTa71 (18S-5.8S-26S rDNA or NOR) and pTa794 (5S rDNA) on chromosomes of 3 diploid wheat species revealed 2 major rDNA loci with equal or slightly different sizes on chromosomes 1A and 5A, which is in accordance with previous observations [Jiang and Gill, 1994b; Dubcovsky and Dvořák, 1995; Megyeri et al., 2012]. In some accessions, however, we observed a significant size reduction at one of the loci (*T. boeoticum* – K-20741, *T. urartu* – PI 428311, K-58495, online suppl. fig. S3a) up to a complete locus loss (5A^m in *T. boeoticum*, K-40118 from Iraq, online suppl. fig. S3c).

Besides major NORs, the karyotypes of most accessions showed 1–3 pairs of minor 45S rDNA sites (online suppl. fig. S3, online suppl. table S2). One of these, described earlier as *Nor-A7* by Jiang and Gill [1994b], was found in the subterminal region of chromosome 5AL. The second minor NOR, designated *NOR-Ax* [Jiang and Gill, 1994b; Dubcovsky and Dvořák, 1995], was located in a distal part of 7A^{bL}. This locus was found in *T. boeoticum* and *T. monococcum* only (fig. 2, online suppl. fig. S3a–d, h, online suppl. table S2). The third, smallest 45S rDNA

locus, designated *NOR-Ay* [Jiang and Gill, 1994b], was detected in the distal region of chromosome 6AS, thus confirming the assumption of Dubcovsky and Dvořák [1995]. The *NOR-Ay* locus could have derived as a result of a significant reduction of 45S rDNA copy number in the respective locus on group 6 chromosomes that has probably occurred in a common ancestral form of einkorn species. This locus was found in all studied accessions of *T. boeoticum*/*T. monococcum* and in approximately half of *T. urartu* accessions (fig. 2, online suppl. fig. S3e, g, online suppl. table S2). Intraspecific heterogeneity could explain the fact that other authors [Jiang and Gill, 1994b] have not revealed 45S rDNA loci on chromosome 6A^u of *T. urartu*.

Two 5S rDNA loci with equal to sharply different sizes were located in the short arms of 1A and 5A distal to the major NORs (fig. 2, online suppl. fig. S3). The 5S rDNA locus was absent on chromosome 5A^b of *T. boeoticum* accession K-61600 from Greece (online suppl. fig. S3h). In the majority of *T. boeoticum*/*T. monococcum* accessions the size of 5S rDNA loci on chromosomes 1A^b and 5A^b were nearly the same (online suppl. fig. S3a, c, d) or the locus on 1A^b was slightly larger (online suppl. fig. S3b). By contrast, nearly half of *T. urartu* accessions carried a significantly larger 5S rDNA site on chromosome 1A^u (online suppl. fig. S3e), and in only few accessions both loci had approximately the same sizes (online suppl. fig. S3e).

Conclusions

Application of different methods of chromosome analysis of einkorn wheat shows that FISH with particular probe combinations exhibits obvious advantages over the C-banding technique with respect to chromosome identification and discrimination between species with different genome constitutions. Among 10 DNA probes tested, the best informative probe was pTa535 (which generates marker signals on all chromosomes allowing chromosome identification and species discrimination). Chromosomes 1A and 5A can be identified also by using either one of the rDNA probes, while 6A and 7A^b are marked by the presence of minor 45S rDNA loci. The pAs1 clone can be applied as an additional probe specific for chromosomes 4A, 6A, and 7A. Similarly, microsatellite sequences GAA_n and GTT_n will be useful for the detection of chromosomes 6A and 7A. Although DNA probes pSc119.2 and Spelt52 were considered not informative for the analysis of einkorn wheat genomes, they can be

utilized for the identification of chromosome 2A in some accessions. Comparison of C-banding and FISH data revealed a conflict between these systems in the classification of 4A and 6A chromosomes. Similarity of pTa535 FISH patterns on the A and A^t genome chromosomes of tetraploid *T. dicoccoides* and *T. araraticum* with the A^u genome chromosomes of *T. urartu* further confirmed the origin of all polyploid wheat from this einkorn species. Some differences observed between chromosomes of diploid and polyploid species could be caused by species-specific translocations that occurred during the formation of primary wheat allotetraploids.

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