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Cytogenetic analysis of *Aegilops* chromosomes, potentially usable in triticale (X *Triticosecale* Witt.) breeding

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Abstract Chromosome identification using fluorescence in situ hybridization (FISH) is widely used in cytogenetic research. It is a diagnostic tool helpful in chromosome identification. It can also be used to characterize alien introgressions, when exercised in a combination with genomic in situ hybridization (GISH). This work aims to find chromosome identification of *Aegilops* species and *Aegilops* × *Secale* amphiploids, which can be used in cereal breeding as a source of favourable agronomic traits. Four diploid and two tetraploid *Aegilops* species and three *Aegilops* × *Secale* hybrids were analysed using FISH with pSc119.2, pAs1, 5S rDNA and 25S rDNA clones to differentiate the U-, M-, S^{sh}- and D-subgenome chromosomes of *Aegilops* genus. Additionally, GISH for chromosome categorization was carried out. Differences in the hybridization patterns allowed to identify all U-, M-, S^{sh}- and D-subgenome chromosomes. Some differences in localization of the rDNA, pSc119.2 and pAs1 sequences between analogue subgenomes in diploid and tetraploid species and *Aegilops* × *Secale* hybrids were detected. The hybridization pattern of the M and S genome was more variable than that of the U and D genome. An importance of the cytogenetic markers in plant breeding and their possible role in chromosome structure, function and evolution is discussed.

Keywords *Aegilops* species · *Aegilops* × *Secale* hybrids · Cytogenetic markers · Fluorescence and genomic in situ hybridization

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Introduction

Interspecific hybridization is the most common method used to transfer genes of agronomic value from wild relatives into cultivated cereals (Benavente et al. 1996). This is of great importance for human nutrition, since cereal species are a staple of diet. One of the main domains in this field of research is resistance breeding. *Aegilops* (goatgrass) species are rich germplasm sources of resistance and tolerance to biotic and abiotic stresses. The genus *Aegilops* belongs to the tribe Triticeae, subtribe Triticinae, where we can also distinguish the genera *Triticum*, *Secale* and *Brachypodium*. Some of the species of *Aegilops* genus like: *Ae. speltoides* Tausch., *Ae. sharonensis* Eig., *Ae. bicornis* (Forsk.) Jaub. & Spach., *Ae. longissima* Schweinf. & Muschl., and *Ae. searsii* Feldman & Kislev ex K. Hammer (S-genome species), are proposed to be the donors of the B-genome of bread wheat (*Triticum aestivum* L.) (Feldman et al. 1995; Belyayev et al. 2001; Raskina et al. 2002), while *Ae. tauschii* Coss. is assumed to be a D-genome donor (Hedge et al. 2002). There are two proposals for B genome origin. The first, called the polyphyletic theory, states that permanent hybridizations and exchanges of genetic information between ancestral AASS amphiploids and related S-genome diploid progenitors singled out the B genome. The second theory assumes that the B-genome originated from a single S-genome ancestor, probably *Ae. speltoides* (Raskina et al. 2002; Belyayev and Raskina 2012). Taking into consideration the close relationship within Triticeae tribe, many genes have been transferred from *Aegilops* species to cultivated wheat, such as those for resistance to leaf rust, stem rust, yellow rust and powdery mildew (Schneider et al. 2008). In interspecific hybridization analysis, it is fundamental to be able to describe the genomic composition of plants carrying the desired traits (Sepsi et al. 2008). Molecular cytogenetics has revolutionized the genetic

analysis of plant genomes and has also provided plant breeders with new tools to identify chromatin involved in resistance to stresses and quality traits. Genomic in situ hybridization (GISH) is successfully used to discriminate the genome composition of hybrids (Mahelka et al. 2011), whereas fluorescence in situ hybridization (FISH), coupled with observations of chromosome morphology (Castilho and Heslop-Harrison 1995), is a method for chromosome identification using specific probes, such as repetitive DNA sequences (Cuadrado et al. 2000). Also Giemsa C-banding and N-banding are successful methods in chromosome identification and detection of chromosome rearrangements (e.g. Gill and Kimber 1977; Łukaszewski and Gustafson 1983; Apolinarska et al. 2010). The most common repetitive DNA sequences used in physical mapping in the genus *Triticum* are: pSc119.2, pSc200 (derived from rye), pAs1 (Afa family, from wheat), spelt 1 and spelt 52 (from *Ae. speltoides*) (Salina et al. 2006). The vast variability of goatgrass species is expressed in a different location of repetitive sequences, used as probes in FISH analysis, as reported in many papers so far. For example, the chromosomes of *Ae. biuncialis* Vis. and its ancestors: *Ae. umbellulata* Zhuk. and *Ae. comosa* Sm., were examined by Schneider et al. (2005) in order to identify wheat addition lines. There are also several other papers discussing the distribution of repetitive sequences with regard to genome differentiation (e.g. Badaeva et al. 1996a, b; Cuadrado and Jouve 2002; Cuadrado et al. 2008; Raskina et al. 2008; Salina et al. 2006, 2009). From our research perspective, this indicated a need for creating physical maps of cytogenetic markers of *Aegilops* accessions used in a further widening of the genetic diversity of triticale. The present work is a continuation of a research cycle (Kwiatek et al. 2012), that deals with genetic diversification of cultivated triticale by introgressing alien *Aegilops* chromatin. Considering the progressive loss of effective resistance genes in triticale because of the appearance of new virulent disease pathotypes (Arseniuk 1996), the adaptation of methods used in gene transfer from alien species is a key issue. The main goal of this research was: (1) to generate physical maps using four repetitive DNA sequences (pSc119.2, pAs1, 5S rDNA, 25SrDNA) of U, M, S and D subgenomes; and (2) to categorize the amphiploid chromosomes with respect to the subgenomes. This paper reports FISH/GISH chromosome characterization and categorization of *Aegilops kotschyi* Boiss., *Ae. ovata* Roth., *Ae. tauschii* and amphiploids derived from *Aegilops* spp. × rye crossing. The ancestors of the introduced subgenomes, namely *Ae. umbellulata*, *Ae. comosa* and *Ae. sharonensis*, were analysed to help to identify the specific signal patterns. It is hoped that the development of these amphiploid forms will allow us to transfer agronomically useful traits (drought and salt tolerance, disease resistance) from goatgrasses into triticale.

Materials and methods

Plant material

Seeds of *Aegilops umbellulata*, *Ae. comosa* and *Ae. sharonensis* were kindly supplied for the study from the National Small Grains Germplasm Research Facility, National Small Grains Collection (Aberdeen, Idaho, USA). Seeds of *Ae. kotschyi*, *Ae. ovata*, *Ae. tauschii* were received by Wojciechowska (1996) from a collection of Professor M. Feldman (Weizmann Institute of Science, Rehovot, Israel). Seeds of *Secale cereale* were provided by a collection of the Institute of Plant Genetics, Polish Academy of Sciences in Poznań, Poland (Table 1). Amphiploids were generated from interspecific crosses between these *Aegilops* spp. and rye (Wojciechowska and Pudelska 1999, 2002a, b, 2005). Twenty seeds of each accession were germinated on moist filter paper in Petri dishes for 3–4 days. The root-tips were collected and ice-cooled for 26 h for metaphase accumulation. The fixation was made using ethanol and acetic acid (3:1, v/v). The chromosome preparations were made according to Pijnacker and Ferwerda (1984).

DNA isolation

The DNA isolation from leaf tissue was made after Lombard and Delourme (2001). The pSc 119.2, 25S rDNA and 5S rDNA were amplified using M13 sequencing primers. The pAs1 (*Afa* family) was amplified according to Nagaki et al. (1995).

In situ hybridization

Three in situ hybridizations on the same chromosome preparations were carried out. First FISH was made according to Książczyk et al. (2010) with minor modifications, using 25S (used for detection of 35S rDNA loci) (Unfried and Gruendler 1990) and 5S rDNA (pTa794) (Gerlach and Dyer 1980) labelled by PCR with digoxygenin-11-dUTP and tetramethyl-rhodamine-5-dUTP, respectively (ROCHE). The hybridization mixture (40 μL per slide) contained 90 ng of each probe in the presence of salmon sperm DNA. After documentation of the FISH sites, the slides were washed (2×45 min in 4 × SSC Tween, 2×5 min in 2 × SSC, at room temperature).

During the second FISH, pSc119.2 and pAs1 (labelled with digoxygenin-11-dUTP and tetramethyl-rhodamine-5-dUTP, respectively) were used as probes.

After second reprobing, GISH was carried out according to Książczyk et al. (2010) with modifications. GISH on chromosomes of tetraploid *Aegilops* species was carried out using U probes (generated from *Ae. umbellulata*), which were labelled with tetramethyl-rhodamine-5-dUTP using nick translation kit (ROCHE) and M probes (*Ae. comosa*) and S probes (*Ae. sharonensis*) labelled with digoxigenin-

Table 1 Plant material used for fluorescence in situ hybridization polymorphism analysis

Accession/form	Number of plants	Genome structure	Source
Aegilops umbellulata	20	2n=2x=14, UU	National Small Grains Germplasm Research Facility, National Small Grains Collection (Aberdeen, Idaho, USA)
Aegilops comosa	20	2n=2x=14, MM	National Small Grains Germplasm Research Facility, National Small Grains Collection (Aberdeen, Idaho, USA)
Aegilops sharonensis	20	2n=2x=14, $S^{sh}S^{sh}$	National Small Grains Germplasm Research Facility, National Small Grains Collection (Aberdeen, Idaho, USA)
Aegilops tauschii	20	2n=2x=14, DD	National Small Grains Germplasm Research Facility, National Small Grains Collection (Aberdeen, Idaho, USA)
Secale cereale	n/a[a]	2n=2x=14, RR	Institute of Plant Genetics, Polish Academy of Sciences
Aegilops kotschyi	20	2n=4x=28, UUSS	Weizmann Institute of Science, Rehovot, Israel
Aegilops ovata	20	2n=4x=28, UUMM	Weizmann Institute of Science, Rehovot, Israel
Aegilops tauschii × *Secale cereale*	20	2n=4x=28, DDRR	Institute of Plant Genetics, Polish Academy of Sciences
Aegilops kotschyi × *Secale cereale*	20	2n=6x=42, UUSSRR	Institute of Plant Genetics, Polish Academy of Sciences
Aegilops ovata × *Secale cereale*	20	2n=6x=42, UUMMRR	Institute of Plant Genetics, Polish Academy of Sciences

[a] Leaf tissue for DNA isolation

11-dUTP. GISH on amphiploids (*Aegilops* ssp. × *S. cereale*) forms was carried out using properly probes, generated from *Ae. kotschyi*, *Ae. ovata*, *Ae. tauschii* genomic DNAs, labelled by nick translation with digoxigenin-11-dUTP. The blocking DNA was obtained by autoclaving the total DNA of rye. The GISH mixture (40 mL per slide), containing 50 % formamide, 2 × SSC, 10 % dextran sulphate, 90 ng each of the genome probes, and 4.5 mg blocking DNA, was denatured at 75 °C for 10 min and stored on ice for 10 min. Chromosomal DNA was denatured in the presence of the hybridization mixture at 75 °C for 6 min and allowed to hybridise overnight at 37 °C. For detection of the hybridization signals, anti-digoxigenin-rhodamine conjugated with FITC (ROCHE) was used. Mitotic cells were examined with an Olympus XM10 CCD camera attached to an Olympus BX 61 automatic epifluorescence microscope. Image processing was carried out using Olympus Cell-F imaging software and Micrographx Picture Publisher software.

Results

U chromosomes

In *Ae. umbellulata*, 5S rDNA and 35S rDNA were found in short arms of two pairs of chromosomes: 1U and 5U (Fig. 1a). The 5S rDNA landmarks were more distant from the centromere than 35S rDNA in 1U chromosomes, but in chromosome pair 5U their location was reversed. The same arrangement was observed in *Ae. kotschyi* (Fig. 1b), *Ae. ovata* (Fig. 1c), and amphiploids: *Ae. kotschyi* × *S. cereale* and *Ae. ovata* × *S. cereale* (Fig. 2). There was only one exception: in chromosome $5U^o$ of plant no. 3/4 of *Ae. ovata* × *S. cereale* a deletion of the 5S and 35S rDNA sites was detected (Fig. 2a, d). The pSc 119.2 labelling was detected at telomeric locations of each U chromosome of *Ae. umbellulata* except short arm of 6U chromosome and long arms of 1U and 2U chromosomes. Moreover, a subtelomeric site in 7U chromosomes was observed. In tetraploid accessions containing the U subgenome, the labelling pattern of pSc 119.2 was similar as in *Ae. umbellulata* but additionally in chromosome $6U^o$ of *Ae. ovata* and amphiploids in the middle of the long arm a considerable signal was detected. Furthermore, in $1U^o$ chromosomes in *Ae. ovata* (and amphiploid) only short-arm signals were found, when in $1U^k$ chromosome of *Ae. kotschyi* two sites were observed (telomeric and distal region of long arm). In the long arm of $7U^o$ chromosome of *Ae. ovata* and *Ae. ovata* × *S. cereale*, two subtelomeric sites were observed.

The pAs1 signals were observed in each chromosome of *Ae. umbellulata*. In 1U, 2U, 3U, 6U and 7U chromosomes, the pAs1 probe was detected in the interstitial region of the long arm. Faint signals were also observed in the distal part of the long arm of 7U chromosomes. The most significant pAs1 signal was detected in the pericentromeric region of 6U chromosomes. The pAs1 labelling patterns in tetraploids and amphiploids were similar to those in *Ae. umbellulata* with the exception of *Ae. kotschyi* and amphiploids, where short arms of 3U had faint signals of the *Afa* family. Furthermore, $7U^k$ chromosomes of *Ae. kotschyi* carried more signals of pAs1 than 7U chromosomes of other species taken into consideration.

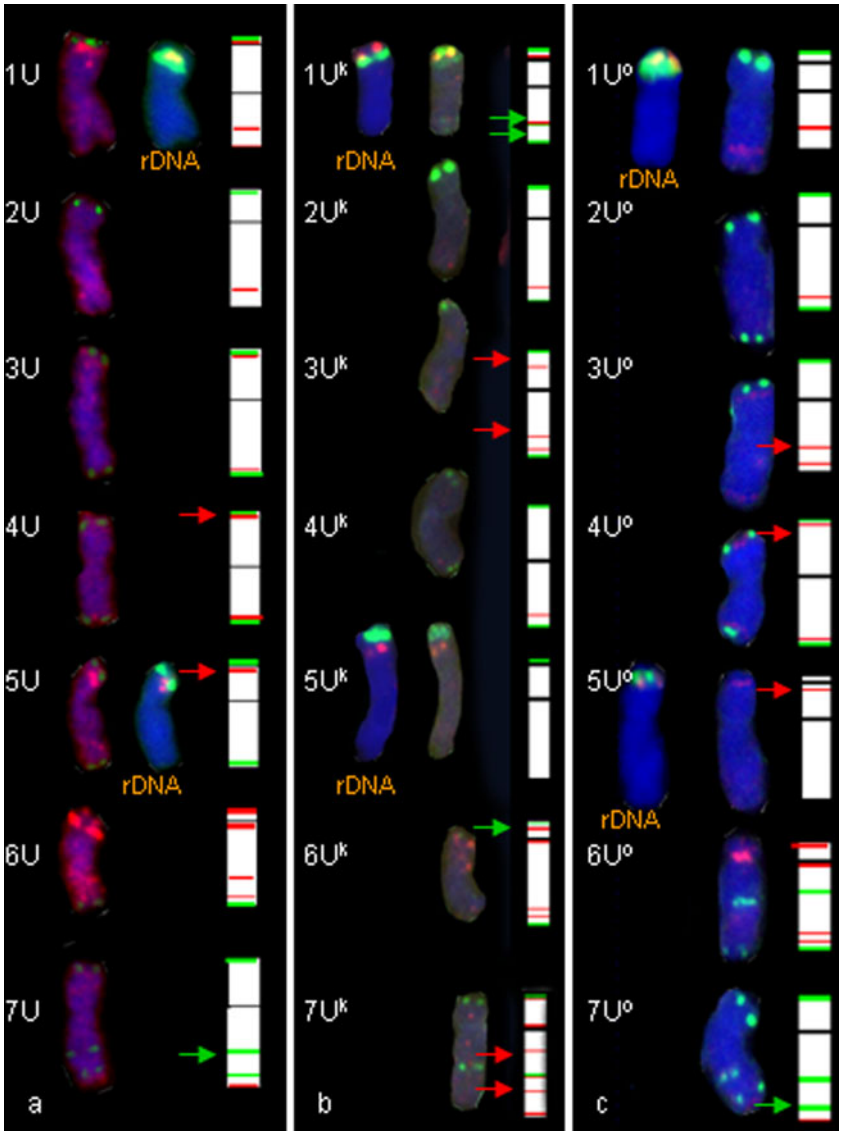

Fig. 1 Fluorescence in situ hybridization (FISH) pattern and graphical presentation showing the location of 5S (*red*) and 35S (*green*) rDNA, pSc119.2 (*green*) and pAs1 (*red*) repetitive clones on the individual somatic chromosomes of *Ae. umbellulata* (**a**), *Ae. kotschyi* (**b**) and *Ae. ovata* (**c**)

M chromosomes

The FISH analysis of *Ae. comosa* using 5S and 35S rDNA probes showed 2 pairs of chromosomes carrying these landmarks (Fig. 3a). In 1M chromosome satellites, 5S rDNA was more distant than the 35S rDNA site, whereas in 5M the location of the 35S rDNA site was telomeric (on the satellite) and the 5S rDNA signal was situated close to nucleolus organizer region (NOR). However chromosome $5M^o$ in *Ae. ovata* and the amphiploids (containing M chromosomes inherited from the above-mentioned accessions), carried only the 5S signal.

All *Ae. comosa* chromosomes with the exception of 2M and 3M were labelled by the pSc119.2 probe. The telomeric region of both arms was labelled in 4M, 5M and 6M chromosomes. The same labelling pattern appeared in *Ae. ovata* and *Ae. ovata* × *S. cereale* with the exception of $7M^o$ and the long arm of $4M^o$ chromosomes in *Ae. ovata* and its amphiploids, where no signals were observed. The hybridization of the pAs1 probe in *Ae. comosa* was present in each chromosome. The most recognizable were 2M chromosomes with two bold sites at telomeric locations of the short arm and dispersed signals in the distant region of the long arm. The pAs1 pattern of 7M chromosome was similar to the 2M one, but the signals in 7M chromosome in the long arm were located in the telomeric region. The most significant differences were in 5M and 6M chromosomes. These chromosomes in *Ae. ovata* carried more pAs1 landmarks than their analogues in *Ae. comosa*.

S chromosomes

The 5S rDNA sites were observed in the short arm of two 1S and 5S chromosomes of *Ae. sharonensis* (Fig. 4a). Two chromosomes ($5S^{sh}$ and $6S^{sh}$) carried 35S rDNA sites.

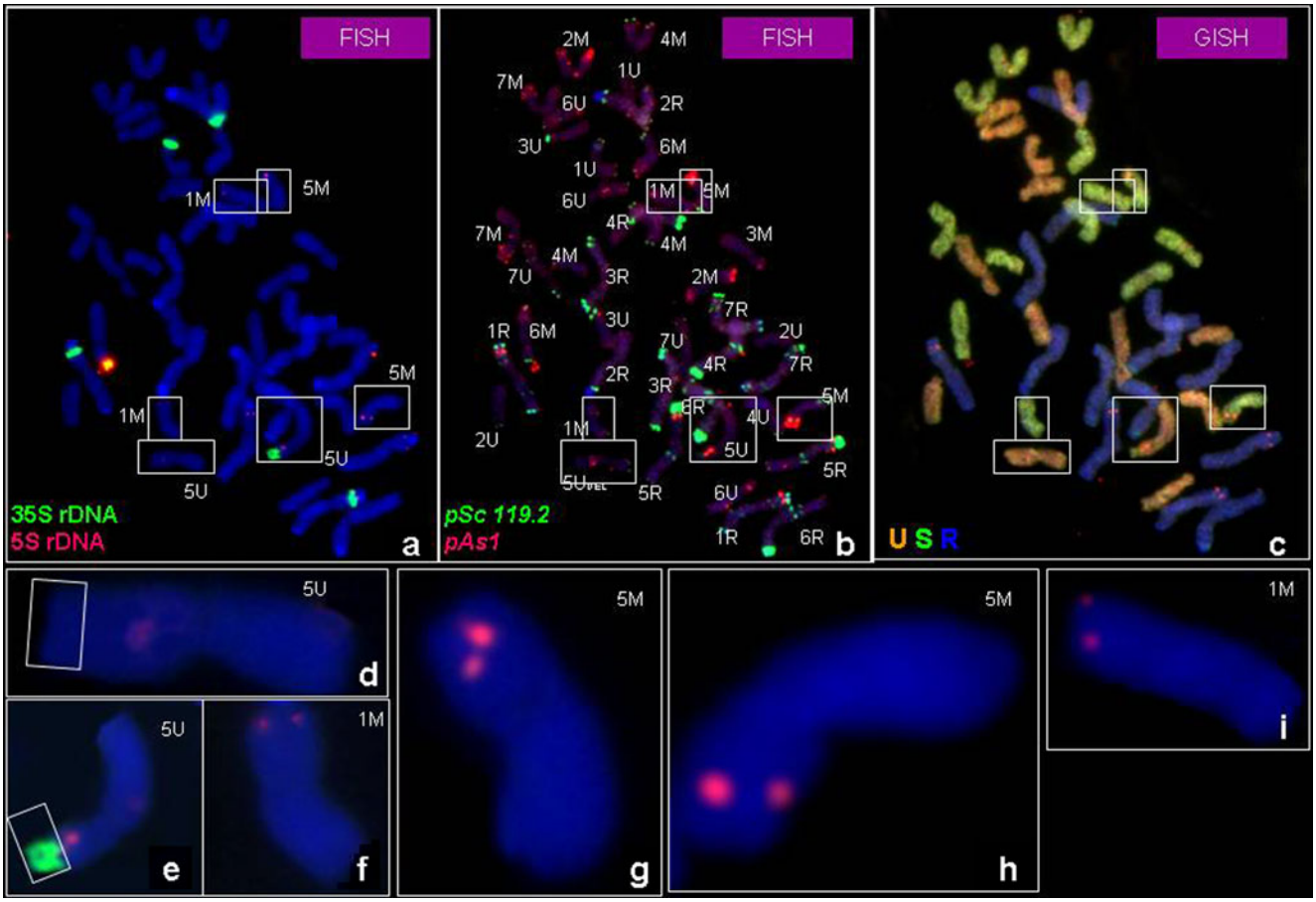

Fig. 2 **a**–**i** Mitotic metaphase cell division of *Ae. ovata* × *S. cereale* (plant 3/4) analysed using: **a** FISH pattern showing the location of 5S rDNA (*red*) and 35S rDNA (*green*), followed by **b** FISH pattern showing the location of pSc119.2 (*green*) and pAs1 (*red*) repetitive clones and **c** genomic in situ hybridization (GISH) with total genomic DNA of *Ae. umbellulata* (*red*), *Ae. comosa* (*green*) used as probes and *S. cereale* (*blue*) used as blocking DNA. **d** Chromosome 5U with 35S rDNA deletion, **e** chromosome 5U, **f**, **i** chromosome 1M, **g**, **h** chromosome 5M

However, in *Ae. kotschyi*, (Fig. 3a, b) and amphiploids, only 5S rDNA signals were observed in 1S and 5S chromosomes.

In *Ae. sharonensis*, *Ae. kotschyi* and amphiploids, chromosomes were labelled with the pSc119.2 probe at telomeric regions. The most significant site is located in the short arm of $6S^{sh}$ chromosome.

No pAs1 labelling was detected in *Ae. sharonensis* chromosomes. Very weak and dispersed but comparable signals were observed in distant regions of long arms of $2S^{k}$, $3S^{k}$, $6S^{k}$ and $7S^{k}$ in *Ae. kotschyi* and amphiploids: *Ae. kotschyi* × *S. cereale*. Significant signals were also observed in the short arm of $2S^{k}$ chromosome of *Ae. kotschyi* (Fig. 4).

D chromosomes

The 5S and 35S rDNA sites were observed in short arms of the 5D chromosome of *Ae. tauschii* (Fig. 5a). The 35S rDNA site was wide and more distal than 5S rDNA signals. On the short arm of 1D only 5S rDNA signals were found. Three chromosomes (2D, 3D and 4D) carried pSc119.2 signals in telomeric regions but only chromosomes 4D had signals in telomeric regions of both arms. The *Afa* family labelling in *Ae. tauschii* was very strong and characteristic. The most recognizable was chromosome 3D, with a large site at a distal location (including the telomere) of the short arm. Well-labelled sites were also observed in telomeric parts of both arms of chromosome 7D. Interstitial sites were found in long arms of chromosomes 2D, 4D, 5D and 6D. In chromosome 4D, significant pericentromeric signals were observed. The same labelling pattern of the pAs1 probe was observed in *Ae. tauschii* × *Secale cereale* amphiploids (Fig. 5b).

Discussion

This study focused on U, M, S and D genomes, which are fundamental in the genus *Aegilops* (Van Slageren 1994). Diploid goatgrass species were compared with their polyploid evolutionary descendants to capture differences in the

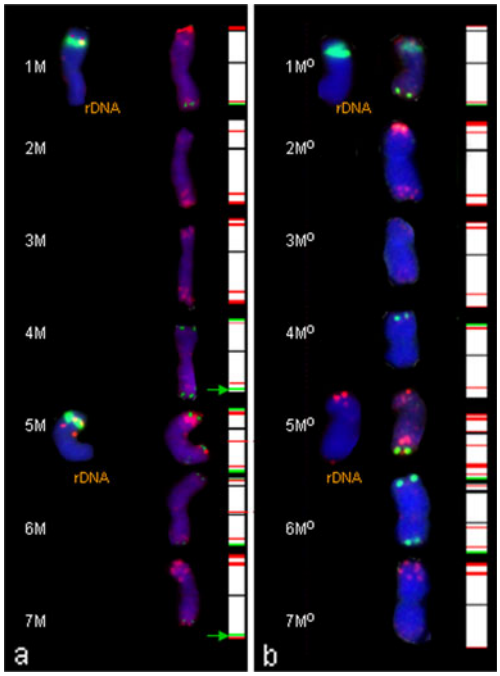

Fig. 3 Fluorescence in situ hybridization (FISH) pattern and graphical presentation showing the location of 5S (*red*) and 35S (*green*) rDNA, pSc119.2 (*green*) and pAs1 (*red*) repetitive clones on the individual somatic chromosomes of *Ae. comosa* (**a**), *Ae. ovata* (**b**)

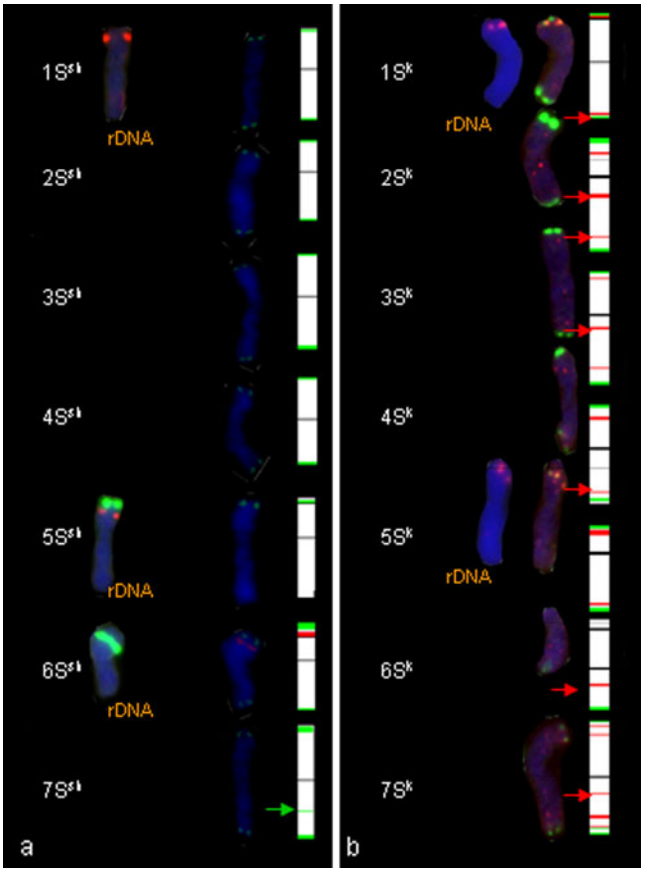

Fig. 4 Fluorescence in situ hybridization (FISH) pattern and graphical presentation showing the location of 5S (*red*) and 35S (*green*) rDNA, pSc119.2 (*green*) and pAs1 (*red*) repetitive clones on the individual somatic chromosomes of *Ae. sharonensis* (**a**), *Ae. kotschyi* (**b**)

distribution of repetitive sequences. Creation of physical maps of cytogenetic markers could be a very useful tool helping to identify *Aegilops* chromosomes in *Aegilops* × *Secale* amphiploids and *Aegilops* × triticale crosses (in the future stages of this experimental program). Mukai et al. (1993) combined pAs1 and pSc119.2 for 2-colour FISH and were able to identify 17 of the 21 wheat chromosome pairs (Pedersen and Langridge 1997). In our study, pAs1 and pSc119.2 with addition of rDNA-FISH were used to identify *Aegilops* chromosomes and to track them in polyploid descendants and *Aegilops* × *Secale* amphiploids. Taking into consideration the subgenomic structure of the majority of tetraploid goatgrasses, it can be assumed that the U genome is pivotal in the genus *Aegilops*. According to the theory of pivotal-differential evolution, several polyploid species contain the U genome, which was derived from the diploid *Ae. umbellulata* (Zohary and Feldman 1962). This genome is considered to be unmodified in the diploids, based on chromosome pairing analysis (Talbert et al. 1993). It is suggested that the pivotal genome is a buffer to provide hybrid fertility, while genetic exchange can occur between the differential genomes (Chee et al. 1995). The present study corroborates this hypothesis, taking into account the small number of differences in the distribution of repetitive sequences of the U genome in *Ae. umbellulata* in comparison with *Ae. ovata* (UUMM), *Ae. kotschyi* (UUSS) and amphiploids (UUMMRR, UUSSRR). Insignificant dissimilarities appeared in 1U, 3U, 4U 6U and 7U, but have not resulted in chromosome identification. This tendency has also been reported in a similar study in which *Ae. umbellulata* and *Ae. comosa* were compared with *Ae. biuncialis* (Schneider et al. 2005). The hybridization patterns with repetitive DNA probes showed that the M and S genomes are much more diverse. Badaeva et al. (2004) showed an elimination of NOR and 35S rDNA site in chromosome 1M and the same arrangement of chromosome 5M (with 5S rDNA and 35S rDNA sites) of *Ae. ovata* (syn. *Ae. geniculata*) in comparison with *Ae. comosa*. 35S rDNA signals was also observed in chromosome 6M in both species, however chromosome 6M of *Ae. comosa* had a major NOR exclusively (Badaeva et al. 2004). On the other hand, Molnár et al. (2011) describe 1M and 6M as satellited chromosomes with strong pTa71 (18S–5.8S–26S rDNA) signals present in *Ae. comosa*, while *Ae. ovata* had no satellited M chromosome and no pTa71 signals were detected. Regarding the quoted studies, in this research we have additionally detected an elimination of 35S rDNA in chromosome 5M of *Ae. ovata* and *Ae. ovata* × *S. cereale* (Fig. 5a, b, g, h). It is possible that rDNA loci change their position by the same process, responsible for a dispersion of heterochromatic sequences through a genome (Dubcovsky and Dvořák 1995). Furthermore, rDNA elimination in S-genome chromosomes in the synthetic polyploid *Ae.*

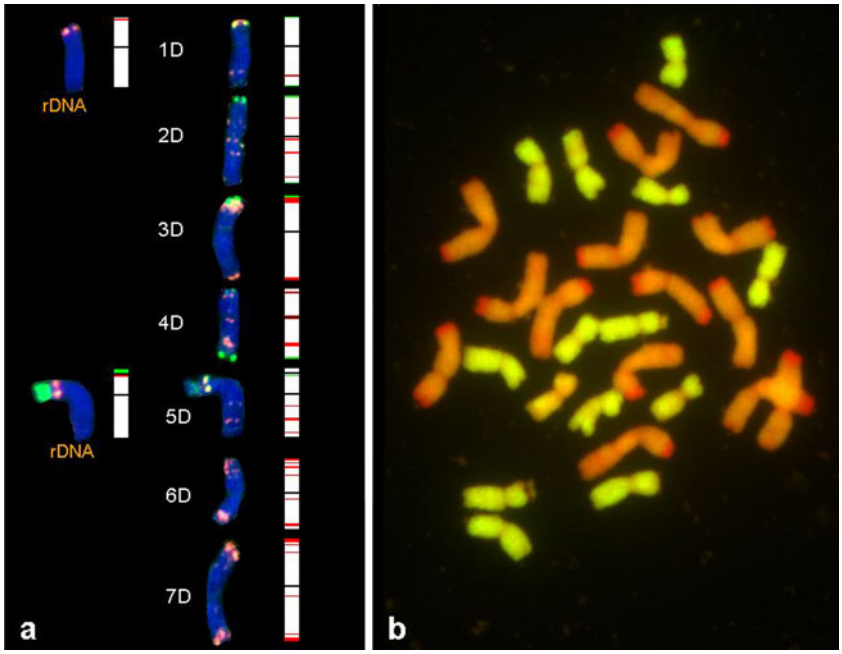

Fig. 5 **a** Fluorescence in situ hybridization (FISH) pattern and graphical presentation showing the location of 5S (*red*) and 35S (*green*) rDNA, pSc119.2 (*green*) and pAs1 (*red*) repetitive clones on the individual somatic chromosomes of *Ae. tauschii*; **b** genomic in situ hybridization (GISH) of *Ae. tauschii* × *S. cereale* analysed using total genomic DNA of *Ae. tauschii* (*green*) used as probe and *S. cereale* (*red*) used as blocking DNA

umbellulata × *Ae. comosa* appears to be similar to rDNA changes in natural allotetraploids (Shcherban et al. 2008). Such deletions of major rDNA sites and their replacements can lead to sudden fluctuations in the rDNA consensus sequence in an evolutionary lineage (Dubcovsky and Dvořák 1995). The differences in location of pSc 119.2 and pAs1 landmarks in 5M, 6M and 7M indicate a large variability in the M genome, which is comparable with molecular research made by Resta et al. (1996). An analysis reported by Kihara (1963) showed that *Ae. comosa* (MM) is less evolutionary stable than *Ae. umbellulata*, as revealed by the morphological variability and greater number of subspecies of *Ae. comosa*.

Ae. sharonensis, like *Ae. longissima*, *Ae. searsii* and *Ae. bicornis*, belong to the section *Sitopsis* of the genus *Aegilops*. This species is considered as a donor of the S-genome in *Ae. kotschyi* and *Ae. variabilis*. Cytogenetic analysis made in this study confirms the conclusions drawn by Badaeva et al. (1996a) that a similar labelling pattern of the pSc119.2 probe was present in *Ae. sharonensis* as well as in polyploid descendants. However, there were some differences in pAs1 labelling patterns between the ancestral S-genome and descendant ones, which suggests that this genome is evolutionally variable. That confirms the pivotal theory. Moreover, the lack of 35 rDNA landmarks in *Ae. kotschyi* was observed, which is novel according to the results reported by Badaeva et al. (1996a).

Another species in the genus *Aegilops* is *Ae. tauschii* (syn. *Ae. squarossa*). The distribution of repetitive sequences in the D-genome is quite conservative and similar to previous reports. However, the presence of pSc119.2 sites is variable. In previous papers, presence of small telomeric pSc119.2 sites in the short arms of 2D, 3D and 5D (inconsistent appearance) chromosomes of *Ae. squarossa* was reported by Badaeva et al. (1996a). Furthermore, the small telomeric pSc119.2 sites were also observed in both arms of 1D, 2D and 4D chromosomes (Schneider et al. 2003) and 7D chromosomes (Kwiatek, unpubl. data) of *T. aestivum*. Combining pAs1 and pSc119.2 with 5S and 35S rDNA probes is an effective cytogenetic tool for identification of complete *Aegilops* chromosomes and detection of their small rearrangements. However, some difficulties may appear during translocation analyses. The labelling landmarks were faint or localized only in the telomeric region in some chromosomes. In the case of a translocation, it might be impossible to distinguish and identify such a chromosome fragment. Taking this into consideration, it is advisable to develop other cytogenetic markers, such as repetitive sequences, synthetic oligonucleotides or bacterial artificial chromosomes (BACs) that can secure the mapping of each chromosome region. In accordance with Zhang et al. (2004), the use of genomic DNA cloned in large-insert vectors, such as BACs (Shizuya et al. 1992), in combination with FISH, called BAC-FISH, has been an effective approach for physical mapping of such specific DNA sequences and identifying individual chromosomes. On the other hand, our study shows that repetitive DNA probes could be considered as a subspecies discrimination factor, especially in variable species containing less stable genomes, such as S or M.

The distribution of cytogenetic landmarks reported in this paper may contribute to study the evolution in Triticeae and breeding research aiming at widening of genetic diversity by distant crosses.

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