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Cytomolecular discrimination of the A^m chromosomes of *Triticum monococcum* and the A chromosomes of *Triticum aestivum* using microsatellite DNA repeats

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Abstract

The cytomelecular discrimination of the A^m- and A-genome chromosomes facilitates the selection of wheat-*Triticum monococcum* introgression lines. Fluorescence *in situ* hybridization (FISH) with the commonly used DNA probes Afa family, 18S rDNA and pSc119.2 showed that the more complex hybridization pattern obtained in *T. monococcum* relative to bread wheat made it possible to differentiate the A^m and A chromosomes within homoeologous groups 1, 4 and 5.

In order to provide additional chromosomal landmarks to discriminate the A^m and A chromosomes, the microsatellite repeats $(GAA)_n$, $(CAG)_n$, $(CAC)_n$, $(AAC)_n$, $(AGG)_n$ and $(ACT)_n$ were tested as FISH probes. These showed that *T. monococcum* chromosomes have fewer, generally weaker SSR signals than the A-genome chromosomes of hexaploid wheat. A differential hybridization pattern was observed on $6A^m$ and 6A chromosomes with all the SSR probes tested except for the $(ACT)_n$ probe. The $2A^m$ and 2A chromosomes were differentiated by the signals given by the $(GAA)_n$, $(CAG)_n$ and $(AAC)_n$ repeats, while only $(GAA)_n$ discriminated the chromosomes $3A^m$ and 3A. Chromosomes $7A^m$ and 7A could be differentiated by the lack of $(GAA)_n$ and $(AGG)_n$ signals on 7A. As potential landmarks for identifying the A^m chromosomes, SSR repeats will facilitate the introgression of *T. monococcum* chromatin into wheat.

Key words: *Triticum monococcum*, microsatellite repeats, karyotypic analysis, FISH

Introduction

Triticum monococcum (2n=2x=14, A^mA^m), (known as einkorn), is one of the most valuable sources of resistance to biotic and abiotic stresses in wheat (Jing et al. 2007).

An efficient crossing programme aim to transfer genes from einkorn into wheat depends on the ability to identify the introgressed A^m chromatin in the wheat background. Fluorescence *in situ* hybridization (FISH) with repetitive DNA probes results in chromosome-specific hybridization patterns, making this technique an excellent tool for the karyotypic analysis of cereals (Mukai et al. 1993; Pedersen and Langridge 1997). One of the most popular combinations of probes for the cytomolecular analysis of wheat consists of the satellite repeats pSc119.2, Afa family and pTa71 (Rey et al. 2015). Unfortunately, these probes only produced a small number of weak diagnostic signals on the A genome chromosomes of wheat. Simple sequence repeats (SSRs), or microsatellites are wide-spread in the genomes of the *Triticum* / *Aegilops* taxa (Cuadrado et

al., 2008). As SSR repeats are able to form large clusters in the genome, they can be used as FISH probes for chromosome identification in wheat and its related species (Cuadrado et al. 2000; 2008).

The FISH karyotype of *T. monococcum* was elaborated by Megyeri et al. (2012) using the probes Afa family and pTa71. More recently, several FISH probes, including two SSR probes (GAA and GTT), were tested on diploid *Triticum* species by Badaeva et al. (2015). However, a comprehensive comparison of the FISH karyotype of diploid *T. monococcum* and hexaploid wheat has not yet been published, a fact that significantly limits the identification of A^m-genome chromosomes in the wheat genetic background. A comparison of the hybridization pattern of diploid and hexaploid wheat would facilitate the transfer of A^m chromosomes and the exploitation of wild genetic diversity in wheat breeding programmes.

The aim of the present work was to investigate the ability of six trinucleotide SSR motifs to discriminate A genomes from *T. monococcum* and *T. aestivum* when used as FISH probes in sequential FISH, together with commonly used repetitive DNA probes.

Results and discussion

The microsatellite repeats $(GAA)_n$, $(CAG)_n$, $(AAC)_n$, $(AGG)_n$ and $(ACT)_n$ were tested as probes in the two-step FISH experiments, where the SSR sequences were first hybridized to the slides. After documentation of the SSR hybridization patterns, the slides were rehybridized using a mixture of repetitive DNA probes 18S rDNA, pSc119.2 and Afa family. The SSR hybridization patterns were assigned to the A^m chromosomes by comparing the results of two FISH experiments (Fig. 1a-b). FISH was also carried out on the chromosomes of hexaploid wheat (*T. aestivum* 'Mv9kr1') in order to compare the hybridization patterns on A^m and A chromosomes within the same homoeologous groups. To visualise differences between the A^m and A chromosomes idiograms were constructed based on the hybridization patterns obtained with different probes (Fig. 1c-i).

Distribution of repetitive DNA probes on the A genome chromosomes

The cytomolecular analysis of *T. monococcum* and *T. aestivum* showed that the A-genome chromosomes have more complex hybridization patterns in the diploid *Triticum* species than in hexaploid wheat. The 18S rDNA probe gave a strong fluorescent signal on the telomeric region of $1A^mS$ in *T. monococcum*, while this signal was missing in hexaploid wheat. A similar phenomenon was observed for the chromosomes of group 5. This could be related to an evolutionary change, where the activity of the NOR regions in the A and D genomes was

suppressed by the activity of the NOR region of the B genome, causing the elimination of the ribosomal genes on chromosomes 1A and 5A of hexaploid wheat (Gerlach et al. 1980; Miller et al. 1983).

The A^m chromosomes, especially those in groups 4 and 7, have more complex Afa family hybridization patterns than those of hexaploid wheat. Han et al. (2005) also observed that the pGc1R-1 repetitive sequence is present in the B and G genome donors of the *Triticum* species but is absent in polyploid wheats. All these results support the idea that allopolyploidization was accompanied by the rapid, extensive elimination of parent-specific repetitive DNA sequences, which presumably played an important role in the initial stabilization of the nascent amphiploid plants through a cytological diploidization process.

Badaeva et al. (2015) obtained the same results with the 5S and 18S rDNA probes, but reported quite different results for the pAs1 probe, which belongs to the Afa family (strong pAs1 signals only on $4A^m$ and $7A^m$ and weak signals on $6A^m$), which can be attributed to divergence in the sequences of the pAs1 and Afa family probes (Badaeva et al. 2015). On the other hand, the pSc119.2 signals present on chromosomes 4A and 5A of bread wheat were missing from the A chromosomes of diploid species, which could be attributed to the evolutionary chromosome rearrangements occurring between 4A, 5A and 7B in polyploid wheat species (Devos et al. 1995).

The results showed that the A^m and A chromosomes can be clearly differentiated within homoeologous groups 1, 4 and 5. However, in the case of chromosome groups 3 and 7, only differences in the intensity of the Afa family signals could be detected. These tend to depend on the quality of the hybridization, but still make it possible to differentiate the A^m and A chromosomes. In the case of chromosome groups 2 and 6, discrimination of A^m and A chromosomes failed to give acceptable results.

Distribution of microsatellite repeats on the A genome chromosomes

Five of the six SSR probes tested, $(AAC)_n$, $(GAA)_n$, $(AGG)_n$, $(CAC)_n$ and $(CAG)_n$, gave signals on the chromosomes of *T. monococcum* (Fig. 1d-i), while signals were only observed on *T. aestivum* with the probe $(ACT)_n$. The results showed that *T. monococcum* has fewer, generally weaker SSR signals than the A-genome chromosomes of hexaploid wheat (Fig. h). One exception was chromosome $6A^m$, where $(GAA)_n$, $(CAG)_n$, $(CAC)_n$, $(AAC)_n$ and $(AGG)_n$ resulted diagnostic signals, allowing the discrimination of $6A^m$ and 6A.

Adonina et al. (2015) reported that changes in the distribution of $(GAA)_n$ sequence on the A-genome chromosomes of diploid and polyploid wheats were associated with chromosomal

rearrangements / modifications that took place during evolution. The $(GAA)_n$ microsatellite probe gave strong pericentromeric and intercalary signals on all the B chromosomes of hexaploid (Cuadrado et al. 2000) and tetraploid (Kubaláková et al. 2005) wheats. The higher frequency of $(GAA)_n$ signals on the A chromosomes of hexaploid wheat compared to those of the diploid *T. monococcum* might be due to intergenomic chromosome rearrangements between the A and B genomes. The expansion of the SSR sequences in hexaploid wheat could be the results of several mutation mechanisms, such as slippage during DNA replication, which could generate new alleles for short SSR regions (Levnison and Gutman 1987; Hancock 1996), and gene conversion and transposition, which may produce longer SSR clusters (Dover 1993; McMurray 1995

More recently, Badaeva et al. (2015) tested a large set of 10 DNA probes, including $(GAA)_n$ and $(GTT)_n$ microsatellite probes, in order to identify the A chromosomes of diploid *Triticum* species. They found that pTa535, $(GAA)_n$, $(GTT)_n$, pAs1(≈Afa family), pTa71 and pTa794 (rDNAs) and Aesp_SAT86 are the most informative for the analysis of the A genomes of diploid and polypoid *Triticum* species. The present study confirmed these results and complemented them by the use of the additional SSR probes $(CAG)_n$, $(CAC)_n$, $(AGG)_n$, $(ACT)_n$, leading to the better discrimination of the A and A^m chromosomes.

In conclusion, fluorescence *in situ* hybridization using the repetitive DNA probes Afa family and 18S rDNA is a perfect tool to identify chromosomes $1A^m$, $4A^m$ and $5A^m$ of *T. monococcum* and to discriminate them from the homoeologous A chromosomes in a wheat genetic background. When used as FISH probes SSR repeats can be considered as potential landmarks to identify the remaining A^m chromosomes during the introgression process.

Figure captions

Fig. 1 Cytomolecular comparison of A-genome chromosomes in *T. monococcum* ('MVGB1306') and bread wheat ('Mv9kr1').

a-b. Sequential FISH (Molnár et al. 2011) on the same cell: a. with microsatellite probe $(AAC)_n$ (red), b. re-probing and identification of chromosomes using the probes Afa family (red) and 18S rDNA (yellow). c. Idiogram of the A^m genome of *T. monococcum* and the A genome of *T. aestivum* showing the genomic distribution of repetitive DNA probes (Afa family, 18S rDNA, pSc119.2). Chromosomal distribution of the SSR clusters in the A^m – and A genomes of *T. monococcum* and *T. aestivum*, respectively: d. $(AAC)_n$ (red), e. $(ACT)_n$ (red), f. $(AGG)_n$ (red), g. $(AGG)_n$ (red), h. $(GAA)_n$ (green), i. $(CAC)_n$ (green). The chromosomes were counterstained by DAPI.

Table 1. Primers and labelling of the SSR probes (Kubaláková et al. 2005) tested

Probes	Primers	Labeling	
		digoxigenin-11-dUTP	biotin-11- dUTP
$(AAC)_n$	5' $(AAC)_7$ 3', 5' $(TTG)_7$ 3'	+	
$(ACT)_n$	5' $(ACT)_7$ 3', 5' $(TGA)_7$ 3'	+	
$(AGG)_n$	5' $(AGG)_7$ 3', 5' $(TCC)_7$ 3'	+	
$(CAG)_n$	5' $(CAG)_7$ 3', 5' $(GTC)_7$ 3'	+	
$(GAA)_n$	5' $(GAA)_7$ 3', 5' $(CTT)_7$ 3'		+
$(CAC)_n$	5' $(CAC)_7$ 3', 5' $(GTG)_7$ 3'		+

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