RESEARCH NOTE

Polymorphism of the simple sequence repeat $(AAC)_5$ in the nucleolar chromosomes of Old Portuguese wheat cultivars

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Introduction

Simple sequence repeats (SSRs) or microsatellites have been widely used as cytogenetic markers in cereals. SSRs could be present in coding and noncoding regions, contributing to genome dynamics and evolution. Previous studies by our research group detected molecular and cytogenetic ribosomal DNA (rDNA) polymorphisms in Old Portuguese bread and durum wheat cultivars. Considering the rRNA genes expression, a maximum number of four Ag-NORs were consistently observed in all durum wheat cultivars while bread wheat cultivars showed a maximum of four or six Ag-NORs per metaphase cell (Carvalho *et al.* 2010, 2011a). In addition, the PCR-RFLP technique revealed rDNA polymorphism in the intergenic spacer (IGS) and the internal transcribed spacer (ITS) regions of these cultivars (Carvalho *et al.* 2009, 2011a,b). In the present work, we characterized cytogenetically the nucleolar chromosomes (1B, 6B, 1A and/or 5D) of 10 Old Portuguese wheat cultivars (five of durum wheat and five of bread wheat) with the fluorescence *in situ* hybridization (FISH) technique performed with 10 SSR probes. Only the SSR $(AAC)_5$ presented clear hybridization patterns in all cultivars. Its hybridization pattern was similar for all durum wheat cultivars but polymorphic among the bread wheat, probably due to spontaneous chromosomal rearrangements. Considering the putative occurrence of insertion events developed by retrotransposons in the proximity of the SSR $(AAC)_5$, based on the present results, this cytogenetic marker could be related to the on/off alternative states of the rRNA genes in bread wheat (Carvalho *et al.* 2010), and it could explain the consistent number of four Ag-NORs (located on the chromosomes 1B and 6B) among durum wheat cultivars (Carvalho *et al.* 2011a).

SSRs constitute a highly repetitive class of DNA widely dispersed in the eukaryote genomes, particularly in animals, and show high levels of polymorphism (Wang *et al.* 1994; Chun-Li *et al.* 2002). Due to their abundance, efficiency and ability for detecting variation among different taxa (Schmidt *et al.* 1993; Depeiges *et al.* 1995), SSRs have been successfully used for evolutionary studies. Members of a particular subclass of SSRs, flanked by DNA sequences, that are present only once in the genome, could provide valuable genetic markers (Weber and May 1989; Powell *et al.* 1996), being described as 'microsatellite markers' (Cuadrado and Schwarzacher 1998). According to these authors, the distinctive hybridization patterns of various SSR motifs showed specific characteristics to both chromosomes and genomes, and could be helpful for understanding the evolution of the genome and for chromosome identification.

FISH experiments performed with SSR probes have been used to study the distribution of these tandem repetitive sequences in different plant species, including wheat (Cuadrado and Schwarzacher 1998; Bardsley *et al.* 1999; Castilho *et al.* 2000; Cuadrado *et al.* 2000).

Materials and methods

The wheat cultivars studied here were kindly given by the National Plant Breeding Station (Elvas, Portugal) and consisted of five bread wheat (*Triticum aestivum* L. em Thell.; $2n=6x=42$; AABBDD) cultivars—'Alentejo', 'Almadense', 'Grécia', 'Tremês Branco' and 'Restauração', and five durum wheat (*Triticum turgidum* L.; $2n=4x=28$; AABB) cultivars—'Alentejo', 'Asa de Corvo', 'Bagudo', 'Pombinho' and 'Rubião de Barba Preta'.

We allowed the germination of seeds from each cultivar and collected root-tips with about 1.5 cm, for further treatment in ice cold water for 24 h, and fixation in ethanol–acetic acid

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(3:1) solution. Ten SSR probes [$(AC)_8$; $(AG)_{12}$; $(AAG)_5$; $(AAC)_5$; $(CAC)_5$; $(CAT)_5$; $(GGC)_5$; $(GATA)_4$; $(GGAT)_4$ and $(GACA)_4$] were labelled with digoxigenin-11-dUTP (Roche Applied Science, Mannheim, Germany) using the Random Primed DNA Labelling Kit (Roche Applied Science), according to the manufacturer's instructions. The 45S rDNA sequence pTa71 (Gerlach and Bedbrook 1979) was labelled with biotin-16-dUTP (Roche Applied Science) by nick translation. For FISH experiments, we followed the Cuadrado and Schwarzacher (1998) procedure with minor modifications, including 1% SDS and 100 ng of each probe in the hybridization mixture. Hybridization signals were observed on an epifluorescence microscope AxioPlan 2 (Zeiss, Göttingen, Germany), and the images were captured by a digital camera (AxioCam, Zeiss) using the AxionVision 3.2 software (Axionvision, Zeiss, Göttingen, Germany). After triple exposure with appropriate filters, images were prepared for printing with the ADOBE PHOTOSHOP 6.0 software (http://www.adobe.com).

The nucleolar chromosomes were identified based on the pTa71 hybridization patterns, previously described by Mukai *et al.* (1991).

Results and discussion

Among the 10 SSR probes tested, only the $(AAC)_5$ probe showed clear and intense hybridization patterns in some chromosomes, including the nucleolar ones, of bread (figure 1a) and durum wheat cultivars (figure 1b).

Cuadrado and Schwarzacher (1998) reported a weak dispersed pattern and diffuse pericentromeric sites for the $(AAC)_5$ probe in the three wheat genomes. Later, Cuadrado *et al.* (2000) elaborated an ideogram for the SSRs $(AAC)_n$ and $(AAG)_n$ for all wheat chromosomes from the cultivar 'Chinese Spring'.

The hybridization patterns schematized in figure 2 were based on the analysis of five metaphases per plant, for three plants per cultivar. No polymorphic distribution of the SSR $(AAC)_5$ was found among plants of the same cultivar.

In the present study, we observed clear and intense patterns distributed through the centromeric, pericentromeric and telomeric regions of both bread and durum wheat chromosomes (figure 1). These hybridization patterns were partially similar to those proposed by the $(AAC)_5$ ideogram described by Cuadrado *et al.* (2000), in respect to the chromosomes 1B and/or 6B of the bread wheat cultivars ('Alentejano', 'Almadense' and 'Grécia') (figure 2). Other similarity was constituted by the absence of $(AAC)_5$ hybridization in the chromosome 5D of all bread wheat cultivars (figure 2). The rDNA *locus* of the chromosome 1A of all bread wheat cultivars was not detected by pTa71 (figure 1). In contrast, this probe detected chromosome 1A of all durum wheat cultivars, that was characterized by an intense $(AAC)_5$ hybridization in the centromeric region and a weakly dispersed hybridization in its long arm (figure 2). No differences was found in the distribution of the SSR $(AAC)_5$ among the nucleolar chromosomes of the durum wheat cultivars. Thus, the $(AAC)_5$ hybridization patterns of the cultivar 'Asa de Corvo' (figure 2) represented those observed in the remaining durum wheat cultivars.

The $(AAC)_5$ hybridization patterns achieved here reflected the high genetic variability of the Old Portuguese wheat cultivars evaluated here as well as a polymorphic physical distribution of this SSR relatively to the bread wheat 'Chinese Spring' previously studied by other authors (Cuadrado and Schwarzacher 1998; Bardsley *et al.* 1999; Cuadrado *et al.* 2000).

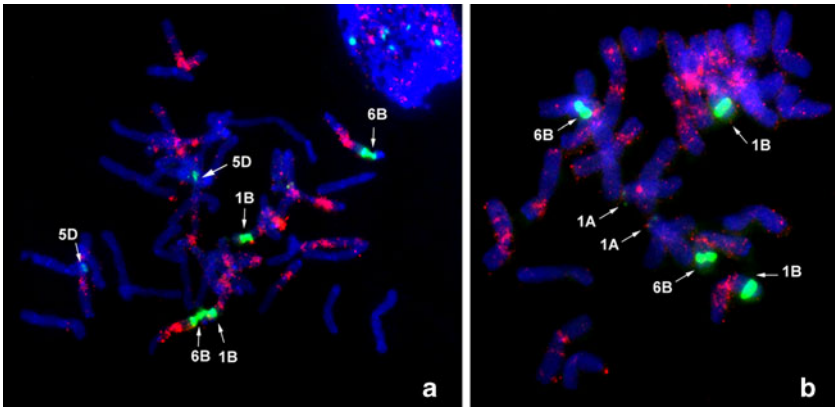

Figure 1. Mitotic metaphase cells from: (a) bread wheat cultivar 'Restauração' (AABBDD; $2n=6x=42$) and (b) durum wheat cultivar 'Asa de Corvo' (AABB; $2n=4x=28$), showing the simultaneous hybridization of the probes SSR $(AAC)_5$ (red) and pTa71 (green). The chromosomes were counterstained with DAPI (blue). The nucleolar chromosomes are identified (arrows).

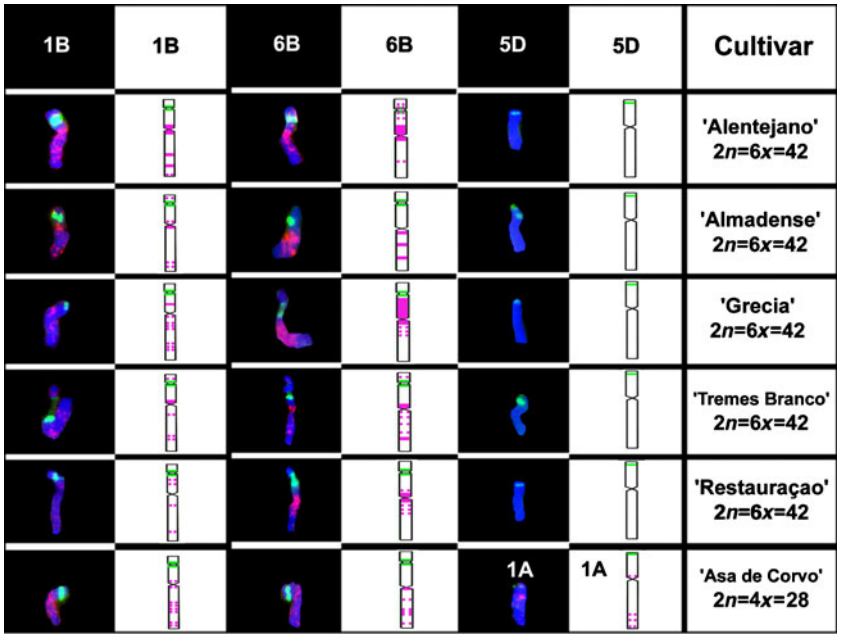

Figure 2. Schematic representation of the nucleolar chromosomes of the Old Portuguese bread and durum wheat cultivars hybridized with the probes SSR $(AAC)_5$ (red) and pTa71 (green), and respective ideogram.

The cultivars studied here were collected in the 1930's from farmers in north to south of Portugal. Based on our recent studies, this germplasm contains huge genetic diversity, particularly, at the rDNA level. Regarding the rRNA genes expression, no polymorphic number of NORs was detected among durum wheat cultivars (Carvalho *et al.* 2011a). In the present study, the physical distribution of the SSR $(AAC)_5$ was also similar among the durum wheat cultivars. Instead, different $(AAC)_5$ hybridization patterns were detected among the bread wheat cultivars. This feature could arise from spontaneous chromosome rearrangements or insertion events derived from retrotransposition, both contributing for genome evolution and increasing genetic variability. Besides, our previous studies revealed polymorphic number of NORs, different NOR methylation levels and rDNA polymorphism among the bread wheat cultivars (Carvalho *et al.* 2009, 2011a, b). Considering, the putative occurrence of insertion events developed by retrotransposons in the proximity of the SSR $(AAC)_5$, based on the present results, this cytogenetic marker could be related to the on/off alternative states of the rRNA genes in bread wheat, and it could explain the consistent number of four Ag-NORs (located on the chromosomes 1B and 6B) among durum wheat cultivars. Our results are supported by previous evidence showing that SSRs could be distributed through coding and noncoding regions of the genome; could have evolutionary significance and dynamics in their genomic distribution; could influence gene expression and genetic disorder, chromatin organization, cell cycle and DNA metabolic processes, and they might be associated with repair DNA mechanisms, recombination and mutation (reviewed by Chun-Li *et al.* 2002).

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