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Short Communication

Distribution of rDNA loci and genome differentiation in tetraploid *Cynodon*

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Abstract

The 45S rDNA and 5S rDNA loci encodes for ribosomal RNA. Mapping of rDNA loci has not been reported in *Cynodon*.We used FISH technique to locate the loci coding for rDNA. Our results showed that the centromeres of three chromosomes had signals of different intensities in the tetraploid bermudagrass line C121. The tetraploid line, C299, had four signals at the centromeres of four chromosomes, with two being strong and two relatively weaker. We found three signals from C121 and four from line C299 about the 5S rDNA sequence. The rDNA signal numbers and sizes indicated existence of genome differentiation in bermudagrass. Combined results of staining pollen mother cells at meiosis indicated that tetraploid bermudagrass has at least two genome types.

Keywords: *Cynodon*, tetraploids, rDNA, FISH, genome differentiation

Bermudagrass (*Cynodon* spp.) is one of the most globally important warm season turfgrasses, and is also widely used as an excellent forage grass [1]. Its basic chromosome number is 9 (x=9). Tetraploidy is common in the bermudagrasses. Cytological identification of tetraploid hybrids in *Cynodon* showed that genome differentiation did not exist between the parental lines [2]. However, there are a few studies that reported genomic differentiation in tetraploid parental lines.

In plants, ribosomal DNAs (45S rDNA and 5S rDNA), which exist as repeated sequences, are valuable cytological chromosome landmarks. The distribution and numbers of rDNA repeats in the genome vary in different plant species. Locating rDNA loci on chromosomes using FISH (fluorescent *in situ* hybridization) is a useful means to study chromosome identification and genomic differentiation [3].The 45S rDNA repeats have been studied in many species [3-6]. The distribution of 45S rDNA and 5S rDNA repeats in the genome of polyploid bermudagrass has not yet been reported. In the present study, we examined the distribution of 45S rDNA and 5S rDNA repeats on metaphase chromosomes of tetraploid *Cynodon* using the FISH technique. It may be possible to use rDNA FISH to detect genome differentiation in *Cynodon* spp.

Plant materials

Two tetraploid bermudagrass lines, C121 and C299, were planted in a test field at Yangzhou University (Yangzhou, Jiangsu Province, China), and used.

Methods

Roots of the two tetraploids were harvested from field-grown plants. The roots were pretreated in 0.002 M 8-hydroxyquinoline at 20°C for 2 h to accumulate prometaphase cells, fixed in methanol-acetic acid (3:1), and stored at –20°C until use. Root tips were digested with 2% cellulase and 1.0% pectinase at 37°C for 1.5 h. Squashes were made in the fixative on a glass slide and flame-dried according the protocol of Kurata *et al*. [7]. Young panicles of the original line were harvested and fixed in a 3:1 solution of 100% ethanol-

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glacial acetic acid (Carrnoy's solution). Pollen mother cells (PMC) at the pachytene stage were crushed in an acetocarmine stain solution according to Wu [8]. The FISH procedure applied to mitotic chromosomes was as described by Gong *et al*. [9].

Distribution of 45S rDNA and 5S rDNA loci on the chromosomes of tetraploid Cynodon

There were different numbers of 45S rDNA hybridization signals in the two tetraploid lines of *Cynodon*. In C121, there were three yellow-green hybridization signals located at the centromeric regions (Fig. 1A), with different signal intensities. In the other tetraploid line C299, the 45S rDNA probe gave four hybridization signals located at the centromeric regions, two strong and two weak (Fig. 1B), and the extent of the hybridization signals could be easily visualized when the chromosomes were extended (Fig. 1C). Among the four hybridization signals, a strong and a weak signal were observed in the extended chromosomes, and the linear signal was discontinuous. By comparing the distributions of 45S rDNA in the two tetraploid genomes, differences in the two species were observed.

Another rDNA, 5S rDNA in tetraploid *Cynodon* C121 gave three hybridization signals (Fig.1D), with large differences in intensities, strong and very weak. In line C299, four hybridization signals were observed (Fig. 1E), two strong and two weak.

Analysis of meiosis in tetraploid Cynodon

In order to confirm the existence of genome differentiation in polyploid Cynodon, we performed cytological examination of pollen mother cells in the tetraploid Cynodon line C299 at meiosis. During pachytene, *Cynodon* homologous chromosomes appeared in pairs (bivalents) (Fig. 2A). Bivalents were condensed and formed 18 bivalents at diakinesis (Fig. 2B). Based on absence of multivalents and the distribution of the two rDNA loci, it was proposed that C299 is an allotetraploid.

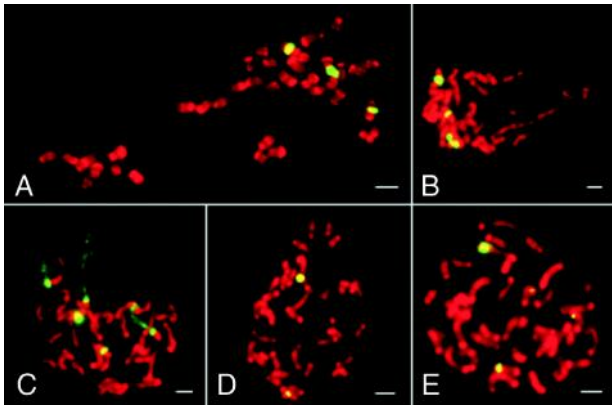

Fig. 1. FISH with rDNA probes on metaphase chromosomes of tetraploid *Cynodon* in mitosis (all scale bars = 5 µm). A. Probe: 45S rDNA, line C121; B & C. Probe: 45S rDNA, line C299; D. Probe: 5S rDNA, line C121; E. Probe: 5S rDNA, line C299

Chromosomal locations of the 45S rDNA loci have been reported in many species. Lima [10] reported that 45S rDNA loci were on the short arms of chromosomes in 87% of the species examined. In some species, 45S rDNA is located not only at the chromosome ends, but also at the centromeric regions [11]. In this study, we found that all 45S rDNA loci were located in the centromeric regions of tetraploid *Cynodon* chromosomes, and no hybridization signals were observed at the ends of the short arms. Thus, the positions and sizes of the 45S rDNA loci differ in *Cynodon* from other plant species examined.

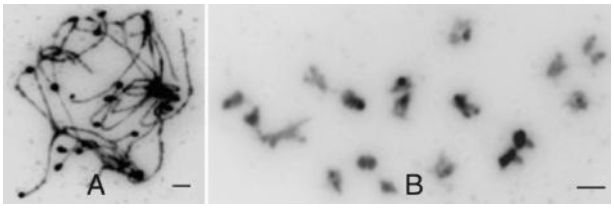

Fig. 2. Meiotic chromosomes of tetraploid C299 (scale bars = 5 µm). A. pachytene; B. diakinesis

As an effective chromosome marker, rDNA loci can provide clues for the study of genome evolution at the molecular and chromosomal levels. In this study, the location of rDNA loci indicates the possible existence of genome differentiation in *Cynodon*. Combined with our data from PMCs at meiosis, *Cynodon* may represent a clear case of the existence of at least two genome differentiations. Whether other genome differentiations are present within the genome of bermudagrasses could be the subject of further study.

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