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Physical and genetical mapping of rDNA sites in *Pennisetum* (pearl millet)

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Two pairs of rDNA sites, with significantly different signal strength, were detected on two pairs of chromosomes of *Pennisetum glaucum* and *P. violaceum*. Both sites were physically located at the distal ends of the short arms of these two chromosome pairs, with the major pair being located on the two shortest chromosomes. Restriction fragment length polymorphism (RFLP) analysis enabled one of the two pairs of rDNA sites, designated *Nor-P1*, to be mapped on the fifth linkage group in *Pennisetum*.

Keywords: genetic mapping, *in situ* hybridization, *Pennisetum*, physical mapping, rDNA.

Introduction

High-resolution fluorescence *in situ* hybridization has been widely used for the physical mapping of DNA sequences. Although only a limited number of experiments have reported the detection of single- and low-copy DNA sequences in plant species, this technique has been used successfully to detect alien chromosomes and chromosome segments and to locate repetitive sequences (see Jiang & Gill, 1994 for a summary). Such studies have not only contributed to our understanding of plant genome organization, but also provided a powerful means for monitoring and characterizing alien chromosome segments introduced from their wild relatives (e.g. King *et al.*, 1993).

In this paper, we report our mapping results of rDNA sites in *Pennisetum*, which is part of our effort to link the RFLP-based genetic map of pearl millet (Liu *et al.*, 1994) with its seven pairs of chromosomes. To date, only one rDNA site located on the short arms of the shortest chromosome pair has been observed in pearl millet (Pantulu & Krishna-Rao, 1982). In this work, we confirm this observation and also report a second pair of rDNA sites located on a different pair of chromosomes.

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Materials and methods

pTa71, which was isolated from wheat and contains the coding sequences for the 18S, 5.8S and 26S ribosomal genes and spacer sequences (Gerlach & Bedbrook, 1979), was used as a probe for both *in situ* hybridization and RFLP analysis. The *in situ* hybridization was carried out as described by Reader *et al.* (1994) using two genotypes: *P. glaucum* accession Tift 23DB and the F_1 from the cross of *P. violaceum* accession IPW2 × *P. glaucum* accession IP6271. The RFLP analysis was carried out using the method described by Liu *et al.* (1994) with the following six restriction enzymes: *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Bam*HI and *Taq*I. Eight individuals from each of the following three segregating populations, *P. glaucum* accession LGD-1-B-10 × *P. glaucum* accession ICMP 85410, Tift 23DB × (IPW2 × IP6271)F_1, and Tift 23DB × (*P. glaucum* accession IP12070 × *P. mollissimum* accession IPW250)F_1, were screened for polymorphism, and 107 three-way F_1 plants from Tift 23DB × (IPW2 × IP6271)F_1 were used for the mapping experiment. The program MAPMAKER (version 3) (supplied by E. S. Lander, Whitehead Institute for Biomedical Research, Cambridge, MA, U.S.A.) was used for linkage analysis.

Results and discussion

As shown in Fig. 1, two pairs of rDNA sites were consistently observed in *P. glaucum* and *P. violaceum*

when the F_1 seeds of IPW2 × IP6271 were analysed using the *in situ* hybridization technique. The intensity of the signal, located at the distal ends of the chromosomes, of one of these pairs was much stronger than that of the other. The intensity and physical position of the signal on each of the chromosomes carrying either the major or minor rDNA sites appeared to be the same even though each of the chromosomes making up the two homologous pairs in question was derived from two different subspecies. This lends further support to the supposition that the rDNA sites may be consistent across different genotypes in pearl millet, despite the considerable karyotypic variation in the species (Chandola & Jain, 1970). This hypothesis is also supported by the observation that Tift 23DB also carries two pairs of rDNA sites of the same intensities and physical positions as observed in *P. glaucum* and *P. violaceum* (not shown).

Although the chromosomes of pearl millet differ in length and arm ratios, it is very difficult to identify consistently and reliably individual chromosomes using conventional light microscopy (Pantulu & Krishna-Rao, 1982). However, in the work reported here we have shown that both pairs of rDNA sites are located at the distal ends of the short arms of two pairs of chromosomes. The observation that the major rDNA site is located on the short arm of the smallest chromosome is in accordance with the previous observation of Pantulu & Krishna-Rao (1982). The second pair of rDNA sites, which has not previously been reported, appears to be located on the second shortest pair of chromosomes, which have a slightly larger short-to-long arm ratio (Fig. 1).

The genome of pearl millet is highly polymorphic, as revealed by RFLP analysis (Liu *et al.*, 1994). However, this seems not to be the case for the rDNA sites. Of all the genotype–enzyme combinations used in this study, only one revealed polymorphism. This was revealed among the *Eco*RI digests of the progeny from the cross of Tift 23DB × (IPW2 × IP6271)F_1. Some six bands were detected and only one segregated. Thus, it was not possible to ascertain whether this was derived from the major or minor pair of rDNA sites, although the former is the more likely candidate because of its much higher copy numbers. Segregation of this single rDNA fragment in the progeny of Tift 23DB × (IPW2 × IP6271)F_1 was tested against segregation of the 50 RFLP loci mapped in this population (Liu *et al.*, 1996). The analysis showed that this rDNA locus, designated *Nor-P1*, was distally located on linkage group 5 (Fig. 2). Only one locus, *Xpsm215.1*, was distally located to *Xpsm815* in the millet map (Liu *et al.*, 1994). Unfortunately, this locus did not segregate in the Tift 23DB × (IPW2 × IP6271) progeny, and thus its location relative to that of *Nor-P1* could not be established.

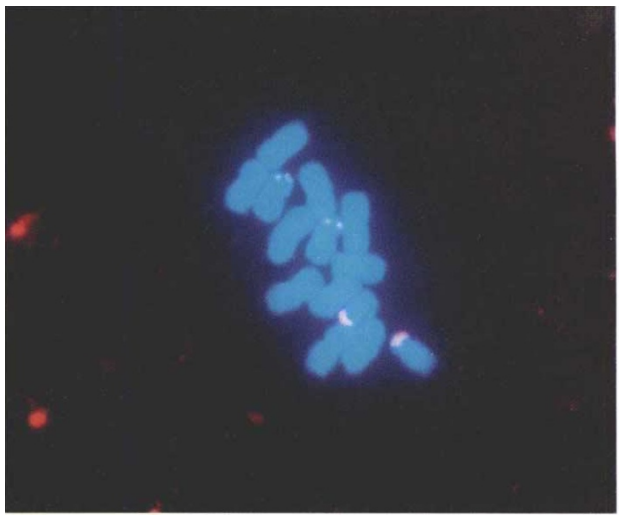

Fig. 1 Mitotic metaphase chromosomes from a root tip of the hybrid between *Pennisetum violaceum* accession IPW2 and *P. glaucum* accession IP6271 after *in situ* hybridization with pTa71, showing the two pairs of rDNA sites.

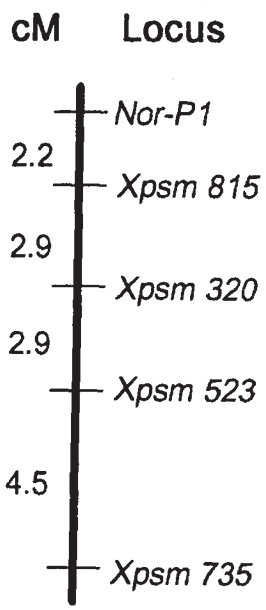

Fig. 2 A linkage map of linkage group 5 of pearl millet, showing the location of the *Nor-P1* locus.

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