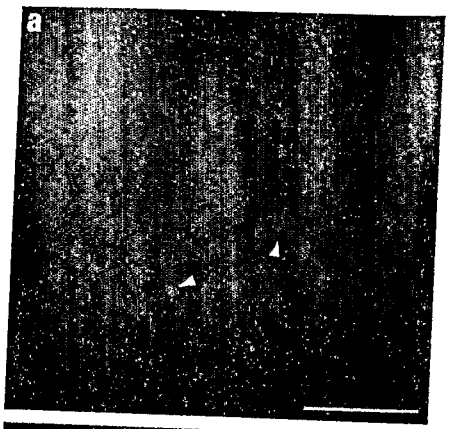

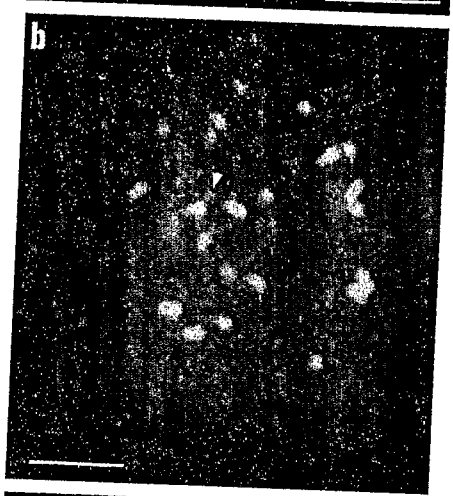

b'

Materials and methods

Plant material

Six F_1 hybrids and seven G2 hybrids were studied. The G2 hybrids consisted of four $F_1 \times$ DEW (BCDEW) and three $F_1 \times$ PSE (BCPSE). G2 hybrids were chosen in order to cover uniformly the range of qDNA between the parental species: 1.13–1.42 pg (unpublished data and Barre et al. 1996). Plants were grown in a greenhouse held at tropical climate (24°C during the day, 18°C at night, relative humidity of 70%).

Flow cytometric measurements

qDNA was estimated as described in Barre et al. (1996). Nuclei were extracted by the leaf chopping method (Galbraith et al. 1983). A modified (0.5% Triton X-100 and pH = 9) version of the lysis buffer of Dolezel et al. (1989) was used. The solution was filtered through nylon cloth (50-µm mesh size) and kept on ice. Just before measurement, saturating propidium iodide (330 µg/µl) was added. *Petunia hybrida* was used as the internal standard (2C = 2.85 pg; Marie and Brown 1993). Three nuclei extractions were done for each plant.

A FACScan cytometer (Becton Dickinson) was used with an argon laser (15 mW) at 488 nm, taking a pulse area of emission at 585 ± 22 nm.

Fluorescent in situ hybridisation

Chromosome preparations, rDNA in situ hybridisations and genomic in situ hybridisations (GISH) were performed as described in D'Hont et al. (1996). For rDNA in situ hybridisation, 5 ng/µl of

Results

rRNA genes

In PSE and DEW, two 18S-5.8S-25S rDNA sites were localised on terminal segments of 2 chromosomes (Fig. 1a). In both species, signals appeared that were associated with a nucleolar constriction. These terminal segments were often broken and separate from the chromosomes in our preparations.

F_1 hybrids

qDNA of F_1 hybrids varied from 1.28 to 1.32 pg, with an average of 1.30 pg, which was close to the average value of the parental species, 1.29 pg. No significant difference between hybrids was noted ($F_{5,12} = 2.22$, $P = 0.12$). The six F_1 hybrids analysed had 22 chromosomes. Their genomic composition was determined using GISH. Eleven red-orange chromosomes originating from PSE and 11 yellow chromosomes originating from DEW, 1 of which had a red terminal signal on a nucleolar constriction, were detected (Fig. 1b).

G2 hybrids

qDNA ranged from 1.20 to 1.39 pg in G2 hybrids ($F_{6,14} = 110$, $P = 0.000$). The seven G2 hybrids had 22

Discussion

Identification of parental chromosomes

GISH efficiently identified the parental origin of all the chromosomes in F_1 and G2 hybrids between DEW and PSE. Yellow chromosomes with a red terminal signal on a nucleolar constriction were interpreted to be chromosomes from DEW. Indeed, it has been shown in sugarcane using the same technique that major rDNA sites fluoresce red even on a yellow chromosome (D'Hont et al. 1996). The highly conserved sequences of rDNA hybridised to DNA of both species, but the red fluorescence appeared more intense than the yellow-green fluorescence. Moreover, the shape and the

species or genic factors (Reiseberg et al. 1995). In this case, it would be difficult to dissociate the interesting and unfavourable traits of wild species from each other and an analysis of large progenies would be required.

Nuclear DNA content and number of PSE chromosomes

The between-species difference in qDNA was equally distributed on all chromosomes, irrespective of the chromosome size, as in *Allium cepa* × *A. fistulosum* hybrids (Narayan 1988). In our G2 hybrids, the fact that qDNA varied gradually and all chromosomes were

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