3. Perlak, F. J., Fuchs, R. L., Dean, D. A., McPherson, S. L. and Fischoff, D. A., *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 3324–3328.
4. Kumar, P. A., Sharma, R. P. and Malik, V., *Adv. Appl. Microbiol.*, 1996, **42**, 1–43.
5. Talekar, N. S. and Shelton, A. M., *Annu. Rev. Entomol.*, 1993, **38**, 275–301.
6. Hofgen, R. and Willmitzer, L., *Plant Sci.*, 1990, **66**, 221–230.
7. Hoekema, A., Hirsch, P. R., Hooykaas, P. J. J. and Schlperoot, R. A., *Nature*, 1983, **303**, 179–180.
8. Hofgen, R. and Willmitzer, L., *Nucleic Acids Res.*, 1988, **16**, 9877.
9. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
10. Chilton, M., Currier, T. C., Farrand, S. K., Bendich, A. J., Gordon, M. P. and Nester, E. W., *Proc. Natl. Acad. Sci. USA*, 1994, **71**, 3672–3676.
11. Doyle, J. J. and Doyle, J. L., *Phytochem. Bull.*, 1987, **19**, 11–15.
12. Sambrook, J., Fritsch, E. F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988.
13. Laemmli, U. K., *Nature*, 1970, **227**, 680–685.
14. Asubel, F. M., Brent, R., Kingston, R. E., Moore, B. D., Seidman, J. G., Smith, J. A. and Struhl, K., *Current Protocols in Molecular Biology*, Current Protocols, Wiley, 1994.
15. Ding, L. C., Hu, C. Y., Yeh, K. W. and Wang, P. J., *Plant Cell Rep.*, 1998, **17**, 854–860.
16. Mazier, M., Pannetier, C., Tourneur, Jouanin, J. L. and Giband, M., *Annu. Rev. Biotechnol.*, 1997, **3**, 313–347.
17. Sutton, D. W., Havstad, P. K. and Kemp, J. D., *Transgen. Res.*, 1992, **1**, 228–236.
18. Kumar, P. A. *et al.*, *Mol. Breed.*, 1998, **4**, 33–37.
19. Hayakawa, T., Zhu, Y., Itoh, K., Kimura, Y., Izawa, T., Shimamoto, K. and Toriyama, S., *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 9865–9869.
20. Tanaka, A., Mita, S., Ohta, S., Kyozuka, J., Shimamoto, K. and Nakamura, K., *Nucleic Acids Res.*, 1990, **18**, 6767–6770.
21. Cheng, J., Bolyard, M. G., Saxena, R. C. and Sticklen, M. B., *Plant Sci.*, 1992, **81**, 83–91.
22. van der Salm, T. *et al.*, *Plant Mol. Biol.*, 1994, **26**, 51–59.

ACKNOWLEDGEMENTS. This project was supported by DBT, Government of India. We are thankful to Dr Mohan, Department of Entomology, IARI, New Delhi for his help in the insect bioassays.

Received 18 December 2001; revised accepted 4 May 2002

Chromosome identification in *Plantago ovata* Forsk. through C-banding and FISH

M. K. Dhar[†,]*, S. Kaul[†], B. Friebe[#] and B. S. Gill[#]

[†]Department of Biotechnology, University of Jammu, Jammu 180 006, India
[#]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502, USA

***Plantago ovata*, the source of Psyllium husk has a haploid complement of just four chromosomes. Somatic chromosomes of this species were studied to delineate C-banding pattern and physical mapping of repetitive, telomeric and rDNA sites to provide landmarks for chromosome identification. Whereas C-banding facilitated identification of all the chromosomes, the fluorescence *in situ* hybridization (FISH) with 5S rRNA gene probe helps in the identification of chromosome 1, and 45S rRNA probe identifies chromosomes 3 and 4. Chromosome 2 is unique in the sense that it does not bear any rDNA FISH sites. The major portion of the genome is comprised of repetitive DNA sequences.**

OF the 280 species in the genus *Plantago*[1], *P. ovata* is the only one which is economically important as a source of Psyllium (Isabgol) of commerce. Psyllium constitutes the seed husk which is mainly used as a laxative, and to small extent in the cosmetic and food industry. Its oral use helps reduce blood cholesterol levels. The diploid chromosome number for the species is $2n = 2x = 8$, with chromosome size ranging from 2.5 to 2.9 µm in Feulgen-stained preparations. The haploid chromosome complement consists of two nucleolar and two non-nucleolar chromosomes. Although pachytene analysis does help in further chromosomal identification on account of linear differentiation, it is tedious and time-consuming, and as such not amenable for routine analysis[2]. Several structural and numerical variants have earlier been induced in this species[3,4], but characterization of such variants at the somatic level could be possible only in such individuals where nucleolar chromosomes were involved. Generally, detailed analysis of meiotic pairing behaviour has to be performed to ascertain the nature and origin of altered/extra chromosomes[5]. Recently-developed molecular cytogenetic techniques of DNA:DNA fluorescence *in situ* hybridization (FISH) have provided powerful tools to microscopically visualize structural and functional organization of genes, chromosomes and genomes, and even physical mapping of transgenes[6–9]. Since chromosome identification constitutes the first step in genetic manipulation of a species, the present investigation was therefore undertaken to provide meaningful landmarks to facilitate unequivocal chromosome identification of individual chromosomes employing C-banding and DNA:DNA *in situ* hybridization.

Root tips from fast-growing germinating seeds of *P. ovata* were excised, and pretreated in 0.002 M 8-hydroxyquinoline for 4 h at 4°C for metaphase arrest, and subsequently fixed for 24 h in Carnoy's fixative. Somatic chromosomes were analysed from Fuelgen-stained root-tip squashes. For C-banding, the protocol standardized by Gill *et al.*[10] on wheat was followed, with minor modifications. Genomic DNA was isolated from young leaves according to Saghai-Maroof *et al.*[11] and Cot-1 fraction was prepared following the method of Britten and Kohne[12], with slight modifications.

*For correspondence. (e-mail: manojkdhar@rediffmail.com)

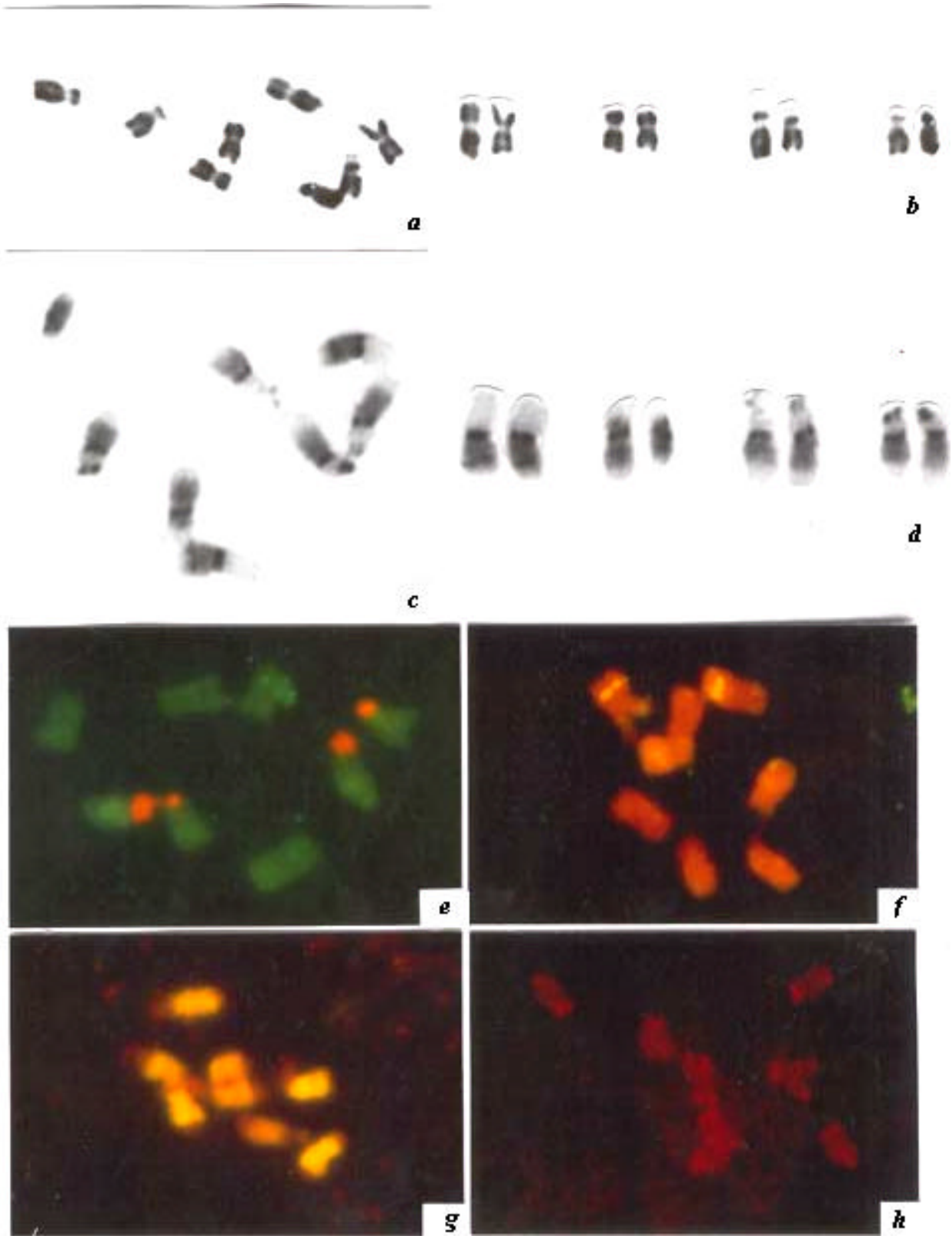

Figure 1. C-banding and FISH in *Plantago ovata*. ***a*** and ***b***, Somatic metaphase of *P. ovata* and photoidiogram thereof showing four nucleolar and four non-nucleolar chromosomes; ***c*** and ***d***, Root-tip metaphase spread showing banding linear differentiation of chromosome through C-banding. Note the short arm of chromosome 1 which is entirely euchromatic; ***e***–***h***, Physical localization of 45S, 5S rDNA, Cot1 fraction and telomeric sequences in *P. ovata*. ***e***, 45S probe identifies chromosomes 3 and 4; ***f***, 5S probe identifies chromosome 1; ***g***, Cot1 fraction shows heavy labelling on all chromosomes; and ***h***, mapping of telomeric sequences.

For *in situ* hybridization, the protocol outlined in Kynast *et al*.[13] was followed. For mapping 18S–25S rRNA genes, we used the probe pTa71 isolated from wheat[14]. The entire plasmid was directly labelled by nick translation with tetramethyl-rhodamine-6-dUTP (Roche Molecular Biochemicals, USA) following the manufacturer's instructions. For FISH mapping of telomeric sequences, the probe pAtT4 (ref. 15) was used. For FISH with 5S rRNA gene, the probe pPov1 (cloned from *P. ovata* details on cloning, etc. in Dhar *et al*., unpublished data) was labelled with digoxigenin-11-dUTP (Roche Molecular Biochemicals, USA) according to the manufacturer's instructions. The signals were visualized using Zeiss Axioplan microscope equipped for phase contrast and epifluorescence.

C-banding reveals the distinct pattern of each chromosome of the haploid complement. A brief description of the individual chromosome C-banding pattern is as follows (Figure 1 *a–d*): Chromosome 1: The largest non-nucleolar chromosome is metacentric and most peculiar. One of its arms is completely devoid of heterochromatin. In addition to the prominent centromeric band, it has a dark band towards the terminal end of the arm. Chromosome 2: This is a submetacentric, non-nucleolar chromosome and shows prominent centromeric band. Chromosome 3: This is distinct in morphology, being nucleolar and subtelocentric. It has the centromeric band. Chromosome 4: Although this chromosome is also subtelocentric and nucleolar, it is distinguishable from chromosome 3 in having a centromeric band and a dark band at the end of the short arm. This is a report of the application of C-banding in identification of chromosomes in the genus *Plantago*. The technique is highly reproducible and can be routinely used for characterization of various numerical (trisomics) and structural variants (translocations), isolated in this species[5].

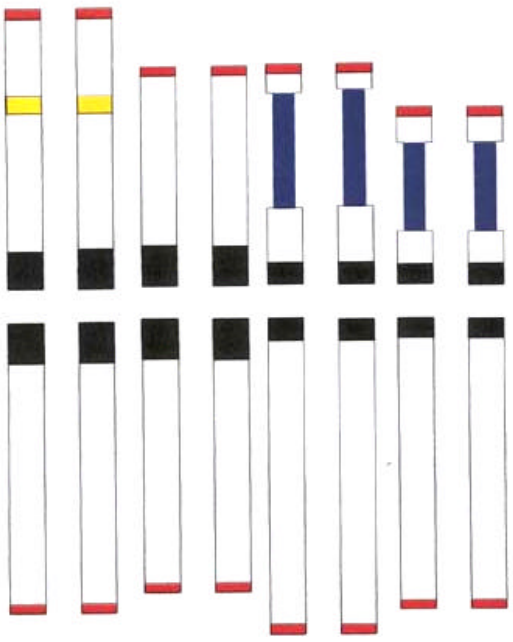

Figure 2. Diagrammatic representation of C-banding pattern (in black), and mapping of ribosomal RNA (5S in yellow and 45S in blue) and telomeric sequences (in red).

FISH with pTa71 revealed distinct signals on the four subtelocentric chromosomes, i.e. chromosomes 3 and 4 (Figure 1 *e*). On the other hand, 5S rRNA sites as revealed by FISH with pPov1 were detected on one of the arms of chromosome 1 (Figure 1 *f*). In addition, FISH was also tried by direct labelling of Cot1 DNA. Major portions of all the chromosomes get labelled (Figure 1 *g*). This result is in complete agreement with C-banding. Hybridization with pATt4 revealed TTTAGGG repeat sequences at the ends of all the chromosomes (Figure 1 *h*). The overall results obtained through C-banding and FISH mapping are shown in Figure 2.

P. ovata being commercially important, efforts have been made to develop high-yielding varieties[2]. However, the results have not been encouraging. Genetic improvement of the plant through manipulation holds the key to a lasting solution. However, genetic manipulation can be carried out efficiently if the genetic basis of the trait of interest is known. Identification of chromosomes constitutes the first step in understanding the genome organization of a species. The present study has clearly demonstrated that application of C-banding together with *in situ* hybridization can help in achieving this target.

1. Rahn, K., *Bot. J. Linn. Soc.*, 1996, **120**, 145–198.
2. Koul, A. K. and Sharma, P. K., *Genetics and Crop Improvement* (eds Gupta, P. K. and Bahl, J. R.), Rastogi and Co, Meerut, 1986, pp. 359–366.
3. Sareen, S. and Koul, A. K., *Crop Improv.*, 1991, **18**, 144–147.
4. Koul, A. K., Sareen, S. and Dhar, M. K., in *Plant Cytogenetics in India* (ed. Ghosh, S.), Cell and Chromosome Research, Kolkata, 1994, pp. 77–88.
5. Padha, L., Sareen, S., Mangotra, R. and Koul, A. K., *Caryologia*, 1998, **51**, 149–158.
6. Gill, B. S. and Friebe, B., *Curr. Opin. Plant Biol.*, 1998, **1**, 109–115.
7. Lavania, U. C., *Curr. Sci.*, 1998, **74**, 126–133.
8. Lavania, U. C., *Methods Cell Sci.*, 2001, **23**, 149–154.
9. Schubert, I., Fransz, P. F., Fuchs, J. and deJong, J. H., *ibid*, 2001, **23**, 57–69.
10. Gill, B. S., Friebe, B. and Endo, T. R., *Genome*, 1991, **34**, 830–839.
11. Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, R. A. and Allard, R. W., *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 8014–8018.
12. Britten, R. J. and Kohne, D. E., *Science*, 1968, **161**, 529–540.
13. Kynast, R. G., Friebe, B. and Gill, B. S., *Chromosome Res.*, 2000, **8**, 133–139.
14. Gerlach, W. L. and Bedbrook, J. R., *Nucleic Acids Res.*, 1979, **7**, 1869–1885.
15. Richards, E. J. and Ausubel, F. M., *Cell*, 1988, **53**, 127–136.

ACKNOWLEDGEMENTS. The senior author thanks the Department of Biotechnology, Government of India for financial assistance to conduct part of this work and the Department of Plant Pathology, Kansas State University, USA for necessary facilities.

Received 29 January 2002; revised accepted 20 April 2002