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# Mapping of DNA markers to arms and sub-arm regions of *Nicotiana sylvestris* chromosomes using aberrant alien addition lines

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**Abstract** Seven monosomic addition plants, each containing the full complement of *Nicotiana plumbaginifolia* (2n = 20, genome constitution PP) and an aberrant chromosome of *Nicotiana sylvestris* (2n = 24, SS), were produced from backcrosses of hyperdiploid derivatives of the sesquidiploid hybrid PPS to *N. plumbaginifolia*. The *N. sylvestris* chromosomes in these plants were characterized by karyotype analysis, Southern hybridization with DNA markers previously localized on *N. sylvestris* chromosomes and a 269-bp fragment from the 3′ end of 25S rDNA, and fluorescence in situ hybridization using 25S rDNA, 5S rDNA and telomere repeats $(TTTAGGG)_n$ as probes. The *N. sylvestris* chromosomes in these plants were identified to be telocentrics 6S, 7S and 8S, and deletions 7S, 10, 12S and 12L, respectively. The successful identification of aberrant chromosomes in these lines enabled us to assign DNA markers to arms and sub-arm regions of *N. sylvestris* chromosomes. All aberrant chromosomes in the addition lines could be transmitted through mitosis and meiosis. The potential applications of the addition lines in high-resolution physical mapping, the isolation of *N. sylvestris* chromosomes by flow cytometry, and an understanding of the chromosomal distribution of 45S rDNA in *N. sylvestris* are discussed.

**Keywords** *Nicotiana sylvestris* · Aberrant monosomic addition line · RFLP analysis · Deletion mapping · Fluorescence in situ hybridization · Ribosomal DNA

## Introduction

Monosomic alien addition lines, in which a single chromosome from one species is added to the genome of another species, are useful tools for molecular analysis of plant chromosomes. They have been used for gene/marker assignment (McGrath et al. 1990; Biyashev et al. 1997), study of the structure and composition of specific chromosome regions such as knobs and centromeres (Ananiev et al. 1998a, b, c), chromosome isolation by flow cytometry (Li et al. 2001), and the production of radiation hybrids for physical mapping (Riera-Lizarazu et al. 2000).

*Nicotiana sylvestris* (2n = 2*x* = 24, genome constitution SS) is the maternal parent of cultivated allotetraploid *Nicotiana tabacum* (2n = 4*x* = 48, SSTT) (Gray et al. 1974; Bland et al. 1985; Olmstead and Palmer 1991). To understand the genome organization of this species, we have dissected its genome by generating a series of *Nicotiana plumbaginifolia–sylvestris* monosomic addition lines and mapped 64 RFLP markers to 9 of its 12 chromosomes (Suen et al. 1997). In that study, we found that in the genetic background of *N. plumbaginifolia* (PP, 2n = 2*x* = 20) the chromosomes of *N. sylvestris* are unstable, manifested by breakage, fusion and centromere misdivision. This provides an opportunity to recover additional plants with deficient and telocentric *N. sylvestris* chromosomes in the progenies of the sesquidiploid hybrid PPS. We report here cytological and molecular characterization of some aberrant addition lines obtained from backcrosses of hyperdiploid derivatives of PPS to *N. plumbaginifolia*. The availability of these lines enabled us to assign DNA markers to chromosome arms and regions.

Recently, Lim et al. (2000a) reported that when *N. sylvestris* genomic DNA was digested with *Bst*NI and hybridized with a 220-bp fragment from the 3′ end of tomato 25S rDNA, three distinct bands of 3.1, 2.7 and 2.4 kb were observed. Based on this result, they suggested that there was no homogenization of 45S rDNA units in *N. sylvestris*. According to our numbering system, chromosomes 5, 6 and 7 of *N. sylvestris* are sat-chromosomes (Suen et al. 1997) and the terminal ends of their short arms are sites of 45S rDNA (Chang 1996). The availability of addition lines containing normal and aber-

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**Table 1** *Nicotiana plumbaginifolia–sylvestris* monosomic addition lines used in this study and summary of results from traditional cytological and Southern blot analyses

| Plant No. | *N. sylvestris* chromosome | DNA markers mapped |
|---|---|---|
| 318-47[a] | 5 | 1812, 1842, 18253, 19119-1, 19147 |
| 88[a] | 6 | 18126, 18217, 1932, 1943, 19125, 19149 |
| 318-37-10[b] | Telo 6S | – |
| 387-4[a] | 7 | 1850, 18124-2, 18238, 19109 |
| 387-5-7[b] | Telo 7S | 18238 |
| 318-5-2[b] | Del 7S | 18238 |
| 297-25[a] | 8 | 1874, 18166-1, 18173, 18186-3, 18203, 1949, 19148, c237, c525, pNR |
| 357-29-1[b] | Telo 8S | 18186-3 |
| 387-26[a] | 10 | 18110, 18133, 18145, 18208, 18241, 18242, 18255-2, 1931, 1993, 19145, c378 |
| 387-32-7[b] | Del 10 | 18133, 18145, 18241, 18255-2 |
| 389-40[a] | 12 | 1851, 1882, 18157, 18162, 18186-2, 18243, 18255-1, 18255-3, c749-2 |
| 318-32-15[b] | Del 12S | 1851, 18255-3, c749-2 |
| 389-4[a] | Del 12-1 | 1851, 18255-3, c749-2 |
| 389-49[a] | Del 12-2 | 1851,1882, 18157, 18162, 18186-2, 18243, 18255-1 |
| 389-2-5[b] | Del 12L | 18162 |
| 297-9[a] | T5-8 | (1812, 1842, 19119-1) (1874, 18166-1, 18173, 18203, 1949, 19148, c237, c525, pNR) |
| 318-24[a] | T5-12 | (18253, 19147) (1851, 18255-3, c749-2)[c] |

[a] Suen et al. 1997
[b] This study
[c] Markers in parenthesis belong to one synteny group

rant chromosomes 5, 6 and 7, obtained in a previous (Suen et al. 1997) and the present study, provides an opportunity to understand the chromosomal distribution of the different types of 45S rDNA in *N. sylvestris*.

## Materials and methods

### Plant material

Seven monosomic addition plants, each containing the normal *N. plumbaginifolia* genome and an aberrant *N. sylvestris* chromosome, were obtained from backcrosses of hyperdiploid derivatives of a sesquidiploid hybrid PPS (Suen et al. 1997) to *N. plumbaginifolia*. These plants, together with some monosomic addition lines obtained previously (Suen et al. 1997), were used in this study (Table 1). All plants were maintained vegetatively through shoot culture (Huang and Chen 1988) and sexually through self-fertilization.

### Feulgen staining

Somatic chromosomes were prepared from root tips of in vitro cultured plants and stained by basic fuchsin according to the method of Lin and Chen (1990).

### Southern hybridization

The 64 RFLP markers previously assigned to *N. sylvestris* chromosomes (Suen et al. 1997) and a 269-bp fragment from *N. plumbaginifolia* 25S rDNA were used as probes. The 269-bp fragment, corresponding to the region between nucleotides 2,876 and 3,145 of *Lycopersicon esculentum* 25S rDNA (Kiss et al. 1989), was amplified and labeled with digoxigenin-11-dUTP by the polymerase chain reaction (PCR) using pNP25S (a 1-kb fragment of *N. plumbaginifolia* 25S rDNA cloned into pBluescript, GenBank accession number AF375999) as a template and oligonucleotides 5′-TGTCGGCTCTTCCTATCATTGTG-3′ and 5′-CTTCTAGCCCGGATTCTGACTTA-3′ as primers. Plant DNA extraction, restriction endonuclease digestion, DNA blotting, labeling of RFLP markers, hybridization, washing, and signal detection were as described by Suen et al. (1997).

### Fluorescence in situ hybridization

The probes for fluorescence in situ hybridization (FISH) were pNP25S, pRTy5S (a 3-kb tandem array of rice 5S rDNA cloned into pTZ19R, Chung et al. 1993), and telomere sequence repeats $(TTTAGGG)_n$ generated by PCR using primers $(5′\text{-}TTTAGGG\text{-}3′)_4$ and $(5′\text{-}CCCTAAA\text{-}3′)_4$ in the absence of a template (Cox et al. 1993). Probes were labeled with digoxigenin-11-dUTP or biotin-16-dUTP by nick translation following the instructions of the manufacturer (Roche Molecular Biochemicals). Chromosomes for FISH were prepared from root tips as described by Chen et al. (1997). The hybridization mixture contained 50% formamide, 2 × SSC, 10% dextran sulfate, 0.1% SDS, 5 ng/µl of probe DNA, and 1 µg/µl of herring sperm DNA. Hybridization was carried out at 37 °C overnight. Slides were washed in 20% formamide, 0.2 × SSC at 42 °C for 10 min, in 2 × SSC at 42 °C for 10 min, and in 2 × SSC at room temperature for 3 × 5 min. Digoxigenin-labeled probes were detected with fluorescein-conjugated antibodies (Roche Molecular Biochemicals) and signals were amplified with fluorescein-conjugated anti-sheep IgG (Vector Laboratories). Biotin-labeled probes were detected with avidin-Texas red (Vector Laboratories). Chromosomes were counterstained with propidium iodide (PI) or 4,6-diamidine-2-phenylindole dihydrochloride (DAPI). Slides were visualized under an Olympus AX70 fluorescence microscope with appropriate filter sets and the images were photographed on Fujicolor Supera 200 ASA print film.

## Results

### Karyotype analysis

Feulgen-stained somatic metaphase chromosomes of haploid *N. plumbaginifolia* and *N. sylvestris* have been described (Lin and Chen 1990; Suen et al. 1997). Briefly, all the ten chromosomes of *N. plumbaginifolia* are telocentric or acrocentric and differ markedly in size. The 12 chromosomes of *N. sylvestris* are more uniform in size (Fig. 1A) and on average are smaller than those of *N. plumbaginifolia*. The two largest chromosomes of *N. sylvestris* are metacentric and are difficult to distin-

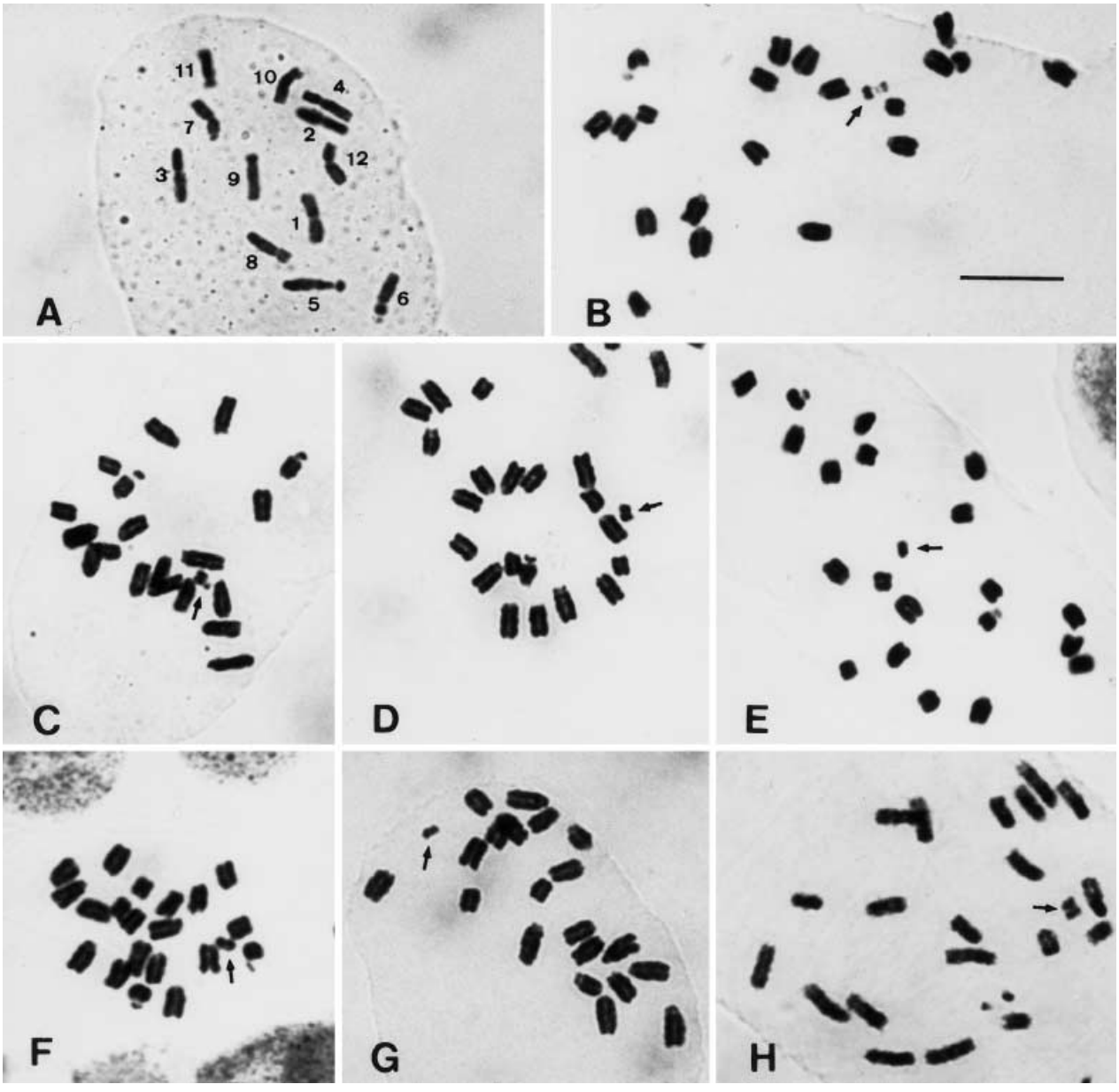


**Fig. 1** Somatic metaphase chromosomes of haploid *N. sylvestris* (**A**) and seven monosomic alien addition lines (**B–H**), each line containing 20 *N. plumbaginifolia* chromosomes and an aberrant *N. sylvestris* chromosome. The aberrant chromosomes in the addition lines are: **B** telocentric 6S, **C** telocentric 7S, **D** deleted 7S (deletion of the satellite), **E** telocentric 8S, **F** deleted 12S, **G** deleted 12L, **H** deleted 10. Numbering of the *N. sylvestris* chromosomes in Fig. 1A is somewhat arbitrary (see text). The aberrant chromosomes (arrows) were identified based on their morphology and DNA markers they carried (see Table 1). Bar = 10 μm.

guish from each other. Similarly, chromosomes 9, 10 and 11 are difficult to distinguish, as they are all subtelocentric and do not differ appreciably in length. Chromosomes 5, 6 and 7 are sat-chromosomes, which could be distinguished from each other by length, arm ratio, and size of the satellite. The secondary constriction of chromosome 5 is less prominent compared with those of chromosomes 6 and 7.

The aberrant *N. sylvestris* chromosomes in the seven addition plants were small in size; of these, all except that in plant 387-32-7 were telocentric (Fig. 1B–H). The *N. sylvestris* chromosomes in plants 318-37-10 and 387-5-7 were identified to be the short arms of chromosomes 6 and 7 (telo 6S and telo 7S), respectively, judged by size and presence of a satellite (Fig. 1B, C). The identification of *N. sylvestris* chromosomes in other plants was not possible based on chromosome morphology alone (Fig. 1D–H).

## Assignment of RFLP markers by Southern-blot analysis

To identify the aberrant chromosomes in addition plants, we probed total genomic DNA of these plants with 64 RFLP markers previously assigned to 9 of the 12 *N. sylvestris* chromosomes (Suen et al. 1997). In each hybrid-

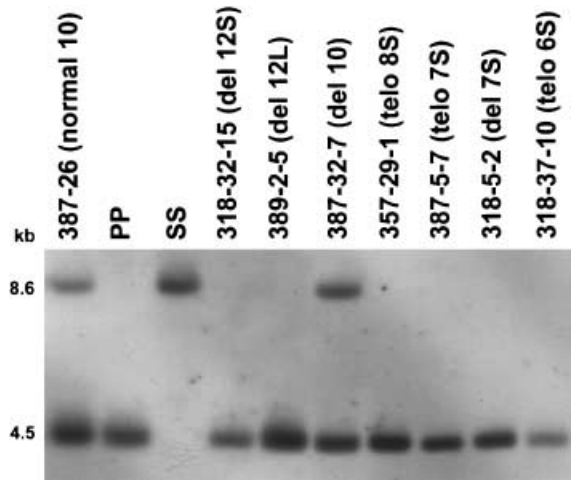


**Fig. 2** Southern blot analysis of *N. plumbaginifolia* (PP), *N. sylvestris* (SS), and *N. plumbaginifolia–sylvestris* monosomic addition lines. Genomic DNA was digested with *Hin*dIII and probed with marker 18145. Only addition lines containing a normal 10 and a deleted 10 have the *N. sylvestris*-specific band.

ization experiment, the normal addition line carrying the tested marker was included as a reference. Figure 2 is an example of the analysis using marker 18145 as a probe. The patterns revealed that only plant 387-26 containing a normal chromosome 10 and plant 387-32-7 containing a biarmed deficient chromosome (Fig. 1H) showed the *N. sylvestris*-specific band. From this we conclude that marker 18145 is located in a region including the short arm, the centromere, and a small proximal segment of the long arm of chromosome 10 (del 10). Other markers were analyzed in a similar way and the results are summarized in Table 1.

## Fluorescence in situ hybridization

FISH of somatic metaphases of *N. sylvestris* using pNP25S and pRTy5S as probes revealed that 25S rDNA signals were clustered at the terminal ends of the short arms of chromosomes 5, 6 and 7, and that 5S rDNA signals appeared as a narrow band in the proximal region of the long arm of a submetacentric or subtelocentric chromosome (Fig. 3A). FISH of pRTy5S to metaphases of the individual addition lines identified *N. sylvestris* chromosome 8 to be the carrier of 5S rDNA (Fig. 3B), coincident with the result of Lim et al. (2000b).

In a previous study (Suen et al. 1997), we found that the *N. sylvestris* chromosomes in two addition lines were the result of translocations between chromosomes 5 and 8 (T5-8) and between chromosomes 5 and 12 (T5-12), respectively. From the markers they carried (Table 1), we suspected that each of these translocated chromosomes contained a different arm of chromosome 5. To test this, we hybridized somatic metaphases of the two lines simultaneously with probes pNP25S and pRTy5S. The results showed that 25S rDNA signals clustered at the terminal end of the short arm and 5S rDNA signals in the proximal region of the long arm of chromosome T5-8 (Fig. 3C); no signal was detected on chromosome T5-12 (data not shown). From this we conclude that T5-8 may be made up of the short arm of chromosome 5 and the long arm of chromosome 8 (T5S-8L). Because T5-12 carries the same markers (1851, 18255-3, c749-2) as del 12S (Table 1), it is conceivable that chromosome T5-12 may consist of the long arm of chromosome 5 and the short arm of chromosome 12 (T5L-12S).

Cytological and Southern-blot analyses identified the *N. sylvestris* chromosomes in addition plants 318-37-10, 387-5-7 and 318-5-2 to be telo 6S, telo 7S and del 7S (telo 7S without a satellite), respectively (Table 1). To confirm the identification and to know whether the broken ends were healed by telomeric sequences, we hybridized somatic metaphases of these plants simultaneously with pNP25S and telomere sequence repeats $(TTTAGGG)_n$. As shown in Fig. 3D–F, 25S rDNA signals occurred at one end of telo 6S and telo 7S, but were absent in del 7S. However, all the three aberrant chromosomes showed hybridization signals of telomere repeats at the two ends. FISH of telomere repeats to metaphases of other lines indicated that the broken ends of all aberrant chromosomes in these lines were healed by telomeric sequences (data not shown).

## Distribution of 45S rDNA in parental species and chromosome addition lines

The results from Southern hybridization of a 269-bp fragment from the 3′ end of 25S rDNA to *Bst*NI-digested genomic DNA from *N. plumbaginifolia*, *N. sylvestris*, their hybrid, and the relevant monosomic addition lines are shown in Fig. 4. In addition to the three 3.1-, 2.7- and 2.4-kb major bands reported by Lim et al. (2000a), three short minor bands were observed in *N. sylvestris*. *N. plumbaginifolia* showed two major bands of 4.6 kb and 3.7 kb and a few minor bands of various sizes. The PS hybrid displayed a combination of bands of the two parents. All addition lines except telo 7S and del 7S (see below) showed normal *N. plumbaginifolia* bands; the bands of *N. sylvestris*, however, were more variable. The patterns of bands in lines with a normal 5, 7, T5S-8L or telo 7S were essentially similar, but the intensities of the bands below 3.1 kb differed; the NOR of chromosome 5 appeared to have more copies of rDNA units than chromosome 7. In all of these lines, the 2.4-kb *N. sylvestris* band disappeared and was replaced by a weak band of slightly larger in size. Lines with a normal 6 and a telo 6S had the major 2.4-kb band and a weak band slightly larger than the 3.1-kb band originally present in *N. sylvestris*. Consistent with the cytological observation and Southern analysis, lines carrying del 7S and T5L-12S, which do not have the NOR and 45S rDNA, lacked the *N. sylvestris* bands. An interesting result of this study was the change of a *N. plumbaginifolia* band from 4.6 kb to 5.0 kb in the line carrying the short arm of *N. sylvestris* chromosome 7 (telo

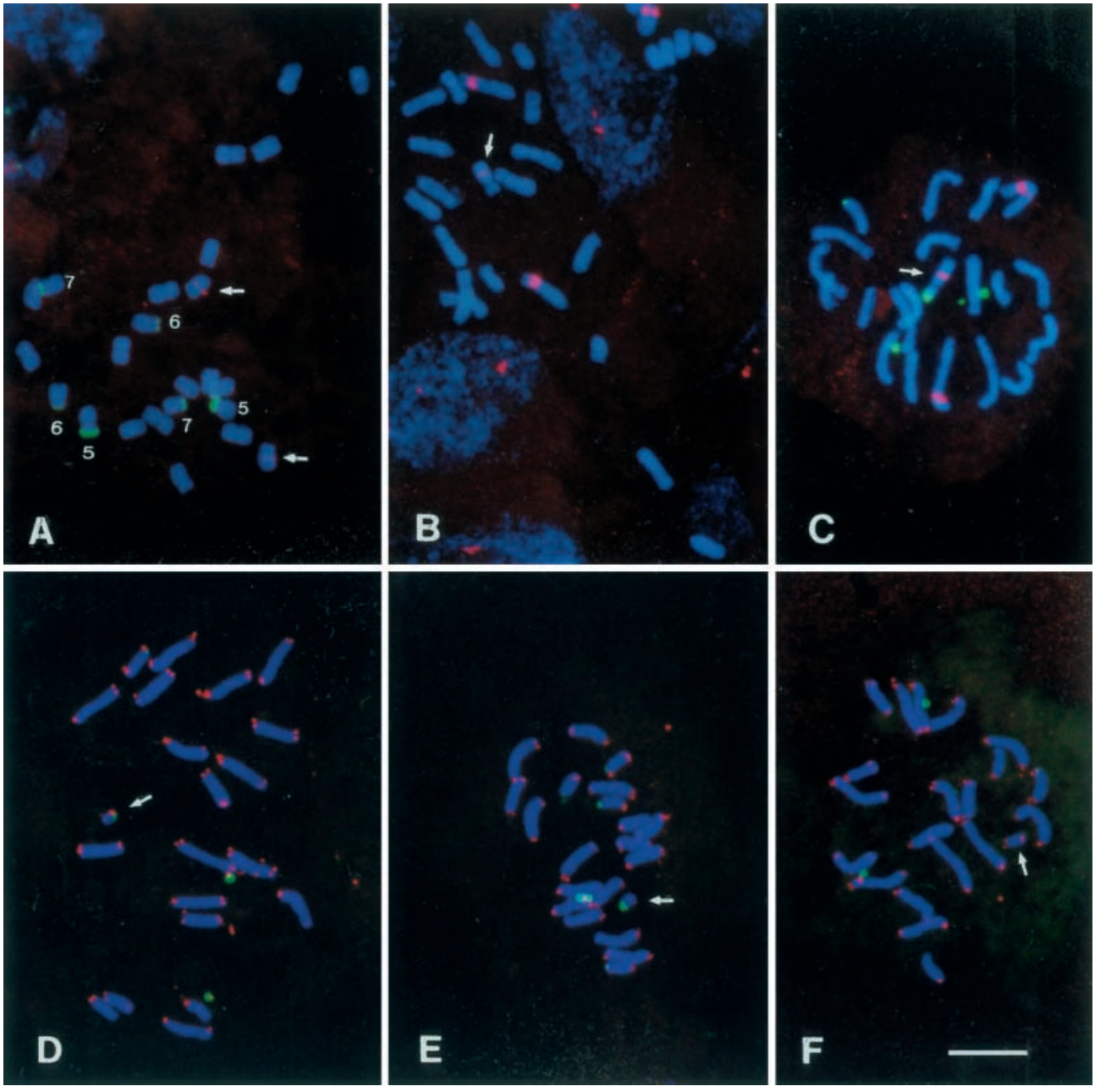


**Fig. 3** Characterization of *N. plumbaginifolia-sylvestris* monosomic addition plants by FISH. **A** *N. sylvestris* chromosomes (blue) showing six 45S rDNA sites (green) at the terminal ends of the short arms of chromosomes 5, 6 and 7, and two 5S rDNA site (red) in the proximal region of the long arm of one pair of submetacentric/subtelocentric chromosomes (arrows). **B** chromosomes (blue) of plant 297-25 showing *N. sylvestris* chromosome 8 (arrow) to be 5S rDNA (red) carrier. **C** chromosomes (blue) of plant 297-9 showing 45S rDNA (green) at the terminal end of the short arm and 5S rDNA (red) in the proximal region of the long arm of T5-8 (arrow). **D** Chromosomes (blue) of plant 318-37-10 showing 45S rDNA (green) in the satellite and telomere repeats (red) at both ends of telo 6S (arrow). **E** Chromosomes (blue) of plant 387-5-7 showing 45S rDNA (green) in the satellite and telomere repeats (red) at both ends of telo 7S (arrow). **F** Chromosomes (blue) of plant 318-5-2 showing telomere repeats (red) at both ends of del 7S (arrow) but absence of 45S rDNA. Bar = 10 μm.

7S) but not in the line with a normal 7. More interesting was the partial change of the size of this band in the line in which the 45S rDNA site on the short arm of *N. sylvestris* chromosome 7 was deleted (del 7S).

## Discussion

All aberrant chromosomes in the addition lines obtained in this study are smaller compared with those of the normal complement of *N. sylvestris*. Of these, only one has two arms, while all the others lack a visible primary constriction. However, all these aberrant chromosomes could be transmitted through mitosis and meiosis, implying that they have a functional centromere and telomeric sequences at the two ends.

The chromosomes identified as telo 6S, telo 7S and telo 8S may originate from centromere misdivision of univalents in the sesquidiploid hybrid PPS and its hyperdiploid derivatives during gametogenesis. For the formation of del 7S, del 12S and del 12L, in addition to centromere misdivision, a second break at sites proximal to the nucleolar organizer region (NOR) on the short arm of chromosome 7, and in the interstitial regions of the short and long arms of chromosome 12, respectively, has to occur. The del 10 chromosome may result from a single break in the proximal region of the long arm. FISH with the probe $(TTTAGGG)_n$ indicated that the broken ends of all aberrant chromosomes were healed by the addition of telomeric sequences. The molecular structure of the healed ends could not be analyzed, because DNA sequences at the breakpoints are not known. In wheat, deletion stocks, in which breakpoints occurred in the NOR of chromosome 1B, were available (Endo and Gill 1996). Tsujimoto et al. (1997) analyzed the DNA sequence at the breakpoint of one of these stocks and found the occurrence of a breakage-fusion-bridge cycle(s) before the addition of telomere repeats. Chromosomes T5S-8L and T5L-12S obtained in our previous study (Suen et al. 1997) may result from fusion of the arms of two different chromosomes immediately after centromere misdivision.

From cytological and Southern-blot analyses of the aberrant addition lines, we tentatively assign DNA markers to arms and specific regions of *N. sylvestris* chromosomes 5, 6, 7, 8, 10 and 12 (Fig. 5). Among the 16 breakages involved in the formation of aberrant chromosomes, ten occurred in the centromere. This is not surprising, as *N. sylvestris* chromosomes in the sesquidiploid hybrid PPS and its hyperdiploid derivatives formed univalents (Lin and Chen 1990; Chen, unpublished results), whose centromeres are subjected to occasional misdivision during meiosis (Darlington 1939). However, one question is why all telocentric chromosomes in the aberrant lines consisted of only short arms? One likely explanation may be that telocentric long arms did actually exist but, because they were morphologically similar to the small telocentric chromosomes of *N. plumbaginifolia*, they were therefore neglected during the screening process. Of the six breakages occurring in interstitial regions, one was between the NOR and the distal region of the short arm of chromosome 7, one in the proximal region of the long arm of chromosome 10, and two in each arm of chromosome 12 (Fig. 5). It seems that the distribution of breakpoints in the *N. sylvestris* genome is not random; chromosome 12 appears to be particularly liable to breakage.

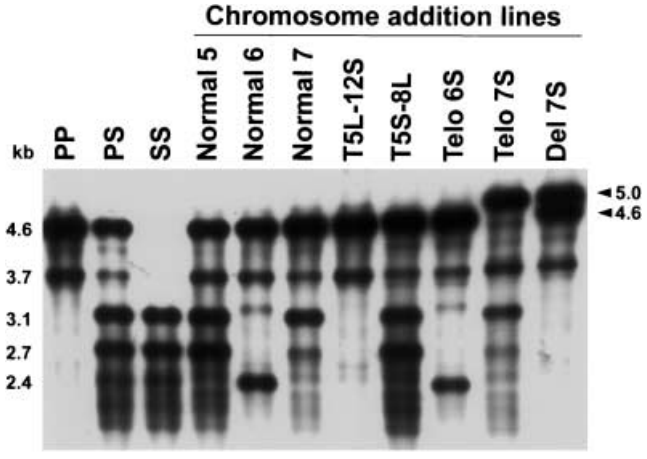


**Fig. 4** Southern blot analysis of *N. plumbaginifolia* (PP), *N. sylvestris* (SS), somatic hybrid (PS), and eight *N. plumbaginifolia-sylvestris* monosomic addition lines. Genomic DNA was digested with *Bst*NI and probed with a 269-bp fragment from the 3′ end of *N. plumbaginifolia* 25S rDNA.

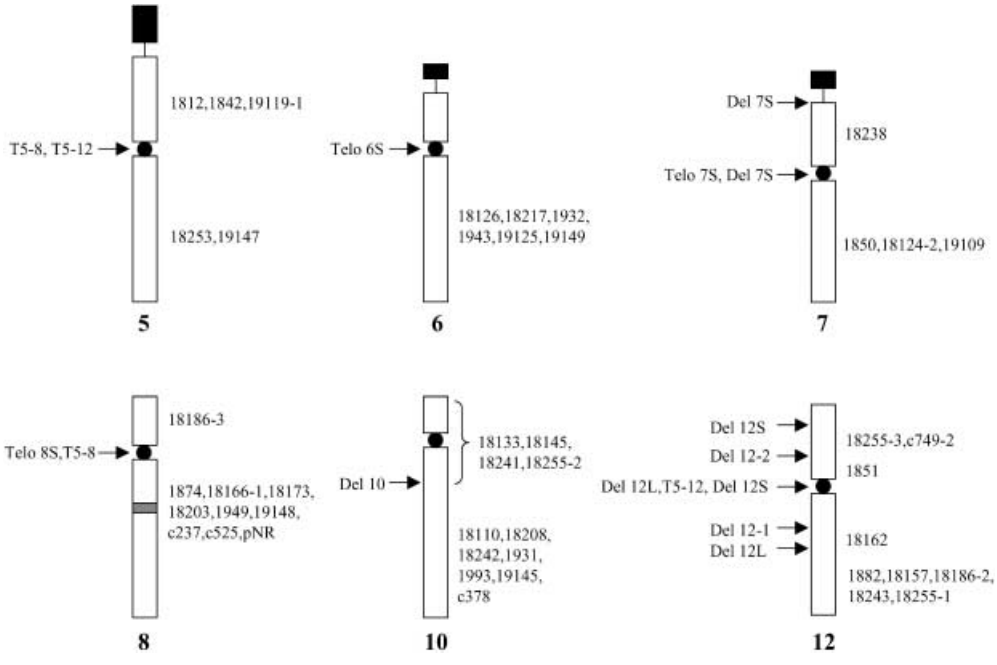


**Fig. 5** Assignment of DNA markers to arms and regions of *N. sylvestris* chromosomes based on data in Table 1 and FISH using 25S and 5S rDNAs as probes. The chromosomes were drawn based on Fig. 1A, with the short arm placed on the top. Arrows on the left-hand side of chromosomes indicate the putative breakpoints in aberrant chromosomes of the addition lines. DNA markers are placed on the right-hand side of chromosomes within the regions between the breakpoint and telomere or between two breakpoints. Orders of markers in the same regions and of those above the breakpoint of del 10 are arbitrary. Black and hatched areas indicate 45S rDNA and 5S rDNA sites, respectively. Black circles indicate centromeres.

Endo (1988) found that when a certain chromosome from *Aegilops cylindrica* is added to wheat, chromosome breakages occur in the gametes that lack the *A. cylindrica* chromosome. Using this unique genetic system, Gill and colleagues obtained over 400 deletion stocks, in which breakages occurred in all the 42 arms of wheat chromosomes (Endo and Gill 1996). These stocks have been used extensively in wheat genome mapping (see Faris et al. 2000 and references therein). Although the chromosomes of *N. sylvestris* tend to break in the genetic background of *N. plumbaginifolia*, the frequency of breakages is not high enough to generate sufficient numbers of aberrant chromosomes for high-resolution physical mapping as in wheat. Parokonny et al. (1992) reported the production of asymmetric somatic hybrids through fusions of gamma-irradiated protoplasts of *N. sylvestris* with non-irradiated protoplasts from *N. plumbaginifolia*. About 47% of the asymmetric somatic hybrids contained 1 to 3 minichromosomes, which could be stably transmitted through mitosis. One way to increase the frequency of aberrant chromosomes, therefore, would be irradiation of the protoplasts of haploid *N. sylvestris* before fusion with those of *N. plumbaginifolia* and a backcross of the resultant asymmetric somatic hybrids to *N. plumbaginifolia*. Alternatively, the monosomic addition lines obtained in this and previous studies could be irradiated directly to produce progenies with short segments of *N. sylvestris* chromosomes integrated into the genome of *N. plumbaginifolia*. The latter method is similar to that practiced in mammalian hybrid-cell systems (Cox et al. 1990), and has recently been applied to maize genome mapping (Riera-Lizarazu et al. 2000).

Recently, Lim et al. (2000a) detected three distinct bands of 3.1, 2.7 and 2.4 kb when *Bst*NI-digested genomic DNA from *N. sylvestris* was probed with a 220-bp fragment from the 3′ end of tomato 25S rDNA. In the present study, we mimicked the hybridization experiments (see Materials and methods) of Lim et al. (2000a) and observed three short minor bands besides the major bands described by Lim et al. (2000a). Our results are more consistent with the work of Volkov et al. (1999), who isolated three long and two short variants of the 45S rDNA from *N. sylvestris*. The presence of multiple hybridization bands supports the conclusion of Lim et al. (2000a) that there is little or no homogenization of the length variants of 45S rDNA in *N. sylvestris*.

Southern-blot analysis of addition lines revealed that the length variants of *N. sylvestris* 45S rDNA differed in chromosome distribution. Although chromosomes 5 and 7 showed similar patterns of bands, the former appeared to have more copies of variants than the latter; chromosome 6 had fewer major variants compared with the other two sat-chromosomes, and lacked all minor variants. These results are in agreement with the observations of differences in the size of satellites revealed by Feulgen staining and the strength of 25S rDNA signals detected by FISH.

In the present study, we found that deletion of the long arm of sat-chromosome 7 in the telo 7S addition line caused almost a complete shift of a *N. plumbaginifolia* band from 4.6 kb to 5.0 kb. Moreover, even in the del 7S addition line, in which the 45S rDNA site and the long arm were deleted, a partial shift of the 4.6-kb band occurred. From a comparison of the distribution of RFLP markers in *N. sylvestris* (2n = 24) and *N. plumbaginifolia* (2n = 20), Lin et al. (2001) found that there have been considerable chromosome reorganizations between these two species during evolution. It is possible that chromosome 7 of *N. sylvestris* may be homoeologous to two *N. plumbaginifolia* chromosomes, the short arm to one sat-chromosome and the long arm to a chromosome without a NOR. Based on this assumption, we postulate that physical contact between donor and recipient 45S rDNA during gametogenesis may be a necessary process for changes in the length of repeat units. This process may be absent in the addition line containing a normal *N. sylvestris* chromosome 7, but present when the long arm of this chromosome was deleted as in telo 7S. The addition line containing a del 7S was obtained from two rounds of backcrosses of a hyperdiploid derivative of PPS to *N. plumbaginifolia*. It might be that the addition chromosome was a telo 7S after the first backcross, but its NOR was deleted after the second backcross. Thus, the partial change of the 4.6-kb band was a residual effect in the previous generation when the NOR was present. If this explanation is correct, then the change in length of the 45S rDNA repeat units may be considerably rapid.

Flow cytometry is an efficient technique, which has been used to separate human chromosomes from one another for various applications (Ferguson-Smith 1993). However, the technique has not been successfully applied to plants. One problem with flow karyotyping in plants is the lack of a significant difference in chromosome size within a karyotype, which makes discrimination of single chromosome types difficult (Doležel et al. 1994). The aberrant *N. sylvestris* chromosomes in addition lines obtained in this study are much smaller than those of the recipient *N. plumbaginifolia*; therefore, they could be easily isolated by flow cytometry for the construction of arm and region-specific libraries.

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