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Species-specific evolution of telomeric and rDNA repeats in the tobacco composite genome

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Abstract In order to investigate possible interactions between parental genomes in the composite genome of *Nicotiana tabacum* we have analyzed the organization of telomeric $(TTTAGGG)_n$ and ribosomal gene (rDNA) repeats in the progenitor genomes *Nicotiana sylvestris* and *Nicotiana tomentosiformis* or *Nicotiana otophora*. Telomeric arrays in the *Nicotiana* species tested are heterogeneous in length ranging from 20 to 200 kb in *N. sylvestris*, from 20 to 50 kb in *N. tomentosiformis*, from 15 to 100 kb in *N. otophora*, and from 40 to 160 kb in *N. tabacum*. The patterns of rDNA repeats (18*S*, 5.8*S*, 25*S* RNA) appeared to be highly homogeneous and species-specific; no parental rDNA units corresponding to *N. sylvestris*, *N. tomentosiformis* or *N. otophora* were found in the genome of *N. tabacum* by Southern hybridization. The results provide evidence for a species-specific evolution of telomeric and ribosomal repeats in the tobacco composite genome.

Key words Evolution · Tobacco · Telomeres · Ribosomal genes

Introduction

Nicotiana tabacum is a natural amphidiploid plant commonly used in plant genetics. Its genome is composed of two ancestral genomes derived from species close to modern *N. sylvestris* (S) and *N. tomentosiformis* or *N. otophora* (T). It is estimated that the tobacco genome originated from the ancestral S and T genomes by sexual hybridization and subsequent chromosomal diploidization 5–6 million years ago (Okamura and Goldberg 1985). Contradictory data, however, exist on the extent of the independent evolution of both S and T compartments of the genome. Analysis of crosses of tobacco with *N. sylvestris* and *N. tomentosiformis* (Gerstel 1960, 1963), bimodality in the GC composition of the tobacco genome (Matassi et al. 1991), and the presence of homeologous genes without apparent modifications (e.g. nitrate reductase) (Vaucheret et al. 1989), suggest that both compartments evolved relatively independently without extensive interactions. On the other hand, most recent data provide evidence that the tobacco genome was subjected to substantial evolutionary pressures involving interactions and genetic exchanges between its S and T parts: (1) intergenomic exchange was shown to occur in a glucan endo-1,3-D-glucosidase gene family (Sperisen et al. 1991) (2) the copy number of T-genome-specific GRS repetitive sequences was found to be about ten times higher in *N. tomentosiformis* (*otophora*) than in *N. tabacum* (Gazdova et al. 1995) (3) an excellent cytogenetic study revealed the presence of recombinant chromosomes originating from both T and S genomes using total genomic DNA from tobacco progenitors and also species-specific repetitive probes (Kenton et al. 1993). The authors of this latter study suggested that the telomeric sequences $(TTTAGGG)_n$ and the clusters of rDNA genes might represent the only high-copy repetitive sequences common to both parts of genome. It may be expected that these sequences would have been primary candidates for genetic exchanges between the evolutionary diverged S and T components.

To resolve some of these issues we have compared the structure of telomeric and ribosomal repeats in tobacco and its putative progenitors corresponding to *N. sylvestris*, *N. tomentosiformis* or *N. otophora* using pulsed-field gel electrophoresis (PFGE) and Southern-blot hybridization.

Materials and methods

Plant material

The *Nicotiana* species and cultivars used are listed in Table 1. Total DNA from leaves and, in some experiments, from leaf-derived calli

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Table 1 List of *Nicotiana* species used. Sources of seeds were as follows: the Tobacco Research Institute, Báb, Slovakia (B), seed collection of Max-Planck-Institut für Züchtungsforschung, Köln, Germany (K), SEITA, Institut du Tabac, Bergerac, France (S), Seed Bank, Wakehurst Place, Royal Botanic Gardens, Kew, UK (KW), and Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany (G)

Species	Cultivar	Source
N. tabacum L.	Vielblättriger	B
	Samsun	B
	Petit Havana SR-1	K
	095-55502	KW
N. sylvestris		S
N. tomentosiformis	Goodsp.	S
N. otophora Griseb.	406/76	G

was isolated by a modified cetyl ammonium bromide method (Saghai-Maroof et al. 1984).

DNA probes

Internal *Eco*RI fragments of the 18*S* and 25*S* rDNA genes cloned from the tomato genome (Kiss et al. 1988, 1989) were used as probes. These were ^{32}P-labelled using the Decaprime kit, Ambion. For the detection of the telomeric DNA repeats, a $(CCCTAAA)_6$ oligonucleotide was employed after end-labelling with [γ-^{32}P]ATP and T4 polynucleotide kinase (NEB).

Evaluation of restriction-fragment length

DNA samples were digested with an excess of the enzymes (10 U/µg of DNA) *Bst*NI, *Eco*RI, *Hin*dIII, *Bam*HI, *Pvu*II, and *Taq*I (NEB). DNA digests were size-separated in 0.8% agarose gels and, after Southern blotting, hybridized with a labelled probe. After washing at high stringency (65 °C, 0.2 × SSC + 0.1% SDS), (Sambrook et al. 1989) the restriction fragments of the 18*S* and 25*S* rDNA genes were visualized by autoradiography.

Preparation of samples for pulsed-field gel electrophoresis (PFGE)

Cell nuclei from various *Nicotiana* species were isolated from leaves according to Espinás and Carballo (1993). The nuclei were suspended in a minimal volume of buffer containing 10 mM HEPES-NaOH pH 7.5, 20 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 2 mM EDTA, 0.5 mM EGTA, 0.2 mM PMSF, 250 mM sucrose, and mixed at 40 °C with an equal volume of molten 1.5% LMP agarose. The mixture was transferred to an agarose block former (Bio-Rad) and left at 4 °C for 10 min. The agarose blocks were then placed in a solution containing 0.5 M EDTA pH 9.0, 1% N-lauroyl sarcosine and 500 µg/ml of Proteinase K (Boehringer), and incubated for 48 h at 50 °C with one exchange of the solution after 24 h. The blocks were then equilibrated with TE (10 mM Tris. HCl, pH 8.0, 1 mM EDTA) and 0.2 mM PMSF. Finally, the blocks were equilibrated with restriction buffer, and digested with 20 U of restriction enzyme (NEB) in a new portion of restriction buffer for 16 h. PFGE was performed on a CHEF-DRII aparatus (Bio-Rad). The conditions of electrophoresis were: 1% agarose gel, 0.5 × TBE, 190 V, pulse time ramped from 1 to 20 s during 20 h, 15 °C. After electrophoresis, the gel was stained with ethidium bromide and alkali-blotted onto Hybond N+ membrane.

Results

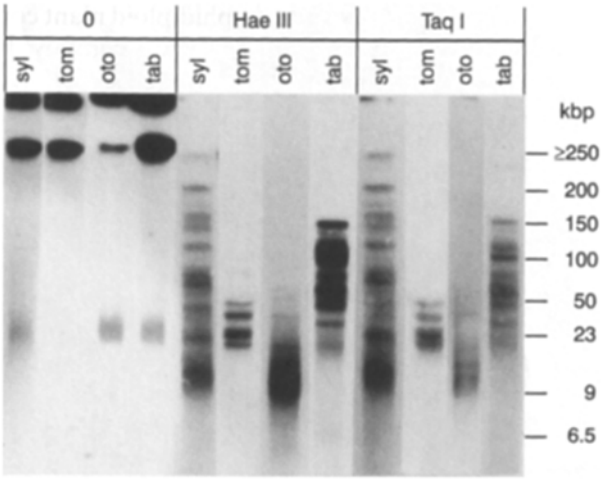
Distinct lengths of telomeric TTTAGGG repeats in tobacco and its progenitors

To determine the size of the telomeric repeats, high-molecular-weight DNA was isolated from cell nuclei of *N. sylvestris*, *N. tometosiformis*, *N. otophora* and *N. tabacum*. DNAs in agarose blocks were digested with frequently cutting methylation-insensitive restriction enzymes and subjected to PFGE under conditions enabling the separation of 10–250 kb fragments. The blots were hybridized to the $(CCCTAAA)_6$ probe. There was no detectable material on gels of more than 20 kb in length after ethidium bromide staining, suggesting the completion of the digestions (data not shown). As shown in Fig. 1 the telomeres of each *Nicotiana* species were well separated in distinct bands after *Taq*I or *Hae*III digests. In *N. sylvestris* the hybridization bands were detected in 20–200 kb, in *N. tomentosiformis* in 20–50 kb, in *N. otophora* in 15–100 kb and in *N. tabacum* in 40–160 kb regions.

Distinct units of rDNA genes in tobacco and its progenitors

Genomic DNA was isolated from leaves of different cultivars of *N. tabacum* as well as from its putative progenitors *N. sylvestris*, *N. tomentosiformis* and *N. otophora*. Isolated DNAs were subjected to restriction-

Fig. 1 Pulsed-field gel electrophoresis (PFGE) analysis of telomeres in *Nicotiana* species: syl *N. sylvestris*, tom *N. tomentosiformis*, oto *N. otophora*, tab *N. tabacum*. Agarose-embedded leaf DNAs were digested with *Hae*III or *Taq*I and fractionated on a 1% agarose gel using a CHEF-type electrophoresis apparatus (for conditions see Materials and methods). The separated DNA was transferred to a Hybond N+ membrane and hybridized to the end-labelled $(CCCTAAA)_6$ probe. Intact DNAs from each species are shown in the first four lanes. Positions of lambda concatemers and lambda/*Hin*dIII markers are indicated



enzyme digestions and subsequent analysis by Southern hybridization using 25*S* and 18*S* rDNA probes. Most enzymes (*Bst*NI, *Hin*dIII, *Bam*HI, *Pvu*II, *Rsa*I, *Taq*I) revealed distinct patterns of rDNA genes in tobacco and its progenitors. In the *Bst*NI digests shown in Fig. 2 the species-specific diagnostic fragments were of 1.6, 1.8 and 2.1 kb in *N. sylvestris*, 3.1 and 3.2 kb in *N. tomentosiformis*, 4.2 and 4.4 kb in *N. otophora* and 1.5 and 2.7 kb in *N. tabacum*. The lower-molecular-weight fragments (0.38 and 0.9 kb) were common to all species tested and were probably derived from highly conserved regions of the 25*S* rDNA gene. The species-specific patterns were neither influenced by variations among different cultivars (vars. Vielblättriger, Petit Havana SR-1, Samsun and 095-55 were tested) nor by cultivation conditions (callus cultures provided the same patterns as the leaf). *Eco*RI has two highly conserved recognition sites close to the 5′ and 3′ ends of the 25*S* rDNA gene of tobacco (Borisjuk et al. 1988) and tomato (Kiss et al. 1989). The probe hybridized to two fragments of the same length in all *Nicotiana* species tested suggesting that the polymorphisms observed with other enzymes arose due to variations in the non-transcribed spacer region.

Hybridizations of 25*S* and 18*S* rDNA probes (Figs. 2 and 3) to the *Hin*dIII-restricted DNA produced the same simple patterns of bands of about 9–14 kb length. In tobacco, both probes hybridized to two bands of approximately 10 and 11 kb which is close to the predicted size of the tobacco major rDNA units (Borisjuk et al. 1988). It is therefore probable that most *Nicotiana* rDNA repeats contain a single *Hin*dIII site. Based on this supposition the shortest rDNA units appear to be present in the genome of *N. sylvestris* (8.5 kb) while the longest ones are in *N. otophora* (14 kb).

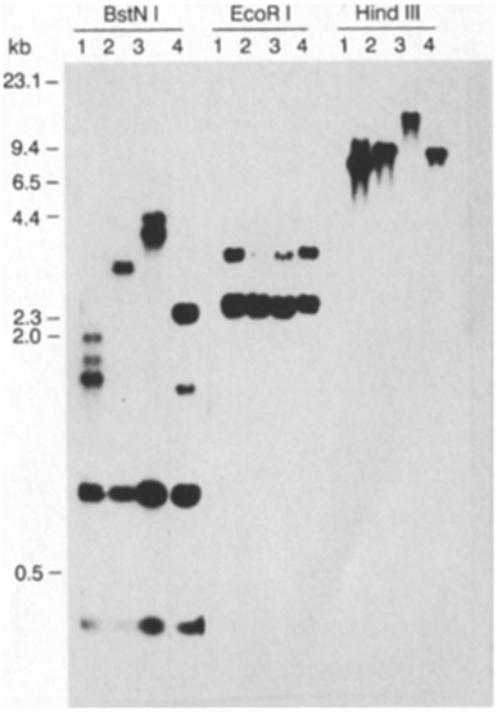


Fig. 2 Autoradiogram of restriction-enzyme digests of total DNA from *N. sylvestris* (*lanes 1*), *N. tomentosiformis* (*lanes 2*), *N. otophora* (*lanes 3*) and *N. tabacum* (*lanes 4*) hybridized to the random prime-labelled 25*S* rDNA probe. The fragments were separated on a 0.8% agarose gel and blotted to a Hybond N+ membrane. *Hin*dIII-digested lambda DNA was used as a size marker

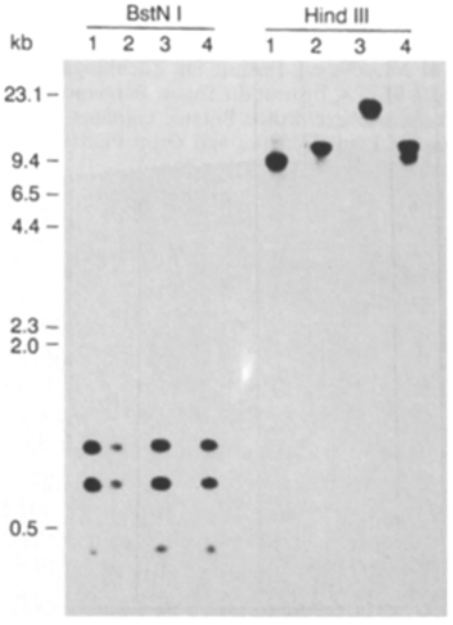


Fig. 3 Autoradiogram of restriction-enzyme digests of total DNA from *N. sylvestris* (*lanes 1*), *N. tomentosiformis* (*lanes 2*), *N. otophora* (*lanes 3*) and *N. tabacum* (*lanes 4*) hybridized to the random prime-labelled 18*S* rDNA probe. The experiment was performed as described in Fig. 2

Discussion

The evolution of parental genomes within composite genomes remains a topic of considerable interest in plant genetics. In order to examine possible interactions between both ancestral genomes in the composite genome of *N. tabacum* we have analyzed the structure of two regions with high intergenomic homology, namely telomeric repeats and ribosomal gene clusters. We show that telomere lengths in *N. tabacum* are distinct from those found in its modern progenitors *N. sylvestris*, *N. tomentosiformis* and *N. otophora*. Since cytogenetic studies have failed to reveal any interstitial localization of TTTAGGG repeats we suggest that the hybridization bands on PFGE blots represent true blocks of terminal chromosomal repeats. The profiles appeared to be slightly more complex in tobacco, which might reflect the double number of chromosomes (2n = 48) in comparison with the ancestral genomes. Thus it seems likely that in plants, as in mammals [e.g. mouse (Kipling and Cooke, 1990)], the telomere lengths may differ even among closely related species within the genus.

The length of the rDNA repeat unit varies significantly between species due to the variability of non-coding intergenic spacers (Flavell 1986). To reveal possible interactions between rDNA clusters of alien genetic compartments we have performed restriction-enzyme analysis of ribosomal genes in the putative progenitors and compared the patterns obtained with those in tobacco. In the absence of extensive interaction an additive pattern of the rDNA units inherited from both ancestral S and T genomes would be expected. However, the results presented in this work show that the dominant rDNA units in tobacco differ remarkably from those found in its putative progenitors. Thus it seems that during the 5–6 million years of the existence of the tobacco genome the parental rDNA units were subjected to genetic drive, were homogenized, and fixed (Dover and Tautz 1986) so that today the original parental units are not detectable by conventional Southern hybridization techniques. Interestingly, the morphology and position of individual NORs seem to correspond to both parental genomes despite their extensive recombination and homogenization (Kenton et al. 1993). An open question remains why some composite genomes are vulnerable to recombination events while others are apparently not. For example, in the composite genome of *Brassica napus* the parental rDNA units of *Brassica oleracea* and *Brassica campestris*, respectively, remain largely unchanged (Bennet and Smith 1991; our unpublished results). Understanding the mechanisms governing gene interactions in composite genomes might help to improve the breeding programs of many agronomically important plants.

In conclusion we find that the *N. tabacum* genome contains a species-specific organization of telomeric and ribosomal repeats that is different from that found in its modern progenitors. *N. sylvestris*, *N. tomentosiformis* or *N. otophora*. While the blocks of telomeric repeat are largely heterogeneous in size, the ribosomal clusters have converged to apparent homogeneity during the evolution of the composite tobacco genome.

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