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Variability of the 5S and 45S rDNA Sites in *Passiflora* L. Species with Distinct Base Chromosome Numbers

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Cytologically, the species of *Passiflora* with known chromosome number can be divided into four groups: (1) $2n$ = 12, 24, 36; (2) $2n$ = 24; (3) $2n$ = 18, 72; and (4) $2n$ = 20. The base chromosome number proposed for the genus is x = 6, with x = 9, x = 10 and x = 12 being considered secondary base numbers. In the present study, variability of 5S and 45S rDNA sites was investigated in 20 species of these four groups to check the reliability of this hypothesis. In the group with x = 6, five diploid species ($2n$ = 12) exhibit two 5S rDNA sites and two (*P. capsularis*, *P. morifolia* and *P. rubra*) or four (*P. misera* 2*x* and *P. tricuspis*) 45S rDNA sites. The hexaploid cytotype of *P. misera* had 12 45S rDNA sites and six 5S rDNA. A tetraploid species, *P. suberosa*, had ten 45S rDNA sites and four 5S rDNA sites, both in the same chromosomes as the 45S rDNA sites. In the group with x = 9, *P. actinia*, *P. amethystina*, *P. edmundoi*, *P. elegans*, *P. galbana*, *P. glandulosa* and *P. mucronata* displayed six 45S rDNA sites, whereas *P. alata*, *P. cincinnata*, *P. edulis* f. *flavicarpa*, *P. edulis* var. roxo and *P. laurifolia* had four sites. In this group, all species were diploid ($2n$ = 18) and had only two 5S rDNA sites. *Passiflora foetida*, the only species with $2n$ = 20, had six 45S rDNA sites and four 5S rDNA sites. The species with x = 12 ($2n$ = 24), *P. haematostigma* and *P. pentagona*, showed four 45S rDNA sites and two 5S rDNA. In general, the number and location of 5S and 45S rDNA sites were consistent with the hypothesis of x = 6 as the probable ancestral genome for the genus, while the groups of species with x = 9, x = 10 and x = 12 were considered to be of tetraploid origin with descending dysploidy and gene silencing of some redundant gene sites, mainly those of 5S rDNA.

Key words: *Passiflora*, chromosome base number, FISH, rDNA, karyotype.

INTRODUCTION

Passiflora L. is the largest genus of the family Passifloraceae, with approx. 465 species distributed mainly in tropical regions (Vanderplank, 1996; Cervi, 1997). Most of these species are herbaceous, although some representatives are shrubs or trees. Many species of *Passiflora* are cultivated as ornamentals or for their edible fruits and medicinal properties.

Cytologically, the species of *Passiflora* for which chromosome numbers are known can be divided into four karyological groups, represented by $x = 6$, $x = 9$, $x = 10$ and $x = 12$. Most species of *Passiflora* are diploid, with $2n = 12$, $2n$ = 18 or $2n$ = 20, although some tetraploids ($2n$ = 24), hexaploids ($2n$ = 36) and octoploids ($2n$ = 72) have been recorded (Snow and MacDougal, 1993; Melo *et al*., 2001). Different chromosome base numbers (x = 3, 6, 9) have been proposed for the genus, without a clear understanding of this variation and of the phylogenetic relationships among the species (Storey, 1950; Raven, 1975; Morawetz, 1986; Snow and MacDougal, 1993). Recently, Melo *et al*. (2001), revising the cytotaxonomy of the group, considered $x = 6$ as the most probable base number for the genus, whereas $x = 9$, $x = 10$ and $x = 12$ were considered secondary base numbers. However, the second-most probable base number, x = 12, appears to have played an important role in the evolution of the group because it is better represented in other genera of the family. Therefore, with regards to the cytotaxonomy of this group, it is important to know whether x = 6 is derived from x = 12, or *vice versa*. The mechanisms of chromosome alteration most probably related to these changes are descending dysploidy (x = 12 $\rightarrow$ 6) or polyploidy (x = 6 $\rightarrow$ 12) (Guerra, 2000). In this case, any additional indication of polyploidy or dysploidy may be important for understanding the evolution of this group.

In the genus *Aloe*, Adams *et al*. (2000) found a correspondence between the number of 5S rDNA sites revealed by FISH (fluorescent *in situ* hybridization) and the ploidy level of several species. Diploid species displayed two 5S rDNA sites, tetraploids four sites and hexaploids six sites. A similar correspondence was found in *Saccharum* (D'Hont *et al*., 1998). However, more often, this relationship has been not observed, mainly because the number of rDNA sites can vary among diploid species. In diploid species of *Paeonia*, for instance, the number of chromosome pairs carrying 45S rDNA sites varied from three to five (Zhang and Sang, 1999). Exceptionally, the number of 5S and 45S rDNA sites can vary widely within a single species, as in *Crocus vernus* (Frello and Heslop-Harrison, 2000). Nevertheless, the number of 5S or 45S rDNA sites is generally larger in tetraploids than in related diploids. In

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TABLE 1. Passiflora *species analysed with respective chromosome numbers, number and position of 45S and 5S rDNA sites, herbarium vouchers and provenances*

Taxon	2*n*	Number and position of 5S rDNA sites	Number and position of 45S rDNA sites	Voucher number	Provenance*
Subgenus *Astrophea* (DC.) Mast.					
Section *Pseudoastrophea* (Harms) Killip					
P. haematostigma Mart. ex Mast.	24	2 (st)	4 (st)	PAS-1641	Caeté, MG
P. pentagona Mast.	24	2 (st)	4 (st)	TSAH-1736	Barra da Estiva, BA
Subgenus *Plectostemma* Mast.					
Section *Cieca* (Medic.) Mast.					
P. morifolia Mast.	12	2 (st)	2 (st)	UFP-31195	Águas de Lindóia, SP
P. suberosa L.	24	4 (st)	10 (st; p)	TSAH-1742	Porto Alegre, RS
Section *Decaloba* (DC.) Mast.					
P. misera Kunth	12	2 (st)	4 (p)	TSAH-1737	Foz do Iguaçu, PR
	12	2 (st)	4 (p)	TSAH-1746	Cachoeira do Sul, RS
	36	6 (st)	12 (st; p)	TSAH-1741	Recife, PE
	36	6 (st)	12 (st; p)	UFP-31197	Caruaru, PE
P. tricuspis Mast.	12	2 (st)	4 (p)	UFP-31201	São José do Rio Preto, SP
Section *Xerogona* (Raf.) Killip					
P. capsularis L.	12	2 (st)	2 (st)	TSAH-1745	Águas de Lindóia, SP
P. rubra L.	12	2 (st)	2 (st)	TSAH-1744	Mata do Saltinho, Rio Formoso, PE
Subgenus *Distephana* (Juss.) Killip					
P. glandulosa Cav.	18	2 (st)	6 (st)	UFP-31196/1	Cultivated: Embrapa CNPMF, Cruz das Almas, BA
	18	1 (st)	6 (st)	UFP-31196/2	Cultivated: Embrapa CNPMF, Cruz das Almas, BA
Subgenus *Passiflora*					
Series *Quadrangulares* (Harms) Killip					
P. alata Curtis	18	2 (st)	4 (st)	–	Cultivated: Embrapa CNPMF, Cruz das Almas, BA
Series *Laurifoliae* Killip ex Cervi					
P. laurifolia L.	18	2 (st)	4 (st)	–	Cultivated: Embrapa CNPMF, Cruz das Almas, BA
Series *Passiflora*					
P. cincinnata Mast.	18	2 (st)	4 (st)	UFP-17402	Buíque, PE
P. edulis Sims. f. *flavicarpa* Deg.	18	2 (st)	4 (st)	–	Cultivated: Embrapa CNPMF, Cruz das Almas, BA
P. edulis Sims var. roxo	18	2 (st)	4 (st)	–	Cultivated: Embrapa CNPMF, Cruz das Almas, BA
Series *Kermesinae* Killip ex Cervi					
P. edmundoi Sacco	18	2 (st)	6 (st)	UFP-31199	Palmeiras, BA
Series *Simplicifoliae* (Harms) Killip					
P. actinia Hook.	18	2 (st)	6 (st)	PAS-1592	Porto Alegre, RS
P. galbana Mast.	18	2 (st)	6 (st)	UFP-31200	Mamanguape, PB
P. mucronata Lam.	18	2 (st)	6 (st)	TSAH-1740	Igarassu, PE
Series *Lobatae* (Harms) Killip					
P. amethystina Mikan var. *amethystina*	18	2 (st)	6 (st)	UFP-31202	Águas de Lindóia, SP
	18	2 (st)	6 (st)	–	Domingos Martins, ES
P. elegans Mast.	18	2 (st)	6 (st)	PAS-1591	Porto Alegre, RS
Subgenus *Dysosmia* (DC.) Killip					
P. foetida L.	20	4 (st)	6 (p)	TSAH-1735	Petrolina, PE

Species are organized according to the infrageneric division proposed by Killip (1938).
St, Subterminal; p, proximal.
* BA, Bahia; ES, Espírito Santo; MG, Minas Gerais; PB, Paraíba; PE, Pernambuco; PR, Paraná; SP, São Paulo, RS, Rio Grande do Sul.

Rhynchospora, the number of 45S rDNA site pairs varied from two to four among diploids, and from four to 15 among polyploids (Vanzela *et al*., 1998). A similar variation in number of 5S and 45S rDNA sites has been described in diploid and tetraploid species of *Hordeum* (Taketa *et al*., 1999). Therefore, in general, polyploids have a higher average number of rDNA sites (mainly 45S rDNA) than diploids.

In the present study, the variability of 5S and 45S rDNA sites was investigated in 20 species of *Passiflora*, representing different base numbers, to help identify the base number of the genus and relationships among the haploid numbers.

MATERIALS AND METHODS

Material was collected in the field or was obtained from the germplasm bank of Embrapa Mandioca e Fruticultura Tropical—CNPMF (Table 1). Voucher specimens of all materials are deposited at the UFP Herbarium (Federal University of Pernambuco, Brazil), or at TSAH of Embrapa Semi-Árido (Petrolina-PE, Brazil).

For cytological analysis, young root tips were pre-treated with 0·002 M 8-hydroxyquinoline at 8 °C for 24 h, and fixed overnight at room temperature in Carnoy 3 : 1 (ethanol : acetic acid). Floral buds of *P. pentagona* Mast. were collected in the field and fixed directly in Carnoy. Root tips were then stored at –20 °C. Root tips were digested in 2 % cellulase (Sigma, St Louis, Mo, USA) and 20 % pectinase (Sigma) solution for 2 h at 37 °C, and squashed in 45 % acetic acid. Coverslips were removed by freezing in liquid nitrogen and the slides were air-dried. Slides were stained briefly with DAPI (4′,6-diamidino-2-phenylindol)/glycerol and the best ones were re-fixed in Carnoy 3 : 1 for 30 min, dehydrated in 100 % ethanol and stored at –20 °C until required for *in situ* hybridization.

To locate the 45S rDNA sites, probes SK18S and SK25S were used, containing 18S and 25S rDNA of *Arabidopsis thaliana* L. (Unfried *et al*., 1989; Unfried and Gruendler, 1990), kindly supplied by Professor Dieter Schweizer (University of Vienna, Austria). The 5S rDNA was obtained from total genomic DNA of *P. edulis* Sims by PCR using the primers 5′-GTGCGATCATACCAGC(AG)(CT)TAATG-CACCGG-3′ and 5′-GAGGTGCAACACGAGGA-CTTCCCAGGAGG-3′. The *in situ* hybridization procedure followed that of Moscone *et al*. (1996). Probes SK18S and SK25S were labelled with biotin-11-dUTP and detected with TRITC (tetramethyl rhodamine isothiocyanate), while the 5S rDNA probe was labelled with digoxigenin-11-dUTP and detected with FITC (fluorescein isothiocyanate). Chromosomes were counterstained with DAPI and the slides mounted in Vectashield H-1000.

Cells were photographed with a DMLB Leica epifluorescence microscope, using Kodak Ultra colour film ASA 400, or their images captured with a Cohu-CCD video camera using Leica QFish software.

RESULTS

Karyologically, the species were distributed into four groups, in agreement with the base number: six species with $x = 6$ ($2n = 12, 24, 36$), 11 with $x = 9$ ($2n = 18$), one with $x = 10$ ($2n = 20$) and two with $x = 12$ ($2n = 24$). Chromosome morphology varied from metacentric to submetacentric. Size chromosome asymmetry was more evident in the group of species with $x = 6$, especially in *P. suberosa*, *P. capsularis* and *P. morifolia*. Most of the populations had not been analysed previously by Melo *et al*. (2001), but they exhibited the same number of chromosomes and chromosome morphology as previous samples. Only *P. haematostigma*, with $2n = 24$ (Fig. 1M), was investigated for the first time.

The positions of 5S and 45S rDNA sites are summarized in Table 1. Figures 1 and 2 illustrate the main results. Note that filter Leica I3 used to excite FITC (green) also excites TRITC (red), changing the colour of the signals captured with filter N2·1 for TRITC. Consequently, the 45S rDNA sites are shown twice and in different colours.

The number of 5S rDNA sites was almost constant, usually a pair for each species, except in *P. foetida* ($2n = 20$) and in the tetraploid *P. suberosa* L., with four sites, and *P. misera* Kunth (6*x*), with six sites (Table 1). The position of these sites often seemed to be subterminal and, at least in the species with $x = 6$, it was always observed on chromosome VI (the smallest pair) or V. In several species, visualization of the 5S rDNA site was limited by its small size. For instance, in *P. alata* Curtis, the 5S rDNA sites were observed as minuscule points inside the chromosome mass (Fig. 2O). In a single individual of *P. glandulosa* Cav., the 5S rDNA site of one homologue was not detected, possibly owing to its very small size (Fig. 2K). The interphase nuclei of this sample also displayed only one fluorescent spot. In another individual of this same species, the two 5S rDNA sites showed size heteromorphism (Fig. 2L).

The small chromosome size and the tendency of fluorescence to expand beyond the region marked by the probe did not allow us to distinguish clearly between terminal and subterminal rDNA sites. Since, in some cases, the 45S rDNA sites were located within a subterminal secondary constriction, all sites are referred to as subterminal in Table 1, although some of these may be terminal. In general, 45S rDNA signals were larger, more numerous and more variable than 5S rDNA signals.

In the group of diploid species with $x = 6$, the 45S rDNA sites varied from one pair in *P. capsularis*, *P. morifolia* and *P. rubra*, to two pairs in *P. misera* 2*x* and *P. tricuspis* (Fig. 1A–I; Table 1). In polyploids, this number was higher. In *P. misera* 6*x*, the number and position of rDNA sites were the expected multiple of the diploid form, with 12 45S rDNA sites and six 5S sites. However, while all 45S rDNA sites in *P. misera* 2*x* were the same size (Fig. 1H), *P. misera* 6*x* showed six large and six small blocks of 45S rDNA (Fig. 1J), and six 5S rDNA sites distributed in three very small chromosome pairs. DAPI bands observed in the diploid cytotype were also observed in the hexaploid one (Fig. 1G).

The tetraploid *P. suberosa* displayed an asymmetrical karyotype, with eight to 12 larger chromosomes and 12 to 16 smaller ones. It had ten 45S and four 5S rDNA sites. Six 45S rDNA sites were located on the smallest chromosomes: two in the proximal region and four in the subterminal region. These latter four chromosomes also showed the 5S rDNA sites (Fig. 1K and L). The largest 45S rDNA site was located on the second largest chromosome pair. *Passiflora tricuspis*, although showing a karyotype very similar to that of *P. misera* 2*x*, displayed 45S rDNA in chromosome pairs II and III, and 5S rDNA in pair V (Fig. 1F), whereas in *P. misera* these sites were located in pairs II, IV or V (45S) and VI (5S). *Passiflora capsularis*, *P. rubra* and *P. morifolia* Mast. had only two 45S rDNA sites, located on the short arm of chromosome pair I in the first two species (Fig. 1B and D), and on the metacentric pair V in *P. morifolia* (Fig. 1E).

In the two species of the group with $x = 12$, *P. haematostigma* and *P. pentagona*, both with $2n = 24$, four 45S rDNA sites and two 5S rDNA sites were observed, all terminally located (Fig. 1M and N). They had larger chromosomes than the species with $x = 6$.

Chromosomes of *Passiflora foetida* ($x = 10$; $2n = 20$) were larger than those of species with $x = 6$ and smaller than those of species with $x = 9$. Six proximal 45S rDNA sites and four terminal or subterminal 5S rDNA sites were observed (Fig. 1P and Q).

In the group of $x = 9$, the number of 45S rDNA sites varied from two to three pairs (Table 1), all apparently subterminals (Fig. 2). Of the species with three pairs of sites, *P. edmundoi*, *P. elegans* and *P. mucronata* were similar in

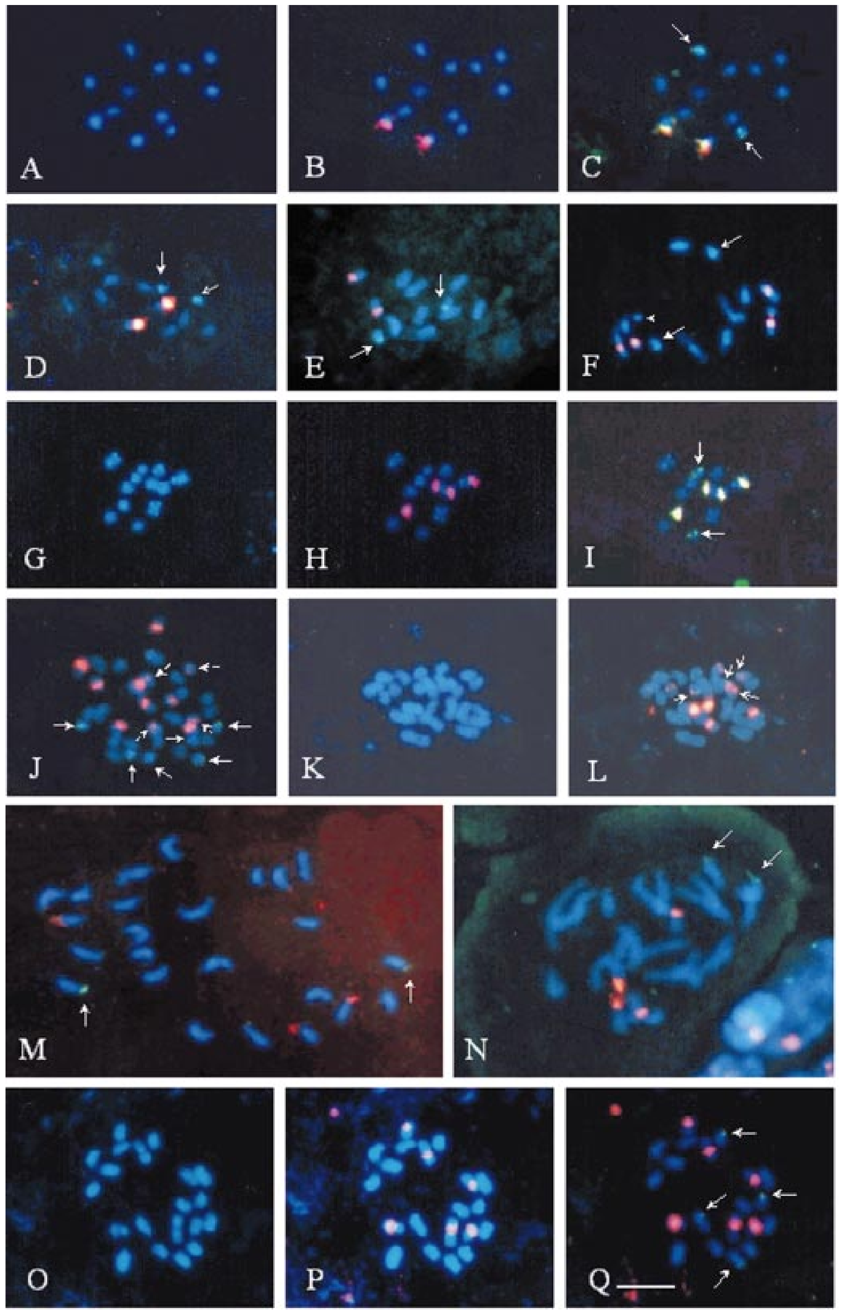

FIG. 1. Fluorescent *in situ* hybridization with 5S (green) and 45S (red or yellow) rDNA probes, in *Passiflora* species with $x = 6$, $x = 10$ or $x = 12$. A–C, *P. capsularis*. Note chromosomes stained with DAPI in A, 45S rDNA sites in B and a pair of 5S rDNA sites in C (arrows). D, *P. rubra*. Arrows indicate 5S rDNA. E, *P. morifolia*. Arrows indicate 5S rDNA. F, *P. tricuspis*. Note a longer pair of chromosomes with terminal regions characteristically less condensed, two pairs with proximal blocks of 45S rDNA, with one of the chromosomes being distended (arrowhead), and a pair with sub-terminal 5S rDNA (arrows). G–I, *P. misera* 2*x*. Note the terminal DAPI⁺ bands in almost all chromosomes in G, 45S rDNA sites apparently sub-terminal in one pair of chromosomes and proximal in the other one in H, and 5S rDNA sites in I (arrows). J, *P. misera* 6*x*. Broken arrows and arrowhead indicate small 45S rDNA sites. K and L, *P. suberosa*. Note that three of the five pairs of 45S rDNA sites are located in the smallest chromosomes, two of which also have 5S rDNA (arrows). M, *P. haematostigma*. Arrows indicate terminal 5S rDNA sites. N, *P. pentagona*. Arrows indicate 5S rDNA sites. O–Q, *P. foetida*. Note the secondary proximal constrictions in three chromosome pairs in O, three pairs of 45S rDNA proximal sites in P, and two pairs of 5S rDNA sub-terminal sites in Q (arrows). All chromosomes were counterstained with DAPI (blue). Bar in Q represents 5 µm.

having 45S rDNA sites in chromosome pairs I and III, whereas the third pair was located on chromosome VII, IX or VIII, respectively (Fig. 2A, C and E). The 5S rDNA was located on the short arm of pair II in *P. edmundoi* (Fig. 2B), on the long arm of pair VI in *P. elegans* (Fig. 2D) and on the long arm of pair VII in *P. mucronata* (Fig. 2F).

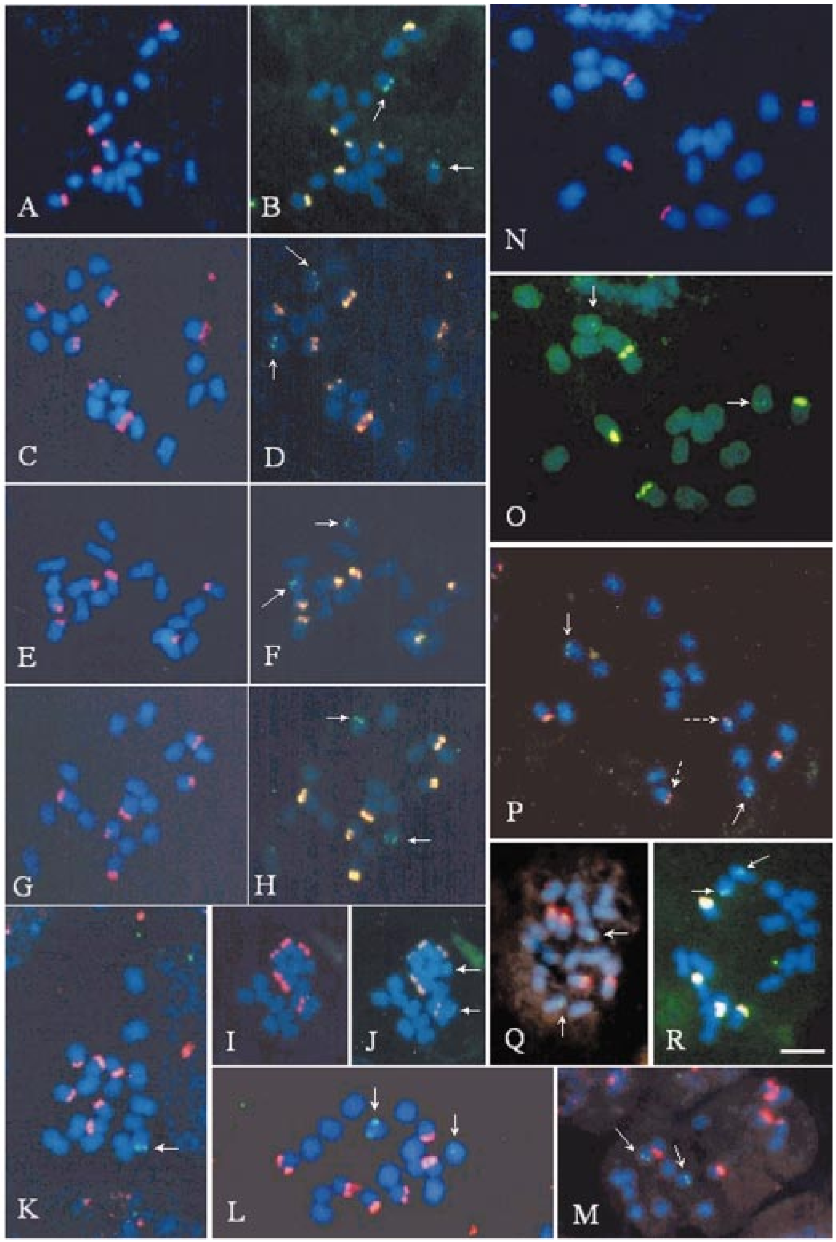

FIG. 2. Fluorescent *in situ* hybridization with 5S (green) and 45S (red or yellow) rDNA probes, in *Passiflora* species with *x* = 9. *P. edmundoi* (A and B); *P. elegans* (C and D); *P. mucronata* (E and F); *P. galbana* (G and H); *P. actinia* (I and J); *P. glandulosa* (K and L); *P. amethystina* (M); *P. alata* (N, O); *P. edulis* (P); *P. laurifolia* (Q); and *P. cincinnata* (R). Arrows indicate 5S rDNA sites while broken arrows indicate weak signals of 45S rDNA. All chromosomes were counterstained with DAPI (blue). Bar in R represents 5 µm.

In *P. galbana* two larger blocks of 45S rDNA were observed, located on pairs II and IV, and a smaller one on the pair V (Fig. 2G and H). *Passiflora actinia* had a symmetrical karyotype with 5S rDNA signals observed as very weak dots (Fig. 2I and J). In *Passiflora glandulosa*, 45S rDNA sites (Fig. 2K and L) were located on chromosomes V, VII and VIII, and 5S rDNA sites on one or two chromosomes of pair VI. In *P. amethystina* (Fig. 2M), the 45S rDNA sites were located on pairs V, VI and IX, whereas the 5S rDNA sites were observed in the sub-terminal region of pair VII.

Passiflora alata had the largest chromosomes of the group with *x* = 9, with 45S rDNA located on the terminal region of pairs V and VIII, and 5S rDNA subterminally located on the short arm of pairs III or IV (Fig. 2N and O). In *Passiflora edulis* f. *flavicarpa* there was a large and a small 45S rDNA site, located on the long arm of pairs VII and IX, respectively, and 5S rDNA was located on the long arm of pair V (Fig. 2P). In *P. edulis* var. roxo, the number and position of the rDNA sites were the same as those in *P. edulis* f. *flavicarpa* although it was impossible to define the chromosome morphology. *Passiflora cincinnata* and

P. laurifolia had 45S rDNA sites of similar size; these were located in pairs IV and VI in the former species, and in pairs V and VIII in the latter (Fig. 2Q and R).

DISCUSSION

In general, both the number and position of 45S rDNA sites detected by FISH coincided with the CMA+ bands and secondary constrictions observed in the karyotypes of *Passiflora* (Melo *et al*., 2001). However, some 45S rDNA regions not detected as secondary constrictions could be identified as CMA+ bands. In *P. tricuspis*, for example, there were two chromosome pairs with CMA+ blocks and 45S rDNA sites but only one secondary constriction (Melo *et al*., 2001). On the other hand, the number of 5S rDNA sites did not correlate with any other cytological parameter.

Among the species that are of agronomic importance (all concentrated in the group with $x = 9$), variation in the number and position of rDNA sites was very limited and did not constitute an important cytological marker to characterize species. Nevertheless, inter-specific hybrids among representatives of the series *Quandrangulares* (Harms) Killip, *Laurifolia* Killip ex Cervi or *Passiflora*, and species of the series *Kermesinae* Killip ex Cervi, *Simplicifoliae* (Harms) Killip or *Lobatae* (Harms) Killip can be easily identified by the difference in the number of 45S rDNA sites.

Variation in the number of rDNA loci among species with different base numbers

The species with $x = 6$ in this study were restricted to the subgenus *Plectostemma* Mast. (Table 1), with representatives in three of the seven sections proposed by Killip (1938). In this subgenus, most species are diploid ($n = 6$), although the frequency of polyploidy is higher than that in the other subgenera of *Passiflora* (Snow and MacDougal, 1993; Melo *et al*., 2001). Variation in the location of 45S rDNA seems to correlate with the infrageneric division of Killip (1938). Species of section *Decaloba* (DC.) Mast. had two chromosome pairs with proximal 45S rDNA sites, whereas species of the sections *Cieca* (Medic.) and *Xerogona* (Raf.) Killip had only one subterminal site. In species of *Xerogona* (*P. capsularis* and *P. rubra*), the site was found in the largest chromosome pair, whereas in the only species belonging to *Cieca* studied (*P. morifolia*), it was located on one of the smallest chromosome pairs.

The only two species analysed that showed intraspecific polyploidy, both with $x = 6$, had more 45S rDNA sites than the related diploid species. The hexaploid form of *P. misera* had 12 sites, whereas the tetraploid *P. suberosa* had ten sites, six more than expected based on the only diploid of the section investigated. Regarding the 5S rDNA sites, in the group of species with $x = 6$, the number of sites was clearly related to the ploidy level: all diploids had two marked chromosomes, tetraploids had four and the hexaploids had six.

Passiflora suberosa was the only species that had 5S and 45S rDNA loci in the same chromosome, suggesting that the increase in number of 45S rDNA sites in this species occurred by transposition of 45S rDNA repeats from original chromosomes to others that did not possess these sites. Schubert and Wobus (1985) had previously related the capacity of the 45S rDNA repeats to be transposed to different regions of a chromosome complement. The eventual linking of 5S and 45S rDNA sites in the same chromosome has been observed as a derivative characteristic in *Hypochaeris* (Cerbah *et al*., 1998), *Allium* (Lee *et al*., 1999), *Clivia* (Ran *et al*., 2001) and some other genera.

The finding of $2n = 24$ in *P. haematostigma* and *P. pentagona* reinforces the separation of these species of the subgenus *Astrophea* into a different karyological group with $x = 12$ (Melo *et al*., 2001). The only other species cytologically known in this subgenus, *P. lindeniana* Triana & Planch., also has $n = 12$ (Berry, 1987). Although *P. haematostigma* and *P. pentagona* have $n = 12$, like *P. suberosa*, they possess a more widely diploidized karyotype, with only one pair of 5S rDNA site and two pairs of 45S rDNA sites.

The 11 species analysed of the group with $x = 9$ are included in the subgenera *Distephana* (Juss.) Killip and *Passiflora*. They are diploids with one pair of 5S rDNA sites and two or three pairs of 45S rDNA sites. The karyotype with three pairs of 45S rDNA was found in representatives of subgenera *Passiflora* and *Distephana*, whereas that with two pairs was restricted to subgenus *Passiflora*. Variation in the number of 45S rDNA sites among related diploid species with the same chromosome number has been observed in several other genera (see, for example, Moscone *et al*., 1999; Široký *et al*., 2001).

The only species with $n = 10$, *P. foetida*, of the subgenus *Dysosmia* (DC.) Killip, had two pairs of 5S rDNA sites and three pairs of 45S rDNA, and was therefore more similar to tetraploid than to diploid karyotypes of the genus. Thus, *P. foetida* might be derived from a tetraploid karyotype with $n = 12$ ($x_1 = 6$), changing to $n = 10$ by descending dysploidy. The same could have occurred to species with $x = 9$, which have, on average, more 45S rDNA sites than species with $x = 6$. Based on the alignment of ITS-1 and ITS-2 sequences from 45S rDNA (18S + 5·8S + 25S), Scherer *et al*. (2000) positioned *P. foetida* closer to species of the subgenus *Passiflora* than to those of the subgenus *Plectostemma*, which is compatible with the evolutionary route suggested by these data. Using molecular phylogenetic analysis of ITS and *trn*L-*trn*F intergenic spacers, Muschner *et al*. (2003) also placed *P. foetida* (subgenus *Dysosmia*) in the same clade of the subgenus *Passiflora*.

Base chromosome number of the genus

The number of 45S rDNA sites in the present sample varied from one to three per haploid complement (except in the infraspecific polyploids), with a single pair only being found amongst species with $x = 6$. Given that diploids generally have fewer rDNA sites than the related polyploids, it is reasonable to suppose that the group of species with $x = 6$ is the originally diploid, single group whereas those with $x = 9$, 10 and 12 are of tetraploid origin, with descending dysploidy ($12 \rightarrow 10 \rightarrow 9$), and reduction of redundant sites, mainly the 5S rDNA ones. Polyploids have a tendency to reduce the number of duplicated sites owing to diploidization mechanisms and gene silencing (Leitch and Bennett, 1997). The selection pressure to reduce the rDNA site number to a single pair seems to be smaller in 45S rDNA owing to the existence of more efficient inter-loci homogenization mechanisms for this sequence than for 5S rDNA (Cronn *et al*., 1996). Therefore, the probability of finding the duplicated 5S rDNA sites would be larger in infraspecific polyploids or in neopolyploids, than in paleopolyploids. Vogel *et al*. (1999) observed that silencing isoenzyme markers also is more evident in the paleopolyploids than in the neopolyploids.

The only two samples analysed here that showed site duplication proportional to the ploidy level (*P. misera* and *P. suberosa*) were also the only examples of infraspecific polyploidy. Multiplication of the 5S rDNA site number has also been found in other intraspecific polyploids, such as *Aloe* (Adams *et al*., 2000), or in neopolyploids, as in common wheat (Mukai *et al*., 1990). On the other hand, the occurrence of a single 5S rDNA site in *P. haematostigma* and *P. pentagona* seems to reflect an oldest origin of polyploidy in these species, as observed in typical paleopolyploids, such as *Hevea* (Leitch *et al*., 1998) and *Manihot* (Carvalho and Guerra, 2002).

Although these data corroborate the hypothesis of $x_1 = 6$ as being the primary base number of *Passiflora* and Passifloraceae, the hypothesis that the base number is 12 still has in its favour the fact that this is the best represented haploid number among other genera and it is also the base number of *Astrophea*, a subgenus of shrubby and arboreal species, considered one of the most primitive of *Passiflora*. However, since polyploidy is a highly recurrent phenomenon in angiosperms, this widespread representation may be due to independent paleopolyploid lines. Guerra (2000) observed that in many taxa, the most primitive representatives are paleopolyploids that conserve ancient characteristics of the group. The polyploid origin of these representatives is suggested by the frequent occurrence of the chromosome number corresponding to the haploid ($n = 6$, in the case of *Passiflora*) in close taxa, which would hardly be justified by descending dysploidy (see, for example, Félix and Guerra, 2000).

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