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Cytogenetic Studies in Some Species of *Passiflora* L. (Passifloraceae): A Review Emphasizing Brazilian Species

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ABSTRACT

The Passifloraceae is represented by species of tropical and subtropical origin. The Passiflora *is the richest genus with approximately 450 species, 200 of them being native to Brazil. Recent karyological studies have reported the basic chromosome number for the* Passiflora *genus as* $x = 6$*, whereas* $x = 9$, $x = 10$ *and* $x = 12$ *were established as secondary basic numbers. High rates of fertility occur in most* Passiflora *species, since both meiotic index and pollen viability are above 90%. Unusual meiotic behavior has been described in some taxa. Unviable pollen were observed in some diploids species. The genome size varies from 1.83 to 5.36 pg, and significant interspecific variance has been observed. Studies using the FISH methodology have shown that there are two to three rDNA 45S sites and one 5S site in the species analyzed. In this review, information about the above-mentioned studies is presented and discussed in detail.*

Key words: Karyotype, banding, meiotic behavior, pollen viability, nuclear DNA content, FISH

INTRODUCTION

The Passifloraceae family contains more than 600 species of tropical and subtropical origin (Vanderplank, 1991). The *Passiflora* is the richest genus; new species are still being described (McDougal, 2001) and 90% of them are native to the Americas (Leitão Filho and Aranha, 1974; Lopes, 1991). There is a great inter and intraspecific genetic variability in the *Passiflora* (Fajardo et al., 1998; Viana et al., 2003; Vieira and Carneiro, 2004) and more than 200 species are natives to Brazilian regions, putting the country in a privileged position in relation to the *Passiflora* genetic resources (Ferreira, 1994). The *Passiflora edulis* f. *flavicarpa*, the yellow passion fruit, is the species of the highest economic importance in the America and the *P. edulis* f. *edulis*, the purple passion fruit, is marketed in Australia. Brazil and Ecuador are important world producers and consumers. A dozen wild species have edible fruits and are planted in the Latin America, but not widely exploited commercially.

Interspecific crosses were carried out as an alternative for introducing the genes from the wild to the cultivated species. Some sexual hybrids have been obtained in order to increase the ornamental value of the plants (Vanderplank, 1991), to tolerate low temperatures (Knight, 1991), or improved agronomic traits (Knight, 1991;

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Ruberté-Torres and Martin, 1974). Somatic hybrids were also produced to obtain disease or low-temperature resistant plants (Dornelas et al., 1995; Otoni et al., 1995; Barbosa and Vieira, 1997). Self-incompatibility is a somewhat common mechanism in the *Passiflora* (Payán and Martin, 1975; Rêgo et al., 2001; Souza et al., 2006).

Although *Passiflora* cytogenetics has been poorly studied and the chromosome numbers fine known for a very few number of species considering the size of the genus, this kind of analysis would be of importance as a contribution to the breeding and evolutionary studies. Nuclear DNA content, meiotic behavior and pollen viability, karyotype establishment and chromosome markers as banding and *in situ* hybridization are important tools that may be useful to develop the strategies for the germplasm conservation and for understanding the relationships among the *Passiflora* species. Concerning the breeding, the absolute cytological knowledge cooperates for planning the interspecific hybridizations and also giving the information related to the effect of the individual genome on the hybrid pairing.

Chromosome number and ploidy level

The chromosome number is known in less than 30% of the *Passiflora* species (Soares-Scott et al., 2005). Of around of 200 species that are native to Brazil (Oliveira, 1987), the chromosome number of only a few has been reported (Table 1). The *Passiflora* species can be divided into four karyological groups, represented by $x = 6$, $x = 9$, $x = 10$ and $x = 12$ (Melo and Guerra, 2003). The major part presented $2n = 12$ or 18, but $2n = 14$, 20, 22, 24, 36, 72 and 84 were also found in the native and introduced species. Aneuploidy and polyploidy have been reported as the evolutionary mechanisms (Melo et al., 2001), although most of the *Passiflora* species are diploid. Cytotypes were described, *P. misera* Kunth with $2n = 12$ and 36, and *P. suberosa* L. with $2n = 12$, 24, 36. The chromosome number as 22 was reported for *P. foetida*, but according to Melo et al. (2001), this species presented the distended proximal secondary constrictions in prophase and prometaphase that might be confused with two chromosomes leading to wrong interpretation of $2n = 20$. The species presenting $2n = 12$ are considered primitive, or of ancestral *Passiflora* genus (Stebbins, 1950). Storey (1950) examined different species, interspecific hybrids and polyploid races of the *Passiflora* and concluded that the basic chromosome number of this genus was $x = 3$ or $x = 6$. For Darlington and Wylie (1956, quoted by Mehetre and Dahat, 2000), the basic chromosome number was $x = 9$. Recent studies suggested that the basic chromosome number of the genus could be $x_1 = 6$ whereas $x_2 = 9$, $x_2 = 10$ and $x_2 = 12$ are only secondary basic numbers (Melo et al., 2001; Melo and Guerra, 2003). However, $x = 12$ appeared to play an important role in the evolution of the group because it was better represented in the other genera of the family. In this case, the mechanisms of the chromosome alteration most probably related to these changes would be descending dysploidy ($x = 12 \rightarrow 6$) or polyploidy ($x = 6 \rightarrow 12$).

Karyotype studies

Karyotype analysis of the *Passiflora* genus has made possible the observation of intra and interspecific variation in the morphology of the chromosomes. The analysis indicated that the number and position of the satellites, secondary constrictions, number and length of chromosomes, and the position of the centromere were probably characteristics of the subgenera and sections (Beal, 1973a, b; Snow and MacDougal, 1993; Mayeda, 1997; Vieira et al., 2004; Table 2). Some authors have observed intraspecific polymorphism, mainly in relation to the satellite number and the location (Oliveira and Coleman, 1996; Mayeda, 1997; Souza et al., 2003c). Mayeda (1997) suggested the presence of two larger pairs of the chromosomes (asymmetric karyotype) and two satellites as the characteristics of the *Passiflora* genus. However, some species have shown gradual size variation among the chromosomes and only one satellited chromosome pair (Melo et al., 2001; Souza et al., 2003c). According to Melo et al. (2001), the *Xerogona*, *Decaloba* and *Cieca* sections of the *Plectostemma* subgenus presented very characteristic secondary constrictions (Table 3) and their number and position were the most important karyological features, mainly in the *Decaloba* section. Basically species presented the metacentric and submetacentric chromosomes, with variable karyotype symmetry (Melo et al., 2001; Beal, 1973a, b; Vieira et al., 1997; Oliveira and Coleman, 1996; Soares-Scott et al., 1999). The subgenus *Plectostemma* is considered as the most evolved in the *Passiflora* genera (Mayeda, 1997; Vieira et al., 2004) because it presented more asymmetric karyotypes, in agreement with studies related to the plant and herbivore co-evolution (Benson et al., 1975). The chromosome complement length varied significantly from 32.9 to 62.3 µm in the species studied (Beal, 1973a, b; Mayeda, 1997; Souza et al., 2003c; Vieira et al., 2004). Understanding such diversification on genome length is important because it is a significant process during the plant speciation (Greilhuber, 1998).

Table 1 - Chromosome numbers in species of genus *Passiflora*, occurring in Brazil.

Classification (according to Killip 1938)	**Taxon**	***n***	**2*n***
Subgenus *Astrophea* (DC.) Mast.	*P. pentagona* Mast.	12 (Melo et al., 2001; Souza, 2002)	24 (Melo et al., 2001)
Section *Pseudoastrophea* (Harms) Killip	*P. haematostigma* Mart. Ex Mast.	-	24 (Melo and Guerra, 2003)
Subgenus *Calopathanthus* (Harms) Killip	*P. racemosa* Brot.	9 (Heitz, 1926; Melo et al., 2001)	18 (Heitz, 1926; Bowden, 1945; Beckett, 1960)
Subgenus *Distephana* (Juss.) Killip	*P. coccinea* Aubl.	9 (Oliveira and Coleman, 1996)	18 (Beal, 1971; Oliveira and Coleman, 1996; Mayeda, 1997; Melo et al., 2001; Soares-Scott et al., 2001)
	P. glandulosa Cav.	-	18 (Melo et al., 2001)
	P. vitifolia Kunth	9 (Storey, 1950)	18 (Storey, 1950; Snow and McDougal, 1993)
Subgenus *Dysosmia* (DC.) Killip	*P. foetida* Linn.	10 (Storey, 1950; Beal, 1969b, 1971; Oliveira and Coleman, 1996; Melo et al., 2001)	18 (Heitz, 1927; Janaki Ammal, 1945) 20 (Nishiyama and Kondo, 1942; Storey, 1950; Guerra, 1986; Melo et al., 2001) 22 (Bowden, 1945; Harvey, 1966)
Subgenus *Passiflora* Serie *Incarnatae*	*P. incarnata* Linn.	9 (Beal, 1969b) 18 (Lloyd, 1963)	18 (Heitz, 1926; Bowden, 1940, 1945; Storey, 1950; Mayeda, 1997) 36 (Lloyd, 1963)
Serie *Kermesinae* Killip ex Cervi	*P. edmundoi* Sacco	9 (Souza et al., 2003a)	18 (Melo et al., 2001; Souza et al., 2003a)
	P. kermesina Link and Otto	-	18 (Guerra, 1986; Melo et al., 2001)
Serie *Laurifoliae* Killip ex Cervi	*P. nitida* Kunth	9 (Oliveira and Coleman, 1996)	18 (Passos, 1999; Melo et al., 2001)
	P. laurifolia Linn.	9 (Storey, 1950)	18 (Storey, 1950; Simmonds, 1954)
Serie *Lobatae* (Harms) Killip	*P. amethystina* Mikan	9 (Souza, 2002)	18 (Mayeda, 1997; Barbosa and Vieira, 1997a; Melo et al., 2001)
	P. caerulea Linn.	9 (Nakajima, 1931; Simonet and Miedzyrzecki, 1932; Beal, 1969b)	18 (Heitz, 1926; Nakajima, 1931; Simonet and Miedzyrzecki, 1932; Bowden, 1940, 1945; Melo et al., 2001)
	P. elegans Mast.	-	18 (Melo et al., 2001, Melo and Guerra, 2003)
	P. giberti N.E. Brown	9 (Oliveira and Coleman, 1996; Souza, 2002)	18 (Oliveira and Coleman, 1996; Mayeda, 1997)
Serie *Passiflora*	*P. cincinnata* Mast.	9 (Oliveira and Coleman, 1996; Melo et al., 2001; Souza, 2002)	18 (Beal, 1971; Guerra, 1986; Oliveira and Coleman, 1996)

(Cont. ...)

(Cont. Table 1)

Classification (according to Killip 1938)	**Taxon**	***n***	**2*n***
	P. edulis Sims. f. *edulis*	9 (Storey, 1950; Beal, 1969a, b; Gill et al., 1984; Souza, 2002)	18 (Janaki Ammal, 1945; Storey, 1950; Beal, 1969a; Guerra, 1986; Mayeda, 1997)
	P. edulis Sims. f. *flavicarpa* Deg.	9 (Beal, 1969b, Storey, 1950; Souza, 2002)	18 (Storey, 1950; Oliveira and Coleman, 1996; Barbosa and Vieira, 1997a; Melo et al., 2001)
	P. filamentosa Cav	9 (Beal, 1969b)	18 (Beal, 1969b)
Serie *Quadrangularis* (Harms) Killip	*P. alata* Dryand.	9 (Beal, 1969b; Souza, 2002)	18 (Guerra, 1986; Mayeda, 1997; Melo et al., 2001; Souza et al., 2003)
	P. quadrangularis Linn.	9 (Beal, 1969b)	18 (Janaki Ammal, 1945; Storey, 1950; Beckett, 1960; Beal, 1969b; Melo et al., 2001; Souza et al., 2003) 36 (Soares-Scott et al., 1999)
	P. seemannii Griseb.	-	18 (Beal, 1973a)
Serie *Serratifoliae*	*P. malacophylla* Mast.	9 (Souza, 2002)	18 (Souza et al., 2003)
Serie *Setaceae* Killip ex Cervi	*P. setacea* DC.	9 (Oliveira and Coleman, 1996; Melo et al., 2001)	18 (Soares-Scott et al., 1999)
Serie *Simplicifoliae* Harms) Killip	*P. actinia* Hook.	-	18 (Melo et al., 2001; Soares-Scott et al., 2001)
	P. galbana Mast.	-	18 (Melo et al., 2001; Souza et al., 2003)
	P. jilekii Wawra	9 (Melo et al., 2001)	-
	P. mucronata Lam.	9 (Souza et al., 2001)	18 (Guerra, 1986; Melo et al., 2001; Soares-Scott et al., 2001; Souza et al., 2003)
Serie *Tiliaefoliae*	*P. ligularis*	9 (Beal, 1969b; 1971)	18 (Storey, 1950; Beal, 1969b; Soares-Scott et al., 2001)
	P. maliformis Linn.	9 (Storey, 1950; Beal, 1969b)	18 (Storey, 1950; Beal, 1969b; Mayeda, 1997)
Subgenus *Plectostemma* Mast. Section *Cieca* (Medic.) Mast.	*P. coriacea* Juss.	6 (Beal, 1971)	12 (Snow and McDougal, 1993; Oliveira and Coleman, 1996; Melo et al., 2001)
	*P. gracilis*J. Jacq. Ex Link	6 (Beal, 1971)	12 (Beal, 1971) 18 (La Cour, 1951) 20 (Bowden, 1945)
	P. morifolia Mast.	6 (McDougal, 1983; Souza, 2002)	12 (Melo et al., 2001)
	P. suberosa Linn.	6 (Stebbins, 1950; Diers, 1961) 12 (Storey, 1950; Beal, 1969b; Melo et al., 2001; Souza, 2002) 18 (Storey, 1950)	12 (Stebbins, 1950; Diers, 1961) 24 (Storey 1950; Beal, 1969b, 1971; Snow and MacDougal, 1993; Melo et al., 2001) 36 (Storey, 1950)

(Cont. ...)

(Cont. Table 1)

Classification (according to Killip 1938)	**Taxon**	***n***	**2*n***
	P. warmingii Masters	6 (Beal, 1971)	12 (Beal, 1971)
Section *Decaloba* (DC.) Mast.	*P. misera* Kunth	-	12, 36 (Melo et al., 2001)
	P. tricuspis Mast.	-	12 (Melo et al., 2001)
Section *Xerogona* (Raf.) Killip.	*P. capsularis* Linn.	6 (Souza, 2002)	12 (Bowden, 1945; Beal, 1971; Snow and MacDougal, 1993; Mayeda, 1997; Melo et al., 2001)
	P. rubra Linn.	6 (Souza, 2002)	12 (Snow and MacDougal, 1993; Melo et al., 2001)

Table 2 - Some characteristics of karyotypes and nuclear DNA content (2C) in species of genus *Passiflora*, occurring in Brazil.

Taxon	**LHC**	**r**	**NS /SC**	**TF (%)**	**Nuclear DNA content (pg)**
P. coccinea	18.28 (Oliveira and Coleman, 1996) 21.39 (Mayeda, 1997)	-	3/3 (Melo et al., 2001)	40.71 (Mayeda, 1997)	-
P. incarnata	18.56 (Mayeda, 1997)	-	3/3 (Soares-Scott et al., 1999) 1/3 (Mayeda, 1997)	42.26 (Mayeda, 1997)	-
P. edmundoi	23.96 (Souza et al., 2003)	1.12 (Souza et al., 2003)	1/1 (Souza et al., 2003)	46.78 (Souza et al., 2003)	3.43 (Souza, 2002)
P. kermesina			3/3 (Melo et al., 2001)		-
P. nitida	-	-	-/3 (Passos, 1999) 2/2 (Melo et al., 2001)		4.82 (Souza et al., 2003b)
P. laurifolia	-	-	-	-	3.88 (Souza, 2002)
P. amethystina	21.06 (Mayeda, 1997)	-	2/2 (Mayeda, 1997)	43.97 (Mayeda, 1997)	3.36 (Souza et al., 2003b)
P. giberti	20.01 (Oliveira and Coleman, 1996) 22.44 (Mayeda, 1997)	-	2/2 (Oliveira and Coleman, 1996; Mayeda, 1997)	41.91 (Mayeda, 1997) 42.46 (Vieira et al., 2004)	3.92 (Souza, 2002)
P. cincinnata	25.82 (Oliveira and Coleman, 1996)	-	-	-	-
P. edulis f. *edulis*	19.82 (Mayeda, 1997)	-	2/2 (Mayeda, 1997)	44.2 (Mayeda, 1997)	3.16 (Souza, 2002)
P. edulis f. *flavicarpa*	19.3 (Oliveira and Coleman, 1996) 18.8 (Mayeda, 1997)	-	-/1 (Oliveira and Coleman, 1996) 2/2 (Mayeda, 1997) -/3 Soares-Scott et al., 1999)	44.9 (Mayeda, 1997)	3.19 – Brazil 3.21 – Mexico (Souza, 2002)

(Cont. ...)

(Cont. Table 2)

Taxon	LHC	r	NS /SC	TF (%)	Nuclear DNA content (pg)
P. alata	22.36 (Mayeda, 1997) 22.38 (Souza et al., 2003)	1.25 (Souza et al., 2003)	2/2 (Mayeda, 1997) 2/2 (Melo et al., 2001) 3/3 (Souza et al., 2003)	42.82 (Mayeda, 1997) 45.3 (Souza et al., 2003)	-
P. quadrangularis	62.8* (Beal, 1973a) 33.58 (Souza et al., 2003)	1.5 (Beal, 1973a) 1.59 (Souza et al., 2003)	6/6 (Beal, 1973a) 2/2 (Souza et al., 2003)	38.68 (Souza et al., 2003)	5.36 (Souza, 2002)
P. seemannii	55.9 – 66.9 (Beal, 1973a)	-	-	-	-
P. malacophylla	14.67 (Souza et al., 2003)	1.37 (Souza et al., 2003)	1/1 (Souza et al., 2003)	43.42 (Souza et al., 2003)	-
P. galbana	17.9 (Souza et al., 2003)	1.24 (Souza et al., 2003)	2/2 (Souza et al., 2003)	44.41 (Souza et al., 2003)	3.52 (Souza et al., 2003b)
P. mucronata	22.54 (Souza et al., 2003)	1.38 (Souza et al., 2003)	2/2 (Melo et al., 2001; Souza et al., 2003)	42.01 (Souza et al., 2003)	3.4 (Souza, 2002)
P. maliformis	55.9* (Beal, 1973a) 22.54 (Mayeda, 1997) 21.20 (Vieira et al., 2004)	1.2 (Beal, 1973a) -	4/4 (Beal, 1973a) 2/2 (Mayeda, 1997) -	46.21 (Mayeda, 1997) 46.22 (Vieira et al., 2004)	3.78 (Souza et al., 2003b)
P. morifolia	13.43 (Melo et al., 2001)	1.72 (Melo et al., 2001)	1/1 (Melo et al., 2001)	-	-
P. suberosa	55.1* (Beal, 1973a)	1.3 (Beal, 1973a)	5/5 (Beal, 1973b)	-	1.85 (Souza, 2002)
P. misera	9.93 (Melo et al., 2001)	1.87 (Melo et al., 2001)	1/1 (Melo et al., 2001)	-	-
P. tricuspis	10.89 (Melo et al., 2001)	1.67 (Melo et al., 2001)	1/1 (Melo et al., 2001)	-	-
P. capsularis	8.03 (Mayeda, 1997)	-	1/1 (Snow and MacDougal, 1993) 1/2 (Mayeda et al., 1997)	39.55 (Mayeda, 1997)	-

*Total chromosome length in the complement (Beal, 1973a). LHC = length of haploid complement; r = ratio of length of long arms to length of short arms; NS/CS = number of satellites/secondary constrictions; TF (%) = asymmetry index or index total form percent (TF%; Huziwara, 1962).

Table 3 - Karyological features in subgenus of *Passiflora*.

	Subgenus *Plectostemma*		
	Sections		
Authors	***Xerogona***	***Decaloba***	***Cieca***
Melo et al., 2001	SC in the terminal region of the largest chromosome pair. Observed in *P. capsularis* and *P. rubra*.	SC in the proximal region of the One of the smallest chromosome pairs*. Observed in *P. misera* and *P. tricuspis.*	SC in the terminal region of the smallest chromosome pair. Observed in *P. morifolia.*
Mayeda, 1997	Two SC in the largest chromosome pairs and only one pair satellited. Observed in *P. capsularis*.	-	-
	Subgenus *Passiflora*	Subgenus *Distephana*	Subgenus *Tacsonia*
Mayeda, 1997; Vieira et al., 2003	Two pairs of largest chromosomes, being the chromosome 1 submetacentric and 2 metacentric. SC and satellites on two smallest pairs. Observed in *P. alata*, *P. edulis*, *P. amethystina*, *P. giberti*.	Chromosome 1 submetacentric And 2 metacentric. Observed in *P. coccinea*. -	SC in three chromosome pairs. Observed in *P. molissima***. -
Passos, 1999	SC on three chromosome pairs. Observed in *P. nitida*.		

SC = secondary constrictions. *Similar observation reported by Beal (1973b). ** Introduced species (Sousa and Meletti, 1997).

Banding and in situ hybridization

Banding pattern studies have been performed using stains with affinity for A-T or C-G bases (Melo et al., 2001) and with silver to stain the nucleolus organizer region (Mayeda, 1997). Howeverk(□sw species have been included in these studies, which allow the individual classification of the chromosomes and the analysis of the genomic relationships among the related species. The studies involving Ag-NOR banding, where only active NORs were specifically detectable (Scwarzacher et al., 1980), were performed in *P. alata*, *P. amethystina*, *P. coccinea*, *P. edulis* f. *flavicarpa*, *P. incarnata* and *P. maliformis*. In some species, there was more than one pair of the chromosomes carrier of the nucleolus organizer region, as in *P. coccinea* (Mayeda, 1997; Vieira et al., 2004). The CMA-DAPI banding was performed on eight species, *P. amethystina*, *P. caerulea*, *P. capsularis*, *P. edulis* f. *edulis*, *P. foetida*, *P. racemosa*, *P. rubra* and *P. tricuspis*. These species showed from one to three pairs of the CMA$^+$ blocks but did not present the DAPI$^+$ heterocromatin. The CMA$^+$ block positions were in agreement with the morphological features observed on the chromosome pairs in relation to the presence of the secondary constrictions (Melo et al., 2001) and with both the number and position of the 45S rDNA sites detected by the FISH. On the other hand, the number of 5S rDNA sites did not correlate with any other cytological parameter (Melo and Guerra 2003).

The studies aiming at the mapping of the 45S rDNA showed the presence of secondary constrictions and satellites associated to the chromosomes 8 and 9 in *P. edulis* f. *edulis*, with four sites, and *P. amethystina*, with six sites. Both the species were used as the parental, and the somatic hybrid presented two sites more than both the parental (Cuco et al., 2001). Melo and Guerra (2003) analyzed the variation on 5S and 45S rDNA sites in 20 *Passiflora* species (21 taxons) and observed that the diploid species with $x = 6$, in general, presented only two 5S sites and two or four 45S rDNA sites, which were clearly related to the ploidy level, while others species with $x = 9$

and $x = 10$ frequently displayed more than two sites of the 45S and 5S rDNA. According to these authors, in general, the number and localization of 5S and 45S rDNA sites were consistent with the hypothesis of $x = 6$, originally diploid, as the probable ancestral genome for the genus, while the group of species with $x = 9$, $x = 10$ and $x = 12$ were considered to be of tetraploid origin with descending dysploidy (12 → 10 → 9), and reduction (gene silencing) of some redundant sites, mainly those of 5S rDNA.

Meiotic behavior and pollen viability

Meiotic studies performed on the cultivated and wild *Passiflora* species indicated meiotic stability (Barbosa and Vieira 1997a, b; Melo et al., 2001; Souza, 2002; Souza et al., 2003a). The species with $2n = 18$, in general, presented regular meiosis and high fertility (Ferreira, 1994). Some species with $2n = 24$, *P. pentagona* and *P. suberosa*, presented unusual meiotic behavior; many irregularities were observed that could lead these species to a meiotic index below 90% (Souza, 2002). The laggard chromosomes, anaphase bridges (Barbosa and Vieira, 1997b; Souza, 2002), non-oriented segregation at metaphase and anaphase I (Souza et al., 1996), spindle orientation abnormalities and asynchronism were the most common irregularities observed, and consequently, anomalous post-meiotic products as monads, dyads, triads and polyads were formed (Souza, 2002; Souza et al.; 2003a). The species displayed bivalents as the predominant chromosome associations but univalents and quadrivalents in diploid species have also been observed (Melo et al., 2001; Souza, 2002; Souza et al., 2003a). Studies in the interespecific F_1 hybrids have demonstrated meiotic instability. According to Soares-Scott et al. (2003), there were clear differences in the meiotic behavior between the hybrids, and the sexual hybrid showed more regular meiosis than the somatic hybrid.

Studies on the chiasmata frequency and position are rare in *Passiflora*. Souza (2002) observed that the mean total number of the chiasmata observed in *Passiflora* species with $n = 6$ was 6.6 while in species with $n = 9$ and $n = 12$ were 10.5 and 12.1, consecutively. The recombination index ranged up to 16.49% among the species with $n = 6$ and up to 34% among the species with $n = 9$, while in the species with $n = 12$ the variation among species was practically none (only 0.7%). The interstitial chiasmata predominated. The meiotic index was below 89% in $n = 12$ species and above 92% for all the other species studied. The meiotic behavior in the species group with $n = 12$ was the only one considered similar due to the approximately 12 chiasmata per cell. Chiasma tended to take place in the interstitial position and MI of approximately 88%. According to Love (1951), the plants with an MI of 90 to 100% could be considered as stable cytologically, but actually it was impossible to ensure stability in the plants with index of 88 to 92%, since plants with an MI less than 90% were likely to provide difficulties for the plant breeder in the interspecific crosses.

The pollen viability in the cultivated species has been above 80% (Barbosa and Vieira, 1997b; Otoni et al., 1995; Souza, 2002; Souza et al., 1998), while in wild species values were observed between 99.5 to 78.2% (Barbosa and Vieira, 1997a; Ruberté-Torres and Martin, 1974; Souza, 2002). The pollen viability variation was observed in *P. incarnata*, from 55 to 85.3% during two years of observation (Soares-Scott et al., 2003). According to Souza et al. (2004), it was possible to observe two types of unviable pollen: empty (T1) and shrunken (T2), which could be related to the type of the gene action. A higher T1 pollen percentage (small-sized unviable) in relation to T2 suggested that meiotic irregularities occurred during the microsporogenesis, were possibly the main cause of the pollen inviability in the *Passiflora* species analyzed, although post-meiotic genes would also be acting during the microgametogenesis in the passion fruit. Previous meiotic behavior studies in some *Passiflora* species showed meiotic irregularities to the percentage of unviable pollen (Soares-Scott et al., 1995; Souza et al., 1996; Barbosa and Vieira, 1997a). Abnormally large pollen grains (giant cells, up to 54% larger than the mean size of normal pollen) were observed in the *Passiflora*, probably indicating the existence of imbalanced or non-reduced gametes (Souza, 2002).

Nuclear DNA content

In the last decade, studies using the flow cytometry to obtain nuclear DNA content data have intensified. The genome size in picograms (1pg = 10^{-12}; Heslop-Harrison and Schwarzacher, 1996) or megabase pairs (1 pg = 980 Mbp; Bennett et al., 2000), genome organization (base composition; Dolezel et al., 1992), and ploidy analysis have been obtained by this technique.

This methodology has been employed because of the precision and the reproducibility of the analysis, easy sample preparation, small quantity of plant material requirement and rapidity in obtaining the results. It

also contributes with plant breeding programs, which involve interspecific hybridization, since in some cases, when parental species have different nuclear DNA content, the flow cytometer may detect interspecific hybrids according to its intermediary DNA values (Keller et al., 1996).

Species of the *Passiflora* genus present great phenotypic variation, and the few published genome size results show significant differences among the species. Nuclear DNA content was initially determined in pg for *P. antioquiensis* (3.00) using densitometry (Ingle et al., 1975) and *P. menspermifolia* (4.54) using flow cytometry (Arumuganathan and Earle, 1991). Flow cytometry was used to confirm the occurrence of the hybridization and for ploidy determination in *P. edulis* f. *flavicarpa* + *P. incarnata* somatic hybrids (Otoni et al., 1995). In recent studies, this technique has been shown to be efficient to determine the nuclear DNA content in the *Passiflora* species (Souza, 2002; Souza et al., 2003b), which present medium genome size (Tabela 2). Only significant interspecific variance has been observed.

CONSIDERATIONS

In view of the world tendency to eliminate the wild species as a consequence of the deforestation, some *Passiflora* species are endangered, and genotypes which would be useful for the breeding programs have disappeared. Moreover, cytological studies on the wild species are basic for understanding the chromosome evolution of this genus and the Passifloraceae family. For the plants, including wild and cultivated *Passiflora* species that are used in the interspecific hybridization, the ability to manipulate meiosis and recombination would have great commercial importance. However, meiosis is a highly dynamic process, and no technique using fixed, dead material will reveal all its subtleties; imaging techniques that can reveal dynamic events in the living organisms are expected to be applied in the near future (Shaw and Moore 1998). Flow cytometry would play an important role in the genetic studies of the *Passiflora* since species C-values (haploid DNA quantity) might coincide with their crossability relationships (Ohri, 1998) being useful in taxa or genotype selection that would be used in the crosses.

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RESUMO

A família Passifloraceae é representada por espécies de origem tropical e subtropical. *Passiflora* é o gênero mais rico, com aproximadamente 450 espécies, cerca de 200 delas nativas do Brasil. Recentes estudos cariológicos têm relatado o número básico de cromossomos para o gênero *Passiflora* como sendo x = 6, enquanto x = 9, x = 10 e x = 12 foram considerados números básicos secundários. Altas taxas de fertilidade são observadas na maioria das espécies de Passiflora, uma vez que o índice meiótico e a viabilidade polínica apresentam-se acima de 90%.Comportamento meiótico irregular tem sido descrito para alguns taxas. Grãos de pólen inviáveis foram observados em espécies diplóides. O tamanho do genoma varia de 1,83 a 5,36 pg, e variação interespecífica significativa tem sido observada. Estudos usando a metodologia de hibridização *in situ* (FISH) tem demonstrado haver de dois a três sites de DNAr 45S e um site de DNAr 5S nas espécies analisadas. Nesta revisão, informações sobre os estudos acima mencionados são apresentados e discutidos em detalhes.

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