Karyotype determination in three Caricaceae species emphasizing the cultivated form (*C. papaya* L.)

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Abstract — Papaya (*Carica* papaya L.) is the most important species of Caricaceae family. All Caricaceae species are classified as diploids (2n=2x=18 chromosomes) and dioecious, except for C. *papaya*, *V. monoica* e *V. cundinarmacensis*. The plant sexual determination in papaya is due to one gene with three alleles, however, sometimes, the literature reports the presence of a sexual chromosome. The objective of this study was to determine the karyotype of three Caricaceae species, the cultivated form (*C. papaya*) and two wild species (*V. monoica* and *V. cundinarmacensis*). The arm ratio of each chromosome (r), the total haploid complement (THC) and the centromeric index were estimated. Seeds were germinated and root tips were pretreated and stained with Giemsa. Eighteen chromosomes were counted for all three species, confirming the chromosome number previously related for the family. The three species have symmetric and similar karyotypes, with small and metacentric chromosomes. The chromosome sizes ranged from 2.29 µm to 1.52 µm in *C. papaya*, from 2.49 µm to 1.35 µm in *V. monoica* and from 2.45 µm to 1.66 µm in *V. cundinarmacensis*. The THC ranged from 18.69 µm (*V. cundinamarcensis*) to 17.11 µm (*V. monoica*). It was not observed sexual chromosome in this study. Thus, if there are sexual chromosomes in *C. papaya*, they are probably homomorphic. Some sophisticated techniques should be applied in order to elucidate this question. All data presented here have been karyologically unpublished, except for the ploidy number.

Key words: *C. papaya*, homomorphic chromosomes, sexual chromosomes, *V. cumdinamarcensis*, *V. monoica*.

INTRODUCTION

The Caricaceae family consists of six genera: *Carica*, with a single species (C*arica papaya*), *Vasconcellea* (21 species), *Cylicomorpha* (2 species), *Jarilla* (3 species), *Jacaratia* (7 species) and *Horovitzia* (1 species) (Badillo 2000). All species are dioecious, with the exception of *V. monoica* that is strictly monoecious and *V. cundinamarcensis* and *C. papaya* that have dioecious and/or andromonoecious individuals (Badillo 1971; Storey 1976). Up to date, all species studied are diploid with 2n=2x=18 chromosomes (Darlington and Ammal 1945).

Sex determination in papaya (*C. papaya* L.) is due to a single gene with three allelic forms: *m,* M_1 and M_2. The *mm*, M_1m, and M_2m genotypes represent gynoecious, androecious and hermaphrodite individuals, respectively (Hofmeyr 1938; Storey 1938; 1953).

Hofmeyr (1941) established the hypothesis that the sex determination in papaya involves a genetic balance. The M_1 and M_2 symbols represent inert or inactivated regions of different sizes that can be found in the sex chromosomes. Since M_1 is a long inert region, it is phenotypically expressed as staminated and it is greatly influenced by the autossomal genes. The M_2 region smaller than M_1, is less influenced by the autosome genes and thus is phenotypically expressed as hermaphrodite. Thus, the heterozygosity of the M_1m and M_2m genotypes become them vulnerable to alterations in the phenotypic expression or sexual reversion caused by environmental factors. The M_1M_1, M_2M_2 and M_1M_2 genotypes are not found due to the zygotic lethality.

The *m* homologous region is normal and the viable genotypes are M_1m (male plant), M_2m

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(hermaphrodite plant) and *mm* (female plant). A large concentration of genes for femaleness is in the sex chromosomes but genes for maleness are in the autosomes. Therefore, the *mm* genotype is pistillated and its homozygote condition confers phenotypic stability (HOFMEYR 1941). HOROVITZ and JIMENEZ (1967) studied other Caricaceae species and proposed a similar hypothesis to that by HOFMEYR (1938) and STOREY (1938), using the sex chromosomes terminology *X, Y* and *Z*. The *XX* genotype represents female plants, XY_1 male plants, XY_2 hermaphrodite plants (only in *C. papaya)* and *ZZ* the monoecious species (*V. monoica*). In hybrids derived from crossing between dioecious and monoecious species, the *XZ* genotype can be monoecious or pistillated, depending on the pisttilated plant used as parent. The Y_1Y_1, Y_1Y_2 and Y_2Y_2 combinations are lethals. This hypothesis also postulates that the *Z* chromosome, homologous to *X* and *Y*, contains an *F* gene that controls the expression of femaleness (gynodioecious) and an *Am* gene that controls the expression of maleness (androdioecious). Although these authors referred to sex chromosomes in the plant sexual determination, they do not reported the existence of sex chromosomes, neither heteromorphic nor homomorphic types in these species. WESTERGAARD (1958) considered that the study of sex genetics in plants should first establish if sex chromosomes actually exist, and, if they do, characterize them as homomorphic or heteromorphic. LIU *et al*. (2004) using molecular markers in papaya concluded that this species has an incipient Y chromosome, with a specific male region that corresponds to 10% of the sex chromosome. This region shows crossing over suppression and degeneration in the DNA sequence.

Considering the lack of reports in the literature about the karyotype and the existence of sex chromosomes in papaya and its related species, this research was carried out to determine the karyotype for three species belong to the Caricaceae family, *C. papaya*, *V. monoica* and *V. cundinamarcensis*.

MATERIAL AND METHODS

Seeds from *C. papaya* and *V. monoica* were germinated in B.O.D. chamber at 27.5°C with eight hours of light and 16 hours of dark. Seeds of two papaya cultivars were used: *Golden* (Solo group) and *Maradol* (Formosa group). Seeds from *V. cundinamarcensis* were germinated in pots and seedlings were grown under greenhouse conditions.

Root tips with 1-2 cm length from the first two species were pretreated with a saturated solution of paradyclorobenzene at 4°C for 8h, while root tips from *V. cundinamarcensis* were pretreated with 2 µM trifluralin solution for 21h at 4°C. The root tips were fixed in 3:1 methanol: acetic acid and stored in a freezer (-20°C) until use.

Four root tips of each species were transferred to 1 ml tubes and submitted to enzymatic digestion, according to JEWELL and ISLAM-FARIDI (1994), with minor modifications. In the last step, the protoplasts were resuspended in 4:1 methanol: acetic acid. Two µl of this suspension was dropped on a slide that was air-dried. The slides were observed under an Olympus BX60 microscope, using phase contrast. The five best slides were selected, stained with 5% Giemsa, sealed, and observed under an optical microscope. For NOR and satellite observation, a protocol using silver nitrate was applied (GUERRA and SOUZA 2002).

High-resolution digital images were captured using a 3.3. MPixel Qcolor3C digital camera connected to the Olympus BX60 optical microscope. They were analyzed using the *Image-Pro Plus Software* (5.1 version, *Media Cybenertics*).

The chromosomes were measured using the MicroMeasure 3.3 software (REEVES and TEAR 2000). The parameters analyzed were: absolute chromosome size (µm), long and short arms length, arm ratio (r = long arm/short arm), total haploid complement (THC = sum of the absolute length of the metaphasic chromosomes), and the centromeric index (CI = (short arm length/total length) x 100).

The absolute size and the arm ratio were observed to determine the homologous pairs. The chromosomes were classified according to GUERRA (1986) who considered the arm ratio (LEVAN *et al*. 1964) and the centromeric index (CI). The ideograms and cariograms were based on chromosome measurements taken from five metaphasic plates of different samples. The data from absolute chromosome size and HLL were analyzed and tested by F test at 5% of probability.

RESULTS AND DISCUSSION

Conventional staining was used in the present study and resulted in the complete staining of the chromosomes. This technique gave good results, showing the spread chromosomes with a little overlapping. Eighteen chromosomes were counted in all observed cells (Fig. 1), confirming the previously reported number for the Caricaceae family (DARLINGTON and AMMAL 1945).

It was not possible to observe the NOR region based on the silver nitrate protocol. However it is possible to suggest that in *C. papaya* the NOR region is on chromosome 6 and in *V. monoica* is on chromosome 3, based on the conventional staining (Fig. 1E and F).

The analysis of variance showed that there was no significant difference among species for the means of the nine chromosome pairs, except for pair 8 (Table 1), where the largest size was observed in *V. cundinamarcensis* and the shortest was observed in *V. monoica*. The chromosome length in the cultivated species ranged from 2.29 µm to 1.52 µm, from 2.49 µm to 1.35 µm in *V. monoica* and from 2.45 µm to 1.66 µm in *V. cundinamarcensis*. Small size chromosomes were prevalent (Table 1).

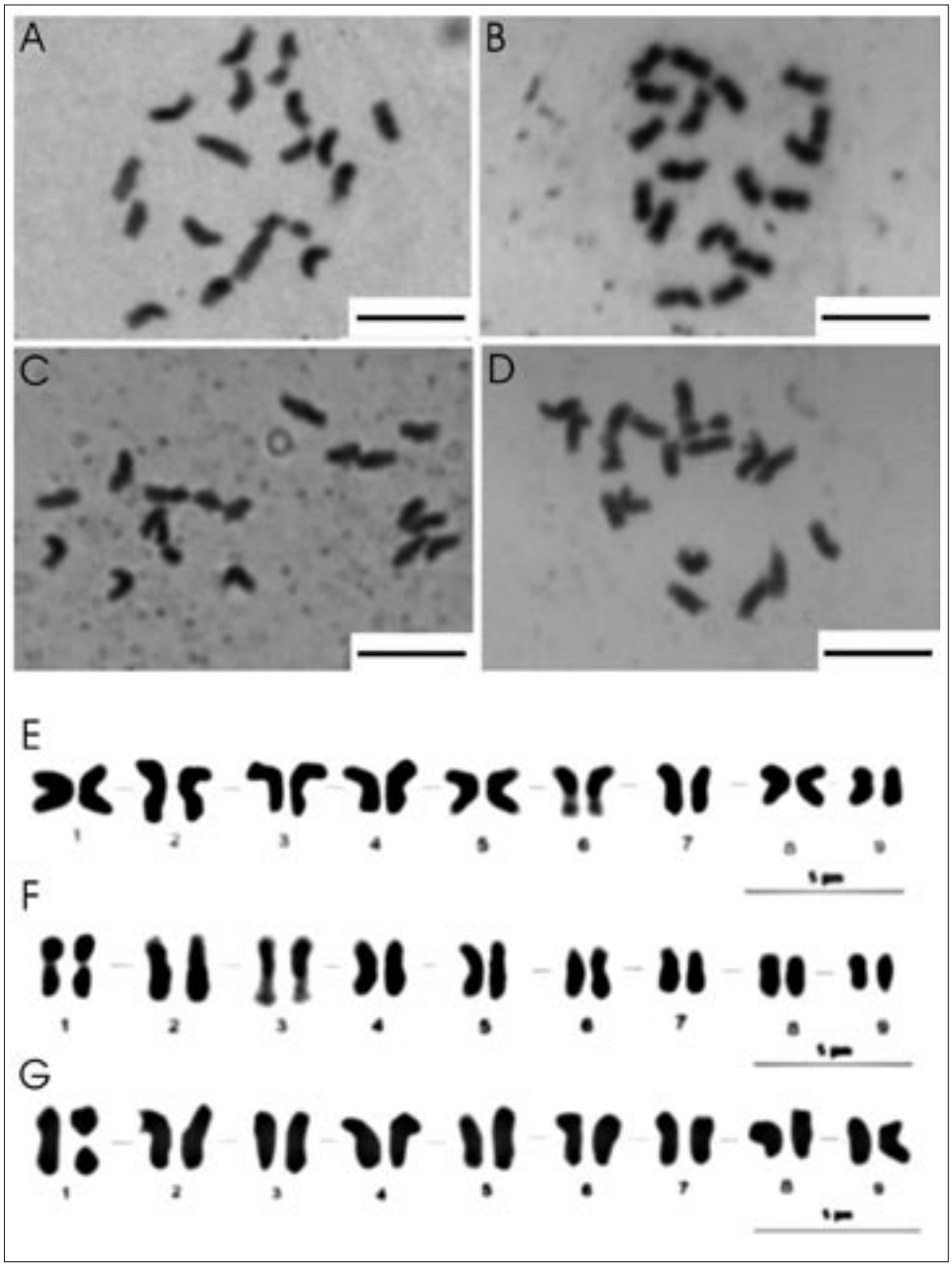

Fig. 1 — Mitotic metaphases and karyograms of three Caricaceae species (2n=18 chromosomes). A) *Carica papaya* (Golden); B) *C. papaya* (Maradol); C) *Vasconcellea monoica*; D) *Vasconcellea cundinamarcensis*. E-G) karyograms of *C. papaya* (E), *V. monoica* (F) e *V. cundinamarcenisis* (G). Bar= 5 µm.

Table 1 — Mean length (µm) and standard error of long arms (LA), short arms (SA), chromosome (C), arm ratio (r), centromeric index (CI), and chromosome type (CT) in Caricaceae species.

		1	2	3	4	5	6	7	8	9	TOTAL
C. papay	LA	1.23±0.12	1.15±0.17	1.15±0.19	1.06±0.17	1.09±0.14	1.01±0.06	0.94±0.08	0.93±0.09	0.80±0.12	9.36
	SA	1.06±0.12	1.00±0.09	0.91±0.07	0.93±0.06	0.85±0.07	0.84±0.10	0.80±0.08	0.71±0.08	0.72±0.09	7.82
	C	2.29±0.19	2.15±0.25	2.06±0.22	1.99±0.17	1.94±0.09	1.85±0.09	1.74±0.17	1.64±0.13	1.52±0.19	17.18
	r	1.16	1.15	1.26	1.14	1.28	1.20	1.18	1.31	1.11	
	CI	46.28	46.51	44.17	46.73	43.81	45.40	45.97	43.29	47.36	
	CT	M	M	M	M	M	M	M	M	M	
V. monoica	LA	1.43±0.18	1.22±0.11	1.18±0.20	1.08±0.08	1.06±0.17	0.97±0.11	0.94±0.14	0.90±0.06	0.76±0.07	9.54
	SA	1.06±0.09	0.99±0.08	0.94±0.07	0.90±0.08	0.82±0.10	0.82±0.08	0.76±0.06	0.69±0.08	0.59±0.07	7.57
	C	2.49±0.20	2.21±0.12	2.12±0.13	1.98±0.13	1.88±0.14	1.79±0.14	1.70±0.15	1.59±0.10	1.35±0.12	17.11
	r	1.34	1.23	1.25	1.20	1.29	1.18	1.23	1.30	1.28	
	CI	42.57	44.79	44.33	45.45	43.61	45.81	44.70	43.39	43.70	
	CT	M	M	M	M	M	M	M	M	M	
V.cundinamarcensis	LA	1.25±0.03	1.27±0.03	1.26±0.19	1.28±0.14	1.11±0.04	1.00±0.02	1.00±0.02	0.93±0.01	0.87±0.04	9.97
	SA	1.20±0.04	1.13±0.07	1.03±0.12	0.90±0.13	0.94±0.07	0.98±0.02	0.88±0.06	0.87±0.01	0.79±0.12	8.72
	C	2.45±0,01	2.40±0.03	2.29±0.07	2.18±0.01	2.05±0.02	1.98±0.05	1.88±0.03	1.80±0.01	1.66±0.16	18.69
	r	1.04	1.12	1.22	1.42	1.18	1.02	1.13	1.06	1.10	
	CI	48.97	47.08	44.97	41.28	45.85	49.49	46.80	48.33	47.59	
	CT	M	M	M	M	M	M	M	M	M	

There were no significant differences among the total haploid complement (THC). The THC values were 18.69 µm, 17.11 µm and 17.18 µm for *V. cundinamarcensis*, *V. monoica, and C. papaya*, respectively.

LEVAN *et al*. (1964) classified the chromosomes in six types, based on their centromeric position: *M, T, m, sm, st,* and *t,* where *M* and *T* are symbols used for chromosomes whose centromeres is located exactly in the mid portion (metacentric) and the terminal portion (telocentric) of the chromosome, respectively. On the other four types, the centromeres would be located in the median (*m*), sub-median (*sm*), sub-terminal (*st*) and terminal (t) regions. GUERRA (1986) revised this classification and suggested the use of the arm ratio (r) and the centromeric index (CI) to classify the chromosomes in four types: metacentric (*M*, r = 1.00 to 1.49; CI = 40.1 to 50.0), submetacentric (*SM*, r = 1.50 to 2.99, CI = 25.1 to 40.0), acrocentric (*A*, r = 3.00 to 7.00, CI = 0.01 to 25.00) and telocentric (*T*, r = ∞, CI = 0). The author reported that this classification was more appropriate for species with small chromosomes.

The ideogram of the three species (Fig. 2) showed that their genomes consisted of metacentric chromosomes, according to Guerra's classification. All species had symmetric and similar karyotypes, where metacentric chromosomes predominated. Based on these results, it is possible infer that although there is a great morphological chromosomal similarity among these species, it is not enough to obtain hybrids between *C. papaya* and the other Caricaceae species.

The symmetrical karyotype makes difficult the distinction among chromosome pairs and other parameters are necessary to designate them. DATTA (1971) used conventional staining to analyze the karyotype of five papaya varieties and observed that the chromosomes were short (from 1 to 4.25 µm) and metacentric or submetacentric. Errors or differences in the measurement of small chromosomes can occur, due to differences in the arm condensation, mechanical distortions, differences in chromosome arm colors and others difficulties (SYBENGA 1959).

No heteromorphic or unpaired chromosomes were observed or even any chromatin corpuscle that would suggest the presence of sex chromosomes. DATTA (1971) also did not observe any heteromorphic pair or even a chromosome without its homologous and concluded that sex determination in papaya may be due to cytoplasmic factors that can not detected by conventional cytological techniques. COSTA *et al*. (2007) using

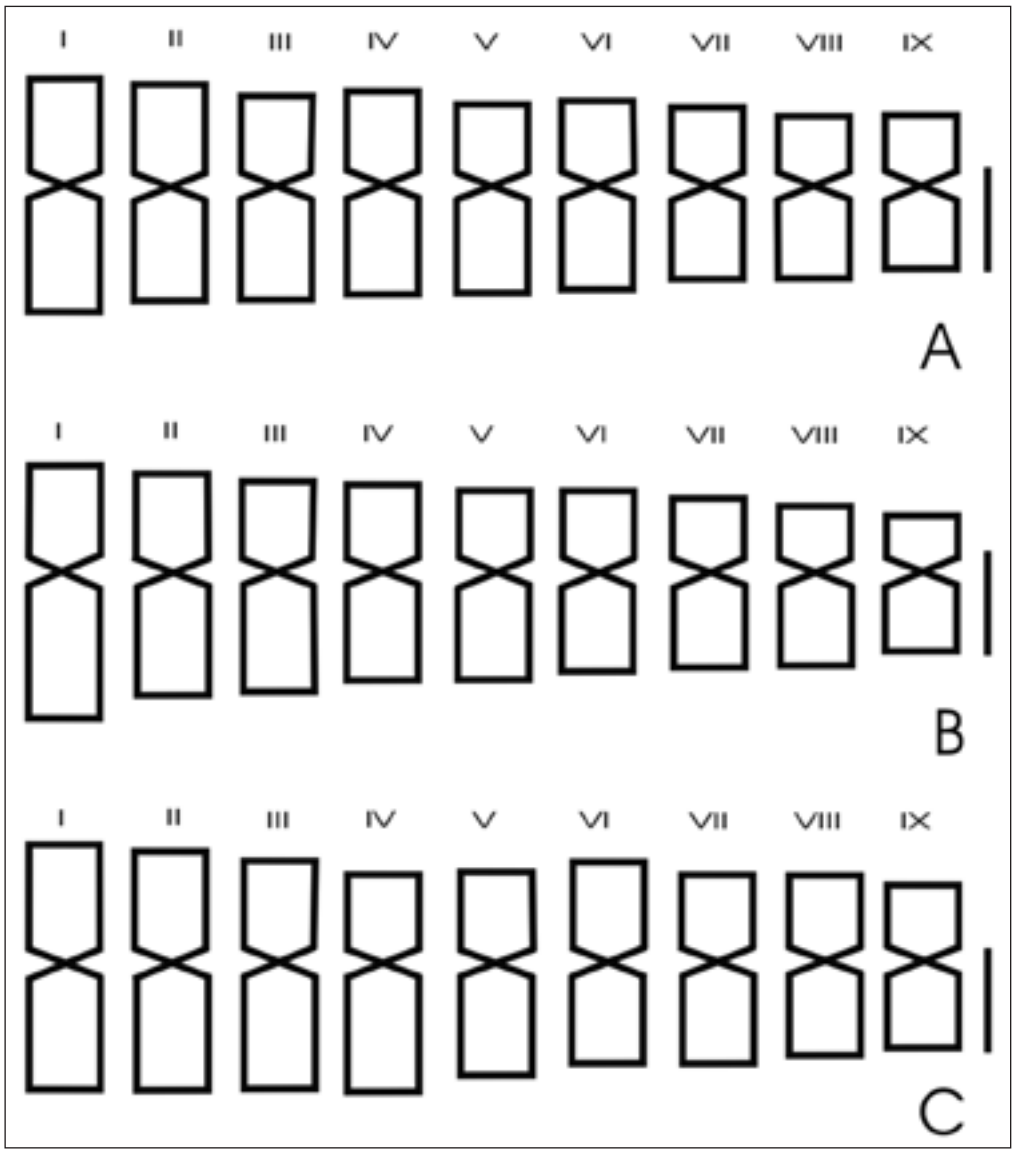

Fig. 2 — Idiograms in Caricaceae species, 2n=18 chromosomes. A) *C. papaya*.; B) *V. monoica*; C) *V. cundinamarcensis*. Bar= 1μm.

FISH technique on papaya chromosomes also did not refer to the existence of heteromorphic sex chromosomes. STOREY (1953) examined several pollen mother cells in male and hermaphrodite papaya plants and observed precocious chromosome migration in several, but not in all cells. This early chromosome separation is a characteristic that distinguishes sex chromosomes from autosome chromosomes (STOREY 1953).

Sex in most angiosperm plants can be due to the presence of an autosome gene and in some cases, it is due to the presence of sex chromosomes. There are species from Cannabinaceae, Caryophyllaceae, Curcubitaceae, Loranthaceae, Vitaceae and Polygonaceae families that present heteromorphic sexual chromosomes. On the other hand, a large number of species presents homomorphic sex chromosomes (CHARLESWORTH and GILMARTIN 1998).

These are the first karyotypes for the three species of Caricaceae family. Their chromosomes are very short and similar and there are no heteromorphic sex chromosomes. More sophisticated staining techniques need to be applied for a complete elucidation of these issues, as in the case of the date palm tree that has small and homomorphic sexual chromosomes related to the nucleolus organizing region (RON) (SILJAK-YAKOVLEV *et al.*

1996). Since these species have similar karyotypes, the lack of viable seed in interspecific hybridizations is not due to a lack of chromosome homology between species, but might be due to a lack of homology between genomic constitutions of the Caricaceae species.

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