Genetic Relationship between *Vasconcellea* and *Carica* Based on Their Chromosome Features

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**Summary**  The objective of this study was to evaluate the genetic relationship between five *Vasconcellea* species (*V. cauliflora*, *V. goudotiana*, *V. quercifolia*, *V. monoica*, and *V. cundinamarcensis*) and *Carica papaya* based on their chromosome features. To do that, five metaphase plates showing well spread and condensed chromosomes were used for the chromosome measurements: the absolute chromosome length (CL) and the lengths of the long (LA) and short (SA) arms. Further, we estimated the ratio between the arms (r), the centromeric index (CI), and the total length of haploid set (TLH). The Total Form index (TF%), the Rec, and the SYi were estimated for symmetry/asymmetry of the karyotype. Most species show karyotype formulae of nine pairs of metacentric chromosomes (9m) except for *V. goudotiana*, which has five pairs of metacentric chromosomes and four pairs of sub-metacentric chromosomes (5m+4sm). *V. goudotiana* had the longest total length of haploid set, whereas *V. quercifolia* showed the shortest one. Based on the estimates of symmetry/asymmetry indexes, four species have symmetric karyotype; moreover, *V. goudotiana* has asymmetric karyotype because it showed the lowest values of Syi and TF% index. The similarity matrix of five parameters for the six species was employed and based on the dendogram four clusters were formed; the first included *C. papaya* and *V. cundinamarcensis*, the second cluster was formed by *V. monoica* and *V. cauliflora*, the third by *V. quercifolia* and fourth by *V. goudotiana*. So, based on chromosome features *V. cundinamarcensis*, *V. cauliflora*, and *V. monoica* are closer to papaya than *V. goudotiana*.

**Key words**  Chromosome, Karyotype, *Vasconcellea* spp., *Carica*.

The genus *Vasconcellea* (Caricaceae) was considered for a long time a section within the genus *Carica* L. (Badillo 1971). However, Badillo (2000, 2001) separated the monospecific section *Carica* from the section *Vasconcellea*, based on morphological and genetic evidence (Aradhya et al. 1999) rehabilitating the *Vasconcellea* section on generic level; since then the genus is formed by 25 species, and the *Carica* by only one, the cultivated form. Most of the *Vasconcellea* species has Ecuador as the center of origin or diversification, but *V. microcarpa* and *V. cundinamarcensis* are found in the Andes while *V. cauliflora* is found in Mexico (Badillo 1971). The *Vasconcellea* species are found at wild or at semi-domesticated conditions and five of them have been placed on

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the International Union for the Conservation of Nature and Natural Resources Red List of Threatened Species (IUCN 2003); however, for Kyndt et al. (2005) more than five Vasconcellea species are endangered in Ecuador.

Compared to papaya (Carica papaya L.), the Vasconcellea differ in some features, such as smaller fruits, fruits with less juice and with high concentration of papain (Scheldeman et al. 2007). Vasconcellea cauliflora, V. goudotiana and V. quercifolia are tolerant to low temperatures, present fruits with smooth skin and firm pulp and are resistant to certain diseases, which are important characteristics for the genetic improvement of the papaya (Van Droogenbroeck et al. 2004, Dillon et al. 2006). So, they can be exploited directly or as a genetic resource for improving the papaya by genetic breeding (Caetano et al. 2008).

Most breeding programs for papaya are based on intraspecific hybridization (Silva et al. 2008), so it is necessary to widen the genetic base of papaya to generate genetic variability and to develop new cultivars resistant to diseases. One way to do that is by using wide hybridization (Hajjar and Hodgkin 2007). However, attempts have been done since 1958 by Jimenez and Horovitz (1958) and Horowitz and Jimenez (1967) and the transference of resistance gene from wild to the cultivated papaya is not well succeeding unless embryo rescue technique is used (Magdalita et al. 1996, Siar et al. 2011).

Before introducing wild species in a hybridization program, it is necessary to know the genetic relationship between the wild and the cultivated form. The genetic relationships among species has to be known to predict the success or failure of a hybridization program since interspecific hybridization presents a set of barriers that can be manifested before or/and after fertilization. The karyotype analysis is good to predict the genetic relationships between species (Singh 2002).

Considering the importance of Vasconcellea species for papaya breeding, this study was set up aiming to determine the chromosome features of five Vasconcellea species (V. monoica, V. goudotiana, V. quercifolia, V. cauliflora, V. cundinamarcensis) and to evaluate the genetic relationship between them and C. papaya.

Materials and methods

This study was done using root tips from C. papaya, V. monoica, V. goudotiana, V. quercifolia, V. cauliflora, V. cundinamarcensis. The root tips were obtained from seedlings or plants propagated by cuttings and grown in a greenhouse.

The slides were prepared according to Costa et al. (2008); the collected root tips, measuring approximately 2 cm in length, were pre-treated with saturated solution of paradichlorobenzene for 8 h at 4°C. After the pre-treatment these tips were rinsed in distilled water, fixed in 3 : 1 (ethanol : acetic acid solution) for 24 h and maintained in the freezer until the slide preparation.

The root tips were submitted to enzymatic digestion (20% pectinase and 2% cellulase) for 1 h and 15 min at 37°C. After the digestion the material was transferred to 1-mL tubes containing distilled water and centrifuged for 10 min at 5,000 rpm for the nuclei suspension. After the centrifugation step the nuclei were resuspended in a 2 : 1 solution (methanol : acetic acid) for 24 h and maintained in the freezer until the slide preparation.

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Slides were mounted using one drop of material and left to dry at room temperature. After drying, the slides were stained with 5% Giemsa solution for 15 min at 37°C, covered with a glass cover slip and observed in an optical microscope under bright field (Olympus BX 60).

The images containing condensed and well-spread chromosomes were captured with a digital camera (3.3 MPixelQcolor3C) mounted to the optical microscope (Olympus BX 60, USA) by using the Image-Pro Plus Software version 5.1. The five best cells displaying metaphasic chromosomes from each species were used for the following measurements: the absolute chromosome length (μm) and the length of long and short chromosome arms by using the MicroMeasure version 3.3.
software (Reeves and Tear 2000).

The ratio between the arms ($r=\text{long arm/short arm}$), total length of haploid set (TLH=sum of the absolute length of the metaphasic chromosomes of each species) and the centromeric index (CI=[length of the short arm/total length]×100) were estimated based on the chromosome measurements.

The chromosomes were classified according to Guerra (1986), who has proposed four chromosome types: metacentric (m, $r=1.00$ to 1.49; CI=40.1 to 50.0), sub metacentric (sm, $r=1.50$ to 2.99, CI=25.1 to 40.0), acrocentric (a, $r=3.00$ to 7, CI=0.01 to 25.0) and telocentric (t, $r=\infty$, CI=0). The centromere position, the absolute chromosome size and the ratio between chromosome arms were observed for the identification of the homologous chromosomes.

The karyotype asymmetry was done based on the Total Form (TF%) (Huziwara 1962) and the Rec and SYi indexes (Greilhuber and Speta 1976). The TF% index is expressed by the ratio between the sum of the lengths of the short arms of individual chromosomes and the total length of the complement (Huziwara 1962). The Rec index expresses the average of the ratios between the length of each chromosome and that of the longest one. The SYi value is expressed by the ratio between the average length of the short arms and the average length of the long arms (Castiglione et al. 2007).

A hierarchical cluster analysis using Euclidean distances was also employed to compare the karyological data (TLH, TF%, Rec index, Syi Index, and total chromosome length) of the Caricaceae species. The dendogram was built by algorithm UPGMA (Unweighted Pair Group Method Using Arithmetic Averages) by using Genes (Cruz 2006).

Results

We observed 18 chromosomes for all species (Fig. 1) as has been reported by the literature for the Caricaceae. The karyotype formulas and the karyological data for *V. goudotiana*, *V. cauliflora*, *V. monoica*, *V. cundinamarcensis*, and *V. quercifolia* are reported in Table 1, in which the TF%, Syi and Rec indices are summarized.

Fig. 1. Methaphase plates of Caricaceae species. (A) *V. cauliflora*, (B) *V. quercifolia*, (C) *V. goudotiana*, (D) *V. monoica*, (E) *V. cundinamarcensis*, (F) *C. papaya*. Bar=5μm.
The length of the chromosomes varied among the species and *V. goudotiana* was the one with chromosome sizes varying from 3.06 to 2.03 μm; on the other hand, *V. quercifolia* had the smallest chromosome sizes varying from 2.27 to 1.47 μm (Table 1). Papaya, the cultivated form, has chromosome sizes varying from 2.29 to 1.52 μm; *V. monoica* from 2.49 to 1.35 μm and *V. cundinamarcensis* from 2.45 to 1.66 μm. Based on the data and according to Guerra (1986), *V. goudotiana* has karyological formula represented by five metacentric chromosome pairs and four sub metacentric chromosome pairs (4sm+5m) while the others species presented all chromosomes as metacentric (9m) (Table 1).

Table 1. Karyotype formulae (KF), chromosome size range (LC: Large chromosome to SC: Short chromosome), centromeric index (CI), total length of haploid set (TLH), Total Form index (TF%), Rec index, and Syi index for six *Caricaceae* species.

<table>
<thead>
<tr>
<th>Species</th>
<th>KF</th>
<th>LC–SC (μm)</th>
<th>CI</th>
<th>TLH (μm)</th>
<th>TF%</th>
<th>Rec index</th>
<th>Syi index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. goudotina</em></td>
<td>4sm+5m</td>
<td>3.06–2.03</td>
<td>39.7</td>
<td>23.08</td>
<td>40.25</td>
<td>83.78</td>
<td>67.32</td>
</tr>
<tr>
<td><em>V. cauliflora</em></td>
<td>9m</td>
<td>2.73–1.57</td>
<td>45.7</td>
<td>18.94</td>
<td>46.00</td>
<td>77.33</td>
<td>85.84</td>
</tr>
<tr>
<td><em>V. quercifolia</em></td>
<td>9m</td>
<td>2.27–1.47</td>
<td>45.9</td>
<td>16.18</td>
<td>46.00</td>
<td>83.00</td>
<td>92.22</td>
</tr>
<tr>
<td><em>V. monoica</em></td>
<td>9m</td>
<td>2.49–1.35</td>
<td>43.7</td>
<td>17.11</td>
<td>44.24</td>
<td>76.44</td>
<td>79.24</td>
</tr>
<tr>
<td><em>V. cundinamarcensis</em></td>
<td>9m</td>
<td>2.45–1.66</td>
<td>46.1</td>
<td>18.69</td>
<td>46.65</td>
<td>84.80</td>
<td>87.39</td>
</tr>
<tr>
<td><em>C. papaya</em></td>
<td>9m</td>
<td>2.29–1.52</td>
<td>45.4</td>
<td>17.18</td>
<td>45.51</td>
<td>82.66</td>
<td>83.65</td>
</tr>
</tbody>
</table>

The similarity matrix of five parameters (TLH, TF%, Rec index, Syi index, and chromosome

Fig. 2. Dendogram of *Caricacea* species using UPGMA algorithm based on THC, TF%, Rec index, Syi index and total chromosome length.

The length of the chromosomes varied among the species and *V. goudotiana* was the one with chromosome sizes varying from 3.06 to 2.03 μm; on the other hand, *V. quercifolia* had the smallest chromosome sizes varying from 2.27 to 1.47 μm (Table 1). Papaya, the cultivated form, has chromosome sizes varying from 2.29 to 1.52 μm; *V. monoica* from 2.49 to 1.35 μm and *V. cundinamarcensis* from 2.45 to 1.66 μm. Based on the data and according to Guerra (1986), *V. goudotiana* has karyological formula represented by five metacentric chromosome pairs and four sub metacentric chromosome pairs (4sm+5m) while the others species presented all chromosomes as metacentric (9m) (Table 1).

Total length of haploid set (TLH) among species varied from 16.18 μm for *V. quercifolia* to 23.08 μm for *V. goudotiana* and (Table 1). These results indicate that the *V. goudotiana* species presents the largest genome among the Caricaceae species studied herein. The TLH for *V. monoica* was 17.11 μm, for *V. cundinamarcensis* 18.69 μm and for *C. papaya* 17.18 μm.

Indices of karyotype symmetry/asymmetry were estimated as the Total Form index (TF%), which ranged from 40.25% for *V. goudotiana* to 46.65% for *V. cundinamarcensis*; the cultivated papaya had 45.51%. The Syi index varied from 67.32% (*V. goudotiana*) to 92.22% (*V. quercifolia*) and the Rec index varied from 76.44% (*V. monoica*) to 84.80% (*V. cundinamarcensis*).

The similarity matrix of five parameters (TLH, TF%, Rec index, Syi index, and chromosome
length) was employed for cluster analysis and a dendrogram was constructed (Fig. 2). The species were grouped into four clusters. Cluster I included the cultivated form, C. papaya, and *V. cundinamarccencis*. Cluster II included *V. cauliflora* and *V. monoica*. Cluster III and Cluster IV were formed by one species each, *V. quercifolia* and *V. goudotiana*, respectively.

**Discussion**

The chromosome number for the *Vasconcellea* species was confirmed as $2n=2x=18$ chromosomes as has been reported for Caricacea family (Datta 1971, Caetano *et al.* 2008, Costa *et al.* 2008). The chromosome size was considered small ($\leq 3 \mu m$) except for *V. goudotiana*, which showed the longest chromosomes among the species. The total length of haploid set (TLH) varied from 16.18 $\mu m$ (*V. quercifolia*) to 23.08 $\mu m$ (*V. goudotiana*) and papaya showed 17.18 $\mu m$, so papaya has one of the smallest genome size among the species studied herein.

Eder-Silva *et al.* (2007) reported that the chromosome sizes in the *Jacaratia spinosa* (*Caricaceae*) varied from 2.5 to 1.5 $\mu m$, and Datta (1971) studying five papaya varieties observed that the chromosomes size varied from 1.0 to 4.25 $\mu m$. Differences in the measurements can occur, especially in species with small chromosomes, due to arm condensation, mechanical distortions, and others (Sybenga 1992). However, all authors agree with the statement that the Caricaceae family present small chromosomes considering that chromosome with size up to 3 $\mu m$ must be considered small (Guerra 2000).

Levan *et al.* (1964) classified the chromosome in six types, according to their centromere position: M, T, m, sm, st, and t, where M and T are used for chromosomes whose centromere is located on the mid portion (metacentric) or at 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. However, this classification is more indicated for species with large chromosomes and based on that 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), sub metacentric (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, $r=\infty$, CI=0). Based on Guerra’s classification, the metacentric (m) chromosome type prevails among the species, except for *V. goudotiana* which show metacentric and sub metacentric, so all species have similar chromosome type showing a relationship on the basis of karyotype formula with homogenous karyotypes, whereas *V. goudotiana* has heterogeneous karyotype with karyotype formula $4sm+5m$. Probably, the metacentric is a common feature in the Caricaceae species, since up to now most of the species show this type of chromosomes (Eder-Silva *et al.* 2007, Costa *et al.* 2008).

The secondary constriction that is connected to the nucleolar organizer region (NOR) was not observed. The NOR is a sequence of DNA that presents multiple copies (from 500 to 1,000 copies) of the two largest rRNA fragments 18S and 28S (Sybenga 1992, Fukui and Nakayama 1996), and it can be located at the terminal or interstitial region of the chromosome. When located in the interstitial region it originates the satellite (Schubert 2007). The fact that this region was not observed in this study may be related to the staining method used, which was not specific for the nucleolar-organizing region (Guerra and Souza 2002).

The karyotype symmetry is a concept very debated by the researchers since Levitsky (1931) established that a symmetrical karyotype is one in which all chromosomes are similar and metacentrics; the manifestation of asymmetry is less median centromeres and less uniform chromosomes (Levitsky 1931). Karyotype asymmetry was evaluated by estimation of three indices TF%, the Syi, and the Rec Index, which are indicated to evaluate the variation in centromere position in a chromosome complement. According to Huziwara (1962) the TF% index can range
from zero to 50% and karyotypes with index value closer to 50% are considered extremely symmetrical and the one closer to zero is asymmetrical. The Syi index might vary from 0, asymmetric, when the mean short arm length is equal to zero (S=0), to 1 or 100%, when the median short arm length is equal to median of long arm length (S=L) a symmetry karyotype (Greilhuber and Speta 1976, Verona et al. 2002, Peruzzi and Erglu 2013). Our results suggest that all species have symmetrical karyotypes. A symmetrical karyotype is mainly characterized by the presence of metacentric and sub metacentric chromosomes with similar sizes, while asymmetric karyotypes are those that suffer a shift of the centromere position or through the accumulation of differences in the relative size of the chromosomes of the complement (Paszko 2006, Castiglione et al. 2007). It is assumed that a determined group of flowering plants with a more asymmetrical karyotype can be derived from a more symmetrical group (Seijo and Fernandez 2003).

Cluster analysis could explain the relationship among the six species. The results of UPGMA based on karyotypic parameters (TLH, TF%, Rec index, Syi Index, and total chromosome length) showed the six species were separated into four clusters. Carica papaya and V. cundinamarcensis are more related compared to the other species. However, they formed a group with V. cauliflora and V. monoica, probably because they have chromosomes with measurements quite similar. V. quercifolia formed a group by itself and it is among the species with the smaller chromosomes, and V. goudotiana, the less related to the five species, has the biggest chromosomes. These results are similar to those obtained by Jimenez and Horovitz (1958) that based on hybridization data reported three gene pools: one with V. monoica, V. cauliflora, V. microcarpa, and V. cundinamarcensis, other with C. papaya and the third one with V. goudotiana. Costa et al. (2008) based on fluorescent in situ hybridization (FISH) observed that V. cundinamarcensis and V. goudotiana were the closest species while C. papaya was isolated from them. Both Vasconcellea species showed only one pair of 5S site whereas three pairs were found in C. papaya. On the other hand, one 18S site was observed in papaya whereas four and five 18S sites were observed in V. goudotiana and V. cundinamarcensis, respectively.

So, even though the six species have similar chromosome features and symmetrical karyotype, V. goudotiana is less related to papaya, so it should be avoided in intergeneric breeding programs for the cultivated form.

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