Meiotic Behavior of Male and Hermaphrodite Genotypes of Papaya (Carica papaya L.)

Lyzia Lemos Freitas, Monique Freitas Neto, Telma Nair Santana Pereira*, and Messias Gonzaga Pereira

Universidade Estadual do Norte Fluminense, Centro de Ciências e Tecnologias Agropecuárias, Laboratório de Melhoramento Genético Vegetal.
Av. Alberto Lamego 2000, Campos dos Goytacazes, 28013–602. RJ, Brazil

Received November 29, 2012; accepted April 10, 2013

Summary The aim of this study was to analyze the meiotic behavior and to estimate the meiotic index and the pollen grain viability of three papaya genotypes, male (Cariflora) and hermaphrodite (Golden and UENF/CALIMAN01). In considering of the fact that the genotypes have different flower sizes, initially, meiotic stages were defined in relation to bud size, and, after that, meiosis was observed in the three genotypes. To observe meiosis, flower buds of different sizes were collected and the length of each bud was measured using graph paper. Once the measurements had been taken, two slides were prepared for each flower bud by squashing the anthers in 1% acetic carmine solution. Slides were observed under an optical microscope and for each bud, size was determined as indicating one of three stages, depending on meiotic phase: pre-meiotic, meiotic stage, or post-meiotic stage. In this way, meiosis was observed for the three genotypes, with particular attention given to possible irregularities in cell division in relation to prophase I. It was observed that there is a relation between flower bud size and meiosis. In general, meiosis was normal, but all genotypes showed some meiotic irregularity, with laggard chromosome being the most common. The Cariflora (male) genotype had a higher frequency of irregularities compared to the hermaphrodite genotypes. The meiotic indices estimated for the Golden, UENF/CALIMAN01, and Cariflora genotypes were 92.6%, 91% and 76.9%, respectively; however, the pollen grain viability, on average, was higher than 90%. In conclusion, the meiotic behavior of male and hermaphrodite genotypes was similar, and all three genotypes had some degree of meiotic abnormality.

Key words Papaya, Sex chromosomes, Meiosis, Laggard chromosomes, Meiotic index.

Papaya (Carica papaya L.) is a diploid species with nine pairs of chromosomes (Damasceno Jr. et al. 2010). It is triocious with female, male, and hermaphrodite plants, and is considered to be sexually polymorphic. The cultivars are ginoandromonoicas, consisting of female and hermaphrodite plants.

The most accepted theory for sex determination in papaya plants is that the sex of plants is determined by a gene with three allelic forms (M1, M2, m); so, the genotype of the female plants is mm, that of the male is M1m and that of the hermaphrodite is M2m (Hofmeyr 1938, Storey 1938). However, Liu et al. (2004) formulated another theory that the sex of the plants is due to the presence of an incipient sex chromosome pair differentiated by a small male-specific region of the Y chromosome (MSY). According to Ming et al. (2007) there are two Y chromosomes in papaya, a controlling male designated as Y and the other, controlling the sex hermaphrodite, designated as Yh. The female plant is homogametic with XX chromosome constitution, the male is XY, and the hermaphrodite is XYh.

* Corresponding author, e-mail: telmasp2012@gmail.com
DOI: 10.1508/cytologia.78.163
During meiotic prophase, homologous chromosomes pairing due to the presence of the synaptonemal complex, a tripartite protein structure which extends along the entire length of the bivalent, is observed (Mercier and Grelon 2008). The heteromorphic sex chromosomes, X and Y are distinct from each other and from the autosomes by size and shape; so, the behavior of the sex chromosomes during meiosis is tremendously affected, including the formation and dynamics of the synaptonemal complex; so, synapses or pairing between X and Y chromosomes are not expected.

Zhang et al. (2008) observed, at the pachytene of hermaphrodite plants (XY\textsuperscript{h}), nine bivalents being that the XY\textsuperscript{h} pachytene bivalent is largely euchromatic based on its DAPI staining pattern; however, brightly stained and knob-like heterochromatin structures were observed in the MSY region. The identification of this MSY region was confirmed by fluorescent in situ hybridization mapping of MSY-specific bacterial artificial chromosomes (BAC) clones. The authors observed five distinct knobs (K1 to K5) in the MSY region. K1 is the largest knob associated with both X and Y\textsuperscript{h} chromosomes and the others were associated to the MSY and were absent in the corresponding regions on the X chromosome. They also observed a unique characteristic of chromosomal pairing in the MSY. The Y\textsuperscript{h} chromosomal domain, associated with K4, consistently protruded away from X and appeared to have a curved shape, similar to the loop caused by the duplication/deletion events associated with one of the two chromosomes.

Thus, in plants where the mechanism of sex determination is controlled by the presence of a heteromorphic pair of sex chromosomes, the analysis of meiosis is extremely important because it is expected that the pairing of this pair is not perfect and that abnormalities will occur during meiosis. The precocious segregation of a pair of chromosomes has been reported at anaphase I of meiosis of pollen mother cells in male and hermaphrodite plants (Kumar et al. 1945, Storey 1953) and this early separation was considered an indication of the presence of a heteromorphic sex chromosome pair.

The objectives of this study were to analyze the meiotic behavior, to estimate the meiotic index, and to estimate pollen grain viability of male and hermaphrodite plants of papaya, thereby seeking to investigate the occurrence of meiotic irregularities that would indicate the presence of sex chromosomes.

Materials and methods

Plant materials

The genetic materials used in this study were buds from the Golden and Carilflora genotypes and the UENF/CALIMAN01, hybrid. The genotype Golden belongs to the Solo Group and the hybrid to the Formosa Group and both segregate for female and hermaphrodite plants. The genotype Carilflora is dioecious and segregates for male and female plants. All three genotypes were grown in 5 L pots and kept in a greenhouse. At flowering time, in each genotype, for both male and hermaphrodite plants, fifty buds were collected randomly, at different developmental stages. The flower buds were fixed in absolute ethyl alcohol and glacial acetic acid in a 3:1 ratio at room temperature. After 24 h the fixative solution was replaced with 70% ethanol until the moment of the slides preparation.

For meiotic comparative analysis, the length of each bud was measured using graph paper. Each bud flower measured was classified into one of the three stages: pre-meiotic stage, when the cells were not dividing yet; meiotic stage, when the cells were in full cell division; or post-meiotic stage, when the cells were already at the final stage of division or at cytokinesis. Based on this classification, the association between the bud length and the meiotic stage was determined for the three genotypes.

After the measurements, slides were prepared by squashing anthers in drops of 1% acetic carmine solution. After remotion of the debris, the slides were covered with coverslips. The slides
were observed under an optical microscope (Olympus BX 60, USA) and special attention was taken to the occurrence of meiotic irregularities for the three genotypes.

The meiotic index (MI) was also estimated for the three genotypes based on the relation between pos-meiotic products, normal and abnormal (Love 1951). The normal pos-meiotic products were considered the tetrads whilst the abnormal pos-meiotic products were dyads, triads, and polyads. Three anthers were squashed in drops of 1% acetic carmine solution to conduct the analysis. The slides were observed under an optical microscope (Olympus BX 60, USA). The MI of each of the three genetic materials was estimated based on a count of 500 pos meiotic products per slide.

For the evaluation of pollen grain viability, flower buds, at anthesis, were collected in 70% ethanol and stored in the refrigerator until the slide preparation. Thereafter, the anthers were squashed in triple solution (Alexander 1969) and slides were observed under a light microscope (Olympus BX60, USA). Viable pollen grains were detected by the presence of red or purple color in the protoplasm, while non-viable pollen grains were identified by the presence of green color. For each genetic material, five slides were prepared, with a single slide per flower bud, with 500 pollen grains counted and classified as viable or non-viable per slide, totaling 2,500 pollen grains being accounted for per genotype.

Results

Based on the flower bud measurement data, the flower buds were classified into three stages (pre-meiotic, meiotic and post-meiotic). Flower bud sizes from 2 to 4 mm (Golden and Cariflora) and from 2 to 5 mm (UENF/CALIMAN01) were classified into the pre-meiotic stage since no meiotic cell division was observed. Flower buds with sizes of 5 mm (Golden and Cariflora) and 6 mm (UENF/CALIMAN01) showed meiotic cells in different stages, especially in prophase I (Fig. 1, A1/B1) and at metaphase I (Fig. 1, A2/B2). Phases of meiosis II (Fig. 1 A3/B3) were observed in flower buds measuring 6 mm (Golden and Cariflora) and 7 mm (UENF/CALIMAN01), which showed a high frequency of dividing cells. The post-meiotic stage was observed in flower buds whose sizes varied between 7 mm and 9 mm for Golden and Cariflora, respectively (Fig. 1, A4/B4), and from 8 to 10 mm for UENF/CALIMAN01 (Fig. 1, A5/B5).

The meiosis in all three genotypes confirmed the diploid condition of the papaya ($2n=2x=18$ chromosomes), with nine pairs of bivalents being observed at diakinesis (Fig. 2A–C) and at metaphase I (Fig. 2D, E). No abnormalities were observed in prophase I in terms of chromosome pairing at diakinesis (Fig. 2A–C). The pairing and the segregation of chromosomes were normal (Fig. 2G–K, L); no differences were therefore observed in chromosome pairing and segregation between the hermaphrodite and male genotypes.

Meiotic abnormalities were observed in all three genotypes, and those most commonly observed were laggard chromosomes in cells in metaphase I (Fig. 2F) and metaphase II (Fig. 2J) and early segregation (Fig. 2E). The percentage of cells with early segregation varied from 4% (Golden) to 8% (Cariflora), and laggard chromosomes varied from 12% (Golden) to 40% (Cariflora). As such, we might conclude that the presence of laggard chromosomes was the most common abnormality.

The number of cells lacking synchrony of chromosomes varied from 2% (UENF/CALIMAN01) to 10% (Cariflora). Lack of synchrony is characterized by having, on the superior pole, chromosomes in metaphase II, and, on the inferior pole, chromosomes in early anaphase II, with separation of the chromosomes to the opposite poles of the cell (Fig. 2I).

Although abnormalities have been observed in all three genetic materials, the male genotype (Cariflora) presented, in general, a higher incidence of irregularities, both meiotic and post-meiotic, compared to the hermaphrodite genotypes (Golden and UENF/CALIMAN01). This meiotic behavior must have been reflected in the formation of abnormal post-meiotic products (Fig. 3A–F).
Cariflora (male) showed a high frequency of abnormal post-meiotic products, such as dyads, triads and polyads, while Golden and UENF/CALIMAN01 showed only triads. Therefore, the meiotic index (MI) was estimated at 92.9% and 91% for Golden and UENF/CALIMAN01, respectively. For the male genotype, the MI was estimated at 76.9%. The pollen grain viability was high for all three genotypes: UENF/CALIMAN01 was 98.6% and of Golden 97.2%, while for the male genotype Cariflora viability was 92.3%.

Discussion

Based on the results, it can be concluded that there is an association between flower bud size and meiotic phases; for Golden and Cariflora, the meiosis can be observed in flower bud size of 5 mm and for UENF/CALIMAN01 in buds with 6 mm. The correlation between pollen grain formation and bud size has been observed in others crops, including sour passion (Souza et al. 2002), soybean (Lauxen et al. 2003), ipecac (Souza et al. 2003) and coffee (Silva et al. 2004); for papaya, the bud size may be used as an indicator to the meiosis.

Overall, the meiosis was quite normal in the three genotypes. Since papaya is a diploid species, it was expected that nine pairs of bivalents would be observed at diakinesis. This result confirms the diploid condition of the species, with $2n=2x=18$ chromosomes, and corroborates what has been
noted in other literature for the Caricaceae family (Darlington and Ammal 1945; Datta 1971, Eder-Silva et al. 2007, Damasceno Jr et al. 2010). At diakinesis and metaphase I, no heteromorphic bivalents were observed, indicating that if sex chromosomes are related to the determination of plant sexes, such chromosomes are homomorphic. Similar results were reported by Datta (1971), who did not observe heteromorphic pairs of chromosomes nor non-paired chromosomes. Costa et al. (2008) also did not observe heteromorphic pairs of chromosome using FISH, although their results indicated that papaya had three pairs of 5S and only one 18S rDNA sequence sites, while V. goudotiana and V. cundinarmacensis, both diocieous, had one pair of 5S rDNA sequence site and four and five pairs of 18S rDNA, respectively. Araujo et al. (2010), working on the karyotypes of hermaphrodite genotypes, concluded that the chromosomes are homomorphic.

The chromosome segregation in the three genotypes was normal for both genotypes, male and hermaphrodite. Chromosome abnormalities were observed in all three genotypes; however, Cariflora, the male genotype, had a higher incidence of meiotic and post-meiotic abnormalities compared to the hermaphrodite genotypes (Golden and UENF/CALIMAN01). Meiotic irregularities have been recorded elsewhere for the Caricaceae species. Bajpai and Singh (2006), working on the meiosis of ten papaya cultivars, observed that meiosis was very disturbed and many meiotic irregularities were recorded, with chromosome stickiness being the most common (44%), followed by chromatin bridges (21.1%), laggard chromosomes (4.8%), chromosome diminution/degenera-
tion (5.2%), and micronucleus (5.9%). Silva et al. (2012). also working with Caricaceae wild species (*V. goudotiana*, *V. quercifolia*, and *J. spinosa*), observed lagging chromosomes, sticky chromosomes, precocious segregation, and fiber disturbance.

The most common abnormality observed was laggard chromosomes in metaphase I and metaphase II, followed by lack of synchroly and early segregation. Usually, laggard chromosomes are lost during meiosis and are entrapped in the micronuclei; they result as a signal of genetic material lost and aneuploidy. However, Huang et al. (2012) observed that this assumption is based on analysis of fixed cells and that those laggard chromosome are not, in fact, lost. This observation is very interesting because laggard chromosomes were observed in this study; however, micronuclei formation was very low and pollen grain viability was high for all three genotypes, meaning that pollen grain development was not hampered by the occurrence of laggard chromosomes. Early or precocious segregation observed in papaya has been considered indicative of sex chromosome presence (Kumar et al. 1945), but according to Schubert (2007) species with small chromosomes do not segregate correctly during meiosis. Papaya chromosomes are considered small in size since they measure, on average, $2 \mu m$ (Damasceno Jr. et al. 2009); Guerra (2000) defined chromosomes with sizes under $3 \mu m$ as small, those between $3$ to $5 \mu m$ as medium and those above $5 \mu m$ as large. Ming et al. (2008) believe that recognizing that papaya has a pair of homomorphic primitive sex chromosomes might explain the earlier precocious separation at anaphase I; since the small MSY region is in the middle of the Y chromosome, the pairing with its X counterpart will not happen, but a weak attraction between this pair of chromosomes would make their separation and migration towards each pole of the dividing cell easier and earlier.

The Golden cultivar (hermaphrodite) presented a lack of synchrony, which is characterized by the cell having two distinct phases during nuclear division. In Golden, metaphase II in the upper pole and anaphase II in the lower pole of the same cell was observed. This kind of abnormality has been reported in *V. cauliflora* and *V. cundinamarcensis* (Caetano et al. 2008) and in *C. papaya* and *V. monoica* (Damasceno Jr. et al. 2010).

Meiotic abnormalities have a direct influence on the formation of post-meiotic products, with monads, dyads, triads and polyads as the resulting products (Horner and Palmer 1995). Different post-meiotic products were observed in all three genotypes (Fig. 3A–F), but the male genotype had more dyads and the hermaphrodite genotypes had more triads. Caetano et al. (2008) found that the meiotic abnormalities observed in accessions of *V. cauliflora* and *V. cundinamarcensis* had a direct influence on post-meiotic products formation, resulting in a high meiotic index and low pollen grain viability. The meiotic indices estimated for Golden and UENF/CALIMAN01 were high, at 92.9% and 91%, respectively, indicating that the genotypes are meiotically stable, according to Love (1951). On the other hand, the male genotype had an MI equal to 76.9%, which is a low value; the genotype can therefore be considered meiotically unstable (Love 1951). Meiotic index below 90% has been reported for Caricaceae as *V. monoica* (Damasceno Jr. et al. 2010) and *V. goudotiana* (Silva et al. 2012).

Pollen grain viability varied from 92.3 to 98.6%, observed in UENF/CALIMAN01 and Cariflora, respectively; thus, it can be considered high for all three genotypes, above 90%. Damasceno Jr. et al. (2010) also observed high pollen grain viability for the Sunrise Solo 7212 genotype. Despite the three genotypes presenting some meiotic abnormalities, those irregularities did not influence the pollen grain viability. This can be explained by the checkpoint mechanism that constitutes a complex of proteins which acts when some kind of cell division problem occurs in order to maintain the integrity of the genome during cell division (Weinert 1998).

The meiotic analysis allowed us to infer that papaya has no heteromorphic sex chromosomes since the synapses were normal in both genotypes, hermaphrodite and male. Meiotic irregularities were observed in all three genotypes but no significant differences were observed, except in the formation of post-meiotic products. Based on meiotic index, the male genotype Cariflora might be
considered cytological unstable. However, the irregularities did not affect the pollen grain fertility since pollen viability was high, above 90%, for all genotypes.

In conclusion, the meiotic behavior in male and hermaphrodite genotypes of papaya did not indicate any possible abnormalities that would suggest the presence of a heteromorphic pair of sex chromosomes in papaya.

References


Storey, W. B. 1938. Segregation sex types in Solo papaya and their application to selection of seed. Proceedings of the
American Society for Horticultural Science 35: 83–85

