Plant Syst Evol (2015) 301:231–237
DOI 10.1007/s00606-014-1068-y

ORIGINAL ARTICLE

Chromosome number and nuclear DNA amount in *Psidium* spp. resistant and susceptible to *Meloidogyne enterolobii* and its relation with compatibility between rootstocks and commercial varieties of guava tree

Aline Das Graças De Souza · Luciane Vilela Resende · Isabela Pereira De Lima · Luiza Suely Semen Martins · Vânia Helena Techio

Received: 7 October 2013 / Accepted: 20 April 2014 / Published online: 20 May 2014

Abstract The objective of this study was to determine the chromosome number and DNA amount of accessions of *Psidium* sp. (araça tree, used as rootstocks) and accessions of *P. guajava* (guava tree) resistant/susceptible to *M. enterolobii,* which holds some morphophysiological affinity and which could enable their use as rootstocks compatible with commercial varieties of guava. Metaphase chromosomes were obtained by the squash technique and stained with 2 % Giemsa. The determination of DNA amount was done by flow cytometry using Mary buffer. The intraspecific variation in DNA amount (2C) was of the order of 9x, with $2n = 22$ observed in only one species of *P. guajava.* The other species presented variations in the ploidy level in relation to the basic chromosome number $x = 11$, including the occurrence of polyploidy, cytotypes and disploidy. In some species, the interspecific variation of 2C showed a direct relationship with the ploidy level. Our data confirm that variations in the number of chromosome and in ploidy level are important events to *Psidium* and can support the understanding of the incompatibility of the graft on rootstocks of guava and araça trees resistant to the nematode *M. enterolobii.*

Keywords Myrtaceae · Cytotypes · Disploidy · Cytogenetics · Nematode

A. Das Graças De Souza · L. V. Resende · I. P. De Lima
Department of Agriculture, University of Lavras, Lavras, Minas Gerais, Brazil

L. S. S. Martins
Department of Fitotecnia, Rural University of Pernambuco, Recife, Pernambuco, Brazil

V. H. Techio (✉)
Department of Biology, University of Lavras, Lavras, Minas Gerais, Brazil
e-mail: vhtechio@gmail.com

Introduction

The guava and araça trees (*Psidium* spp.) are cultivated species that present high genetic diversity, first due to the preferential cross-fertilization of the species, according to Alves and Freitas (2007), besides the use of seeds from heterozygous parents in the production of seedlings that leads to a wide genetic diversity.

In Brazil, due to the increased acreage cultivated with guava trees, the appearance of new pests and diseases becomes common (Rossi and Ferraz 2005). In the São Francisco Valley, in the states of Pernambuco and Bahia, the growing of *P. guajava* is strongly harmed by *Meloidogyne enterolobii* (Maranhão et al. 2001). Losses caused by this plant nematode were and have been always high. Considering that Brazil is one of the centers of origin of the guava tree, at present, researchers have been working in the search for rootstocks resistant to plant nematodes and compatible with graft varieties.

The occurrence of a Myrtaceae species resistant to *M. enterolobii* would enable its use as a rootstock for commercial varieties of guava tree. The existence of a great number of genetically different materials, which maintains some morphological affinity, increases the chance of existing compatibility in grafting between different species in *Psidium* (Hartman et al. 1997). Therefore, the search for resistant materials within Myrtaceae and the viability of using those materials as rootstocks is essential.

Still, in accordance to the same authors, genetic and anatomical factors may affect the compatibility of graft and rootstock. For that reason, it is advisable that both graft and rootstock belong at least to the same family. Within the genus *Psidium,* there is the araça tree (*P guineense; P. cattleaynum*), a wild plant which has shown resistance to the nematode *M. enterolobii* (Pessanha et al. 2011).

In this context, the information relative to cytogenetic features and DNA amount may contribute towards the understanding of the relationship of incompatibility between the rootstock of *Psidium* sp. (araça tree) and *P. guajava* (guava tree), resistant to the nematode *M. enterolobii* with commercial genotypes of guava trees. Especially in this group, data become relevant considering that the basic chromosome number of the family is $x = 11$, but there is variation in most genera and a high frequency of polyploidy (Costa and Forni-Martins 2006b).

In addition to the chromosome counts, the determination of the DNA amount may complement the information about the genomic variations of species. For Myrtaceae, few species have their 2C value estimated (Costa et al. 2008).

Therefore, we intended to determine the chromosome number and DNA amount of accessions of araça and guava trees resistant/susceptible to *M. enterolobii,* which keep some morphophysiological affinity and which would enable their use as compatible rootstocks for commercial varieties of guava tree.

Materials and methods

Plant material

In these studies were evaluated sixteen accessions of araça tree used as rootstocks and two of guava tree commercial (Table 1) maintained in greenhouse in the Hydroponics Sector of the Soil Science Department of the University of Lavras—UFLA, located in the town of Lavras, Minas Gerais State, Brazil. The climate is of the type Cwb.

Preparation of the nuclear suspensions for flow cytometry

For each accession, three leaf samples were evaluated with the purpose of estimating the DNA amount. About 20–30 mg of young leaf tissue along with the same amount of young leaf tissue of *Pisum sativum* (internal reference standard), was ground on a Petri dish containing 1 mL of icy Marry buffer for obtaining the nuclear suspension (Dolezel 1997).

To the nuclear suspension were added 25 µL propidium iodide and 50 µL RNAse. Samples were analyzed after 5 min of incubation in a four Colors BD FacsCalibur™ flow cytometer (Becton–Dickinson). Histograms were generated by the software Cell Quest and analyzed with the software WinMDI 2.9. The nuclear DNA amount (pg) of the samples was estimated by comparing with the position in relation to the G1 peak of the internal standard. The size of the nuclear genome of each sample was estimated according to:

$$\text{DNA2C (pg)} = \frac{\text{Peak channel G1 of the sample X standard DNA amount (9.09pg)}}{\text{Peak channel G1 of the standard}}$$

The Kruskal–Wallis test at 5 % of probability was performed. The statistical analysis of the DNA amount was conducted using the software R. Development core team (2008).

Table 1 Accessions of *Psidium* utilized in the experiments of DNA amount and chromosome counts aiming at the incompatibility factors

Accessions	Common name	Scientific name	Origin
1	Guava tree (Paluma)	*Psidium guajava*	Carrancas-Minas Gerais State
2	Purple Guava tree	*Psidium guajava*	Carrancas-Minas Gerais State
3	Araça tree	*Psidium* sp.	A-Lu1Itumirim-Minas Gerais State
4	Araça tree	*Psidum* sp.	A-Lu2 Itumirim-Minas Gerais State
5	Araça tree	*Psidium* sp.	A-Lu3 Itumirim-Minas Gerais State
6	Araça tree	*Psidium cattleyanum*	R.E Ingaí-Minas Gerais State
7	Araça tree	*Psidium guineense*	A-20.1 Recife-Pernambuco State
8	Araça tree	*Psidium cattleyanum*	A-Mar Recife-Pernambuco State
9	Araça tree	*Psidium cattleyanum*	A-17. 2 Recife-Pernambuco State
10	Araça tree	*Psidium cattleyanum*	A-Pasto Itumirim-Minas Gerais State
11	Araça tree	*Psidum* sp.	A-30. 3 Recife-Pernambuco State
12	Araç atree	*Psidium cattleyanum*	A-roxo-Itumirim-Minas Gerais State
13	Araça tree	*Psidium australe*	A-Carrancas-Minas Gerais State
14	Araça tree	*Psidium cattleyanum*	A-30. 4 Recife-Pernambuco State
15	Araça tree	*Psidium cattleyanum*	A-Ufla-1 Lavras-Minas Gerais State
16	Araça tree	*Psidium cattleyanum*	A-Ufla-4 Lavras-Minas Gerais State
17	Araça tree	*Psidium cattleyanum*	A-Ufla-5 Lavras-Minas Gerais State
18	Araça tree	*Psidium cattleyanum*	A-20.3 Recife-Pernambuco State

Chromosome counts

For obtaining the mitotic metaphases, root tips were pretreated with 0.002 M 8-hydroxyquinoline (8-HQ) for about 24 h at 4 °C (Éder-Silva et al. 2007). Afterwards, roots were fixed in Carnoy 3:1 (absolute ethanol/glacial acetic acid) for a 24-h period at room temperature and next stored in a freezer at −20 °C to be later analyzed. For the preparation of slides, roots were hydrolyzed in 5 N hydrochloric acid for 35 min at room temperature. The slides were prepared according to the squash technique and stained with 2 % Giemsa (Guerra and Souza 2002). Chromosome number was obtained by the count of, at least, ten metaphases of each material analyzed.

Results and discussion

Considering that genetic and anatomical factors may affect the compatibility of graft and rootstock (Hartman et al. 1997), the analysis of chromosomal counts and DNA amount can be important to understand the relationship of incompatibility between the rootstock of *Psidium* sp. (araça tree) and *P. guajava* (guava tree), resistant to the nematode *M. enterolobii* with commercial genotypes of guava trees.

This is the first study on the determination of DNA amount of these accessions of araça and guava trees (Table 2). The range of variation was from 0.99 pg in *P. guajava* to 5.48 pg in *P. cattleaynum* (Table 2). Considering this feature, there were no significant differences between accessions 1 and 2 (*P. guajava*), between accessions 6 and 7 (*P. cattleaynum* and *P. guineense*) and also between the two accessions of *P. cattleaynum* (9 and 10). Accession 18 of *P. cattleaynum* presented the highest amount of DNA among the accessions evaluated.

In the accessions of *P. guajava,* the determination of DNA amount and chromosome number were 2C = 0.99 and 1.02 pg, with $2n = 18$ and 22, respectively (Fig. 1a, b).

For most accessions, a coherent and proportional relationship between the chromosome number, the ploidy level and the DNA amount was found. The lowest values of 2C were found in the diploid species ($2n = 18$ and 22) and have increased according to the ploidy level (Figs. 1, 2, 3).

Among the tetraploid species ($2n = 44$), the variation was from 1.99 to 2.21 pg in the accessions *P. guineense* and *P. cattleaynum*, respectively (Figs. 1f, 2a), about twice the value found for the accession of *P. guajava* ($2n = 22$) (Figs. 1a, f, 2a).

In the metaphases examined in this study were found $2n = 22$ chromosomes for *P. guajava* 'Paluma' and $2n = 18$ for *P. guajava* (purple guava tree). The accessions of *P. cattleyanum* showed variations in the chromosome numbers of $2n = 44, 46, 48, 55, 58, 82$. Other four species of *Psidium* sp. (accessions 3, 4, 5 and 11) presented variations of $2n = 30, 36$ and 48 (Table 2; Figs. 1c–e, 2e). In relation to the two populations of *P. cattleyanum* (accessions 17 and 18), there was a discrepancy between the chromosome number ($2n = 66$ and 82) and the value 2C, which can be explained for the fact that accessions 18 apparently have smaller sized chromosomes compared with accession 17 (Fig. 3e, f; Table 2).

Table 2 Average nuclear DNA amount (pg) and chromosome number in species of *Psidium*

Accessions	Scientific name	Nuclear amount DNA (pg)	Chromosome number 2*n*	CV (%)
1	*P. guajava*	0.99 (2.33)[a] n	18	1.00
2	*P. guajava*	1.02 (4.67) n	22	1.46
3	*Psidium* sp.	1.09 (8.00) m	30	1.06
4	*Psidium* sp.	1.86 (11.00) l	30	1.34
5	*Psidium* sp.	1.95 (14.00) k	36	0.69
6	*P. cattleyanum*	1.99 (17.83) j	44	0.96
7	*P. guineense*	2.02 (19.17) j	44	1.13
8	*P. cattleyanum*	2.20 (23.00) i	46	0.68
9	*P. cattleyanum*	2.54 (26.83) h	46	0.90
10	*P. cattleyanum*	2.70 (28.17) h	46	0.93
11	*Psidium* sp.	2.74 (32.00) g	48	0.82
12	*P. cattleyanum*	2.88 (35.00) f	55	0.64
13	*P. australe*	2.97 (39.17) e	55	0.99
14	*P. cattleaynum*	3.00 (41.50) de	58	0.67
15	*P. cattleyanum*	3.01 (42.33) d	66	0.57
16	*P. cattleyanum*	3.11 (47.00) c	66	0.47
17	*P. cattleyanum*	5.32 (50.00) b	66	0.55
18	*P. cattleyanum*	5.47 (53.00) a	82	0.50

Mean values followed by the same letter are not significantly different by the Kruskal–Wallis test at $p > 0.05$

[a] Ranking: classification obtained according to the Kruskal–Wallis test

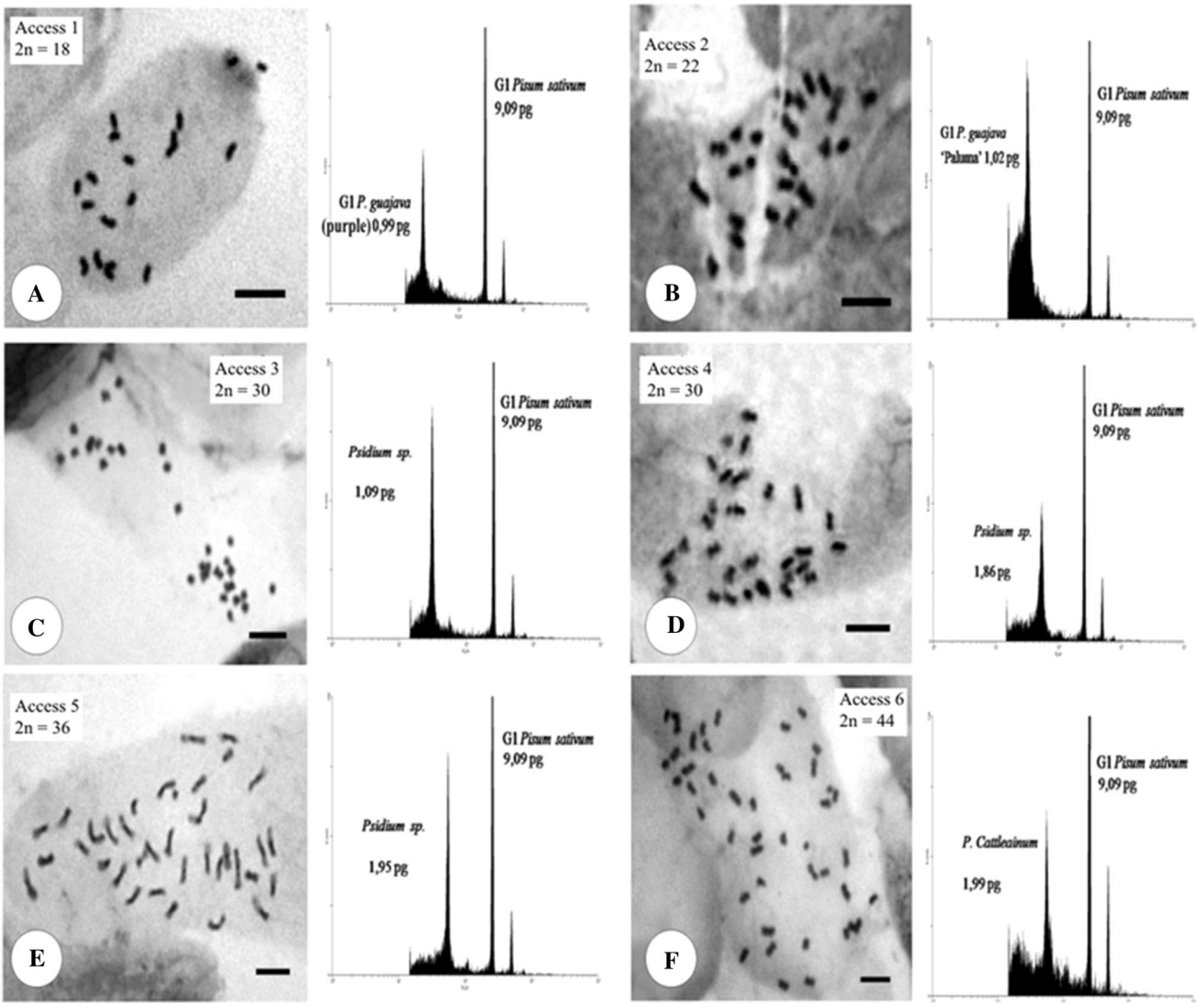

Fig. 1 Mitotic metaphases and histograms of the nuclear DNA amount of accessions of guava tree (*Psidium guajava*) and araça tree (*Psidium* sp.) *Bar* 5 μm

Accessions 15, 16 and 17 of *P. cattleaynum* and *P. guineense* (accession 7) and *P. australe* (accession 13) presented $2n = 66$, $2n = 44$ and $2n = 55$, respectively. The chromosome number of the graft variety Paluma of *P. guajava* ($2n = 22$) and for the seven accessions of *P. cattleaynum* was $2n = 44$, 55 and 66 (Figs. 1b, f, 2a, f, 3a, c).

The chromosome number observed to the accessions 15, 16 and 17 of *P. cattleaynum* and *P. guineense* (accession 7) indicates that they are cytotypes. Costa and Forni-Martins (2006a) and Éder-Silva et al. (2007) had already reported the occurrence of cytotypes in *Psidium*, with variations of $2n = 22$, 33, 44, 55, 66, 77 and 88 chromosomes.

The values obtained for the DNA amount diverge from the number presented by Costa et al. (2008), who observed for *P. guajava* (red guava), *P. guajava* (white guava), *P. cattleyanum* (red fruit), *P. cattleyanum* (yellow fruit), values of $2C = 0.55$ with $2n = 22$, $2C = 0.50$ with $2n = 22$, $2C = 2.91$ with $2n = 66$, and $2C = 1.05$ with $2n = 44$, respectively.

In addition, the results obtained to the graft variety Paluma of *P. guajava* and for the seven accessions of *P. cattleaynum* corroborate with Costa et al. (2008) and Wilson et al. (2005). Costa et al. (2008) had already reported the same chromosome number for the rootstock *P. australe* cambess, whose collection site was also the Carrancas region in Minas Gerais State, Brazil, confirming the chromosome number for this accession (Fig. 2f).

For *P. cattleaynum* Costa and Forni-Martins (2006a) and Costa et al. (2008) found $2n = 44$ in chromosome counts, confirming the results obtained by studies in the access 6 of this species. For the rootstocks of araça tree, *P. cattleaynum*

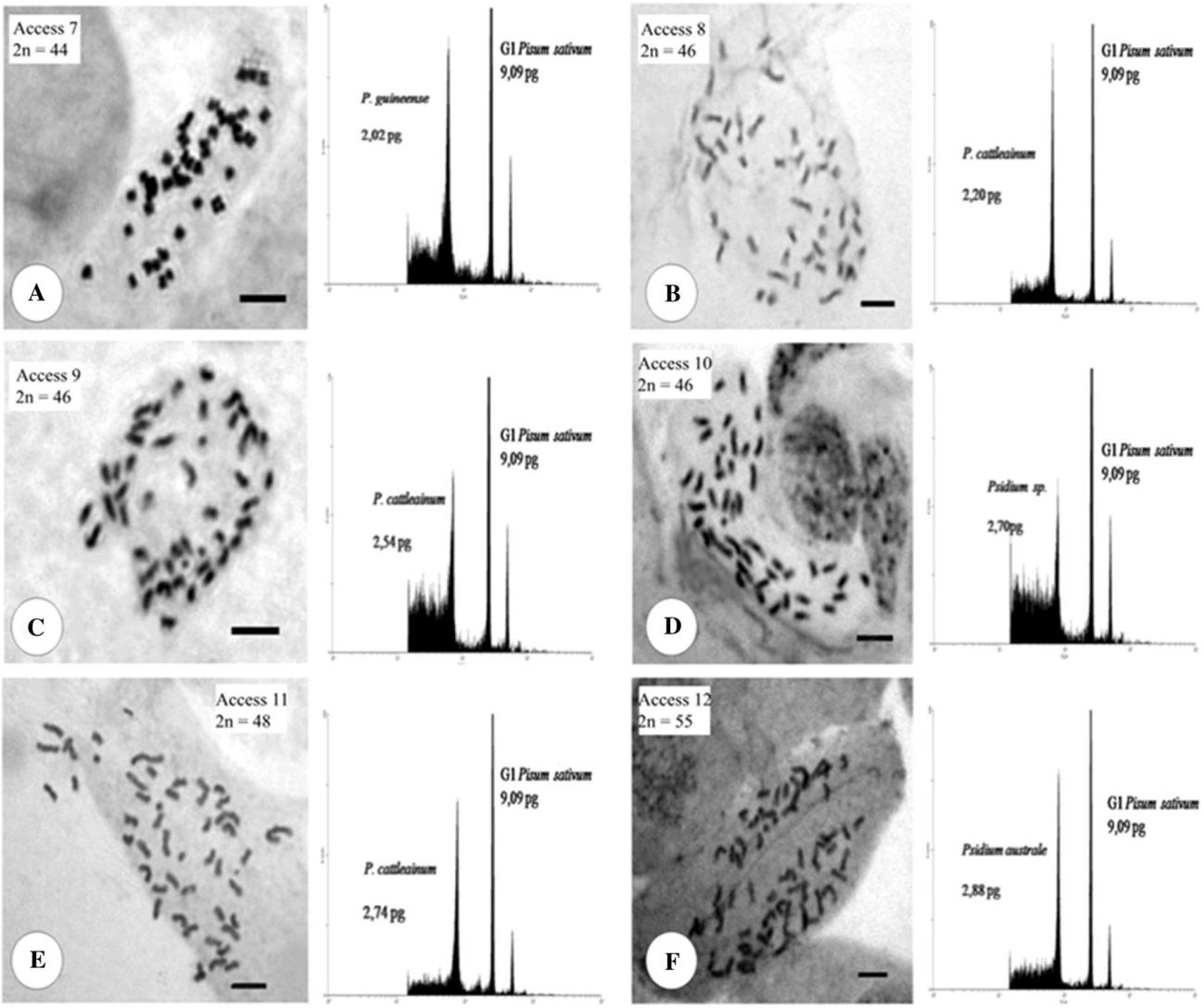

Fig. 2 Mitotic metaphases and histograms of the nuclear DNA amount of accessions of guava tree (*Psidium guajava*) and araça tree (*Psidium* sp.) *Bar* 5 μm

and *P. guineense*, the obtained results were similar to those reported in the literature for the genus.

In addition to the polyploidy series, chromosome counts performed on the accession of purple guava tree (*P. guajava*) with $2n = 18$, *P. cattleyanum* with $2n = 46$, 48, 58 and 82 and *Psidium* sp. $2n = 30$, 36, 46 (Figs. 1, 2, 3), diverge as to the basic number $x = 11$, likely due to the alteration by disploidy.

Possibly, one of the causes for the incompatibility of the rootstocks of *P. cattleyanum* resistant to the plant nematode *M. enterolobii* under the graft 'Paluma' obtained by Almeida et al. (2009) and Carneiro et al. (2007), can be associated with genetic and anatomical factors. In the current study, for example, the accessions of *P. cattleayanum* revealed chromosome number ranging from $2n = 44$ to $2n = 82$, in turn, in *P. guajava*, graft variety Paluma, $2n = 22$ (Figs. 1, 2, 3), underlining a difference in chromosome number and DNA amount among the rootstocks of *P. cattleyanum* and the graft 'Paluma'. This can be a factor that makes difficult the tissue fusion and cell exchange, because each tissue continues to reproduce by mitosis, maintaining its chromosome number, genome size and gene dosages. Thus, it is recommended to use plants as grafts and rootstocks, which present, at least, both similar chromosome number and DNA amount. Grafts performed between plants diverging in relation to the chromosome number and DNA amount will likely be conducted with failure.

In this way, the study of the incompatibility mechanism in association with cytogenetic studies involving species of the genus *Psidium* is of utmost importance, because the chromosome number and DNA amount can be a useful

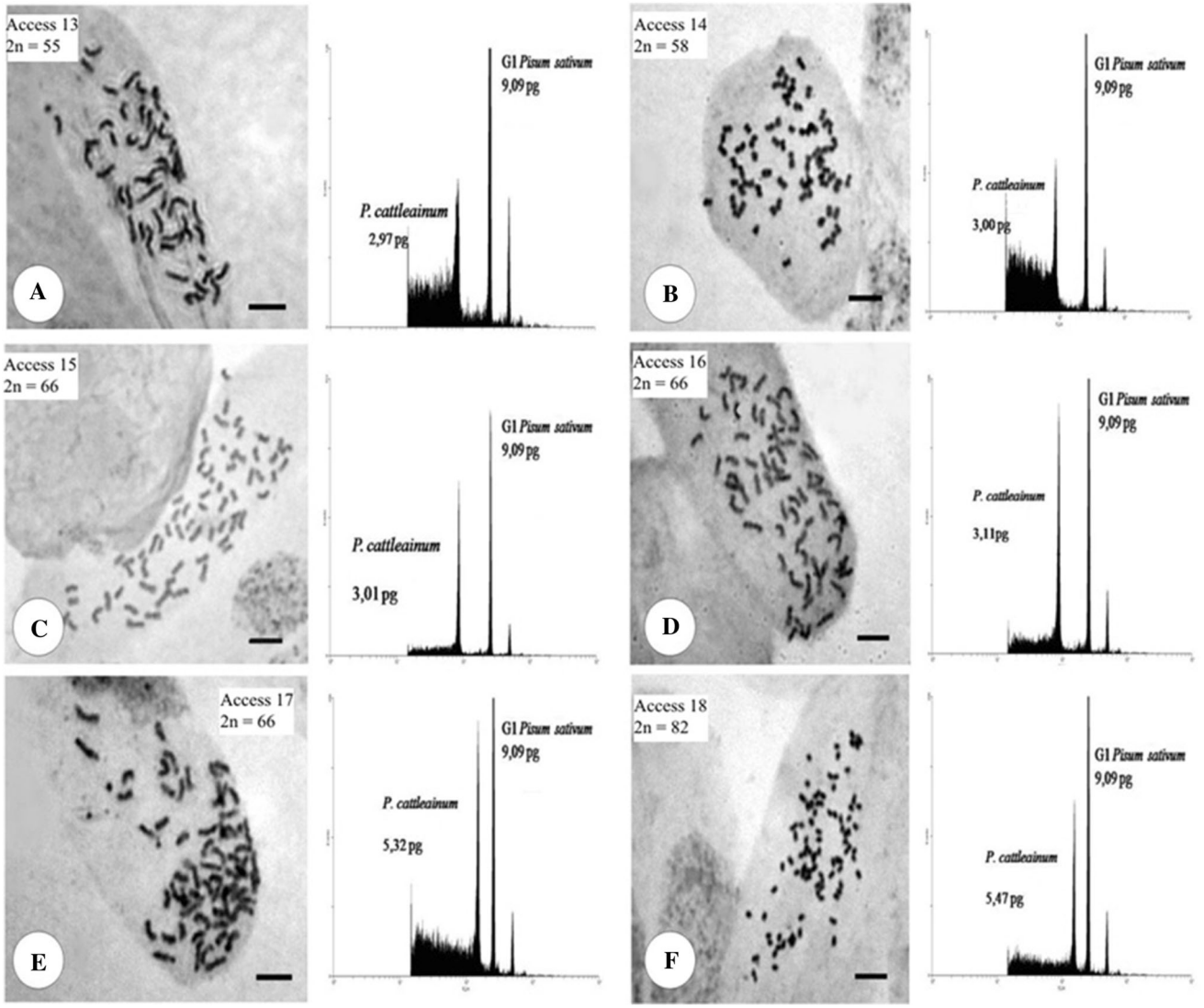

Fig. 3 Mitotic metaphases and histograms of the nuclear DNA amount of accessions of guava tree (*Psidium guajava*) and araça tree (*Psidium* sp.) *Bar* 5 μm

characteristic in the compatibility between rootstocks and grafts. The techniques of flow cytometry and chromosome counts were applied for the first time as a way to evaluate the likely causes of incompatibility in grafting between accessions of guava tree and native araça tree. These techniques proved to be useful for the use of previous selections of *Psidium* spp., used as both graft and rootstock.

Conclusion

The chromosome number of the accessions of *Psidium,* ssp. ranged from $2n = 18$ (*P. guajava*) to $2n = 82$ (*P. cattleyanum*). The interspecific variation of nuclear DNA amount presented a coherent and proportional relationship with the ploidy level in most of the investigated accessions. The chromosome number in plants of *Psidium guajava* that present compatibility with each other is $2n = 22$ and confirms the results obtained in commercial cultivars of this species.

References

Almeida EJ, Soares PLM, Santos JM, Martins ABG (2009) Resistência de goiabeiras e araçazeiros a *Meloidogyne mayaguensis*. Pesquisa Agropecuária Brasileira, Brasília 44:421–423

Alves JE, Freitas BM (2007) Requerimento de polinização da goiabeira. Ciência Rural, Santa Maria 37(5):1281–1286

Carneiro RMDG, Cirotto PA, Quintanilha AP, Silva DB, Carneiro RG (2007) Resistance to *Meloidogyne mayaguensis* in *Psidium* spp. accessions and their grafting compatibility with *P. guajava* cv. Paluma. Fitopatologia Brasileira, Piracicaba 32:281–284

Costa IR, Forni-Martins ER (2006a) Chromosome studies in species of *Gomidesia*, *Marlierea*, *Myrceugenia* and *Myrcia* (Myrtaceae, subtribe Myrciinae). Kew Bull 62:113–118

Costa IR, Forni-Martins ER (2006b) Chromosome studies in Brazilian species of *Campomanesia* Ruiz et Pávon and *Psidium* L. (Myrtaceae Juss.). Caryologia, Firenze 59:7–13

Costa IR, Dornelas MC, Forni-Martins ER (2008) Evolution of nuclear DNA amounts in Neotropical Myrtaceae (fleshy-fruited Myrteae). Plant Syst Evol, Austria 276:209–217

Dolezel J (1997) Applications of flow cytometry for the study of plant genomes. J Appl Genet, Poland 38:285–302

Éder-Silva E, Felix LP, Bruno RLA (2007) Citogenética de algumas espécies frutíferas nativas do nordeste do Brasil. Revista Brasileira Fruticultura, Jaboticabal 29:110–114

Guerra M, Souza MJ (2002) Como observar cromossomos: um guia de técnicas em citogenética vegetal, animal e humana. FUNPEC, Brazil

Hartman HT, Kester DE, Davies JRFT, Geneve RL (1997) Plant propagation: principles and practices, 6th edn. Prentice Hall, New Jersey

Maranhão S, Moura RVL, Pedrosa EMR (2001) Reacão de indivíduos segregantes de araçazeiro a *Meloidogyne incognita* Raça 1, *M. javanica* e *M. mayaguensis*. Nematologia Brasileira, Piracicaba 27:173–178

Pessanha PGO, Viana AP, Junior ATA, Souza RM, Teixeira MC, Pereira MG (2011) Avaliação da diversidade genética em acessos de *Psidium* spp. via marcadores RAPD. Revista Brasileira de Fruticultura, Jaboticabal 33(1):129–136

Rossi CE, Ferraz LCCB (2005) Fitonematoides da superfamília Criconematoidea e Dorylaimoidea associados a fruteiras de clima subtropical e temperado nos estados de São Paulo e Minas Gerais. Nematologia Brasileira, Piracicaba 29(2):183–192

R. Development Core Team (2008). R: A language and environment for statistical computing, reference index version 2.8.0. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL

Wilson PG, O'brien MM, Heslewood MM, Quinn CJ (2005) Relationships within Myrtaceae sensulato based on a matk phylogeny. Plant Syst Evol, Austria 251:3–19