

Meiotic index in Myrtaceae native fruits trees from southern Brazil

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ABSTRACT - This work aimed to evaluate meiotic index in Myrtaceae species, native of southern Brazil. Flower buds were collected and stored in ethylic alcohol:propionic acid (2:1) until evaluation by the smear and squash technique with propionic carmin as a dye. A minimum of 10 buds and 2000 tetrads were observed. *Acca sellowiana* showed a high cytological stability (96%) whereas *Eugenia involucrata*, *E. pyriformis*, *Campomanesia xanthocarpa*, and *E. uniflora* (populations "A" and "B") showed reasonable stability, respectively 87.3%, 89.5%, 89.3%, 84.8%, and 88.3%. Pollen germination of *E. uniflora* was considerably low than of the other species. The most common abnormality was the occurrence of triads. Further evaluations should be conducted in individual plants of each species, in order to enable the characterization of different clones or populations to form the basis of future genetic breeding programs.

Key words: Microsporogenesis, *Eugenia* spp., *Acca sellowiana*, *Campomanesia xanthocarpa*.

INTRODUCTION

The Brazilian fauna is rich in wild species that constitute a patrimony of inestimable value (Mielke et al. 1990). Among many other native species, the Myrtaceae family is outstanding in southern Brazil.

EMBRAPA Temperate Climate Research Center, in Pelotas, RS, Brazil, maintains an active Germplasm Collection to conserve these species, study their potential, and establish a breeding program as well as to develop a production system for their commercial cultivation in the future.

However, the *ex situ* conservation of these plants can bring about some problems such as the lack of adaptation to

the conditions of the collection site. In consequence, meiotic abnormalities may occur during the microsporogenesis.

An assessment of the tetrad meiotic stage is considered a fairly precise and rapid evaluation of a plant's performance during pollen grain formation, suitable for large-scale plant screening. Love (1949) defined the term "meiotic index" as the percentage of normal tetrads at the end of meiosis division. This percentage is an index of the meiotic regularity, which increases with the increasing normality of the chromosomal behavior.

Some authors used the meiotic index to study the cytological stability in wheat varieties (Maan and McCracken 1968, Boyd et al. 1970). Khazanehdari and Jones (1997)

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studied the consequences of meiotic irregularity on fertility, quality, and uniformity in leek. Research with *Brachiaria* showed that meiotic irregularity influenced pollen fertility (Mendes-Bonato et al. 2001, Mendes-Bonato et al. 2002, Risso-Pascotto et al. 2002).

End of meiosis was used by several authors in other studies. In *Prunus* genus, the stage was related to end of the dormancy period (Young and Hauser 1980, Citadin et al. 2002). Cavalcanti et al. (2000) used the meiotic index associated with pollen viability to characterize and identify potential male parents.

However, few papers reporting on the meiotic index of Myrtaceae species are found in literature. Raseira and Raseira (1996) observed that in two populations of strawberry guava (*Psidium cattleianum* Sabine) the meiotic index was related with low pollen viability.

The objective of this research was to evaluate the meiotic index of five native fruit species from southern Brazil of the Myrtaceae family that are maintained at Embrapa Temperate Climate Germplasm Collection to determine the genetic stability of these species.

MATERIAL AND METHODS

The following species were evaluated: Rio Grande cherry (*Eugenia involucrata* DC.), feijoa (*Acca sellowiana* (Berg.) Burr.), uvalheira (*E. pyriformis* Camb.), guabirobeira (*Campomanesia xanthocarpa* Berg.), and Suriname cherry (*E. uniflora* L.). Two populations of the Suriname cherry were studied. Population A was collected from plants obtained from

seeds collected wild and population B consisted of plants originating from urban areas, of unknown origin, brought decades ago to backyards of residencies and schools.

In spring 2003, flower buds of these species were collected from plants of the EMBRAPA Temperate Climate Germplasm Collection. They were fixed in 2:1 solution (ethyl alcohol: propionic acid). Chlorophyll liberation from the buds initially turned the fixative green and was exchanged several times until it ran clear.

Because of the lack of information on the size of buds that would be in tetrad stage, flower buds of different sizes were collected and their diameter (with a caliper) at the mid portion. Diameters of buds and the meiosis stage were recorded.

The smear and squash technique using propionic carmine (1%) was used to assess the meiosis index. One drop of dye was placed onto a slide whereupon a single anther was squashed on it and covered by a cover slide. The slide was slightly heated over a flame to improve the staining of the cells.

At least 2000 tetrads were observed for each species. A normal tetrad has four uniformly sized cells whereas abnormal tetrads have cells of very different sizes. Counts were made of normal and abnormal tetrads as well as dyads (two cells), triads (three cells) and poliads (five or more cells). For each species, 10 buds (100 tetrads per anther and two anthers per flower bud) were observed.

Meiotic index means were compared by the t test, according to Beiguelman (2002).

In vitro germination pollen tests were performed to study a possible relation between the meiotic index and pollen viability (Table 1).

Table 1. Culture media, temperature, and incubation time for *in vitro* germination tests of several native species of southern Brazil from the Myrtaceae family

Species	Common name	Culture media*	Incubation temperature	Incubation time
			— °C —	— hours —
<i>E. uniflora</i> ("population A")	Suriname cherry	Standard + 0.65 mM H ₃ Bo ₃	25	5
<i>E. uniflora</i> ("population B")	Suriname cherry	Standard + 1.3 mM H ₃ Bo ₃	30	5
<i>E. involucrata</i>	Rio grande cherry	Standard	25	3
<i>E. pyriformis</i>	Uvalheira	Not tested		
<i>A. sellowiana</i>	Feijoa	Standard + 1.3 mM H ₃ Bo ₃	25	3
<i>C. xanthocarpa</i>	Guabirobeira	Standard + 0.65 mM H ₃ Bo ₃	30	3

* Standard medium 10% sugar + 1% agar-agar, dissolved in distilled water. The standard medium is used in routine tests in the Breeding Laboratory of the Embrapa Temperate Climate Research Center, under addition of boric acid if necessary (H₃Bo₃)

RESULTS AND DISCUSSIONS

The bud diameter for flowers buds in tetrad stage varied among species. It varied from 2.0 to 2.5 mm for *E. uniflora*, “population B” and from 2.4 to 3.0 mm for *E. uniflora*, “population A”. The diameter was between 3.0 and 3.5 mm for *E. involucrata*, between 6.6 and 7.1 mm for *A. sellowiana*, between 2.6 and 3.0 mm for *E. pyriformis*, and 3.4 to 3.8 mm for *C. xanthocarpa*.

A. sellowiana had a meiotic index above 90%. The other studied species had a lower index but not below 84% (Table 2).

The average meiotic index of *A. sellowiana* was superior to that of other species ($P < 0.05$) and the meiotic index of *E. involucrata* the lowest of all studied species except for population “A” of *E. uniflora*. It was also noted that populations “A” and “B” of *E. uniflora* differed from each other (Table 3).

A. sellowiana, with a meiotic index of 96% can be considered cytologically stable, according to Love’s definition

(1949). The other species had indexes (84.8-89.5%) which showed a reasonable stability. However, according to this same author, indexes below 90% can cause problems in a breeding program due to the instability of a plant or species.

The meiotic indexes found in the studied species are relatively high compared to those found by Raseira and Raseira (1996) in Brazilian guava (*P. cattleyanum*), another Myrtaceae native of southern Brazil and conserved in Embrapa’s collection. These authors found differences among populations as well as among plants of the same population. The indexes varied from 24% to 47% in the population called “Roxo sudeste”, and 42% to 67% in “Amarelo litoral”.

Among the studied species, *A. sellowiana* had the highest *in vitro* pollen germination and *E. uniflora* (population “A” and “B”) had the lowest values (Table 2). The differences in pollen viability among species were high (33% to 86%) whereas the range of meiotic indices was small (96.3% to 84.8%). However, the differences in pollen germination may in part be a consequence of the meiotic index since some flower buds of *E. uniflora*, for instance, had indexes below 80%.

Table 2. Percentage of normal tetrads (meiotic index), abnormal tetrads, dyads, triads, and poliads number of counted cells and percentage of pollen germination for different native fruit species from southern Brazil

Species	Common name	Tetrads		Triads	Dyads	Poliads	N° of counted cells	Pollen germination
		normal	abnormal					
		%						% —* —
<i>E. uniflora</i> (“population B”)	Suriname cherry	88.3	0.9	9.3	0.6	0.9	6000	35.3
<i>E. uniflora</i> (“population A”)	Suriname cherry	84.8	0.6	14.0	0.1	0.5	2000	33.5
<i>E. involucrata</i>	Rio grande cherry	87.3	0.9	10.6	0.4	0.8	2200	71.0
<i>E. pyriformis</i>	Uvalheira	89.5	0.5	9.9	0.1	0.0	2100	**
<i>A. sellowiana</i>	Feijoa	96.3	0.0	3.6	0.1	0.0	3300	85.5
<i>C. xanthocarpa</i>	Guabirobeira	89.3	0.4	9.5	0.6	0.2	2300	61.8

* Percentages of *in vitro* pollen germination in tests under conditions described in Table 1

** Not evaluated.

Table 3. Means and variances of meiotic indexes of Myrtaceae native fruit species from southern Brazil compared by the t test

Species	Means	Variances	t value					
			(1) ¹	(2)	(3)	(4)	(5)	(6)
(1) <i>A. sellowiana</i>	96.3	5.63	-	6.59*	6.76*	9.01*	7.12*	8.19*
(2) <i>E. pyriformis</i>	89.5	7.10		-	0.089	1.20	1.57	3.16*
(3) <i>C. xanthocarpa</i>	89.3	7.31			-	1.02	1.52	3.14*
(4) <i>E. uniflora</i> (population “B”)	88.3	5.32				-	0.74	2.54*
(5) <i>E. involucrata</i>	87.4	12.43					-	1.59
(6) <i>E. uniflora</i> (population “A”)	84.8	15.40						-

* Means differ significantly at 5% probability;

¹The numbers correspond to the species in the first column

Raseira and Raseira (1996) suggest that the low pollen germination in *P. cattleyanum* was related to its low meiotic index, since this is related with abnormalities in chromosome behavior during pollen formation.

Cavalcante et al. (2000) analyzed 70 plants of an open-pollinated *Citrus* population and reported a meiotic index above 90% in 94.1% of the plants. However, all plants with a meiotic index lower than 90% had low pollen viability. When testing pollen for *in vitro* germination, they also noticed signs of non-viable pollen such as the hypertrophy of pollen tubes and the presence of dehydrated pollen grains. The same tendency was observed by Sellito-Boaventura and Pio (1989) in three sweet orange cultivars. The cultivar with the lowest meiotic index was also the one with lowest pollen viability (2.4%), indicating chromosome sterility. According to these authors, this fact could explain the low seed production in this cultivar.

Lee et al. (1994) studied pollen formation in six *Heliconia psittacorum* cultivars and found a relation between their fertility and their regularity in normal tetrad formation during microsporogenesis. Three cultivars with normal pollen formation and a meiotic index above 99% were partially fertile, while three cultivars with 80% of aborted pollen grains and a meiotic index below 70% were completely sterile.

Papers on the meiotic irregularity influencing pollen viability are also found for the *Brachiaria* genus (Mendes-Bonato et al. 2001, Risso-Pascotto et al. 2002), for corn, *Zea mays*, (Taschetto and Pagliarini 1993) and for *Paspalum regnellii* (Pagliarini et al. 1998). Pagliarini (2000) refers to a correlation between irregular chromosome segregation and pollen viability or seed production in several species.

These references show that pollen viability is directly related with normal tetrad formation, that is, the meiotic index. According to Mendes-Bonato et al. (2002), the reduction in pollen viability is the most common consequence of irregularity during the meiotic division.

New evaluations should be carried out in the species referred in the present paper since the meiotic index was studied at species level. In this study, flower buds were collected from several plants of the germplasm collection. Differences in meiotic index and in pollen viability could occur among plants of the same species.

The evaluation of individual plants in each species could aid in the selection of male parents for future breeding programs, especially in the case of native fruit

species. Different clones of various origins, conserved at the Germplasm Bank (repository) of Embrapa Temperate Climate Research Center will be the foundation clones of such programs.

For the studied Myrtaceae species, the most common abnormality was the formation of triads, observed at the end of microsporogenesis (Figure 1C), while dyads, polyads, and abnormal tetrads occurred at rates below 1% (Table 2). Raseira and Raseira (1996) studied the meiotic index for two populations of strawberry guava (*P. cattleyanum* Sabine), “Amarelo litoral” and “Roxo sudeste”, collected in different areas of Rio Grande do Sul State and observed that the occurrence of triads was the most common abnormality. However, in contrast to the present work, the occurrence of dyads, polyads, and abnormal tetrads was relatively high. Triads, polyads, and abnormal tetrads were also observed in 12 populations of *Adesmia*, native of southern Brazil (Coelho and Battistin 1998).

Other meiotic phases were observed in microspores of different anthers of the same bud. This could be due to differences in the anther maturation stage within each bud. However, within one anther, the great majority of microspores were in the same division stage whereas Raseira and Raseira (1996) observed a range of meiotic division stages within the same anther in *P. cattleyanum*.

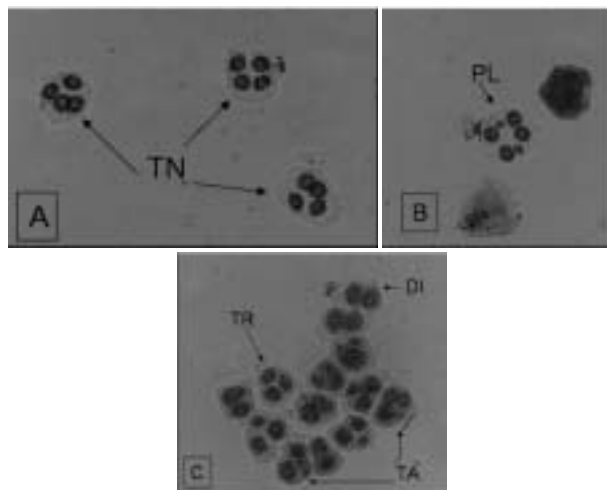


Figure 1. End of pollen meiosis (microsporogenesis) in Suriname cherry (*E. uniflora*). **A)** Formation of normal tetrads (TN) (312.5x); **B)** Formation of polyads (PL) with irregular size cells (312.5x); **C)** Formation of abnormal tetrads (TA), triads (TR), and dyads (DI) (500x)

CONCLUSIONS

1. Feijoa can be considered a cytologically stable species with a meiotic index above 90%.
2. The Suriname cherry, guabirobeira, Rio Grande cherry and uvalheira had satisfactory meiotic indexes with a mean variation between 84.8% and 89.5%.

3. New evaluations should be carried out for individual plants of each species, in order to check differences among clones of a single species.

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Índice meiótico em mirtáceas frutíferas nativas do sul do Brasil

RESUMO - Este trabalho objetivou avaliar o índice meiótico em espécies de mirtáceas nativas do sul do Brasil. Gemas floríferas foram coletadas e armazenadas em álcool etílico:ácido propiônico (2:1) até a avaliação pelo método do maceramento com carmin propiônico como corante. Um mínimo de 10 gemas e 2000 tétrades foram observadas. *Acca sellowiana* mostrou alta estabilidade citológica (96%), enquanto *Eugenia involucrata*, *E. pyriformis*, *Campomanesia xanthocarpa* e *E. uniflora* (populações "A" e "B"), mostraram uma razoável estabilidade, respectivamente 87,3%, 89,5%, 89,3%, 84,8% e 88,3%. A germinação do pólen de *E. uniflora* foi consideravelmente baixa em relação às outras espécies. A anormalidade mais comum foi a ocorrência de tríades. Novas avaliações devem ser realizadas em plantas individuais de cada espécie, a fim de possibilitar a caracterização dos diferentes clones ou populações que serão a base de futuros programas de melhoramento genético.

Palavras-chave: Microsporogênese, *Eugenia* spp., *Acca sellowiana*, *Campomanesia xanthocarpa*.

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