Crop Breeding and Applied Biotechnology 11: 27-35, 2011
Brazilian Society of Plant Breeding. Printed in Brazil

Cytogenetics of *Mimosa bimucronata* (DC.) O. Kuntze (Mimosoideae, Leguminosae): chromosome number, polysomaty and meiosis

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Received 15 April 2010

Accepted 27 June 2010

ABSTRACT - *Chromosome numbers (somatic and/or gametic) were determined in 50 populations of M. bimucronata (DC.) O. Kuntze collected in the species area of distribution in Rio Grande do Sul, south Brazil. All populations were diploid (2n = 2x = 26, n = 13). Polysomatic (mostly tetraploid) cells were detected in the seedlings root-tip cells in 39 out of the 41 populations examined, ranging from 3.0 to 28.2 % among populations, but were absent in the root-tips of grown plants. Polysomaty was as well absent in pollen-mother cells. In* M. bimucronata *pollen-mother cells are joined two-by-two before the onset of meiosis, remaining attached during all the meiotic division until the formation of pollen grain polyads, composed of two sets of four pollen grains each, that are dispersed in this way, which, according to previous suggestions would be an adaptation to ensure high seed set after a single pollination event.*

Key words: *"maricá", intraspecific variability, polyads.*

INTRODUCTION

The genus *Mimosa* L. (Mimosoideae, Leguminosae) comprises around 530 species, most of them native to the Americas (Barneby 1991). Many of *Mimosa* species are multipurpose trees, and among them is *M. bimucronata* (DC.) O. Kuntze, which occurs in Paraguay, Argentina and Brazil (from the State of Alagoas in the northeast to the State of Rio Grande do Sul in the south). Two varieties of the species are described, *M. bimucronata* (DC.) O. Kuntze var. *bimucronata*, the most widespread variety, and *M. bimucronata* var. *adenocarpa* Hasll, restricted to Paraguay, which differs only by the more abundant trichomas in *M bimucronata* var. *adenocarpa.*

M. bimucronata, popularly known in Brazil as "marica", "silva", "espinheiro" or "angiquinho" (Backes and Irgang 2002) is widely used as living and defensive fences (due to its thorns), for medicinal purposes and for honey production and its wood is employed in building, carpentry and as fuel (Burkart 1979, Barneby 1991, Lorenzi 1998). Many authors refer to the species importance in the recovery of degraded lands (Reitz et al. 1983, Marchiori 1993, Bernaci et al. 1998, Nascimento et al. 2003, Barbosa and Faria 2006, Bitencourt et al. 2007).

The basic chromosome number in genus *Mimosa* is suggested as being x = 13, probably derived by dysploidy from the more common x = 14 in the Mimosoideae (Goldblatt 1981). Most of *Mimosa* species studied (around 10 % of the genus) are diploid, with 2n = 2x =26, others are tetraploid (2n = 4x = 52) and a few are octaploid (2n = 8x = 104) (Federov 1969, Alvez and Custódio 1989, Seijo 1993, 1999, 2000, Seijo and Fernández 2001, Goldblatt and Johnson 2009). Intraspecific variability (generally diploid and tetraploid cytotypes) have been reported in *M. pudica*

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L. (Goldblatt and Johnson 2009), *M. somnians* Humb. & Bonpl. ex Willd. (Seijo 1993, 2000), *M. balansae* Micheli (Seijo and Fernández 2001), *M. debilis* Humb. & Bonpl. ex Willd.,and *M. nuda* Benth (Morales et al. 2010). It should be stressed that most chromosome counts are restricted to one or fewer populations, therefore intraspecific variability may be more common than reported. For *M. bimucronata*, Seijo (1999, 2000) reported 2n= 26 for populations from Argentina and Paraguay.

Polysomaty in root tip meristematic cells, the presence of cells with higher ploidy levels, have been reported in some *Mimosa* species by Witkus and Berger (1947) and Seijo (1993, 1999). According to Witkus and Berger (1947) polysomaty could be a way to assure a quick seedling establishment.

Seijo and Neffa (2004) described the formation of polyads, compound pollen grains, in *M. bimucronata*. Compound pollen grains are found in ca. 15 % of the angiosperm families (Kenrick and Knox 1982) and would be a mechanism to reinforce pollination efficiency.

The aim of this paper was to analyze chromosome numbers and meiotic behaviour in *M. bimucronata* populations from the species distribution area in the State of Rio Grande do Sul, South Brazil, to study frequency and distribution of polysomaty.

MATERIAL AND METHODS

All populations analyzed were collected (fruits with mature seeds and/or inflorescences) along the area of distribution of the species in the State of Rio Grande do Sul during the years of 2008 and 2009. Taxonomic vouchers are deposited at the ICN Herbarium (Departamento de Botânica, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil) (Table 1).

For somatic chromosome number determination, the seeds were scarified with sand paper or by a small cut in the testa and germinated in petri dishes lined with moist filter paper. Following Dahmer et al. (2009) with some modifications, root-tips around 1 cm length were pretreated with a saturated solution of paradichlorobenzene for 24 h at 4 ºC, fixed in 6: 3: 1 (ethanol: chloroform: acetic acid) for 12-24 h and stored in 70 % alcohol below 0 ºC until slide preparation. For that, the roots were hydrolyzed with 1 N HCl at 60 ºC for 8-10 min, stained by Feulgen for around 2 h, treated with 2% pectinase for 2 min, and squashed in 2% propionic carmine. At least ten cells with good chromosome spreading and with chromosomes in equivalent degree of contraction were analysed per plant. The best cells were photographed and/or registered by a digital image capturing system.

Percentage of polysomatic cells were determined in the same slides used for chromososome number counting. As a cross check to eliminate the possible cause of polysomaty being a result of pretreatment, slides of the same populations were prepared by the same technique as described above, but with no pretreatment. Additionaly, individuals of the five populations with higher frequency of polysomatic cells in meristematic root- tip cells (4, 9, 11, 24 and 37) were grown in pots with commercial garden soil in a greenhouse. After ca. 11 months, root-tips were collected from these plants and went to the same technical procedures (fixation, pretreatment, staining and squashing) as the seedlings root-tips.

For gametic chromosome number determination and meiotic analysis, young inflorescences, collected at the field, were immediately fixed in Carnoy 6: 3: 1 (ethanol: clorophorm: acetic acid) for 24 h at room temperature and stored in 70% alcohol below 0 ºC until slide preparation. Slides were prepared by squashing the anthers of one flower bud per slide in 2 % propionic carmine. All available cells in all meiotic stages were analyzed. The best cells were photographed and/or registered by a digital image capturing system.

RESULTS AND DISCUSSION

This is the first work where a great number of *M bimucronata* populations (50) were cytogenetically analyzed. Previous works were restricted to a few populations from Paraguay and Argentina (Seijo 1999, 2000).

Somatic chromosome numbers were determined in 193 individuals from 41 populations and gametic chromosome numbers in 49 individuals of 12 populations (Table 1). All individuals were diploid with 2n = 2x= 26 (Figure 1 a to c) or n = 13 (Figure 2b) confirming the diploid nature of *M. bimucronata*, as already reported for two populations of de *M. bimucronata* var. *bimucronata* from Argentina (Seijo 1999) and for one population of *M. bimucronata* var. *adenocarpa* from Paraguay (Seijo 2000) No intraspecific variability for chromosome number was detected among the analyzed populations.

Polysomatic cells were detected in the seedlings root-tip cells, with and without pretreatment, in all the 41 populations examined except populations 13 and 21

Table 1. Population, locality collection, voucher number, geographical coordinates, chromosome number and percentage of polysomatic cells in the *M. bimucronata* poupulations analyzed

Population	Locality and voucher	Geographical coordinates	n=13[a]	2n=26[a]	Polysomatic cells (%)[b]
1	Brazil, Rio Grande do Sul, Porto Alegre. D. Olkoski 01 (ICN 162475)	S30º04'10.46"; W51º08'35.01"	6 (41)	10 (135)	9.6
2	Brazil, Rio Grande do Sul, Porto Alegre. D. Olkoski 02 (ICN 162476)	S30º.04'00.74"; W51º.07'16.09"	5 (25)	10 (64)	4.7
3	Brazil, Rio Grande do Sul, Santa Maria. D. Olkoski 03 (ICN 162477)	-		9 (115)	8.7
4	Brazil, Rio Grande do Sul, Cachoeira do Sul. D. Olkoski 04 (ICN 162478)	S30º15'49.0"; W52º50'48.5"		10 (97)	10.30
5	Brazil, Rio Grande do Sul, Restinga Seca. D. Olkoski 05 (ICN 162479)	S29º44'14.48"; W53º.21'48.07"		4 (43)	18.6
6	Brazil, Rio Grande do Sul, Viamão. D. Olkoski 06 (ICN 162480)	S 30º08'05.5"; W 50º55'06.4"		9 (116)	7.7
7	Brazil, Rio Grande do Sul, Viamão. D. Olkoski 07 (ICN 162481)	S 30º08'05.7"; W 50º55'06.6"		3 (27)	3.7
8	Brazil, Rio Grande do Sul, Viamão. D. Olkoski 08 (ICN 162482)	S 30º05'35.1"; W 50º59'52.1"		7 (65)	6.1
9	Brazil, Rio Grande do Sul, Porto Alegre. D. Olkoski 09 (ICN162483)	S 30º00'51.7"; W 51º19'02.2"		3 (39)	28.2
10	Brazil, Rio Grande do Sul, Eldourado do Sul. D. Olkoski 010 (ICN162484)	S 30º03'09.7"; W 51º25'59.7"		6 (62)	16.1
11	Brazil, Rio Grande do Sul, Eldourado do Sul. D. Olkoski 011 (ICN162485)	S 30º04'47.4"; W 51º36'13.1"		7 (143)	31.5
12	Brazil, Rio Grande do Sul, Arroio dos Ratos. D. Olkoski 012 (ICN162486)	S 30º06'25.1"; W 51º45'15.2"		5 (50)	16.0
13	Brazil, Rio Grande do Sul, Butiá. D. Olkoski 013 (ICN162487)	S 30º07'24.9"; W 51º53'35.4"		3 (25)	0.0
14	Brazil, Rio Grande do Sul, Minas do Leão. D.Olkoski 14 (ICN162488)	S 30º08'23.2"; W 52º01'27.3"		9 (106)	5.6
15	Brazil, Rio Grande do Sul, BR 116, km295. D.Olkoski 15 (ICN162489)	S 30º04'30.5"; W 51º20'27.3"		4 (25)	8.0
16	Brazil, Rio Grande do Sul, Guaíba. D.Olkoski 16 (ICN162490)	S 30º11'04.4"; W51º23'44.0"		6 (65)	12.6
17	Brazil, Rio Grande do Sul, Barra do Ribeiro. D.Olkoski 17 (ICN162491)	S 30º18'53.6"; W 51º25'02.3"		6 (50)	6.0
18	Brazil, Rio Grande do Sul, Capão da Porteira. D.Olkoski 18 (ICN162492)	S30º06'10.7"; W 50º 42'01.8"		3 (31)	6.4
19	Brazil, Rio Grande do Sul, Capivari do Sul. D.Olkoski 19 (ICN162493)	S 30º 07'59.7"; W 50º34'38.7"		5 (41)	14.6
20	Brazil, Rio Grande do Sul, Capivari do Sul. D.Olkoski 20 (ICN162494)	S 30º11'00.4" W 50º30'13.0"		7 (97)	18.5
21	Brazil, Rio Grande do Sul, Palmares do Sul. D.Olkoski 21 (ICN162495)	S 30º22'11.2"; W 50º29'42.3"		4 (42)	0.0
22	Brazil, Rio Grande do Sul, Bacopari. D.Olkoski 22 (ICN162496)	S 30º28'30.3"; W 50º28'07.1"		3 (33)	3.0

To be continued

Table 1, continuation

Population	Locality and voucher	Geographical coordinates	n=13[a]	2n=26[a]	Polysomatic cells (%)[b]
23	Brazil, Rio Grande do Sul, RST 101; km94. D.Olkoski 23 (ICN162497)	S 30º26'07.4"; W050º29'59.1"		8 (78)	16.6
24	Brazil, Rio Grande do Sul, Solidão. D.Olkoski 24 (ICN162498)	S 30º44'31.7"; W 50º36'35.7"		7 (89)	24.7
25	Brazil, Rio Grande do Sul, RST 101; km131,5. D.Olkoski 25 (ICN162499)	S 30º52'47.0"; W 50º42'46.6"		4 (30)	13.3
26	Brazil, Rio Grande do Sul, Mostardas. D.Olkoski 26 (ICN162500)	S 30º59'47.0"; W 50º49'40.2"		3 (32)	9.4
27	Brazil, Rio Grande do Sul, Porto Alegre. N. Dahmer s/n	S 30º01'37.28"; W51º12'02.83"		1 (15)	13.3
28	Brazil, Rio Grande do Sul, Parobé. D.Olkoski 27 (ICN162501)	S 29º38'30.8"; W 50º48'28.0"	3 (12)		
29	Brazil, Rio Grande do Sul, Rodeio Bonito. D.Olkoski 28 (ICN162502)	S 29º31'06.3"; W 50º43'57.9"	3 (24)		
30	Brazil, Rio Grande do Sul, Farroupilha. D.Olkoski 29 (ICN162503)	S 29º24'11.1"; W 51º20'58"	5 (37)		
31	Brazil, Rio Grande do Sul, Farroupilha. D.Olkoski 30 (ICN162504)	S 29º28'10.7"; W 51º21'11.4"	3 (32)		
32	Brazil, Rio Grande do Sul, São Sebastião do Caí. D.Olkoski 31 (ICN162505)	S 29º33'59.4"; W 51º21'37.0"	2 (8)		
33	Brazil, Rio Grande do Sul, São Sebastião do Caí. D.Olkoski 32 (ICN162506)	S 29º37'33.6"; W 51º18'37.2"	4 (23)		
34	Brazil, Rio Grande do Sul, Portão. D.Olkoski 33 (ICN162507)	S 29º42'45.9"; W 51º10'54.6"	4 (22)		
35	Brazil, Rio Grande do Sul, Sapucaia do Sul. D.Olkoski 34 (ICN162508)	S 29º49'13.5"; W 51º10'31.0"	2 (13)		
36	Brazil, Rio Grande do Sul, Esteio. D.Olkoski 35 (ICN162509)	S 29º51'51.9"; W 51º10'50.7"	2 (13)		
37	Brazil, Rio Grande do Sul, Canoas. D.Olkoski 36 (ICN162510)	S 29º52'59.9"; W 51º12'39.6"	10 (46)	10 (103)	25.8
38	Brazil, Rio Grande do Sul, Xangrilá. D.Olkoski 37 (ICN162511)	S 29º46'11.8"; W 50º03'24.1"		2 (18)	5.5
39	Brazil, Rio Grande do Sul, Rondinha. D.Olkoski 38 (ICN162512)	S 29º29'27.1"; W 49º52'15.1"		3 (32)	6.2
40	Brazil, Rio Grande do Sul, Santo Antonio da Patrulha. D.Olkoski 39 (ICN162513)	S 29º52'19.1"; W 50º25'11.3"		2 (24)	12.5
41	Brazil, Rio Grande do Sul, Santo Antonio da Patrulha. D.Olkoski 40 (ICN162514)	S 29º52'59.0"; W050º33'17.8"		3 (26)	11.5
42	Brazil, Rio Grande do Sul, Glorinha. D.Olkoski 41 (ICN162515)	S 29º54'22.0"; W 050º45'26.6"		2 (24)	12.5
43	Brazil, Rio Grande do Sul, Nova Santa Rita. D.Olkoski 42 (ICN162516)	S 29º50'20.3"; W 51º17'54.3"		7 (67)	6.0

To be continued

Table 1. continuation

Population	Locality and voucher	Geographical coordinates	n=13[a] 2n=26[a]	Polysomatic cells (%)[b]
44	Brazil, Rio Grande do Sul, Triunfo. D.Olkoski 43 (ICN162517)	S 29º45'21.1"; W 51º35'48.9"	2 (12)	16.6
45	Brazil, Rio Grande do Sul, Vera Cruz. D.Olkoski 44 (ICN162518)	S 29º42'02.3"; W 52º36'37.7"	1 (11)	9.0
46	Brazil, Rio Grande do Sul, Vale do Sol. D.Olkoski 45 (ICN162519)	S 29º41'23.0"; W 52º41'14.5"	2 (22)	4.5
47	Brazil, Rio Grande do Sul, Candelária. D.Olkoski 46 (ICN162520)	S 29º41'24.5"; W 52º50'56.6"	2 (20)	5.0
48	Brazil, Rio Grande do Sul, Novo Cabrais. D.Olkoski 47 (ICN162521)	S 29º45'58.2"; W 52º58'20.7"	1 (10)	10.0
49	Brazil, Rio Grande do Sul, Cachoeira do Sul. D.Olkoski 48 (ICN162522)	S 29º56'56.4"; W 52º56'52.9"	2 (14)	7.1
50	Brazil, Rio Grande do Sul, Cachoeira do Sul. D.Olkoski 49 (ICN162523)	S 30º04'27.6"; W 52º52'33 7"	3 (27)	7.4

[a] Number of individuals and cells (between brackets) analyzed.
[b] Percentage of polysomatic cells in seedling root-tips.

(Table 1). Almost all of polysomatic cells were tetraploid (Figure 1d) and a few triploid. Percentage of polysomatic cells (in root-tips with pretreatment) among populations ranged from 3.0 to 28.2 among populations. However polysomaty was absent from the root-tip cells of adult plants as well as in pollen-mother cells.

Witkus and Berger (1947) working with *Mimosa pudica* L., detected polysomaty in seedling root-tips from 1 to 4 mm length, but in 10 mm long root-tips no polysomatic cells were found. Seijo (1993, 1999) commented that polysomatic cells up to four times the diploid number of chromosomes were found in root-tips of some *Mimosa* species. Therefore, the results for *M. bimucronata* support Witkus and Berger (1947) suggestion that polysomaty in *Mimosa pudica* L. would be a mechanism for a rapid seedling establishment. On the other hand, absence of polysomatic cells in the germinative line would avoid the potential formation of polyploid gametes (Galbraith et al. 1991).

In *M. bimucronata* pollen-mother cells are joined two-by-two even before the onset of meiosis and remain linked during all the meiotic division (Figure 2a to 2e) until the formation of pollen grain polyads (Figure 2f). The sporogenic cells undergo mitosis and, the two resulting cells (Figure 2a) remain attached during meiotic division, that is synchronous in both sister cells (Figure 2b to 2e). At the end of meiosis the microspores of each set of two sister cells remain linked, originating the polyad, in fact a bitetrad of two groups of four pollen grains, each from one of the sister cells, that are dispersed in that form (Figure 2f).

Seijo and Neffa (2004) described the formation of polyads in *M. bimucronata* and proposed that the number of pollen grains per polyad is determined by the number of cells that remain attached after the last mitotic divisions of the sporogenic cell, that are two in *M. bimucronata*. In *Acacia* Mill., also a Mimosoideae genus, the mechanism of polyad formation was studied in detail (Kenrick and Knox 1979, 1982, Fitzgerald et al. 1993). In *Acacia*, polyads of eight, 16 and up to 32 pollen grains are formed. Polyads with 12 and 16 pollen grains are found in other *Mimosa* species. According to Seijo and Neffa (2004), the number of pollen grains per polyad would correlate to the numbers of ovules per ovary and, due to the dimensions of *M. bimucronata* stigma, only one polyad would fit per stigma. The occurrence of polyads in *M. bimucronata* would be an adaptation to ensure high seed set after a single pollination event (Seijo and Neffa 2004)

Concluding, all the 50 *M. bimucronata* populations analyzed were diploid (2n = 2x = 26, n = 13) and no intraspecific variability for chromosome number was detected. Polysomaty occurred in varied degrees but only in seedlings root-tips and was absent in root-tips of established plants as well as in pollen-mother-cells, supporting the theory that, in *Mimosa*, polysomaty is a way to assure seedling establishment.

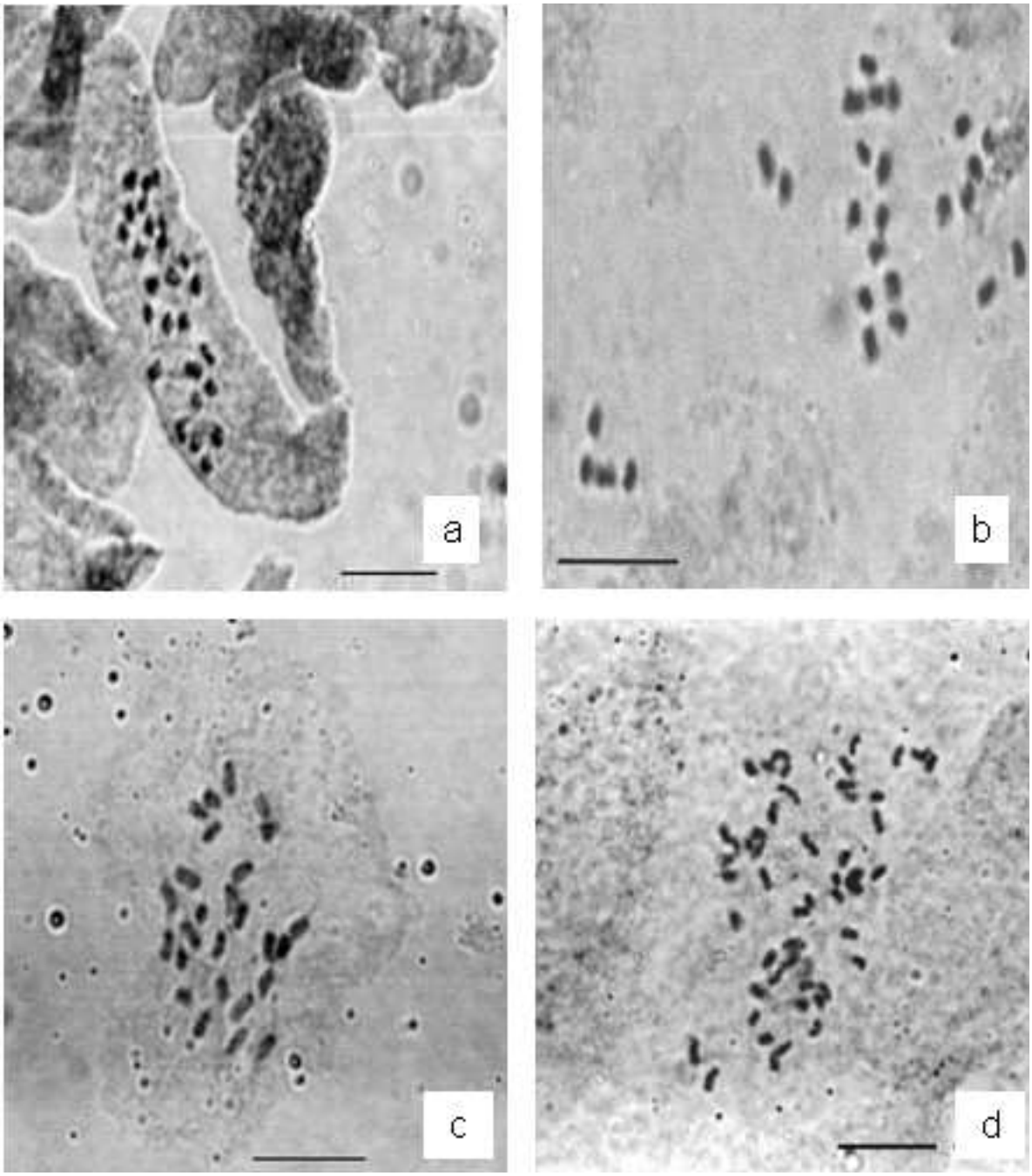

Figure 1. Mitotic metaphases of *M. bimucronata* (DC.) O. Kuntze. (a) population 19; (b) population 1; (c) population 3, all with 2n = 26; (d) root-tip polysomatic cell, with 2n = 52, population 3. Scale bar = 10 μm.

During all the meiotic process pollen-mother cells remain joined two-by-two, leading to the formation of polyads with eight pollen grains (two sets of four) each, probably facilitating pollen dispersion and pollination efficiency.

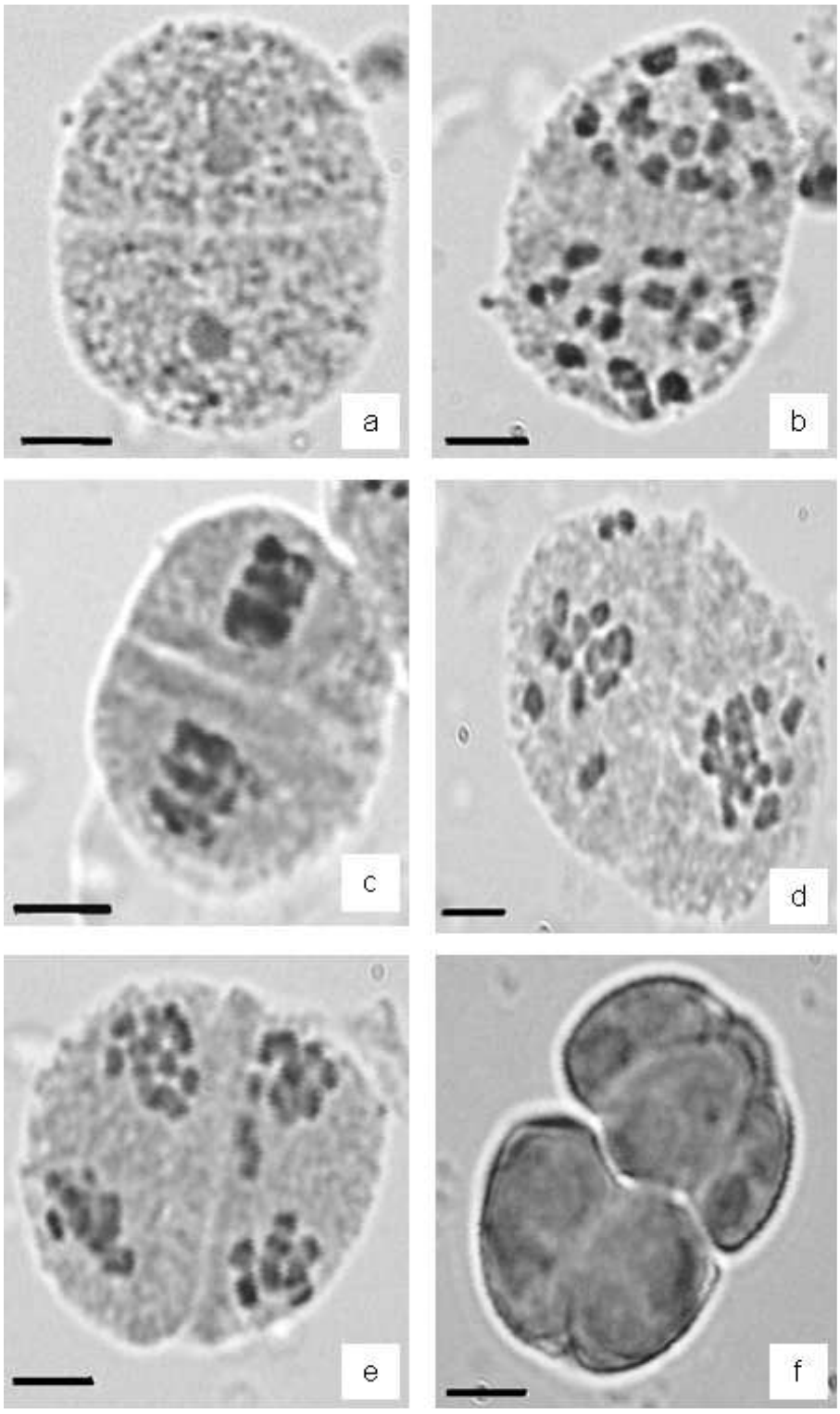

Figure 2. Meiosis in pollen-mother-cells (joined two-by-two) of *M. bimucronata* (DC.) O. Kuntze (a) interphase; (b) diakinesis showing 13 bivalents ; (c) metaphase I; (d) early anaphase I; (e) late anaphase I; (f) equatorial view of a polyad with eight pollen grains. Scale bar = 10 mm.

Citogenética de *Mimosa bimucronata* (DC.) O. Kuntze (Mimosoideae, Leguminosae): número cromossômico, polissomatia e meiose

RESUMO - *Os números cromossômicos (somáticos e/ou gaméticos) foram determinados em plantas oriundas de 50 populations de M. bimucronata (DC.) O. Kuntze coletadas na área de distribuição da espécie no Rio Grande do Sul. Todas as populações analisadas eram diplóides (2n = 2x = 26, n = 13). Células polissomáticas (em geral tetraplóides) foram detectadas em ponta de raiz de plântulas em 39 das 41 populações analisadas, variando de 3 a 28,2% entre populações. mas estavam ausentes nas pontas de raiz de plantas adultas. Polissomatia também estava ausente nas células-mães-de-pólen. Em* M. bimucronata *as células-mães-de-pólen estão juntas duas a duas antes do início da meiose, permanecendo unidas durante toda a divisão meiótica até a formação de políades de graõs de pólen. As políades são compostas de dois conjuntos de quatro grãos cada, que são dispersos desta forma o que, de acordo com sugestões prévias, seria uma adaptação para assegurar alta formação de sementes após um único evento de polinização.*

Palavras-chave: *maricá, variabilidade, políades.*

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