Unreduced gamete formation in *Curculigo capitulata* (Amaryllidaceae)

https://doi.org/10.56238/sevened2024.007-058

Neide da Silva[1], Gabriel Luiz de Melo Sales[2], Ítalo José de Araujo Vidal[3], Maria Fernanda Rodrigues de Tomasi[4], Carlos Eduardo Crippa Coletto[5], Kleber Lopes Longhini[6] and Andréa Beatriz Diverio Mendes[7]

ABSTRACT

Curuculigo capitulata is a perennial species from tropical Asia. The rhizomes have medicinal value and several chemical compounds were isolated from rhizomes. Only a few cytogenetic studies have been developed on this species. The presente study reports the formation of reduced gametes in plants of two Brasilian populations and confirms the chromosome number previously reported.

Keywords: Meiosis, Abnormal cytoKinesis, 2n gametes, Microsporogenesis, Chromosome number.

[1] Specialist. Department of Biotechnology, Genetics and Cell Biology - State University of Maringá - UEM
[2] Doctoral student. Postgraduate in Genetics and Plant Breeding - "Luiz de Queiroz" College of Agriculture - ESALQ
[3] Master's student. Postgraduate in Genetics and Breeding. Department of Agronomy - State University of Maringá - UEM
[4] Agronomy undergraduate student. Department of Agronomy - State University of Maringá - UEM
[5] Undergraduate student in Biological Sciences. Department of Biology - State University of Maringá - UEM
[6] PhD student. Postgraduate in Genetics and Breeding. Department of Agronomy - State University of Maringá - UEM
[7] PhD in Cell Biology. Department of Biotechnology, Genetics and Cell Biology - State University of Maringá - UEM

INTRODUCTION

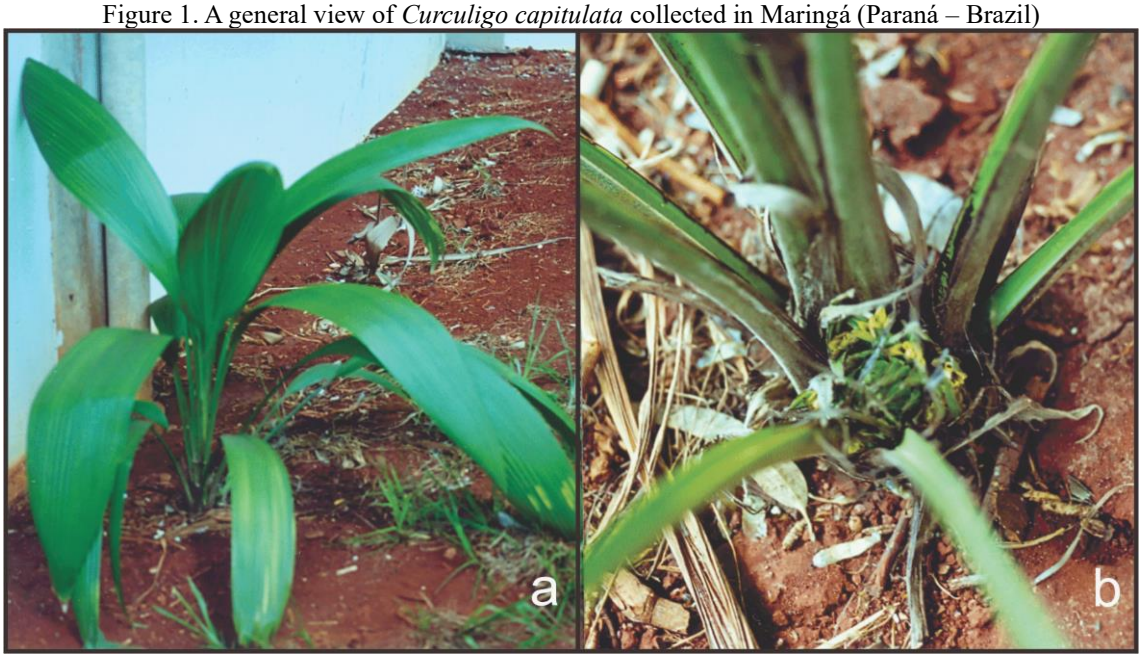
Curuculigo capitulata (Lour.) O. Kuntze (Amaryliidaceae) is a perenial specie from tropical Asia. It grow about 1 meter in lenght and the plant does not develop a stem. Because their leaves resemble palm leaves, it is known as 'palm-grass'. The rizomes have medicinal value. Several chemical compounds were isolated from rhizomes (Chang & Lee 1998, Chang et al. 1999, Galeffi et al 2002, Mujandar & Datta 2007). It is used as tonic, for rheumatism and dismenorrhoea (Galeffi et al. 2002) and also as anti-arrhytmic agente (Chang et al 1999).

Only a few cytogenetic studies have been developed on this species. Yongping et al. (1989) reported 2n = 2x = 18 chromosomes for three species od *Curculigo* analyzed (*C. crassifolia*, *C. capitulata* and *C. sinensis*). The presente study reports the formation of unreduced gametes in plants of two Brasilian population and confirm the chromosome number previously reported.

MATERIALS AND METHODS

Flower buds of C. capitulata (Figure 1) for meiotic studies were collected in Piracicaba (SP, Brazil) and Maringá (PR, Brazil). They were fixed in Carnoy (e ethyl alcohol: 1 acetic acid) for 24 hours at room temperature and then transferred to 70% alcohol and stored in freezer until the time for use. Microsporocytes were prepared by squashing and staining with propionic carmine 1%. The chromosome number and the pairing configuration were determined in meiocytes at diakinesis, and the meiotic behavior was determined up to this phase to the end of meiosis. Photomicrographs were taken using a Wild Leitz microscope with Kodak Imagelink-HQ, ISO 25 black and white film.

Figure 1. A general view of *Curculigo capitulata* collected in Maringá (Paraná – Brazil)



RESULTS AND DISCUSSION

Chromosome counting at diakinesis showed the presence of nine bivalentes (2n = 2x = 18), a number previously reported by Yongping et al. (1989). Univalents chromosomes were recorded in low frequency in this fase (Figure 2a). Table 1 shows the frequency of meiotic abnormalities related to irregular chromosome segregation due to the presence of univalentes. Precocious chromosome migration to the poles was observed in metáfase (Figure 2b), but the chromosome were generally included in the nucleus. Only a few micronuclei were found in telófase I (Figure 2e). Laggards (Figure 2c) and some non positioned chromosomes (Figure 2d) were observed in anaphase I. The percentage of abnormal cells in the second division was lower than that found in the first divison. Some micronuclei were recordeds in telófase II (Figure 2f), but tétrades were absolutely normal.

Figure 2. Meiotic abnormalities related to regular chromosome segregation in *Curculigo capitulata*. a. Meiocyte in diakinesis with 2n= 18 chromosomes. Arrows indicate two pairs as univalentes. b. Metaphase I with precocious chromosome migration to the pole (arrow). c. Anaphase I with laggard chromosomes (arrow). d. Anaphase I with chromosome outside spindle (arrow). e. Telophase I with micronuclei (arrow). f. Telophase I with micronucleus (arrow).

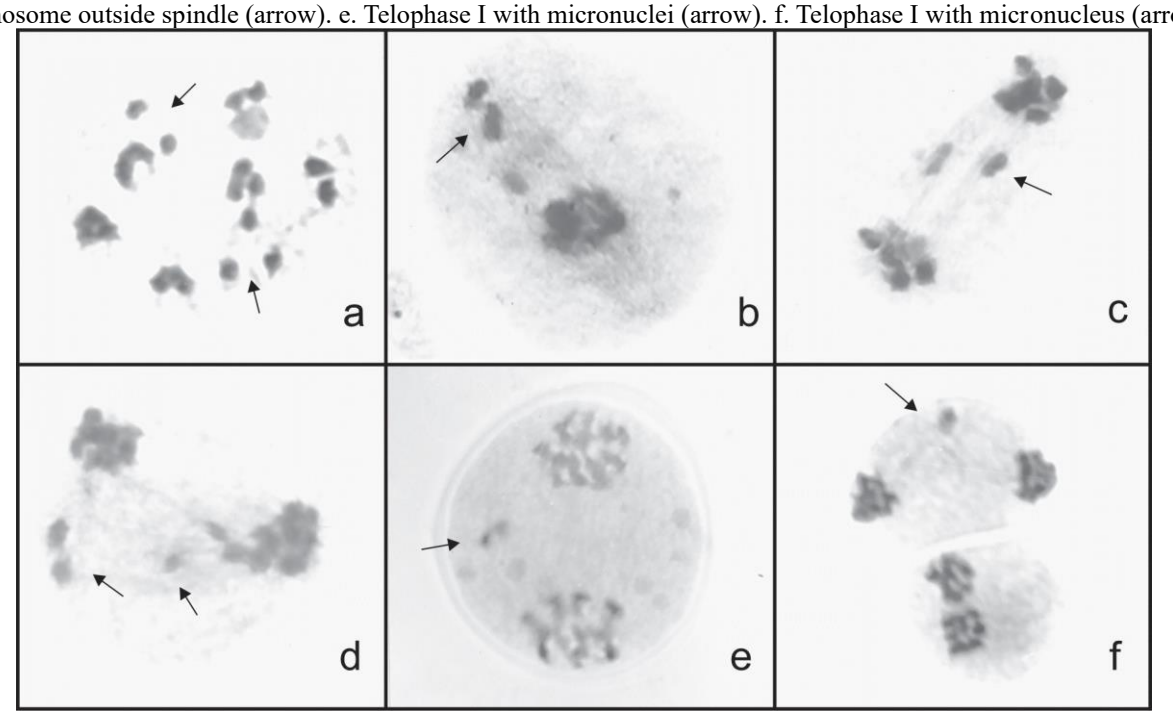


Table 1. meiotic abnormalities realted to irregular chromosome segregation in *curculigo capitulata* colleted in Maringá (Paraná – Brazil) and Piracicaba (São Paulo – Brazil).

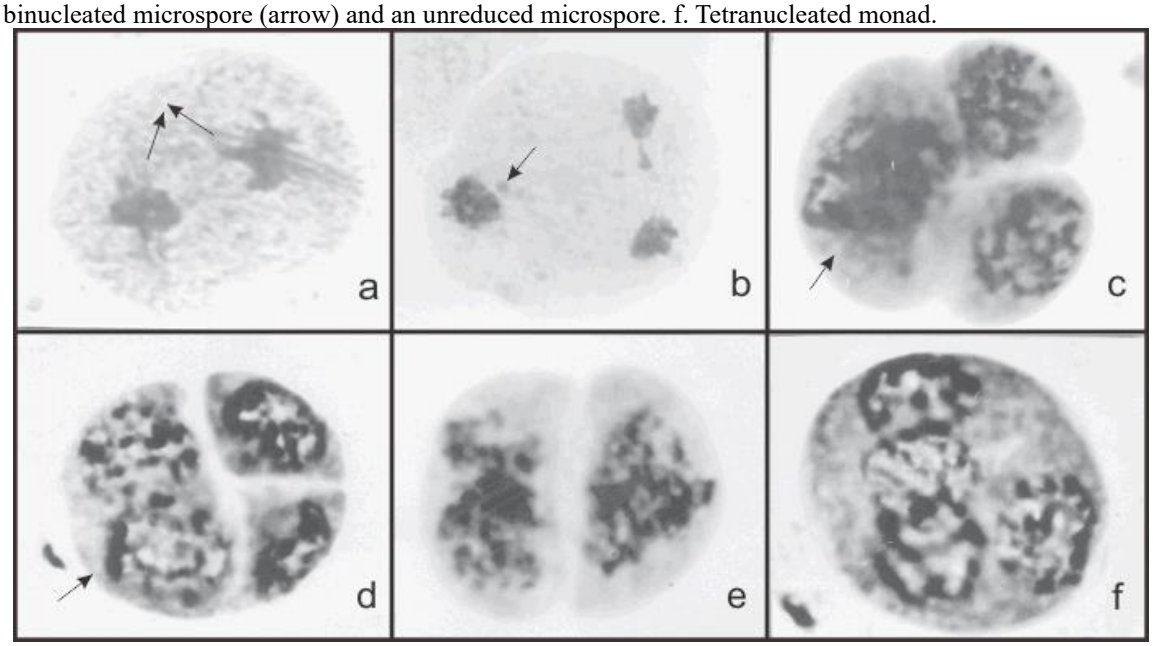
Origin	Plant	Phases of meiossis Number of cells analyzed (% of abnormal cells)							
		MI	AI	TI	PII	MII	AII	TII	TET.
Maringá	1	165 (33.33)	-	189 (0.0)	134 (19.40)	11 (0.0)	-	194 (8.56)	356 (0.0)
	2	03 (0.0)	-	80 (0.0)	505 (8.91)	72 (12.0)	-	18 (7.78)	88 (0.0)
	3	563 (29.84)	75 (26.67)	285 (9.12)	461 (3.47)	217 (0.0)	67 (0.0)	270 (0.0)	-
	4	380 (13.16)	34 (0.0)	319 (4.39)	195 (0.0)	191 (0.0)	30 (0.0)	298 (0.0)	136 (0.0)
	5	88 (23.86)	-	-	14 (0.00	227 (0.0)	18 (0.0)	191 (0.0)	88 (0.0)
	6	338 (37.87)	26 (11.54)	166 (21.69)	185 (0.0)	146 (7.53)	11 (18.18)	103 (0.0)	441 (0.0)
	7	428 (29.43)	29 (0.0)	388 (13.66)	347 (0.86)	262 (3.82)	23 (0.0)	170 (1.18)	12 (0.0)
	8	434 (22.81)	37 (13.51)	354 (8.75)	242 (0.0)	186 (2.67)	20 (10.0)	253 (3.95)	272 (0.0)
	9	38 (13.16)	-	60 (8.33)	308 (0.64)	230 (0.0)	16 (0.0)	291 (3.78)	133 (0.0)
Piracicaba	1	151 (37.56)	50 (8.0)	230 (17.83)	86 (0.0)	157 (0.13)	23 (0.0)	14 (0.0)	216 (0.0)
	2	580 (22.62)	50 (15.38)	401 (23.44)	160 (2.50)	198 (0.0)	241 (0.0)	18 (0.0)	175 (0.0)
	3	97 (19.12)	15 (0.0)	139 (8.63)	255 (0.0)	182 (2.20)	09 (0.0)	143 (0.0)	320 (0.0)
	4	823 (20.29)	170 (0.0)	186 (0.0)	93 (0.0)	106 (0.0)	27 (0.0)	445 (0.0)	120 (0.0)
Total of cells		4058	486	2797	2985	1944	485	2408	2357

Other meiotic abnormality recorded in these plants was relates to abnormal cytokinesis and spindle orientation. The first or the second cytokinesis, or both, failed to occur. A considerable number of meiocytes (Table 2) did not presente the first cytokinesis. In these cells, the spindles were parallel or tripolar (Figure 3a), rejoining or not the segregated chromosome into a restitutional nucleus (Figure 3b). The cells originated a triad with two n micorspores end the one unreduced micorspore (Figure 3c). Triads with one binucleated micorspore (Figure 3d) and a dyads with binucleated and reduced micorspores (Figure 3e) were also observed. When both cytokinesis failed to occur, monads were formed (Figure 3f). Table 2 shows all the types of abnormal meiotic products observed in the analyzed plants.

Table 2. Number of abnormal meiotic products in *Curculigo capitulata* collected in Maringá (Paraná – Brazil) and Piracicaba (São Paulo – Brazil).

Meiotic products	Types	Maringá/Plant									Piracicaba/Plant			
		1	2	3	4	5	6	7	8	9	1	2	3	4
Tetrads	normal	356	88	28	136	195	441	12	272	133	216	175	320	120
Diads	Two unreduced microspores	107	-	44	51	27	31	38	19	10	48	28	60	71
	One unreduced microspore	48	-	-	42	17	-	3	8	4	13	10	-	-
	Two binucleated microspores	-	72	-	6	36	-	21	1	1	10	35	14	-
Triads	One unreduced microspore	128	-	9	-	7	23	5	15	9	66	10	26	105
	One binucleated microspore	36	-	12	-	11	-	1	19	8	6	5	4	-
Monads	Tetranucleated	23	-	9	21	12	-	-	3	4	26	27	-	-
	trinucleated	4	-	17	11	-	-	1	3	3	10	6	-	-
	Binucleated	7	-	15	-	-	51	6	1	1	25	-	-	-
	Uninucleated	16	-	12	-	-	24	14	1	1	11	-	7	-

Figure 3. Abnormalities related to abnormal cytokinesis and meiotic products. a. Meiocyte in metáfase II with adsence of citokinesis and showing tripolar spindle (arrow). b.Telophase II derived from tripolar spindle. Note the unreduced nucleus (arrow). Triad with na unreduced microspore (arrow). d. Triad with a binucleated microspore (arrow). e. Dyad with a binucleated microspore (arrow) and an unreduced microspore. f. Tetranucleated monad.



The number of affected cells in each plant was variable. Some plants presented a high number of unreduced micospores. The trend to form unreduced gametes in plants is highly variable, and it varies among individual within a single taxonomic grou por even among flowers of an individual

plant (Bretagnolle & Thompson, 1995). Meiotic nuclear restitution may be caused by diferente mechanisms (Ramanna, 1979, Veilleux, 1985, Bretagnolle & Thompson, 1995). Absence of cytokinesis and tripolar spindle formation have been reported in some *Brachiaria* species (Risso-Pascotto et al 2003. Gallo et al, 2007).

Polyploidization may be assexual through somatic chromosome doubling and sexual though the formation of unreduced gametes.Actually, unreduced gametes are considered to be the dominant process involved in the origino f polyploidy in plants (Veilleux, 1985, Bretagnolle & Thompson, 1995). Despite the presence of unreduced gamete formation in *C. capitulata*, all plants analyzed were diploid, and polyploidy was never reported in the genus.

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