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A Meiotic Study of the Wild and Semi-domesticated Brazilian Species of Genus Capsicum L. (Solanaceae)

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Summary Meiotic behavior was analysed in 30 accessions of 12 wild (*Capsicum flexuosum*, *C. parvifolium*, *C. buforum*, *C. campylopodium*, *C. cornutum*, *C. pereirae*, *C. friburgense*, *C. schottianum*, *C. villosum* var. *villosum*, and 3 new species) and 2 semi-domesticated (*C. baccatum* L. var *praetermissum* and *C. chinense*) Brazilian *Capsicum* species. This is the first report on meiosis for most of these taxa. Except for *C. baccatum* L. var *praetermissum*, *C. chinense*, *C. flexuosum* and *C. parvifolium*, with $2n=24$ chromosomes, all other taxa had $2n=26$ chromosomes, a number previously considered as not common in the genus. Meiotic pairing was generally regular (12 or 13 bivalents) for most of the species analyzed. However, different irregularities such as univalents, multivalents, bridges, chromosome stickiness, unoriented chromosomes, monads, dyads and polyads, among others, were found at both meiosis I and meiosis II, leading to unbalanced final meiotic products but generally not a high pollen sterility. It was not possible to establish with certainty if these irregularities were due to genetic (meiotic mutations) or environmental (such as pests and diseases) causes.

Key words Brazilian peppers, meiotic behavior.

Chile peppers (genus *Capsicum*), a popular and profitable culture grown in the tropics and temperate regions, are among the main cultivated vegetables and spices. Some species and varieties are also used for ornamental and medicinal purposes. However, there are serious phytosanitary and quality problems that may be overcome by selection and breeding. In *Capsicum*, resistance to pests and diseases is normally an attribute of a given accession and not of the whole species (González and Bosland 1991), therefore the variability found in the wild species represent valuable genetic resources to be explored.

The exact generic and infra-specific delimitations are still controversial but 20 to 30 wild and semi-domesticated species and 5 cultivated ones (*Capsicum annuum* L. var. *annuum*, *C. baccatum* L. var. *pendulum* (Willd.) Eshbaugh, *C. chinense* Jacq., *C. frutescens* L. and *C. pubescens* Ruiz and Pavon) are presently recognized, all of them native to the New World (McLeod *et al.* 1979a, 1979b; McLeod *et al.* 1983a, 1983b).

Different approaches such as morphological, immunological, flavonoid and isozymes patterns and molecular markers analyses, aimed at establishing genetic and evolutionary relations among *Capsicum* species have been disclosing polymorphisms.

As for cytogenetics, karyotype aspects have been studied in wild and domesticated species (Pickersgill 1971, 1977, 1991, Limaye and Patil 1989, Moscone 1990, 1993, 1999, Bertão 1993, Moscone *et al.* 1993, 1995, 1996, Tong and Bosland 1997, 2003, Ferreira 1998, Park *et al.* 2000). In all these studies, $x=12$ (mostly as $2n=24$) have been cited as the predominant basic number in

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the genus, while $x=13$ ($2n=26$) was found in only in 5 taxa (*C. ciliatum* (H., B. & K.) Kuntze, *C. lanceolatum* (Green.) Morton & Stand., *C. mirabile* Martius, *C. campylopodium* Sendtner and 1 unidentified *Capsicum* sp. However, Pozzobon *et al.* (2006) recently found $2n=26$ for 9 more species from Brazil and suggested that $x=12$ and $x=13$ represent different evolutionary trends in the genus. Meiotic behavior evaluation has been performed in the $x=12$ wild and domesticated species as well as in some hybrids, aiming at verifying genomic diversification during evolution as well as possible inter-specific phylogenetic relations (Otha 1961, Lippert *et al.* 1966, Shopova 1966a, 1966b, Carluccio and Saccardo 1977, Pickersgill 1971, 1977, 1991, Saccardo and Ramulu 1977, Egawa and Tanaka 1984, Mirkova and Molchova 1985, Kumar *et al.* 1987, Raghuvanshi and Saxena 1991, Moscone 1992, Bapa Rao *et al.* 1992, Lanteri and Pickersgill 1993, Tong and Bosland 1999, Panda *et al.* 2004). However there are few reports (Tong and Bosland 1997, 2003) on meiotic behavior of the $x=13$ taxa.

Southeastern Brazil is considered to have the largest number of wild *Capsicum* species (at least 10 different taxa), being the biggest diversity center of wild species. (Pickersgill 1984, Bianchetti 1996). Most of them are distributed along the Atlantic Rain Forest and threatened by genetic erosion due to anthropic pressure. These potential genetic resources have not been properly used for *Capsicum* breeding or evolutionary studies mainly due to the bottleneck of lack of basic information as chromosome numbers and meiotic behavior.

Therefore, the objective of the present work was to analyze the meiotic behavior of the EMBRAPA-Hortaliças (Brazilian Agricultural Research Corporation–Horticulture) Distrito Federal, Brazil, collection of wild and semi-domesticated Brazilian *Capsicum* species and accessions.

Material and methods

A total of 30 accessions of 14 species were analyzed: the semi-domesticated *C. baccatum* L. var. *praetermissum* (Heiser and Smith) Hunz., and *C. chinense* Jacq., and the wild *C. flexuosum* Sendtner, *C. parvifolium* Sendtner, *C. buforum* Hunz., *C. campylopodium* Sendtner, *C. cornutum* (Hern.) A. T. Hunziker, *C. schottianum* Sendtner, *C. villosum* Sendtner var. *villosum*, 2 new species *C. pereirae* Hunz. and *C. friburgense* Hunz. (Barboza and Bianchetti 2005) and 3 other putative new species, here referred as *Capsicum* sp 1, *Capsicum* sp 2 and *Capsicum* sp 6 (Table 1). Except for *C. baccatum* L. var. *praetermissum*, *C. chinense*, *C. flexuosum* and *C. parvifolium* ($2n=24$) all other taxa have $2n=26$ (Pozzobon *et al.* 2006).

The material analysed originated from plants directly collected from natural populations, as well as from plants obtained from seeds collected from natural populations, both grown in greenhouse and experimental field at EMBRAPA-Hortaliças. Some accessions were also maintained in pots in a greenhouse at Departamento de Plantas Forrageiras and Agrometeorologia, Universidade Federal do Rio Grande do Sul (Department of Forage Plants and Agrometeorology, Federal University of Rio Grande do Sul), Porto Alegre, RS, Brazil. For some of the accessions, more than 1 individual was grown (identified by letters in Table 2).

Taxonomic vouchers for each accession are kept at EMBRAPA–CENARGEN (National Genetic Resources and Biotechnology Center), Brasília, DF, Brazil.

For meiotic studies, young flowers were fixed in a mixture of 3 : 1 ethanol-acetic acid for 24 h, and stored in 70% ethanol below 0°C until required. Slides were prepared by squashing the anthers in 2% propionic carmine. To analyze chromosome pairing, the highest available number of well-spread cells at diakinesis and metaphase were examined. Besides, to follow all the meiotic process, all available and well-spread cells in any other meiotic phase were examined. Meiotic indexes were estimated following Love (1949). Those tetrads with 4 equal-sized cells were considered as normal, and any deviation as abnormal. Pollen fertility was estimated by analyzing at least 1000 mature pollen grains per plant. Well stained grains were considered as fertile and empty ones as sterile.

Table 1. *Capsicum* species, accession number, collector identification and origin of the accessions examined

Species, CNPH[1] accession number, collector's[2] number	Place of collection[3]	Species, CNPH[1] accession number, collector's[2] number	Place of collection[3]
Semi-domesticated		*C. villosum* var. *villosum*	
C. baccatum L. var.	*praetermissum*	3313, LBB 1538	Estação Biológica da Boracéia, SP
3325, LBB 1553	Caldas, MG	3314, LBB 1539	Estação Biológica da Boracéia, SP
3326, LBB 1555	Maria da Fé, Pedrão, MG	3317, LBB 1543	Estação Biológica da Boracéia, SP
C. chinense		3328, LBB 1557	Parque Nacional do Itatiaia, RJ
3727, LBB 1720	Vila Apiaú, Roraima, RR	*Capsicum* sp 1[4]	
Wild		3301, LBB 1521	Parque Estadual Carlos Botelho, SP
C. flexuosum		*Capsicum* sp 2 (Oito dentes)[4]	
3324, LBB 1552	Caldas, MG	3302, LBB 1523	Parque Estadual Jacupiranga, SP
C. parvifolium		3372, LBB 1525	Estação Ecológica Juréia, Itatins, SP
3331, LBB 1560	Univ. Federal de Viçosa, MG	*Capsicum* sp 6 (Piquete)[4]	
C. buforum		3330, LBB 1559	Lima Duarte, MG
3367, LBB 1550	1 km da Estação Eugene Lefévre, SP	3337, LBB 1568	Parque Nacional do Caparaó, MG
3368, LBB 1554	Maria da Fé, EPAMIG, MG	3370, LBB 1564	Parque Nacional Serra dos Órgãos, RJ
C. campylopodium		*C. pereirae*	
3335, LBB 1566	Santa Maria Madalena, RJ	3329, LBB 1558	Parque Estadual do Ibitipoca, MG
3369, LBB 1562	Parque Nacional da Tijuca, RJ	3336, LBB 1567	Castelo, ES
C. cornutum		*C. friburgense*	
3316, LBB 1542	Estação Biológica da Boracéia, SP	3334, LBB 1565	Nova Friburgo, RJ
3320, LBB 1546	Paraty-Cunha, SP		
3365, LBB 1527	Parque Estadual Serra do Mar, SP		
C. schottianum			
3310, LBB 1535	Estação Biológica da Boracéia, SP		
3311, LBB 1536	Estação Biológica da Boracéia, SP		
3315, LBB 1540	Estação Biológica da Boracéia, SP		
3318, LBB 1544	Natividade da Serra-Bairro Alto, SP		
3319, LBB 1545	São Luiz de Paraitinga SP		

[1] CNPH-Embrapa Hortaliças.
[2] LBB–Luciano de Bem Bianchetti.
[3] ES–Espírito Santo; MG–Minas Gerais; SP–São Paulo; RJ–Rio de Janeiro; RR–Roraima.
[4] Putative new species, numbered and nicknamed following Bianchetti *et al.* (1999).

Results were recorded by photomicrographs and digital image capturing.

Results and discussion

These are the first reports on meiotic behavior of 11 out of the 14 species analyzed. Previous observations on *C. chinense*, *C. flexuosum* and *C. buforum* meiosis have been published and will be discussed further on.

Chromosome pairing at diakinesis and metaphase I, as well as the other phases of meiosis, were predominantly regular for the $2n=24$ species *C. baccatum* var. *praetermissum*, *C. chinense*, *C. flexuosum* and *C. parvifolium* (Table 2). Most of the cells analyzed presented 12 bivalents (II) (Fig. 1A). A few quadrivalentes (IV) were observed in *C. chinense*, *C. flexuosum* and *C. parvifolium* (Fig. 1B), and univalentes (I) em *C. flexuosum*. In some cells chromosome stickiness hampered the interpretation of pairing configurations (Fig. 1C) and in others chromosome superposition and non-oriented bivalents were observed. Even if in reduced frequencies, meiotic abnormalities were seen (Table 2): irregular disjunction and stickiness at anaphase I (Fig. 1D), laggards, bridges and/or fragments at anaphase and telophase I. Pro-metaphase II and metaphase II were regular (12+12) in

Table 2. Meiotic behavior in several Brazilian wild and semi-domesticated *Capsicum* species and accessions

Species/accession	*n*	Associations at diakinesis and metaphase I[1]	Anaphase/ telophase I (% normal)	No. cells	Pro-metaphase/z metaphase II N/Ir[2]	Anaphase/ telophase II (% normal)	No. cells	Pollen tetrads N/M/D/T/Oi[3]	Meiotic index (%)	Pollen viability (%)
Semi-domesticated										
C. baccatum var. *praetermissum*										
3325	12	12 II (2)	75.23	101	5N/4Ir	71.43	70	562N/1D/1T/164Oi	77.20	—
3326	12	—	—	—	—	93.88	49	11N/2Oi	84.61	—
C. chinense										
3727 pl A*	12	—	—	—	—	—	—	230N/6Oi	97.45	89.23
3727 pl B*	12	12 II (36)	—	—	—	96.08	153	438N/11Oi	97.55	81.52
3727 pl C*	12	12 II (3) 10 II+1 IV (1)	88.00	25	4N	78.48	79	219N/7Oi	96.90	95.98
3727 pl D*	12	—	—	—	—	—	—	1087N/41Oi	96.36	95.50
Wild										
C. flexuosum										
3324 A	12	12 II (3)	91.53	118	—	91.96	882	284N/8T/4Oi	95.94	93.70
3324 B (2002)**	12	—	68.18	44	—	81.75	126	133N/3Oi	97.79	92.18
3324 B (2004)**	12	2 I+9 II+1 IV (1)	56.70	97	2N	80.00	180	53N/1M/3T/20Oi	68.83	73.96
C. parvifolium										
3331 pl A*	12	—	84.71	170	113N	98.33	180	472N/2M/1D/9T/8Oi	95.93	81.10
3331 pl B*	12	12 II (5) 10 II+1 IV (1)	—	—	99N	98.48	461	274N/1Oi	99.64	—
Capsicum sp. 1										
3301	13	13 II (36)	100.00	78	—	91.84	98	252N/35Oi	87.80	95.61
Capsicum sp. 2										
3302	13	13 II (68)	98.74	239	58N	100.00	443	799N/6T/51Oi	93.34	72.33
3372	13	—	100.00	2	8N	89.45	218	403N/34Oi	92.23	98.17
Capsicum sp. 6										
3330	13	—	48.15	27	—	89.47	57	149N/5T/7Oi	92.55	94.22
3337	13	26 I (1) Ni (several)	70.76	130	—	78.61	187	252N/86Oi	74.56	75.65
3370	13	26 I (1) 12 I+2 II+2 III+1 IV (1) Ni (37)	51.78	112	—	10.93	183	0N/46M/178D/59T/413Oi	00.00	—
C. pereirae										
3329	13	13II (5) 7 I+8 II+1 III (1) 5 I+6 II+3 III (1) Ni (3)	—	—	—	—	—	—	—	—

3336	13	—	—	—	—	—	—	9N/2Oi	81.81	—
C. friburgense										
3334	13	13 II (2)	77.13	376	—	79.20	452	222N/1M/4D/22T/123Oi	59.68	—
C. buforum										
3367	13	—	55.56	36	6N/4Ir	45.45	11	177N/2D/1T/79Oi	68.34	85.89
3368 pl A*	13	13 II (10)	10.81	37	4N	25.13	199	240N/183D/46T/80Oi	43.72	—
3368 pl B*	13	13 II (26) 1 I+1 III+11 II (1)	55.56	36	—	34.21	114	214N/5M/35D/15T/110Oi	36.09	58.89
C. campylopodium										
3335	13	13 II (58)	94.74	133	—	100.00	279	65N/1T/4Oi	92.86	—
3369	?	—	—	—	—	—	—	598N/10Oi	98.35	96.92
C. cornutum										
3316	13	13 II (26)	100.00	11	—	88.33	120	126N/6Oi	95.45	95.45
3320	13	13 II (12) Ni (17)	93.43	76	22N	42.47	73	463N/8T/73Oi	85.16	84.50
3320 pl A*	13	13 II (66)	100.00	17	21N	93.55	62	160N/6Oi	96.39	92.11
3320 pl B*	13	—	80.77	26	—	73.20	97	420N/2T/179Oi	69.88	79.03
3365	13	13 II (42) 11 II+1 IV (1)	92.04	113	—	97.44	117	177N/65Oi	73.14	—
C. schottianum										
3310	13	13 II (2) Ni (3)	46.15	78	6N	90.91	143	298N/4T/110Oi	72.33	82.33
3311	13	13 II (22) 11 II+1 IV (2)	100.00	30	—	100.00	14	165N/1M/22Oi	87.77	93.35
3315	13	1 I+9 II+1 III+1 IV (1)	78.61	416	29N	96.11	180	268N/1T/54Oi	83.23	94.22
3318	13	—	78.44	167	25N/15Ir	60.00	165	376N/10T/118Oi	74.60	—
3319	13	13 II (29) 2 I+12 II (2)	50.00	62	18N/6Ir	78.94	95	506N/1T/99Oi	83.50	93.19
C. villosum var. *villosum*										
3313	13	13 II (137)	86.13	137	2N	98.70	461	137N/1Oi	99.27	84.62
3314	13	—	—	—	5N	—	—	—	—	—
3317	13	—	—	—	—	—	—	—	—	88.86
3328 pl A*	13	13 II (23)	88.21	195	73N	79.48	268	356N/96Oi	78.76	—
3328 pl B*	13	—	—	—	34N	78.33	203	629N/2M/1D/5T/84Oi	87.24	74.61

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[1] I, I univalents; II bivalents; III trivalents; IV quadrivalents; Ni multiple associations not identified and/or stickiness; ( ) number of cells analyzed.

[2] N normal disjunction 12+12 or 13+13, Ir irregular.

[3] N normal pollen tetrad; M monad; D dyad; T tryad; Oi other irregularities No. cells=number of cells analyzed.

* Different individuals of the same population.

** Different years of collection.

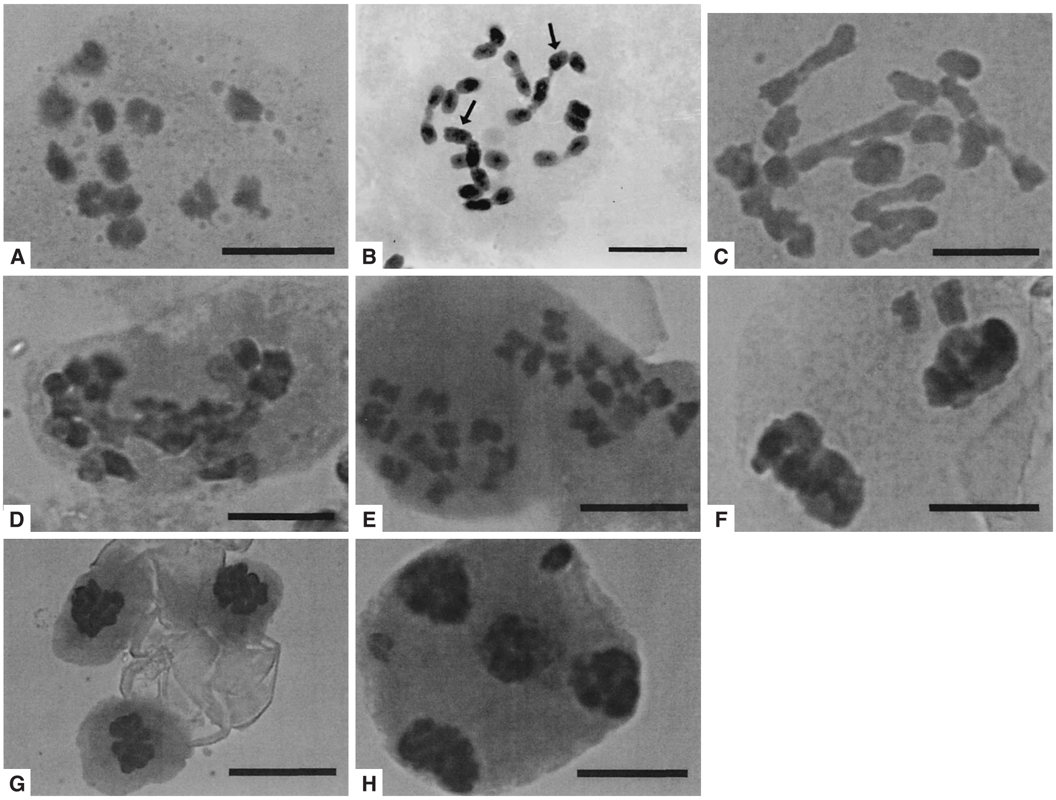

Fig. 1. Meiosis in $2n=24$ *Capsicum* species. A) *C. chinense*, late diakinesis, with 12 bivalents; B) *C. parvifolium*, late diakinesis with two chain quadrivalents (→). C–H) *C. flexuosum*: C) metaphase I, with chromosome stickiness, D) anaphase I, with bridges, E) pro-metaphase II, with a normal 12+12 segregation, F) metaphase II, with two chromosomes outside the plate, G) restitution nuclei, H) telophase II with two micronuclei. Scale bar equal to 10μm.

most of the cells (Fig. 1E) but chromosomes outside the equatorial plate were observed (Fig. 1F) and in telophase II micronuclei (Fig. 1H) and formation of 3 instead of 4 nuclei were found. Monads were found in *C. flexuosum* and *C. parvifolium* (Fig. 2C), dyads in *C. baccatum* var. *praetermissum* (Fig. 2B) and *C. parvifolium* and tryads in *C. baccatum* var. *praetermissum*, *C. flexuosum* and *C. parvifolium*, probably originated from the restitution nuclei observed between pro-metaphase and anaphase II (Fig. 1G and 2A). Some tetrads with unequal-sized cells, polyads with varying microcyte number and size and micronuclei were seen (Fig. 2F). In *C. baccatum* var. *praetermissum* a possible type of chromosome elimination was observed (Fig. 2D and E), in which those chromosomes that appeared as micronuclei in previous meiotic phases were eliminated from the microspore as microcytes. This chromosome elimination have been reported for *C. annuum* L., *C. frutescens* L. and *C. pubescens* Ruiz and Pavon (Shopova 1966b) and is similar to what has been described for *Avena sativa* L. (Baptista-Giacomelli *et al.* 2000), but there is no clear explanation for this phenomenon.

Most of the works with the domesticated *Capsicum* species *C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens* reported regular chromosome pairing (12 II) (Kumar *et al.* 1987, Bapa Rao *et al.* 1992, Panda *et al.* 2004) but some meiotic irregularities were are also found (Lippert *et al.* 1966, Shopova 1966b) and Moscone (1992) observed some abnormalities in 1 accession of the wild $2n=24$ *C. flexuosum*. The possible reasons for these irregularities could be hybrid origin and in-

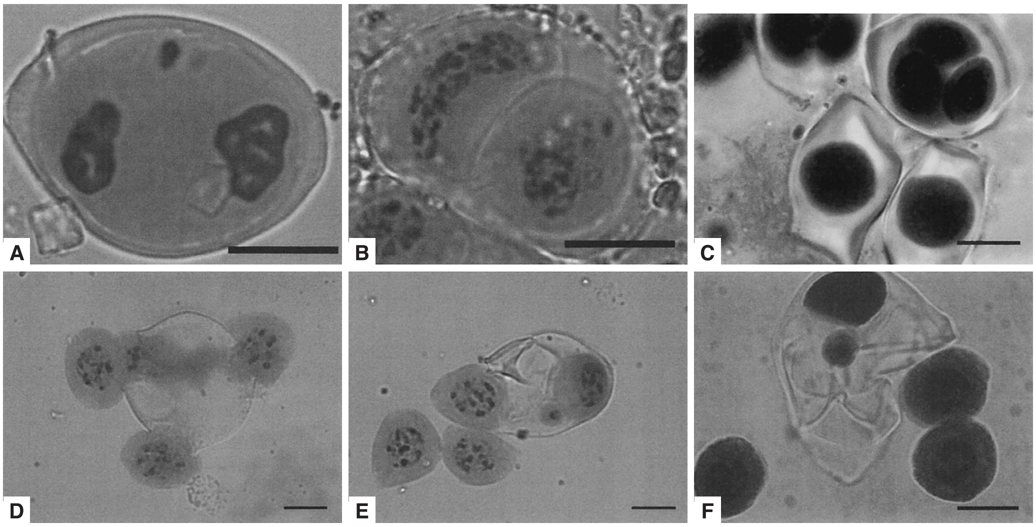

Fig. 2. Meiosis in $2n=24$ *Capsicum* species. A and B) *C. baccatum* var. *praetermissum*: A) formation of two nuclei and one micronucleus, B) dyad, C) *C. parvifolium*, monads. D and E) *C. baccatum* var. *praetermissum* showing chromosome elimination, F) *C. parvifolium*, tetrad and microcyte. Scale bar equal to 10 μm.

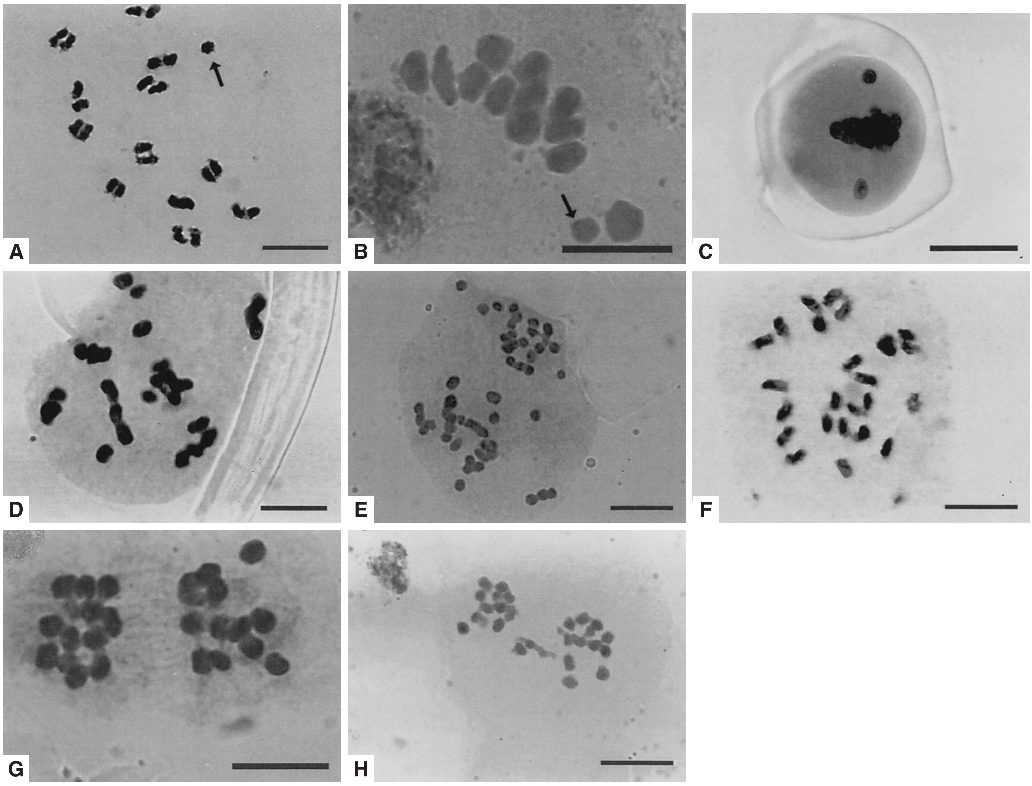

Fig. 3. Meiosis in $2n=26$ *Capsicum* species. A) Diakinesis in *C. sp* 2 with 13 bivalents, B and C) *C. cornutum*: B) metaphase I with 13 bivalents (→small thirteenth pair), C) metaphase I with non-oriented chromosomes, D and E) *C. sp* 6: D) metaphase I, polar view, with univalents and multiple associations, E) anaphase I with chromatid separation, F and G) *C. schottianum*: F) bivalents loosely associate at diakinesis, G) anaphase I with 13+13 chromosome distribution, H) *C. pereirae*, anaphase I with bridge and fragment. Scale bar equal to 10 μm.

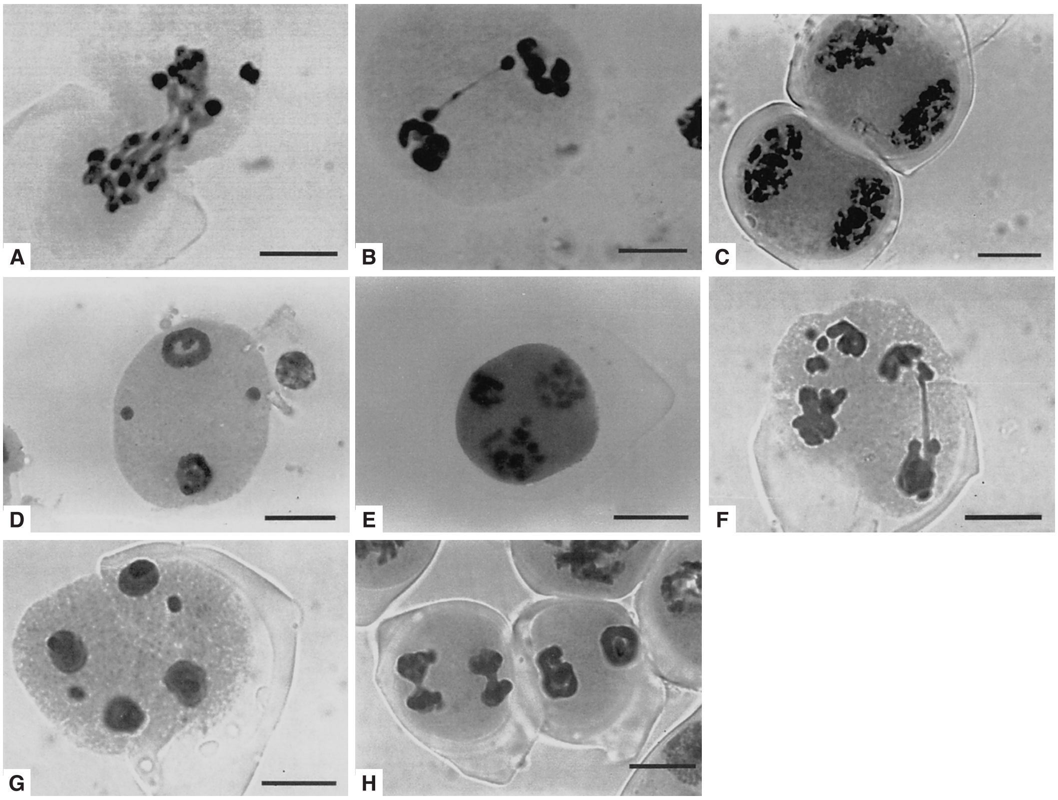

Fig. 4. Meiosis in $2n=26$ *Capsicum* species. A) *C. sp* 6, anaphase I with chromosome stickiness, B) *C. villosum* var. *villosum*, telophase I, with bridge, C) *C. sp* 6, cells with restitution nuclei, D and E) *C. buforum*, cells with formation of two and three nuclei and micronuclei, F–H) *C. friburgense*: F) telophase II with bridge and micronuclei, G) telophase II with micronuclei, H) tellophase II with bridge and formation of two nuclei. Scale bar equal to 10 μm.

tense self-fertilization which would not necessarily influence fertility (Shopova 1966b, Novák and Betlach 1970).

Meiotic indexes most probably reflect what happens during the second meiotic division. In general, most of the plants with $2n=24$ analyzed had meiotic indexes over 90% (Table 2), therefore being considered as meiotically estable and suitable, from the cytological point of view, for plant breeding. *C. baccatum* var. *praetermissum* had relatively lower meiotic indexes (77.20–84.61%) than *C. chinense* and *C. parvifolium* (over 90%). For *C. flexuosum*, a broad variation in meiotic indexes was observed between different individuals of the same accessions analyzed in different years: 68.83–97.79%. This particular species was very susceptible to pests and diseases during the cultivation in the green-house and presented over-branching in young parts as well as dwarfism in vegetative and reproductive parts. That could maybe have caused the observed meiotic anomalies, even if the occurrence of mutations cannot be totally ruled out. Virus and other pathogens may alter cytological processes in plants. In plants of *C. annuum* and *C. pendulum* infected by viruses, several meiotic irregularities similar to those we observed (univalents, laggards, bridges, micronuclei, restitution nucleus, among others) as well as somatic polyploidy have been reported (Swaminathan *et al.* 1959, Mircova and Sotirova 1995).

Meiotic behavior is reported here for the first time for the $2n=26$ species *C. buforum*, *C. campylopodium*, *C. cornutum*, *C. pereirae*, *C. freiburgense*, *C. schottianum*, *C. villosum* var. *villo-*

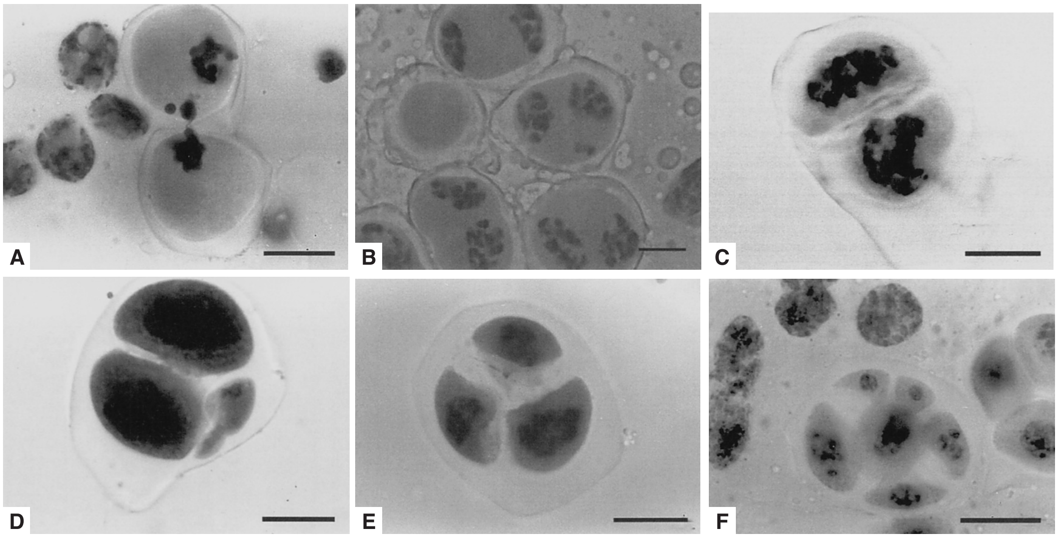

Fig. 5. Meiosis in $2n=26$ *Capsicum* species. A) *C. cornutum*, cells with cytomixis, B and C) *C. buforum*: B) cell with cytoplasm but no chromosomes, C) dyad, D) *C. schottianum*, dyad with microcyte, E and F) *C. buforum*: E) tryad, F) polyad showing micronuceli and microcytes. Scale bar equal to 10 μm.

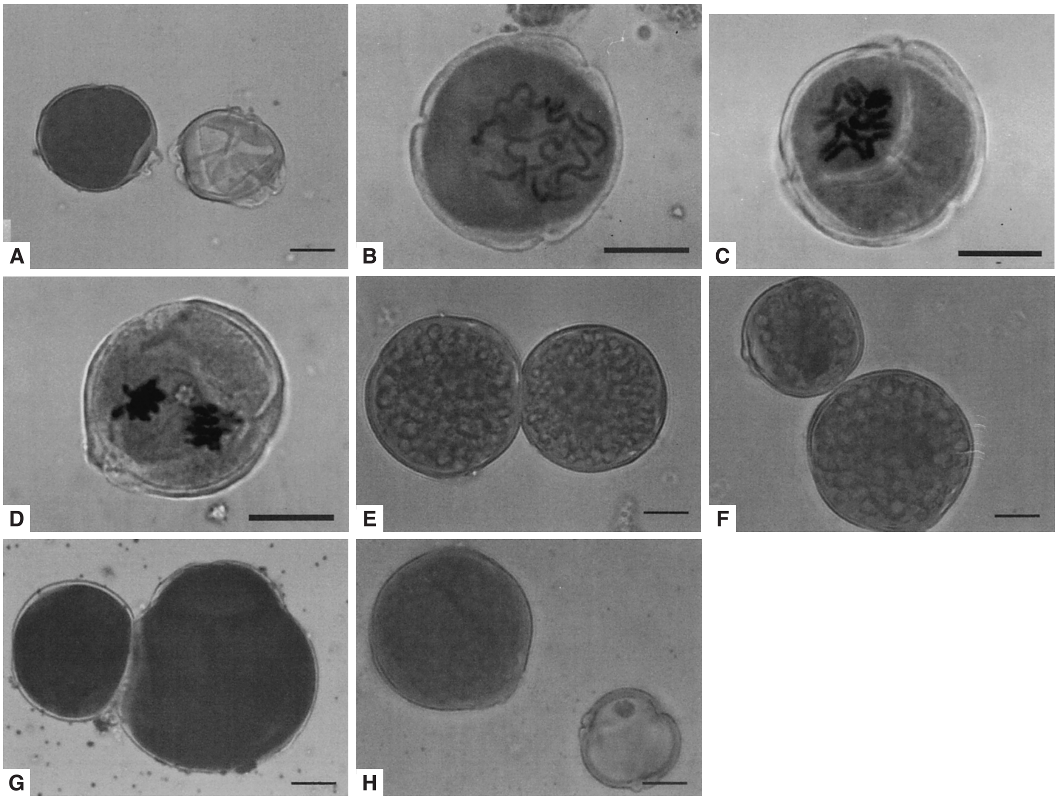

Fig. 6. Potentially viable (stained) and inviable (not-stained) pollen grains of *Capsicum* species: A) *C. flexuosum*. B–D) *C. parvifolium*, showing different phases of pollen mitosis, E) *C. sp* 2, F) *C. sp* 6 with pollen grains of different sizes, G) *C. buforum* with "giant" and normal pollen grains, D) *C. cornutum* with viable and inviable pollen grains. Scale bar equal to 10 μm.

sum, *Capsicum* sp 1, *Capsicum* sp 2 and *Capsicum* sp 6. One of the characteristics of these $2n=26$ taxa, is that the thirteenth chromosome pair is a small acrocentric (Pozzobon *et al.* 2006). As for the $2n=24$ species, meiotic behavior was generally regular in the $2n=26$ taxa (Table 2). In most cells and species 13 bivalents were observed at diakinesis and metaphase I (Table 2, Fig. 3A and B). The small pair formed and easily identified bivalent, normally located apart form the other associations. Secondary associations between bivalents were occasionally seen. In some cells, bivalents loosely associated were observed (Fig. 3F), probably reflecting an earlier chiasma terminalization. Chromosome disjunction at anaphases and telophases I and II were also mostly regular (Table 2, Fig. 3G).

Despite the predominant regular meiotic behavior, some irregularities were found in low frequencies (Table 2) such as non-orientation of chromosome at metaphase I (Fig. 3C), univalents, trivalents, quadrivalents and other possible multiple associations that could not be identified due to chromosome stickiness (Fig. 3D). Separation of sister chromatids at anaphase I were found in *Capsicum* sp. 6 (Fig. 3E), and could be due to desynapsis. Kumar and Raja Rao (1985) observed desynapsis in *C. frutescens* but could not establish its genetic basis, if any. Irregularities observed at anaphase and telophase I (Fig. 3H, 4A, 4B), pro-metaphase, metaphase, anaphase and telophase II (Fig. 4E–H) were of the same type as those found for the $2n=24$ species. Restitution nuclei were observed (Fig. 4C and D), probably due to a reorganization of the chromosomes between pro-metaphase and anaphase II. Tong and Bosland (2003) reported pairing in 12 bivalents in a $2n=24$ accession of *C. buforum* (all the accessions we examined had $2n=26$ chromosomes) and that the presence of laggards at anaphase and telophase I was the possible cause of low pollen stainability.

A wide variation (10.81 to 100.00%) between percentage of cells with normal chromosome disjunction, was verified among accessions within species and between species (Table 2), as for example in *Capsicum* sp. 6 (48.15–70.76 and 10.39–89.47% at anaphase/telophase I and anaphase/telophase II respectively) and *C. buforum* (10.81–55.56 and 25.13–45.45% at anaphase/telophase I and anaphase/telophase II respectively).

Other irregularities such as cytomixis (Fig. 5A), cells with different chromosome number or without any chromosome material at all (Fig. 5B) were also, if rarely, found. Cytomixis have been described for *C. annuum* (Lakshmi *et al.* 1989) and several other plants (Pagliarini 2000) but its cause is not clear.

Meiotic indexes in the $2n=26$ species were generally lower than those found for the $2n=24$ species, and ranged from 0.00 to 99.27% (Table 2), being lower in those accessions with high percentage of irregular disjunction, as for example in accession 3370 of *Capsicum* sp. 6 (0.00%) and in both individuals of accession 3368 of *C. buforum* (43.72 and 36.09%). Species and accessions with more meiotic irregularities in general tended to have lower meiotic indexes than those with a more regular meiosis (Table 2), even if this relation was not necessarily linear. Several types of irregularities were found at the tetrad stage, such as monads, dyads (Fig. 5C and D), triads (Fig. 5E), microcytes with different sizes (Fig. 5F). The higher number of monads, dyads and triads in *Capsicum* sp. 6, *C. friburgense* and *C. buforum* (Table 2) probably results form the restitution nuclei observed at meiosis I and II.

Accession 3370 of *Capsicum* sp. 6 differentiated from the others by its highly irregular meiotic behavior, presenting abnormalities in all phases of meiosis and a meiotic index of 0.00% (Table 2).

Pollen viability ranged from 73.96 to 95.98% for the $2n=24$ species and from 58.89 to 96.92% for the $2n=26$ ones (Table 2, Fig. 6), in most cases over 80%. It should be considered that stainability estimates pollen viability and therefore we cannot be sure that all the stained pollen grains are viable. Pollen grains bigger than normal were found in those species were monads diads and tryads were observed (Fig. 6F and G). “Giant” pollen grains are considered to indicate possibly unreduced gametes (Ramsey and Schemske 1998) or at least gametes with different chromosome numbers, and have been reported also in *Capsicum* (Shopova 1996b, Lippert *et al.* 1966), being the suggested cause for the observation of twin seedlings with different chromosome numbers (Shopova 1966b).

Besides the data presented here, the only reports on meiotic behavior of 2*n*=26 *Capsicum* species are those of Tong and Bosland (1997, 2003) for *C. lanceolatum*, who observed predominance of bivalents, but also occurrence of quadrivalents and pollen fertility of 50.00%.

The irregularities we have detected may be due to genetic (meiotic mutants) or environmental factors (such as pests and diseases) but at the moment there are no sufficient informations to decide for one or the other.

The origin of the small thirteenth pair of chromosomes in the 2*n*=26 species is still controversial and rather speculative. Moscone *et al.* (1993) suggested it could have derived by centric fission from one of the other 12 pairs, but Pozzobon *et al.* (2006), in a comprehensive study on chromosome numbers of 2*n*=24 and 2*n*=26 Brazilian *Capsicum* species, found no cytological evidence for that. Its constancy in number and behaviour during mitosis Pozzobon *et al.* (2006) and meiosis (present work) supports Pickersgill (1977, 1991), who pointed out that this chromosome is not a B chromosome. Bianchetti (1996), examining morpho-ecological characteristics such as fruit colour and type, habitat and geographical distribution of these *x*=13 species, suggested that they represent a primitive condition and that the *x*=12 would be derived. Combining Bianchetti (1996) data and cytological results Pozzobon *et al.* (2006) suggested that *x*=13 is the ancestral basic number and that the 2 basic chromosome numbers represent 2 different evolutionary lines.

Concluding, it is necessary that detailed meiotic behavior studies are extended to the largest possible number of other 2*n*=24 and 2*n*=26 accessions and species, with three main objectives: help to clarify the relations of *x*=12 and *x*=13 species, investigate the origin of the small thirteenth pair (for both aims the use of *in situ* techniques as FISH could be very useful) and to decide if the observed meiotic irregularities are due to genetic or environmental causes.

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