



## Failure of cytokinesis and 2n gamete formation in Brazilian accessions of *Paspalum*

M.S. Pagliarini<sup>1</sup>, S.Y. Takayama<sup>1</sup>, P.M. de Freitas<sup>1</sup>, L.R. Carraro<sup>1</sup>, E.V. Adamowski<sup>1</sup>, N. Silva<sup>1</sup> & L.A.R. Batista<sup>2</sup>

<sup>1</sup>*Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, 87020-900, Maringá-Paraná, Brazil,* <sup>2</sup>*Centro de Pesquisa de Pecuária do Sudeste/EMBRAPA, 13560-970, São Carlos-São Paulo, Brazil*

Received 21 April 1998; accepted 12 March 1999

*Key words:* cytokinesis, 2n gametes, microsporogenesis, *Paspalum*, polyploid

### Summary

Cytological analysis on 112 Brazilian accessions of *Paspalum* showed that 52 were affected by failure of first or second cytokinesis during microsporogenesis. When the failure of cytokinesis occurred only in the first division and in the second division the cytokinesis was normal, a dyad of microspores was formed. In most of them the two cells remained binucleate, but in some a restitutional nucleus was observed. When the first division was normal and the failure of cytokinesis occurred only in the second division, dyads and triads were observed. A restitutional nucleus in one or both cells of the dyad, or in the binucleate cell of the triad was also observed. The percentage of dyads and triads varied among accessions, reaching 15.75 of the sporads in some. The absence of cytokinesis indicates the possibility that the widespread occurrence of polyploidy in the genus *Paspalum* originates from 2n pollen grains.

### Introduction

Meiotic division is characterized by the occurrence of a series of mechanical and biochemical phenomena of considerable complexity which culminate in the reduction of chromosome number. Extensive evidence obtained for different animal and plant species has demonstrated that each step of meiosis is genetically controlled. Baker et al. (1976) described meiotic mutants ranging from lower eukaryotes to higher plants and man. Among genes affecting meiosis are those that promote cytokinesis. The first description of a gene involved in cytokinesis during meiosis was reported for maize (Beadle, 1932). Although callose deposition during microsporogenesis always leads to formation of four haploid microspores, the timing of cytokinesis varies among angiosperms. In general, monocotyledons undergo successive cytokinesis, in which cell plates are formed after both the first and second meiotic division. In plants of this group there is a distinct dyad stage. In most dicotyledons, the two meiotic divisions occur in a common cytoplasm, and

cytokinesis does not occur until after the four nuclei are formed. Absence of cytokinesis in monocotyledons and in dicotyledons leads to formation of 2n gametes. This phenomenon has been detected in many species (Mendiburu & Peloquin, 1977a,b; Ramanna, 1983; Teoh, 1984; Ferris et al., 1992) and has been reported to have an important role in evolution and in breeding.

Although cytogenetic studies have been conducted on some Brazilian *Paspalum* species (Moraes-Fernandes et al., 1968, 1974; Quarín et al., 1996; Pozzobon & Valls, 1997), in many others no cytological information is available. Part of the germplasm collection of Centro Nacional de Recursos Genéticos e Biotecnologia (Cenargen/Empresa Brasileira de Pesquisa Agropecuária) was allocated to the Centro de Pesquisa de Pecuária do Sudeste (CPPSE), located in São Carlos-São Paulo, for breeding purposes. The breeding program includes chromosome counts and evaluation of meiotic behavior. During these studies our group observed that many accessions presented

failure of first or second cytokinesis leading to formation of dyads and triads at the end of meiosis. The importance of this phenomenon for  $2n$  gamete formation and its role in polyploidization are discussed.

### Material and methods

The cytogenetic studies were carried out on accessions from the *Paspalum* collection of CPPSE/EMBRAPA, which comprises 215 accessions. This germplasm collection contains accessions obtained from different regions in the country, especially the South Region.

Inflorescences in the ideal stage for meiotic study were collected from 1994 to 1997 and fixed in Carnoy (3:1 ethyl alcohol:acetic acid) for 24 hours and then transferred to 70% alcohol and stored under refrigeration until the time for use. Microsporocytes were prepared by squashing and stained with 1% propionic carmine. Chromosome number was determined during diakinesis and all phases of meiosis were evaluated. The number of cells analyzed per accession, scored among five plants, ranged from 2000 to 2500.

### Results

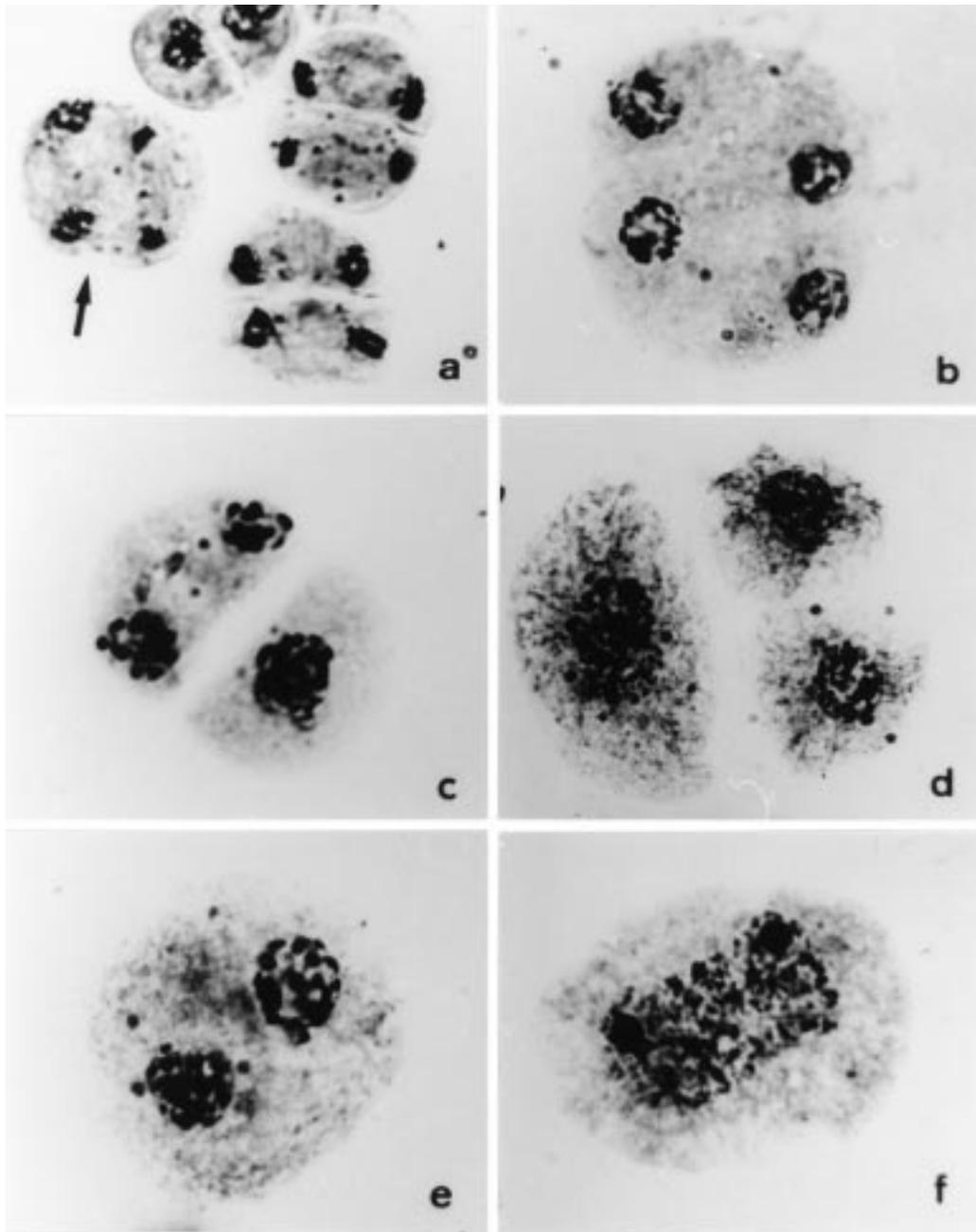
A total of 112 accessions of the CPPSE *Paspalum* collection were analyzed by our group in terms of meiotic behavior. Among them, 52 accessions presented failure of cytokinesis during first or second meiotic division. When the failure of cytokinesis occurred only in the first division and in the second division the cytokinesis was normal, a dyad of microspores was formed. In most of them the two cells remained binucleate, but in some a restitutional nucleus was observed. When the first division was normal and the failure of cytokinesis occurred only in the second division dyads and triads were observed. Triads are formed when cytokinesis occurs in only one cell of the dyad. A restitutional nucleus in one or both cells of the dyad, or in the binucleate cell of the triad was also observed. Figure 1 illustrates some aspects of the failure of cytokinesis observed and the  $2n$  gamete formation. An accession can present only dyads or triads or both types (Table 1). The percentage of dyads and triads was variable, ranging from zero to 8.41 for dyads and zero to 15.75 for triads. The results show that the failure of cytokinesis is more frequent in the second division.

### Discussion

The genus *Paspalum*, comprising more than 400 species, contains several important forage and turf grasses. Essentially all are native to America with the majority occurring in tropical and subtropical Central and South America (Burson, 1997). In Brazil, approximately 220 species can be found in the various ecosystems of the country, especially in the South Region (Valls, 1992).

The chromosome number in this genus ranges from  $2n = 2x = 12$  to  $2n = 16x = 160$ , with the most frequent number being  $2n = 4x = 40$  (Burson, 1997). Polyploidy is an important evolutionary process in higher plants. Stebbins (1971) estimates that 30 to 35% of flowering plant species possess multiple complements of the basic chromosome number in their genus. The polyploidization process can occur by two main modes: (i) somatic chromosome doubling (asexual polyploidization), and (ii) formation of functional  $2n$  gametes (sexual polyploidization). Harlan & De Wet (1975) concluded that spontaneous somatic chromosome is rare in nature, and almost all polyploids originated through sexual polyploidization via  $2n$  gametes, which are widespread in plants (for review see Harlan & De Wet, 1975; Veilleux, 1985; Bretagnolle & Thompson, 1995) and many other cases of  $2n$  gametes have been added to the literature over the last few years (Yan et al., 1997; Nassar & Freitas, 1997; Ortiz, 1997).

Approximately 80% of the *Paspalum* species are polyploid, and more than 70% reproduce by apomixis (Quarín, 1992). The meiotic behavior of the polyploids suggests that polyploidy can originate from chromosome doubling or hybridization. In many species, pentaploidy and hexaploidy have been suggested to originate from the fertilization of  $2n$  eggs in apomictic tetraploid forms (Burson, 1975; Quarín & Burson, 1991; Quarín et al., 1996; Bonilla & Quarín, 1997; Burson, 1997). In pentaploids, apomixis is undoubtedly responsible for evolutionary success. Although the origin of polyploids is attributed, in general, to  $2n$  eggs formed by the apomixis process,  $2n$  pollen as a possible source of polyploidy have been described in some *Paspalum* species (Snyder, 1961; Mehra & Chaudhary, 1981). Irregular meiosis with restitution nuclei has been reported in connection with apospory in *P. secans* (Snyder, 1961) and with diplospory in *P. conjugatum* (Chao, 1980). Both species are pseudogamous, i.e., they require pollination for normal caryopsis development, indicating that



*Figure 1.* Some aspects of failure of cytokinesis in Brazilian accessions of *Paspalum*. a) Microsporocytes in telophase II. The arrow shows a tetranucleate microsporocyte originated by the failure of first cytokinesis. b) Tetranucleate telophase II resulting from failure of first cytokinesis. c) A dyad of microspores showing a binucleate microspore and the other one with a restitutional nucleus. d) A triad showing a binucleate microspore. e) A binucleate microspore. f) A  $2n$  microspore resulting from the restitutional nucleus.

Table 1. Frequency of dyads and triads in some Brazilian accessions of *Paspalum*

Species	Accession code	Chromosome number	N° of sporads analyzed	Frequency and percentage of different sporads					
				Dyad		Triad		Tetrad	
				N°	%	N°	%	N°	%
<i>P. atratum</i>	BRA009610	40	190	0	0.00	0	0.00	190	100.0
<i>P. chacoense</i>	BRA-003841	20	257	0	0.00	0	0.00	257	100.0
	BRA-008311A	20	646	0	0.00	0	0.00	646	100.0
<i>P. compressifolium</i>	BRA-005088	40	306	0	0.00	2	0.65	304	99.35
	BRA-008524	40	303	0	0.00	1	0.33	302	99.67
	BRA-008532	40	404	11	2.72	36	8.91	357	88.37
	BRA-011282	40	349	0	0.00	2	0.57	347	99.43
	BRA-011720	40	381	4	1.05	60	1.75	317	83.20
	BRA-012173	40	239	0	0.00	18	7.53	221	92.47
	BRA-012238	40	294	2	0.68	0	0.00	292	99.32
	BRA-013030	40	368	0	0.00	6	1.63	362	98.37
	BRA-013463	40	349	0	0.00	0	0.00	349	100.0
	BRA-014907	40	492	0	0.00	3	0.61	489	99.39
	<i>P. conspersum</i>	BRA-007480B	60	1108	10	0.90	9	0.81	1089
BRA-008508B		60	1052	0	0.00	9	0.86	1043	99.24
<i>P. fasciculatum</i>	BRA-007498	20	306	0	0.00	0	0.00	306	100.0
	BRA-007501	20	325	0	0.00	0	0.00	325	100.0
<i>P. guenoarum</i>	BRA-003824	40	345	0	0.00	0	0.00	345	100.0
	BRA-006572	40	179	0	0.00	0	0.00	179	100.0
	BRA-014851	40	73	0	0.00	0	0.00	73	100.0
<i>P. indecorum</i>	BRA-007528	20	181	0	0.00	0	0.00	181	100.0
<i>P. ionanthum</i>	BRA-009806	40	456	0	0.00	32	7.02	424	92.98
<i>P. lividum</i>	BRA-003875	40	102	1	0.98	1	0.98	100	98.04
	BRA-006505	40	205	0	0.00	0	0.00	205	100.0
<i>P. malacophyllum</i>	BRA-003077	40	296	0	0.00	12	4.08	284	95.95
<i>P. maritimum</i>	BRA-015041	40	734	0	0.00	0	0.00	734	100.0
	BRA-015059	40	610	0	0.00	0	0.00	610	100.0
	BRA-015067	40	889	0	0.00	0	0.00	889	100.0
<i>P. nicorae</i>	BRA-010782	40	106	2	1.89	4	3.77	100	94.34
<i>P. notatum</i>	BRA-006173	20	270	0	0.00	0	0.00	270	100.0
	BRA-006301	40	139	0	0.00	0	0.00	139	100.0
	BRA-007986	40	289	0	0.00	0	0.00	289	100.0
<i>P. oteroi</i>	BRA-001503	40	291	0	0.00	0	0.00	291	100.0
	BRA-003905	40	368	2	0.54	6	1.63	360	97.83
	BRA-010332	40	283	0	0.00	0	0.00	283	100.0
<i>P. plicatum</i>	BRA-004120	40	346	0	0.00	18	5.20	328	94.79
	BRA-008869	40	545	0	0.00	1	0.18	544	99.82
	BRA-008877	40	263	0	0.00	3	1.41	260	98.59
	BRA-008893	40	304	0	0.00	5	1.64	299	98.36
	BRA-008907	40	570	0	0.00	1	0.18	569	99.82
	BRA-008982	40	655	0	0.00	3	0.46	652	99.54
	BRA-009032	40	922	0	0.00	51	5.53	871	94.47
	BRA-009211	40	435	0	0.00	0	0.00	435	100.0
	BRA-010383	40	417	0	0.00	1	0.24	416	99.76

Table 1. Continued.

Species	Accession code	Chromosome number	N° of sporads analyzed	Frequency and percentage of different sporads					
				Dyad		Triad		Tetrad	
				N°	%	N°	%	N°	%
	BRA-011207	20	293	0	0.00	0	0.00	293	100.0
	BRA-011274	40	329	0	0.00	0	0.00	329	100.0
	BRA-012483	40	287	0	0.00	0	0.00	287	100.0
	BRA-012912	40	334	0	0.00	0	0.00	334	100.0
	BRA-012939	60	246	1	0.41	4	1.63	241	97.97
	BRA-013048	40	725	0	0.00	6	0.83	719	99.17
	BRA-013111	40	622	0	0.00	3	0.48	619	99.52
	BRA-013307	40	670	0	0.00	4	0.60	666	99.40
<i>P. regnellii</i>	BRA-019186	40	664	0	0.00	0	0.00	664	100.0
<i>P. simplex</i>	BRA-007480A	40	392	0	0.00	0	0.00	392	100.0
	BRA-011355B	40	763	0	0.00	0	0.00	763	100.0
<i>P. subciliatum</i>	BRA-014842	40	302	19	6.29	44	14.56	239	79.14
<i>P. yaguaronense</i>	BRA-009300	40	305	0	0.00	0	0.00	305	100.0
	BRA-011002	60	922	0	0.00	0	0.00	922	100.0
	BRA-011371	40	292	0	0.00	0	0.00	292	100.0
	BRA-011398	40	412	0	0.00	29	7.05	383	92.96
	BRA-011401	40	510	0	0.00	0	0.00	510	100.0
<i>Paspalum</i> sp.	BRA-003913	40	561	0	0.00	0	0.00	561	100.0
	BRA-006157	40	432	0	0.00	0	0.00	432	100.0
	BRA-006602	40	380	21	5.53	11	2.89	348	91.58
	BRA-006700	40	516	0	0.00	15	2.91	501	97.09
	BRA-008311B	40	1238	0	0.00	8	0.65	1230	99.35
	BRA-008486	40	380	0	0.00	0	0.00	380	100.0
	BRA-008630	40	260	7	2.69	3	1.15	250	96.15
	BRA-008656	40	114	2	1.75	2	1.75	110	96.49
	BRA-008672	40	554	0	0.00	0	0.00	554	100.0
	BRA-008834	40	385	0	0.00	4	1.04	381	98.96
	BRA-008851	40	523	0	0.00	0	0.00	523	100.0
	BRA-008923	40	941	0	0.00	56	5.95	885	94.05
	BRA-008940	40	433	0	0.00	0	0.00	433	100.0
	BRA-009016	40	616	0	0.00	8	1.30	608	98.70
	BRA-009059	40	659	0	0.00	27	4.09	632	95.91
	BRA-009075	40	315	0	0.00	0	0.00	315	100.0
	BRA-009083	40	83	0	0.00	8	9.64	75	90.36
	BRA-009105	40	673	0	0.00	0	0.00	673	100.0
	BRA-009113	40	622	0	0.00	7	1.13	615	98.87
	BRA-009130	40	562	0	0.00	0	0.00	562	100.0
	BRA-009202	40	352	0	0.00	7	1.99	345	98.01
	BRA-009393	40	214	7	3.27	10	4.67	197	92.06
	BRA-009628	40	500	0	0.00	0	0.00	500	100.0
	BRA-009644	40	554	0	0.00	0	0.00	554	100.0
	BRA-009652	40	768	0	0.00	0	0.00	768	100.0
	BRA-009679	40	1002	0	0.00	0	0.00	1002	100.0
	BRA-009687	40	172	0	0.00	0	0.00	172	100.0
	BRA-009741	40	603	0	0.00	2	0.33	601	99.67
	BRA-010243	40	327	0	0.00	0	0.00	327	100.0
	BRA-010464	40	531	0	0.00	0	0.00	531	100.0

Table 1. Continued.

Species	Accession code	Chromosome number	N° of sporads analyzed	Frequency and percentage of different sporads					
				Dyad		Triad		Tetrad	
				N°	%	N°	%	N°	%
	BRA-010511	40	366	0	0.00	0	0.00	366	100.0
	BRA-010537	40	341	0	0.00	0	0.00	341	100.0
	BRA-011053	40	555	0	0.00	0	0.00	555	100.0
	BRA-011134	40	112	0	0.00	0	0.00	112	100.0
	BRA-011266	40	552	0	0.00	0	0.00	552	100.0
	BRA-011304	40	431	0	0.00	0	0.00	431	100.0
	BRA-012424	40	885	0	0.00	0	0.00	885	100.0
	BRA-012521	40	197	2	1.02	3	1.52	192	97.46
	BRA-012530	40	318	0	0.00	0	0.00	318	100.0
	BRA-012718	40	541	0	0.00	1	0.18	540	99.82
	BRA-012742	40	646	54	8.41	15	2.32	577	89.32
	BRA-012751	40	258	0	0.00	0	0.00	258	100.0
	BRA-013293	40	601	0	0.00	0	0.00	601	100.0
	BRA-013391	40	441	0	0.00	0	0.00	441	100.0
	BRA-013404	40	312	4	1.28	6	1.92	302	96.79
	BRA-013609	40	338	0	0.00	5	1.48	333	98.52
	BRA-014176	40	401	0	0.00	58	14.43	343	85.57
	BRA-014729	40	708	0	0.00	0	0.00	708	100.0
	BRA-014770	40	377	22	5.84	8	2.12	347	92.04
	BRA-014885	40	422	0	0.00	0	0.00	422	100.0
	BRA-015075	40	130	0	0.00	0	0.00	130	100.0

the cytologically 2n pollen grains are physiologically active.

Gametes with the sporophytic chromosome number (2n) can be formed by different mechanisms. Veilleux (1985) summarized three mechanisms: (i) premeiotic disturbances such as endomitoses of archesporial cells, (ii) meiotic disturbances, e.g., premature cytokinesis or co-orientation of spindles in second division, and (iii) abnormal cytokinesis followed by nuclear fusion of multinucleate microspores. The last one is the mechanism observed in *Paspalum*, although it was not always accompanied by nuclear fusion. Abnormal cytokinesis in this genus was described only in some cells of *P. proliferum* (Burson, 1975), while 2n gametes resulting from desynaptic mutants have been described in some species (see Mehra & Chaudhary, 1981). The absence of cytokinesis in some Brazilian accessions of *Paspalum* indicates the possibility that polyploidy originates from 2n pollen grains resulting from nuclear fusion of multinucleate microspores. These results show that chromosomal evolution in the genus *Paspalum* may also occur by sexual polyploidization via 2n pollen. Al-

though the production of 2n gametes is under genetic control (Sala et al., 1989; Katsiotis & Forsberg, 1995; Calderini & Mariani, 1997) it can be also affected by environmental conditions. Stein (1970) listed several species in which the frequency of 2n gametes varied with the cultivation conditions. When the character is genetically controlled, the formation of 2n gametes can occur many times. Over 40 examples of this have been documented by Soltis & Soltis (1993). The occurrence of very high polyploids reported in the genus *Paspalum* could probably be explained on this basis, however genetic analysis is necessary to confirm the assumption.

## References

- Baker, S.B., A.T.C. Carpenter, M.S. Esposito, R.G. Esposito & L. Sandler, 1976. The genetic control of meiosis. *Ann Rev Genet* 10: 53–134.
- Beadle, G.W., 1932. A gene in *Zea mays* for failure of cytokinesis during meiosis. *Cytologia* 3: 142–155.
- Bonilla, J.R. & C.L. Quarín, 1997. Diplosporous and aposporous apomixis in a pentaploid race of *Paspalum minus*. *Plant Sci* 127: 97–104.

- Bretagnolle, F. & J.D. Thompson, 1995. Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. *New Phytol* 129: 1–22.
- Burson, B.L., 1975. Cytology of some apomictic *Paspalum* species. *Crop Sci* 15: 229–232.
- Burson, B.L., 1997. Apomixis and sexuality in some *Paspalum* species. *Crop Sci* 37: 1347–1351.
- Calderini, O & A. Mariani, 1997. Increasing 2n gamete production of diploid alfalfa by cycles of phenotypic recurrent selection. *Euphytica* 93: 113–118.
- Chao, C.Y., 1980. Autonomous development of embryo in *Paspalum conjugatum* Berg. *Bot Notiser* 133: 215–222.
- Ferris, C., R.S. Callow & A.J. Gray, 1992. Mixed first and second division restitution in male meiosis of *Hierochloë odorata* (L.) Beauv (Holy Grass). *Heredity* 69: 21–31.
- Harlan, J.R. & J.M.J. De Wet, 1975. On Ö. Winge and a prayer: The origins of polyploidy. *Bot Rev* 41: 361–390.
- Katsiotis, A. & R.A. Forsberg, 1995. Discovery of 2n gametes in tetraploid *Avena vaviloviana*. *Euphytica* 81: 1–6.
- Mehra, P.N. & J.D. Chaudhary, 1981. Male meiosis in some grasses of the tribe *Panicaceae* from North Eastern India – I. Genus *Paspalum*. *Cytologia* 46: 265–278.
- Mendiburu, A.O. & S.J. Peloquin, 1977a. The significance of 2n gametes in potato breeding. *Theor Appl Genet* 49: 53–61.
- Mendiburu, A.O. & S.J. Peloquin, 1977b. Bilateral sexual polyploidization in potatoes. *Euphytica* 26: 573–583.
- Moraes-Fernandes, M.I.B., I.L. Barreto & F.M. Salzano, 1968. Cytogenetic, ecologic and morphologic studies in Brazilian forms of *Paspalum dilatatum*. *Can J Genet Cytol* 10: 131–138.
- Moraes-Fernandes, M.I.B., I.L. Barreto, F.M.G. Salzano & M.C. Freitas-Sacchet, 1974. Cytological and evolutionary relationships in Brazilian forms of *Paspalum* (Gramineae). *Caryologia* 27: 455–465.
- Nassar, N.M.A. & M. Freitas, 1997. Prospects of polyploidizing cassava *Manihot esculenta* Crantz, by unreduced microspores. *Plant Breed* 116: 195–197.
- Ortiz, R., 1997. Occurrence and inheritance of 2n pollen in *Musa*. *Ann Bot* 79: 449–453.
- Pozzobon, M.T. & J.F.M. Valls, 1997. Chromosome number in germplasm accessions of *Paspalum notatum* (Gramineae). *Braz J Genet* 20: 29–34.
- Quarín, C.L., 1992. The nature of apomixis and its origin in Panicoid grasses. *Apomixis News* 5: 8–15.
- Quarín, C.L. & B.L. Burson, 1991. Cytology of some apomictic *Paspalum* species. *Cytologia* 56: 223–228.
- Quarín, C.L., M.T. Pozzobon & J.F.M. Valls, 1996. Cytology and reproductive behavior of diploid, tetraploid and hexaploid germplasm accessions of a wild forage grass: *Paspalum compressifolium*. *Euphytica* 90: 345–349.
- Ramanna, M.S., 1983. First division restitution gametes through fertile desynaptic mutants of potato. *Euphytica* 32: 337–350.
- Sala, C.A., E.L. Camardo, M.T. Salaberg & A.O. Mendiburu, 1989. Cytological mechanisms of 2n pollen formation and unilateral sexual polyploidization in *Lilium*. *Euphytica* 43: 1–6.
- Snyder, L.A., 1961. Asyndesis and meiotic non-reduction in microsporogenesis of apomictic *Paspalum secans*. *Cytologia* 26: 50–61.
- Soltis, D.E. & P.S. Soltis, 1993. Molecular data and the dynamic nature of polyploid. *Crit Rev Plant Sci* 12: 243–273.
- Stebbins, L., 1971. *Chromosomal Evolution in Higher Plants*. Addison-Wesley Publ. Co., Menlo Park, CA.
- Stein, M., 1970. Polyploidie und Umwelt. *Tagungsberichte Deutschen Akad Wiss* 101: 51–68.
- Teoh, S.B., 1984. Polyploid spore formation in diploid orchid species. *Genetica* 63: 53–59.
- Valls, J.F.M., 1992. Origen do germoplasma de *Paspalum* disponível no Brasil para a área tropical. In: *Red Internacional de Evaluación de Pastos Tropicales/RIEPT 1ª Reunión Sabanas, Brasília, EMBRAPA/CPAC/CIAT*, pp. 68–80.
- Veilleux, R., 1985. Diploid and polyploid gametes in crop plants: mechanisms of formation and utilization in plant breeding. *Plant Breed Rev* 3: 253–288.
- Yan, G., A.R. Ferguson, M.A. McNeilage & B.G. Murray, 1997. Numerically unreduced (2n) gametes and sexual polyploidization in *Actinidia*. *Euphytica* 96: 267–272.

