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Evaluation of microsporogenesis in an interspecific *Brachiaria* hybrid (Poaceae) collected in distinct years

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ABSTRACT. Microsporogenesis in an interspecific *Brachiaria* hybrid, grown in the field under natural environmental conditions in Brazilian savannas, was analyzed in three distinct years of collection. Several types of meiotic abnormalities were recorded during those three years, but varied in type and frequency depending on the year. The average temperature and rainfall 15 days before collection was unusually high in those years. The percentage of abnormal meiocytes recorded was 62% in 2001, 73% in 2004, and 77% in 2005. The abnormalities observed during microsporogenesis compromised pollen viability by generating unbalanced gametes or affecting nucleolus organization. The environmental conditions under which the hybrid was growing could have affected the genetic control of meiosis. More detailed studies, under controlled conditions, are necessary to better understand the effects of environmental factors on *Brachiaria* microsporogenesis hybrids.

Key words: *Brachiaria*; Interspecific hybrid; Microsporogenesis; Environment

INTRODUCTION

Productivity in agricultural ecosystems is severely affected by various biotic and abiotic stresses. Crop yields can be reduced 4- to 20-fold due to abiotic stress when compared to normal conditions (Boyer, 1982). Studies conducted in many species of higher plants have revealed that reproductive processes appear to be much more sensitive to abiotic stresses than is vegetative growth (Baker et al., 1992; Sato et al., 2002, 2006). Several authors declared that meiosis is the most sensitive stage in the life cycle of seed plants (Namuco and O'Toole, 1986; Saini, 1997; Porch and Jahn, 2001; Erickson and Markhart, 2002; Romanova and Tret'yakova, 2005). Among the abiotic stresses capable of inducing abnormalities during meiosis, Sun et al. (2004) cited water availability, temperature, nutrient levels, light, soil salinity, and pollution. These stresses may cause various structural and functional abnormalities in reproductive organs leading to failure of fertilization or premature abortion of seed or fruit. Thus, the damage to productivity from stress at this stage is particularly severe for crops in which the economic yield is the product of sexual reproduction (Saini, 1997).

The better studied abiotic factors affecting reproductive processes are water deficit and temperature. Water deficit ranks as the most significant factor limiting global crop productivity (Boyer, 1982). Water stress interferes with reproductive success of plants by arresting the development of the male gametophyte and, sometimes, the female gametophyte. Drought during stamen initiation up to anthesis causes serious yield reduction in many crops (see Saini, 1997). It has been well demonstrated in several cereals that the main peak of sensitivity is centered on the period from meiosis to tetrad break-up in anthers. On the other hand, sensitivity of flower development, particularly microsporogenesis, to high temperatures has been demonstrated in many crops (Namuco and O'Toole, 1986; Mercado et al., 1997; Saini, 1997; Porch and Jahn, 2001; Erickson and Markhart, 2002), where microspore development, pollen viability, and pollen shed were reduced under high temperatures.

The forage grass *Brachiaria* represents the single most widely planted pasture grass in tropical savannas of South America, especially in Central Brazil where estimated 60 million hectares are cultivated with brachiaria grasses (Macedo, 1995). Some cultivars of this genus have been used as forages since 1980 and have greatly increased beef cattle production, placing Brazil as the major exporter of beef in the world. Brazil is also the largest producer and exporter of *Brachiaria* seeds to Latin America, thus the seed demand is very high both for exportation as well as for national use. Seed production in *Brachiaria* when compared to other crops can be considered low, amounting to less than 20% of inflorescences produced. The universally low percentage of seed set in *Brachiaria* may have genetic origins associated with polyploidy, or could reflect a physiological limitation (Hopkinson et al., 1996). Most of the commercial *Brachiaria* cultivars are tetraploid ($2n = 4x = 36$) and apomictic (Valle and Savidan, 1996) and display many abnormalities during meiosis impairing normal gamete formation (Mendes-Bonato et al., 2001a, 2002). To obtain new variability, intra- and interspecific hybrids have been synthesized at Embrapa Beef Cattle in Brazil, since 1988, to develop new cultivars. Some hybrids are under agronomic and cytological evaluation, but the majority produces low amounts of viable seed. Considering that environmental conditions can impair seed production by affecting many reproductive steps, the meiotic process of a hybrid was evaluated in three different years under natural environmental conditions in the field. The objective was to contribute to a better understanding of the effects of the Brazilian savanna's environment on microsporogenesis in this genus.

MATERIAL AND METHODS

Cytological studies were carried out on one sexual interspecific hybrid (BS03 - HBGC034) collected in three years: March/2001, April/2004, and January/2005. Data of natural environmental conditions in the field of fifteen days before meiosis are presented in Table 1, including average of maximum and minimum temperature, accumulated rainfall, and average hours of sunshine. The weather data were provided by the Embrapa Beef Cattle Weather Station.

The female parent of BS03 (HBGC034) is an artificially tetraploidized sexual accession of *B. ruziziensis* (R30, $2n = 4x = 36$) and the male parent is *B. brizantha* cv. Marandu (B30, $2n = 4x = 36$), the most widely planted apomictic variety of grass in the Brazilian tropics. The hybrid was produced by controlled pollination at the Embrapa Beef Cattle Center (Campo Grande, State of Mato Grosso do Sul, Brazil), in 1988, and is under agronomic evaluation.

Inflorescences for meiotic studies were collected and fixed in a mixture of 95% ethanol, chloroform and propionic acid (6:3:2, v/v) for 24 h; they were then stored under refrigeration until use. Microsporocytes, prepared by squashing, were stained with 0.5% propionic carmine. A total of 690 microsporocytes were analyzed in 2001, 1363 in 2004, and 1486 in 2005. Images were photographed with Kodak Imagelink - HQ, ISO 25 black and white film. Meiotic abnormalities were recorded for the three years of collection and the data were compared by the chi-square test.

RESULTS AND DISCUSSION

The data for environmental factors (maximum and minimum temperature, rainfall, and hours of sunshine) were recorded daily and expressed as means for the fifteen days prior to inflorescence collection (Table 1). In *Brachiaria* there are no scientific reports on the duration of the reproductive cycle, from inflorescence initiation to anthesis, but visual observation of plants growing in the field indicated that this time was around 15 days before inflorescence collection for meiotic studies on anthers. Araújo et al. (2000) studied the development of the female gametophyte in *Brachiaria brizantha* and observed megaspore mother cells in ovaries of both apomictic and sexual plants, two to five days prior to anthesis. Earlier development of the sexual gametophytes up to the end of meiosis was observed, followed by similar rates of development of the embryo sac afterwards.

Table 1. Average maximum and minimum temperature, accumulated rainfall, and hours of sunshine for the fifteen days before inflorescence collection in the three years.

Environmental factor	March/2001	April/2004	January/2005	Amplitude of variation among years
Maximum temperature	30.9°C	30.4°C	29.4°C	1.5°C
Minimum temperature	21.5°C	20.2°C	21.9°C	1.7°C
Amplitude of temperature	9.4°C	10.3°C	7.5°C	2.8°C
Accumulated rainfall	62 mm	95.7 mm	123 mm	61 mm
Hours of sunshine	5.9 h	4.6 h	7.7 h	3.1 h

The timing of tetrad formation in the anther varies among species (Bennett et al., 1971; Bennett, 1977; Luomajoki, 1986). In a study involving 36 species representing 13 angiosperm families, Li and Johnston (1999) concluded that the floral timing varied from

5 to 34 days and detected environmental effects within species. Members of the Poaceae were not evaluated, but in the majority of species studied, the floral timing was estimated to be between 10 to 20 days. Dorion et al. (1996) and Lalonde et al. (1997) verified an effect on wheat microsporogenesis when water stress was applied 4 days before meiosis. On the other hand, Fischer (1973) found poor grain set in wheat when water stress occurred during the last 15 days prior to panicle emergence, which coincides with pollen development. The time to study the effect of stress of high temperature varied among species: 15 days in *Lycopersicon esculentum* (Sato et al., 2002); 5 days in *Capsicum annuum* (Erickson and Markhart, 2002); 13 days in *Phaseolus vulgaris* (Porch and Jahn, 2001), and 14 days in *Brassica napus* (Young et al., 2004).

Several types of meiotic abnormalities were recorded and varied in type and frequency in the three years of collection (Table 2). The chi-square test applied for each abnormality showed a difference among the three years of collection.

Table 2. Abnormalities recorded in hybrid BS03 in each meiotic phase, number of cells analyzed, and number of abnormal cells in the three years of collection.

Phase	Abnormalities	No. of cells analyzed			No. of abnormal cells			Chi-square
		2001	2004	2005	2001	2004	2005	
Metaphase I	Precocious migration to the poles	99	176	221	23	44	19	21.38*
	Irregular cytokinesis				17	-	-	70.59*
	Abnormal nucleolus disorganization				-	-	146	172.43*
Anaphase I	Laggard chromosomes	16	191	104	11	150	27	78.33*
	Chromosome stickiness				-	11	9	2.09**
	Abnormal nucleolus disorganization				-	-	51	121.42*
Telophase I	Micronuclei	38	59	259	11	118	12	133.09*
	Chromosome stickiness				-	56	9	16.38*
	Abnormal nucleolus disorganization				-	-	190	309.89*
Prophase II	Micronuclei	78	128	198	16	81	-	171.28*
	Irregular cytokinesis				22	-	40	36.73*
	Abnormal nucleolus disorganization				-	-	142	227.81*
Metaphase II	Precocious migration to the poles	94	141	251	23	21	-	57.95*
	Irregular cytokinesis				40	-	45	70.84*
	Chromosome stickiness				-	24	-	61.77*
	Abnormal nucleolus disorganization				-	-	136	176.81*
	Absence of cytokinesis				-	-	13	12.50*
	Abnormal spindle				-	-	12	11.52*
Anaphase II	Laggard chromosomes	29	59	55	17	33	16	10.54*
	Chromosome stickiness				-	6	-	8.92**
	Abnormal nucleolus disorganization				-	-	11	19.91*
	Irregular cytokinesis				-	-	11	19.06*
Telophase II	Micronuclei	97	109	131	21	73	-	135.32*
	Chromosome stickiness				16	21	9	8.69**
	Irregular cytokinesis				26	-	15	34.61*
	Absence of cytokinesis				-	-	7	11.24*
	Abnormal nucleolus disorganization				-	-	87	167.67*
Tetrad	Micronuclei	239	326	267	84	142	10	122.09*
	Microcytes				59	58	100	29.77*
	Polyads				35	20	30	11.33*
	Chromosome stickiness				10	5	-	13.64*
Total		690	1189	1486	431 (62%)	863 (73%)	1141 (77%)	

*At 1%, and **at 5% level of probability.

Irregular chromosome segregation, including precocious chromosome migration to the poles in metaphases (Figure 1a,d, and e), laggards in anaphases (Figure 1b and f), and micronucleus formation in telophases (Figure 1c and g) in both meiotic divisions, and chromosome stickiness (Figure 2a to d) were recorded in the three years; irregular cytokinesis (Figure 1e; Figure 2d; Figure 3f and g) was not recorded in 2004, and abnormal spindle orientation and abnormal nucleolus disintegration (Figure 3) were recorded only in 2005. Irregular chromosome segregation and abnormal cytokinesis led to polyad formation (Figure 1h to k), fractionating the genome, and giving rise to pollen grains of different sizes and with micronuclei (Figure 1l). The percentage of abnormal meiocytes was very high and amounted to 62% in 2001, 73% in 2004, and 77% in 2005. The percentage of abnormalities recorded during microsporogenesis can seriously compromise pollen viability in *Brachiaria* by generating unbalanced gametes or by affecting nucleolus organization in the telophase nucleus, since the nucleolus is a structure involved in the rRNA synthesis.

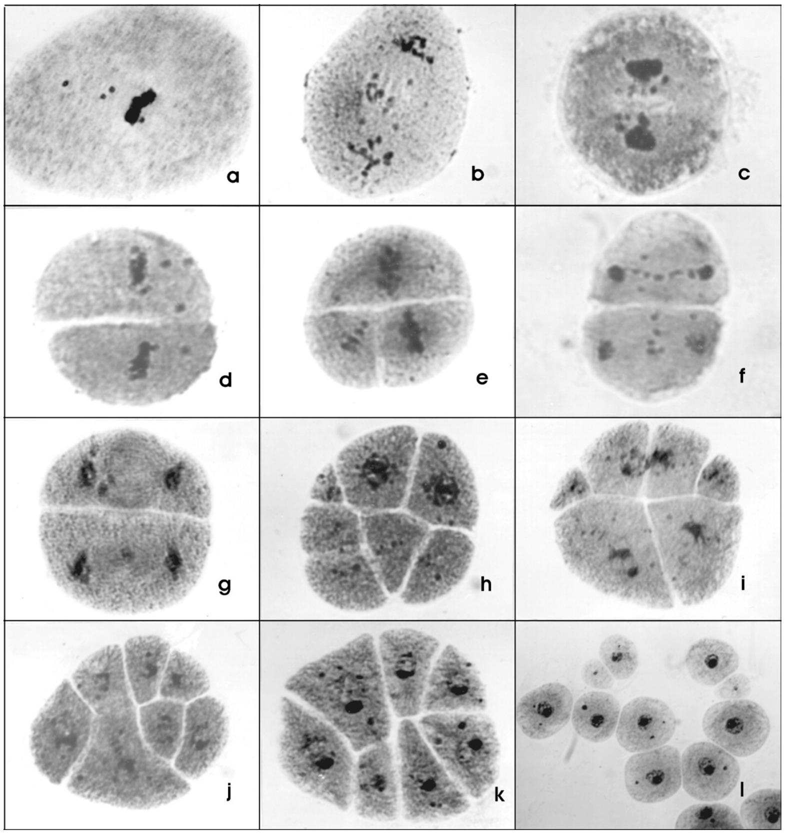

Figure 1. Aspects of irregular chromosome segregation in BS03. **a.** Metaphase I with precocious and irregular chromosome migration to the poles. **b.** Anaphase I with laggard chromosomes. **c.** Telophase I with micronuclei. **d.** Metaphase II with precocious chromosome migration to the poles. **e.** Metaphase II with abnormal cytokinesis and irregular chromosome distribution among cells. **f.** Late anaphase II with laggard chromosomes. **g.** Telophase II with micronuclei. **h** to **k.** Polyads with microspores and microcytes of different sizes. **l.** Pollen grains of different sizes and with micronuclei.

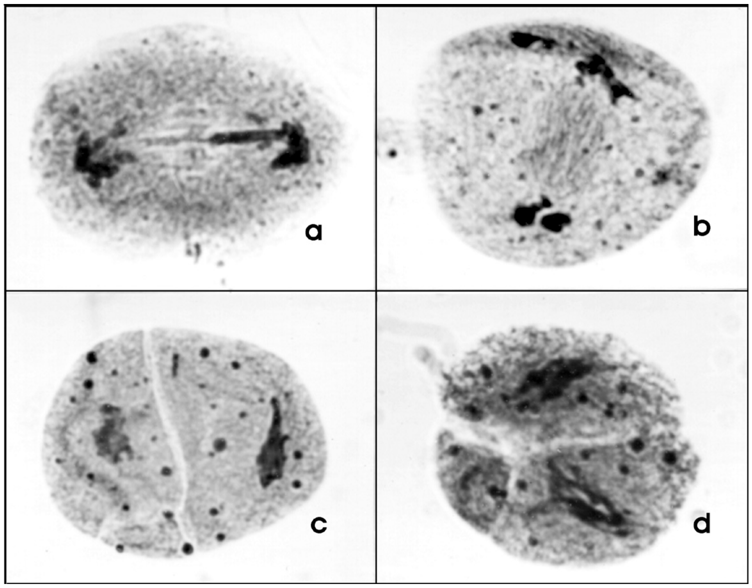

Figure 2. Some aspects of chromosome stickiness. Observe that in b, c and d there are small nucleoli resulting from irregular nucleolus disintegration.

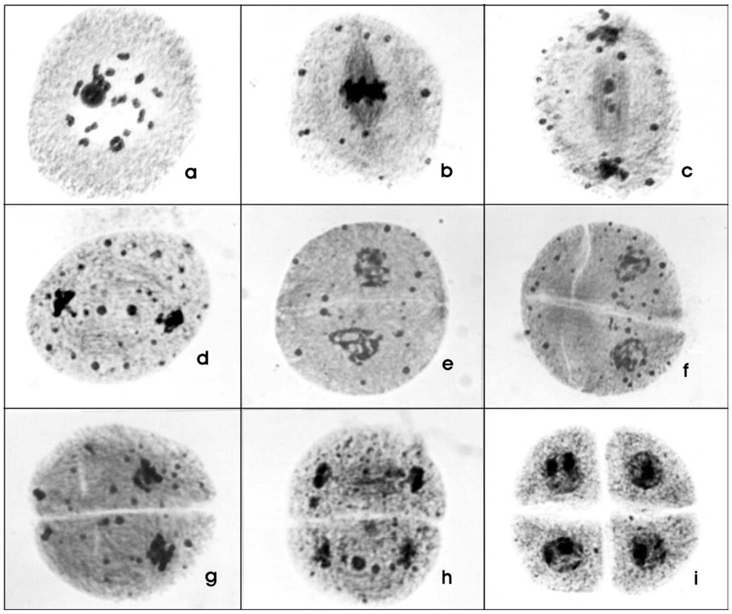

Figure 3. Abnormal nucleolus disintegration. **a.** Diakinesis with a normal nucleolus. **b. c.** and **d.** Metaphase I (a), anaphase I (b), and telophase I (c) with small micronucleoli in the cytoplasm. **e. f.** and **g.** Prophase II with several micronucleoli. Observe signs of abnormal cytokinesis in f and g. **h.** and **i.** Telophase II (h) and tetrad of microspores (i) with micronucleoli.

Irregular chromosome segregation, leading to micronuclei in microspores or microcytes in the tetrad, or polyads, has been widely reported in different polyploidy accessions of several species of this genus (Utsunomiya et al., 2005; Mendes-Bonato et al., 2001a, 2002, 2006; Risso-Pascotto et al., 2003, 2006a,b) as well as chromosome stickiness (Mendes-Bonato et al., 2001a,b; Utsunomiya et al., 2005), abnormal spindle (Risso-Pascotto et al., 2005; Mendes-Bonato et al., 2006), irregular or absence of cytokinesis (Mendes-Bonato et al., 2002; Risso-Pascotto et al., 2005; Boldrini, 2006; Gallo et al., 2007). Abnormal nucleolus disintegration was recorded in *B. decumbens* (Risso-Pascotto et al., 2002).

Environmental conditions prior to meiosis showed a variation of only 1.7°C for maximum temperature, and 1.5°C for minimum temperature among the three years. Rainfall, on the other hand, reflects the differences in precipitation in the seasons: rainy summers (January) and drier autumns (March and April) with 61 mm rain difference in the 15 days prior collection. The difference in hours of sunshine (3.1 h among years) is correlated to precipitation. Temperature seemed to be ideal; however, transitory temperature peaks as well as localized water stress are very common in those summer months, especially with bright sunshine. Thus, specific climatic conditions right before collection could explain the abnormalities observed. The greatest amount of meiotic abnormalities was recorded in 2005 (77% of meiocytes), a year when the maximum temperature was the lowest (29.4°C), and the minimum temperature was the highest (21.9°C). Rainfall was also highest of the three years (123 mm) as well as hours of sunshine (7.7 h). These environmental conditions could have affected the meiotic pathway by altering the function of some genes that control meiosis. Abnormal nucleolus disintegration and abnormal spindle orientation, for example, occurred only under this environmental condition, and the first was the most frequent abnormality in 2005.

In the period of inflorescence collection in January and April, apparently there was no water deficit. In Brazilian savannas, November to April are the summer rain months, and the high temperatures and bright sun may cause temporary stress and stomatal closing, due to high evapotranspiration. In wheat, water deficit during the period of reduction division in pollen mother cells significantly reduces grain set as a result of reduced male fertility, but does not affect female fertility (Bingham, 1966; Saini and Aspinall, 1981, 1982a). The same result was found in rice (Sato, 1954). High temperature, on the other hand, could also account for meiotic abnormalities. In 2005, the inflorescences were collected after five days of maximum temperature (above 30°C). In wheat, short periods of exposure to a relative high temperature (30°C) during pollen mother cell meiosis also resulted in male and female sterility similar to that caused by water deficit (Saini and Aspinall, 1982b).

Global warming is of major concern among scientists and society today. The International Panel on Climate Change (IPCC, 2001) announced that the average global atmospheric temperature may rise 1.8°C by 2050 in relation to 1990, and 4°C by 2100. Because of this, many reports have been dedicated to drawing attention to significant changes in natural and managed ecosystems. The adverse effects of high temperature on plant reproduction have severe implications on worldwide crop production systems. According to Young et al. (2004), a good understanding of the phenomenon will be required, and furthermore, adequate germplasm needs to be developed to cope with change. This is the first study dedicated to understanding the effect of the environment on the *Brachiaria* microsporogenesis.

The *Brachiaria* germplasm collection at Embrapa Beef Cattle encompasses 475 accessions of 15 species collected in Africa in the middle of 1980s. An active breeding program is in effect in Brazil, and selection for tolerance to water and temperature stresses should be emphasized from now on, to prepare for global warming. Seed production in *Brachiaria* hybrids is a major concern in the program, since these wild species are notorious for low seed set. If environmental stresses are to worsen this scenario, some of these genotypes could be discarded as future cultivars or genitors in crosses. This study was carried out under natural environmental conditions that, unfortunately, were very difficult to characterize and impossible to control. Studies under controlled conditions need to be conducted to better understand the true effect of water deficit/excess and high temperature on *Brachiaria* microsporogenesis, pollen fertility, and seed production. This knowledge will be invaluable selecting adequate germplasm to prepare for the future of cattle production in South America and other tropical countries where *Brachiaria* is the single most important cultivated pasture.

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REFERENCES

Araújo ACG, Mukhambetzhanov S, Pozzobon MT, Santana EF, et al. (2000). Female gametophyte development in apomictic and sexual *Brachiaria brizantha* (Poaceae). *Biol. Vegetales - Botaniste* 23: 13-28.

Baker JT, Allen LH Jr and Boote KJ (1992). Temperature effects on rice at elevated CO_2 concentration. *J. Exper. Bot.* 43: 959-964.

Bennett MD (1977). The time and duration of meiosis. *Phil. Trans. R. Soc. Lond. B, Biol. Sci.* 277: 201-224.

Bennett MD, Chapman V and Riley R (1971). The duration of meiosis in pollen mother cells of wheat, rye, triticale. *Proc. R. Soc. Lond. B* 178: 259-275.

Bingham J (1966). Varietal response in wheat to water supply in the field, and male sterility caused by a period of drought in a glasshouse experiment. *Ann. Appl. Biol.* 57: 365-377.

Boldrini KR (2006). Abnormal timing of cytokinesis in microsporogenesis in *Brachiaria humidicola* (Poaceae: Paniceae). *J. Genet.* 85: 225-228.

Boyer JS (1982). Plant productivity and environment. *Science* 218: 443-448.

Dorion S, Lalonde S and Saini HS (1996). Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a xecline in invertase activity and changes in carbohydrate metabolism in anthers. *Plant Physiol.* 111: 137-145.

Erickson AN and Markhart AH (2002). Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. *Plant Cell Environ.* 25: 123-130.

Fischer RA (1973). The effects of water stress at various stages of development on yield processes in wheat. In: Plant responses to climatic factors (Slatyer RO, ed.). UNESCO, Paris, 233-241.

Gallo PH, Micheletti PL, Boldrini KR, Risso-Pascotto C, et al. (2007). 2n gamete formation in *Brachiaria* (Poaceae: Paniceae). *Euphytica* 154: 255-260.

Hopkinson JM, Souza FDH, Diulgheroff S, Ortiz A, et al. (1996). Reproductive physiology, seed production and seed quality of *Brachiaria*. In: The biology, agronomy and improvement of *Brachiaria* (Miles JW, Maass BL and Valle CB do, eds.). CIAT/EMBRAPA, Cali, 124-140.

IPCC Working Group I (2001). Climate change: the scientific basis. Projections of future climate change. Geneva. [http://www.grida.no/CLIMATE/IPCC_TAR/WG1/339.htm].

Lalonde S, Beebe DU and Saini HS (1997). Early signs of disruption of wheat anther development associated with the induction of male sterility by meiotic-stage water deficit. *Sex. Plant Reprod.* 10: 40-48.

Li P and Johnston MO (1999). Evolution of meiosis timing during floral development. *Proc. R. Soc. Lond B* 185-190.

Luomajoki A (1986). Timing of microsporogenesis in trees with references to climatic adaptation. A review. *Acta For. Fenn.* 196: 1-33.

Macedo MCM (1995). Pastagens no ecossistema cerrados. In: Anais do Simpósio sobre Pastagens nos Ecossistemas Brasileiros. In: Simpósio sobre Pastagens nos Ecossitemas Brasileiros: Pesquisas para o Desenvolvimento Sustentável (Andrade RP, Barcellos AO and Rocha CMC, eds.). Soc. Bras. Zootec., Brasília, 28-62.

Mendes-Bonato AB, Pagliarini MS, Silva N and Valle CB (2001a). Meiotic instability in invader plants of signal grass *Brachiaria decumbens* Stapf (Gramineae). *Acta Scient.* 23: 619-625.

Mendes-Bonato AB, Pagliarini MS, Valle CB and Penteado MIO (2001b). A severe case of chromosome stickiness in pollen mother cells of *Brachiaria brizantha* (Hochst) Stapf (Gramineae). *Cytologia* 66: 287-291.

Mendes-Bonato AB, Pagliarini MS, Forli F, Valle CB, et al. (2002). Chromosome numbers and microsporogenesis in *Brachiaria brizantha* (Gramineae). *Euphytica* 125: 419-425.

Mendes-Bonato AB, Risso-Pascotto C, Pagliarini MS and Valle CB (2006). Chromosome number and meiotic behaviour in *Brachiaria jubata* (Gramineae). *J. Genet.* 85: 83-87.

Mercado JA, Mar Trego M, Reid MS, Valpuesta V, et al. (1997). Effects of low temperature on pepper pollen morphology and fertility: evidence of cold induced exine alterations. *J. Hortic. Sci.* 72: 217-226.

Namuco OS and O'Toole JC (1986). Reproductive stage water stress and sterility. I. Effect of stress during meiosis. *Crop. Sci.* 26: 317-321.

Porch TG and Jahn M (2001). Effects of high-temperature stress on microsporogenesis in heat-sensitive and heat tolerant genotypes of *Phaseolus vulgaris*. *Plant Cell Environ.* 24: 723-731.

Risso-Pascotto C, Pagliarini MS and Valle CB (2002). Abnormal nucleolar cycle in microsporogenesis of *Brachiaria decumbens* (Gramineae). *Cytologia* 67: 355-360.

Risso-Pascotto C, Pagliarini MS, Valle CB and Mendes-Bonato AB (2003). Chromosome number and microsporogenesis in a pentaploid accession of *Brachiaria brizantha* (Graminae). *Plant Breed.* 122: 136-140.

Risso-Pascotto C, Pagliarini MS and Valle CB (2005). Multiple spindles and cellularization during microsporogenesis in an artificially induced tetraploid accession of *Brachiaria ruziziensis* (Gramineae). *Plant Cell Rep.* 23: 522-527.

Risso-Pascotto C, Pagliarini MS and Valle CB (2006a). Microsporogenesis in *Brachiaria dictyoneura* (Fig. & De Not.) Stapf (Poaceae: Paniceae). *Genet. Mol. Res.* 5: 837-845.

Risso-Pascotto C, Mendes DV, Silva N, Pagliarini MS, et al. (2006b). Evidence of allopolyploidy in *Brachiaria brizantha* (Poaceae: Paniceae) through chromosome arrangement at metaphase plate during microsporogenesis. *Genet. Mol. Res.* 5: 797-803.

Romanova LI and Tret'yakova IN (2005). Specific features of microsporogenesis in the Siberian larch growing under the conditions of technogenic load. *Russ. J. Develop. Biol.* 36: 99-104.

Saini HS (1997). Effects of water stress on male gametophyte development in plants. *Sex. Plant Reprod.* 10: 67-73.

Saini HS and Aspinall D (1981). Effect of water deficit on sporogenesis in wheat (*Triticum aestivum* L.). *Ann. Bot.* 48: 623-633.

Saini HS and Aspinall D (1982a). Sterility in wheat (*Triticum aestivum* L.) induced by water deficit or high temperature: possible mediation by abscisic acid. *Aust. J. Plant Physiol.* 9: 529-537.

Saini HS and Aspinall D (1982b). Abnormal sporogenesis in wheat (*Triticum aestivum* L.) induced by short periods of high temperature. *Ann. Bot.* 49: 835-846.

Sato K (1954). Relationship between rice crop and water. *Jpn. J. Breed.* 4: 264-289.

Sato S, Peet MM and Thomas JF (2002). Determining critical pre- and post-anthesis periods and physiological processes in *Lycopersicon esculentum* Mill. exposed to moderately elevated temperatures. *J. Exp. Bot.* 53: 1187-1195.

Sato S, Kamiyama M, Iwata T, Makita N, et al. (2006). Moderate increase of mean daily temperature adversely affects fruit set of *Lycopersicon esculentum* by disrupting specific physiological processes in male reproductive development. *Ann. Bot.* 97: 731-738.

Sun K, Hunt K and Hauser BA (2004). Ovule abortion in *Arabidopsis* triggered by stress. *Plant. Physiol.* 135: 2358-2367.

Utsunomiya KS, Pagliarini MS and do Valle CB (2005). Microsporogenesis in tetraploid accessions of *Brachiaria nigropedata* (Ficalho & Hiern) Stapf (Gramineae). *Biocell* 29: 295-301.

Valle CB and Savidan YH (1996). Genetics, cytogenetics, and reproductive biology of *Brachiaria*. In: *Brachiaria*: Biology, Agronomy, and Improvement (Miles JW, Maass BL and Valle CB, eds.). CIAT/EMBRAPA, Cali, 147-163.

Young LW, Wilen RW and Bonham-Smith PC (2004). High temperature stress of *Brassica napus* during flowering reduces micro- and megagametophyte fertility, induces fruit abortion, and disrupts seed production. *J. Exp. Bot.* 55: 485-495.