

## Meiotic behavior of several Brazilian soybean varieties

Nilton Cesar Pires Bione<sup>1</sup>, Maria Suely Pagliarini<sup>1</sup> and José Francisco Ferraz de Toledo<sup>2</sup>

### Abstract

Despite the importance of soybeans little cytogenetic work has traditionally been done, due to the small size and apparent similarity of the chromosomes. Fifteen soybean [*Glycine max* (L.) Merrill] varieties adapted for cultivation in two distinct regions of Brazil were analyzed cytogenetically. A low frequency of meiotic abnormalities was noted in all varieties, although they were not equally affected. Irregular chromosome segregation, chromosome stickiness, cytoplasmic connections between cells, cytomixis and irregular spindles were the main abnormalities observed, none of which had been described previously in soybeans. All of these abnormalities can affect pollen fertility. Pollen fertility was high in most varieties and was correlated with meiotic abnormalities. Although soybean is not a model system for cytological studies, we found that it is possible to conduct cytogenetic studies on this species, though some modifications in the standard methods for meiotic studies were necessary to obtain satisfactory results.

### INTRODUCTION

The genus *Glycine*, which includes the cultivated soybean, comprises predominantly diploid ( $2n = 2x = 40$ ) and tetraploid ( $2n = 4x = 80$ ) species. Soybean contains  $2n = 40$  small (1.42-2.82  $\mu\text{m}$ ), morphologically similar somatic chromosomes (Sen and Vidyabhusan, 1960) that do not show sufficiently different banding patterns to allow chromosome identification (Ladizinsky *et al.*, 1979). Palmer (1976) has pointed out the usefulness of cytogenetic methods for improvement of soybeans. However, information on the cytogenetics of cultivated soybean is minimal when compared with other important crops. The causes of this lack of information include: i) the small but numerous chromosomes which are indistinguishable from each other and ii) the fact that the techniques usually used for cytological studies in other plant species are inadequate for soybean. In recent years, several important sterile male soybean mutants have been described (see Graybosch and Palmer, 1988; Palmer *et al.*, 1992) and a cytogenetic map of the 20 soybean chromosomes has been constructed for the relatively uncondensed pachytene chromosomes (Singh and Hymowitz, 1988). More recently, *in situ* hybridization has been used to characterize individual soybean metaphase chromosomes (Griffor *et al.*, 1991).

Despite its considerable economic importance for Brazil, there have been no detailed cytogenetic studies of soybeans in this country. Paraná State, which is home to the National Center for Soybean Research, is responsible for a large part of Brazilian soybean production. Current research at this center involves the analysis of spontaneous meiotic mutants that cause male sterility which may be useful in hybridization programs. To further our under-

standing of soybean cytology and to improve the technique for meiotic studies we have examined the meiotic behavior and pollen fertility of 15 varieties of soybean adapted for cultivation in two different regions of Brazil.

### MATERIAL AND METHODS

Fifteen soybean varieties from the germplasm collection maintained at the National Center for Soybean Research (Embrapa Soja) were analyzed. Eight of them (group I: EMBRAPA 48, EMBRAPA 58, EMBRAPA 61, EMBRAPA 62, BR-37, BR-16, BR-36, OCEPAR 14) are adapted for cultivation in Paraná State and seven (group II: EMGOPA 308, EMGOPA 314 (Garça Branca), FT-Cristalina, MT/BR-45 (Paiaguás), MT/BR-47 (Canário), MG/BR-48 (Garimpo RCH), BR/IAC-21) for cultivation in the central Brazil. Seeds of each variety were planted at the Technical Irrigation Center of the State University of Maringá (Maringá, PR), where the soil was prepared for soybean cultivation.

Flower buds were collected from five plants of each variety for meiotic analysis and were fixed in FAA (ethanol:formaldehyde:acetic acid, 2:1:1 v/v) for 24 h, after which they were transferred to 70% alcohol and stored at 4°C. Pollen mother cells (PMCs) were prepared by the squash technique and stained with 1% acetic carmine. At least 250 PMCs in different phases of meiosis were evaluated for each plant and any abnormalities seen were recorded. The same procedures and stain used for meiotic analysis were employed with open flowers to test pollen sterility. One thousand pollen grains/plant were examined.

The data were analyzed statistically by analysis of variance in a completely randomized design. Initially, the va-

<sup>1</sup>Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, 87020-900 Maringá, PR, Brasil.  
Send correspondense to M.S.P. E-mail: mspagliarini@uem.br

<sup>2</sup>Centro Nacional de Pesquisa em Soja (Embrapa Soja), 86001-970 Londrina, PR, Brasil.

ieties were compared in the same group, and then the two groups were compared. The mean percentage of normal PMCs/variety in each group was compared using the Duncan test.

## RESULTS

The 15 soybean varieties had a low frequency of meiotic abnormalities (Table I). Analysis of variance revealed significant differences ( $P < 0.05$ ) in meiotic behavior among the varieties in group I cultivated in Paraná State. In this group, the variety EMBRAPA 48 was the most affected by meiotic abnormalities. The varieties in group II, adapted for cultivation in central Brazil, had a more normal meiotic behavior than those in group I, though analysis of variance showed differences among them. There was also a significant difference between the meiotic behavior of the two groups ( $P < 0.05$ ) as determined by analysis of variance.

The meiotic abnormalities observed among the varieties included chromosome segregation, chromosome stickiness, cytoplasmic connections among cells, cytomixis and irregular spindle. The meiotic phases generally most affected by these abnormalities were prophase I and metaphase I.

Precocious migration of univalents to the poles (Figure 1a) was observed in all varieties, with the exception of EMGOPA 314, in which all of the cells had normal meiosis. Another frequent segregational abnormality observed in metaphase I of two varieties was non-oriented bivalents at the equatorial plate (Figure 1b). This abnormality occurred in the varieties OCEPAR 14 and EMBRAPA 48, with a significantly higher frequency in the latter. Laggard chromosomes in anaphases I and II were observed at a low fre-

quency in some varieties. As a consequence of precocious migration of univalents, non-oriented bivalents and laggard chromosomes, some micronuclei (Figure 1c-e) were observed in telophase I and meiosis II. These micronuclei gave rise to microcytes in the tetrads (Figure 1f).

Chromosome stickiness was observed only in the MT/BR-45, EMBRAPA 48 and EMBRAPA 62 varieties, and affected all meiotic phases (Figure 2a-d). The phenomenon ranged from slight stickiness to an indistinct compact chromatin mass involving the entire complement (Figure 2a,b), which impaired chromosome segregation. Bridges were observed in telophase I (Figure 2c) and pycnosis also occurred in some cells (Figure 2d).

The most common abnormality in all varieties was cytoplasmic connections involving two or more microspores (Figure 3a). The mean percentage of cytoplasmic connections ranged from 3.2 to 24.5 (Table II). Analysis of variance showed significant differences ( $P < 0.05$ ) in this characteristic among the varieties. Although cytoplasmic connections were frequent, only one case of true chromosome transfer among cells (cytomixis) was observed (Figure 3b), although evidence of chromosome transfer was found in some cells with extra chromosomes (Figure 3c,d).

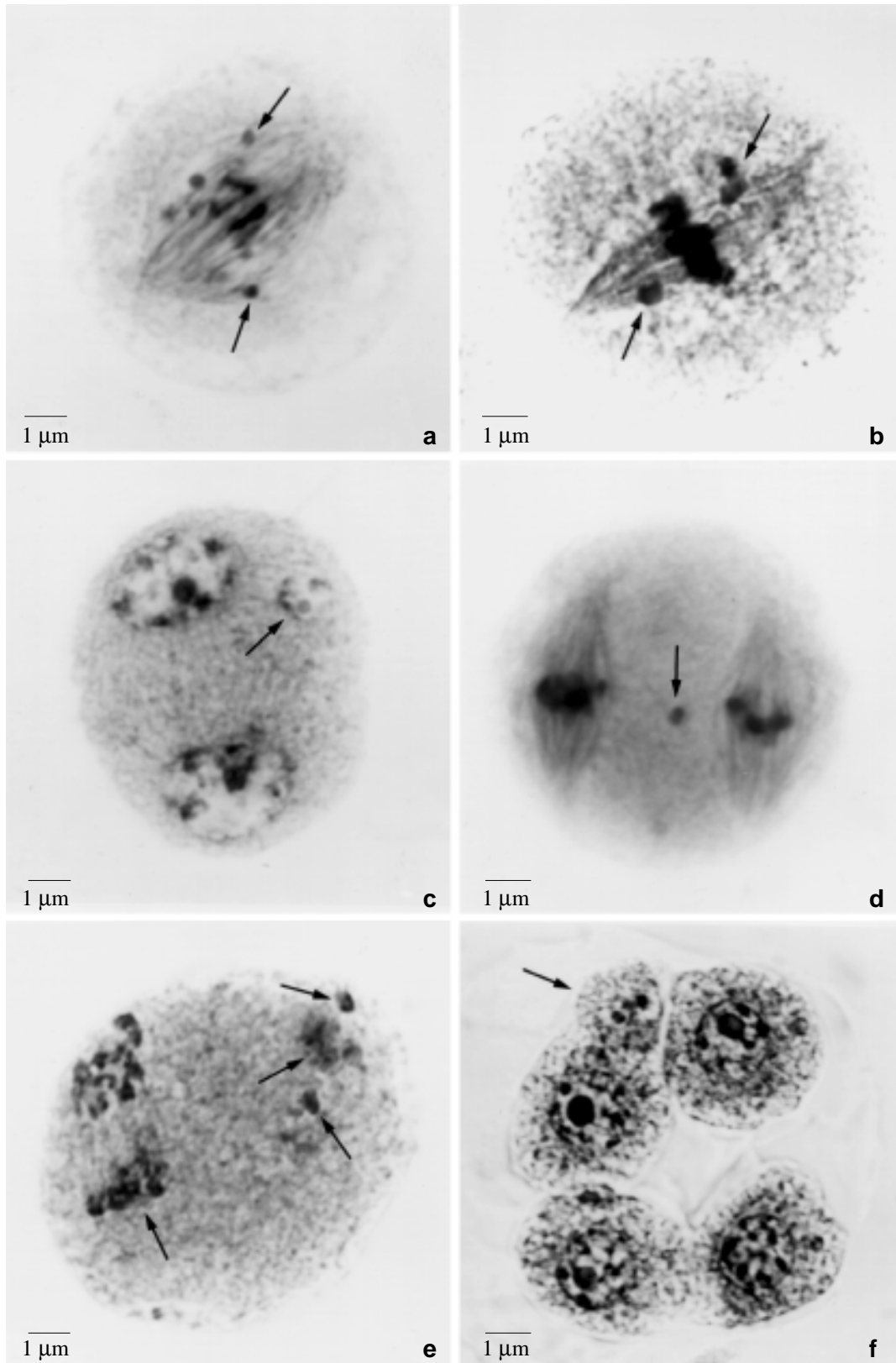
An irregular spindle was observed in only a few cells. In meiosis I, tripolar rather than bipolar spindles were present (Figure 4a,b), whereas in meiosis II the spindles were convergent (Figure 4c).

The test for pollen fertility showed a low percentage of sterile pollen grains (Figure 4d). In most of the varieties, pollen fertility was significantly correlated with meiotic abnormalities, although in a few cases there was no relationship (Table I), as in the case of variety EMGOPA 314, which had the highest meiotic stability but the lowest pollen fertility.

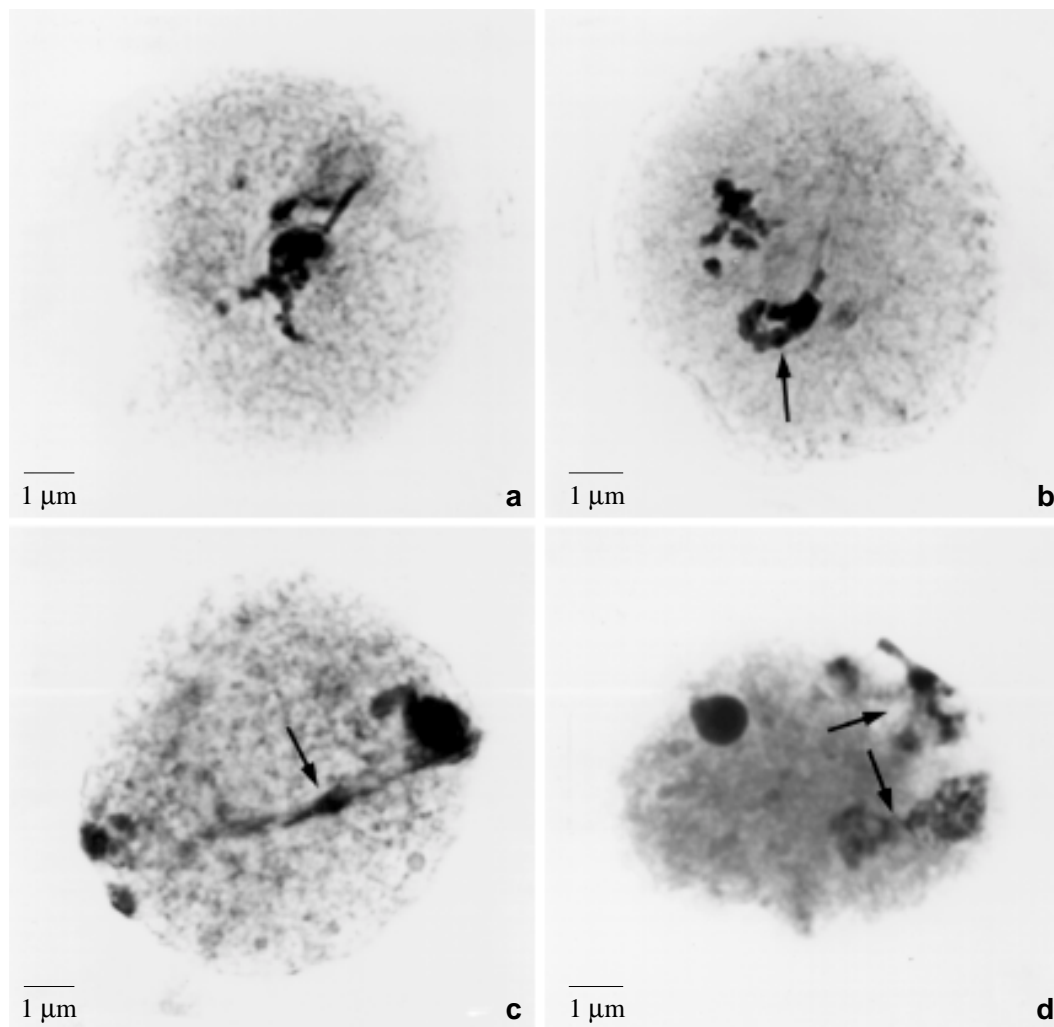
**Table I** - Percentage of meiotically normal pollen mother cells (PMCs)/plant/variety and pollen fertility.

Group	Varieties	No. of PMCs analyzed	Normal PMCs/plant (%)					Mean*	Pollen fertility (%)
			1	2	3	4	5		
I (Paraná State)	EMBRAPA 58	1250	99.55	100.0	99.50	99.33	99.07	99.49a	99.7
	EMBRAPA 62	1381	99.67	98.11	99.19	100.0	99.68	99.33a	99.0
	EMBRAPA 61	1362	100.0	100.0	97.82	99.11	99.62	99.31a	99.7
	BR-37	1098	98.22	98.38	100.0	100.0	99.61	99.24a	99.6
	BR-16	1393	97.69	98.88	99.67	99.66	99.36	99.05a	100
	BR-36	880	99.53	98.97	99.32	97.76	96.88	98.49a	98.8
	OCEPAR 14	1288	90.32	99.50	98.18	96.53	99.63	96.83ab	99.5
	EMBRAPA 48	1067	93.07	99.19	99.12	95.26	91.13	95.55b	99.5
II (Central Brazil)	EMGOPA 314	1128	100.0	100.0	100.0	100.0	100.0	100.0a	95.5
	MT/BR-45	1196	98.10	100.0	100.0	100.0	98.70	99.36a	96.6
	EMGOPA 308	1006	97.23	100.0	99.53	100.0	100.0	99.35a	99.5
	MT/BR-47	1472	100.0	99.07	98.93	98.25	100.0	99.25a	100
	FT-Cristalina	1313	100.0	100.0	99.04	99.65	97.52	99.24a	99.5
	MG/BR-48	1461	99.65	98.37	99.30	99.58	98.79	99.14a	99.4
	BR/IAC-21	1334	100.0	100.0	99.07	97.24	98.81	99.02a	96.6

\*Means with the same letter were not significantly different by Duncan's test ( $\alpha = 0.05$ ).



**Figure 1** - Abnormal chromosome segregation in meiosis. **a)** Metaphase I showing precocious migration of univalents to the poles (arrows). **b)** Metaphase I with non-oriented bivalents (arrows). **c)** Telophase I showing a micronucleus (arrow). **d)** Metaphase II with a micronucleus (arrow). **e)** An unidentified phase with some micronuclei (arrows). **f)** Tetrad with one microcyte (arrow).



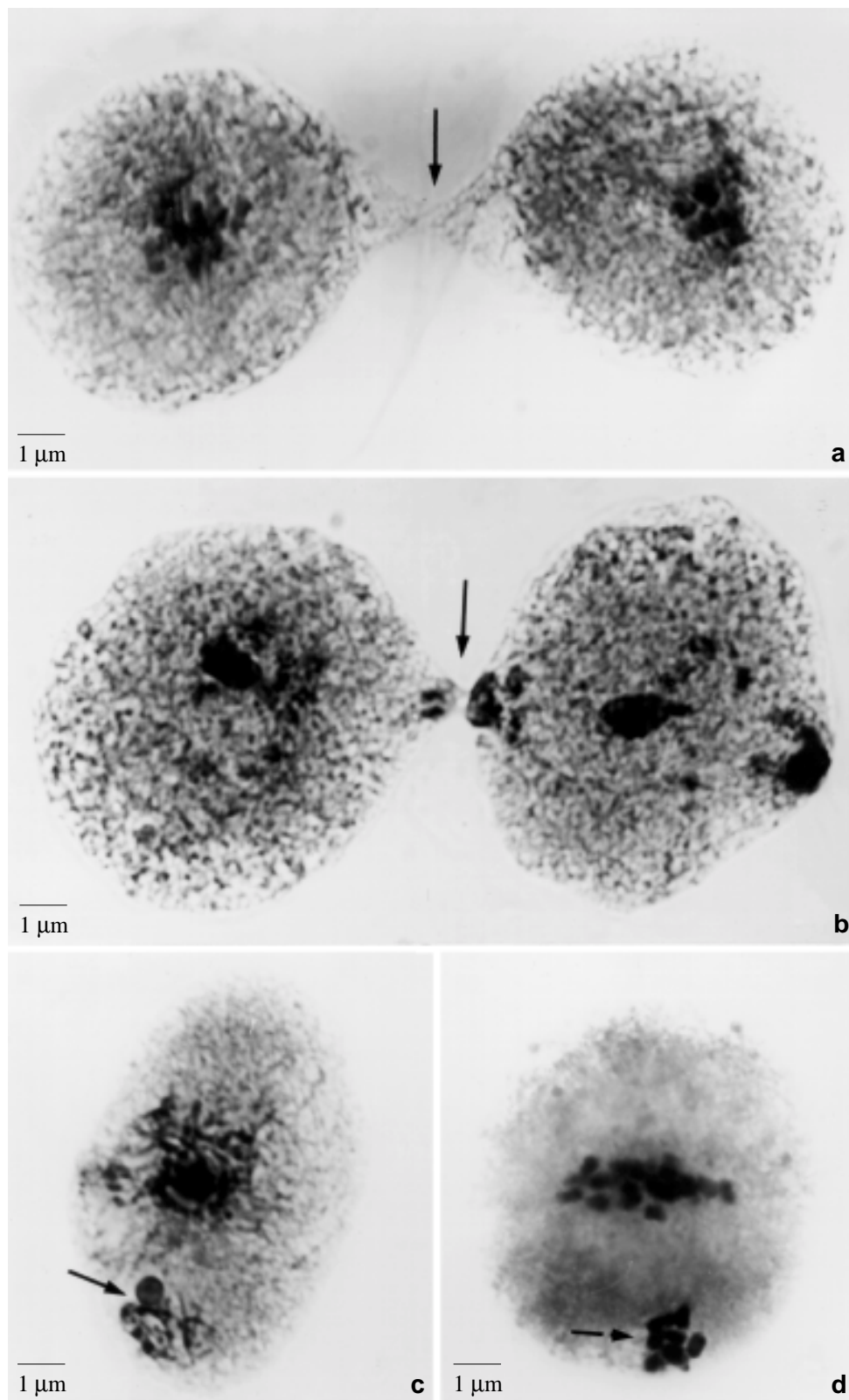
**Figure 2** - Aspects of chromosome stickiness. **a)** Stickiness in prophase I involving the entire chromosome complement. **b)** Metaphase I with some chromosomes linked by stickiness (arrow). **c)** Telophase I with a thick bridge (arrow) caused by chromosome stickiness. **d)** Prophase I showing pycnotic micronuclei (arrows).

## DISCUSSION

Spontaneous chromosomal aberrations are relatively rare in *Glycine* compared with other important genera (Singh and Hymowitz, 1991a) and generally involve polyploidization and aneuploidy. Spontaneous meiotic mutations that cause male sterility have also been reported in soybeans (see Graybosch and Palmer, 1988; Palmer *et al.*, 1992). We found meiosis to be relatively normal in 15 varieties of cultivated soybeans with few abnormalities when compared with other crops (Moraes-Fernandes, 1982; Souza *et al.* 1997; Baptista-Giacomelli, 1999). The abnormalities involved chromosome segregation, chromosome stickiness, irregular spindle formation and connections among cells, and had not been described in soybeans.

The observed precocious chromosome migration to the poles may have resulted from univalent chromosomes

at the end of prophase I or precocious chiasma terminalization in diakinesis or metaphase I. Univalents may originate from an absence of crossing-over in pachytene or from synaptic mutants. However, prophase I stages were not analyzed because of the poor quality of the squash preparations. Chiasmata are responsible for the maintenance of bivalents which permit normal chromosome segregation. This process ensures pollen fertility. While precocious migration of univalents to the poles is a very common abnormality among plants (Pagliarini, 1990; Pagliarini and Pereira, 1992; Defani-Scoarize *et al.*, 1995a,b; Consolaro *et al.*, 1996), the other segregational abnormality (non-oriented bivalents) observed in the varieties OCEPAR 14 and EMBRAPA 48 is rare, but is known to occur in *Chlorophytum comosum* (Pagliarini *et al.*, 1993). The behavior of these and of the laggard chromosomes is characteristic in that they generally lead to micronucleus formation (Koduru and Rao,

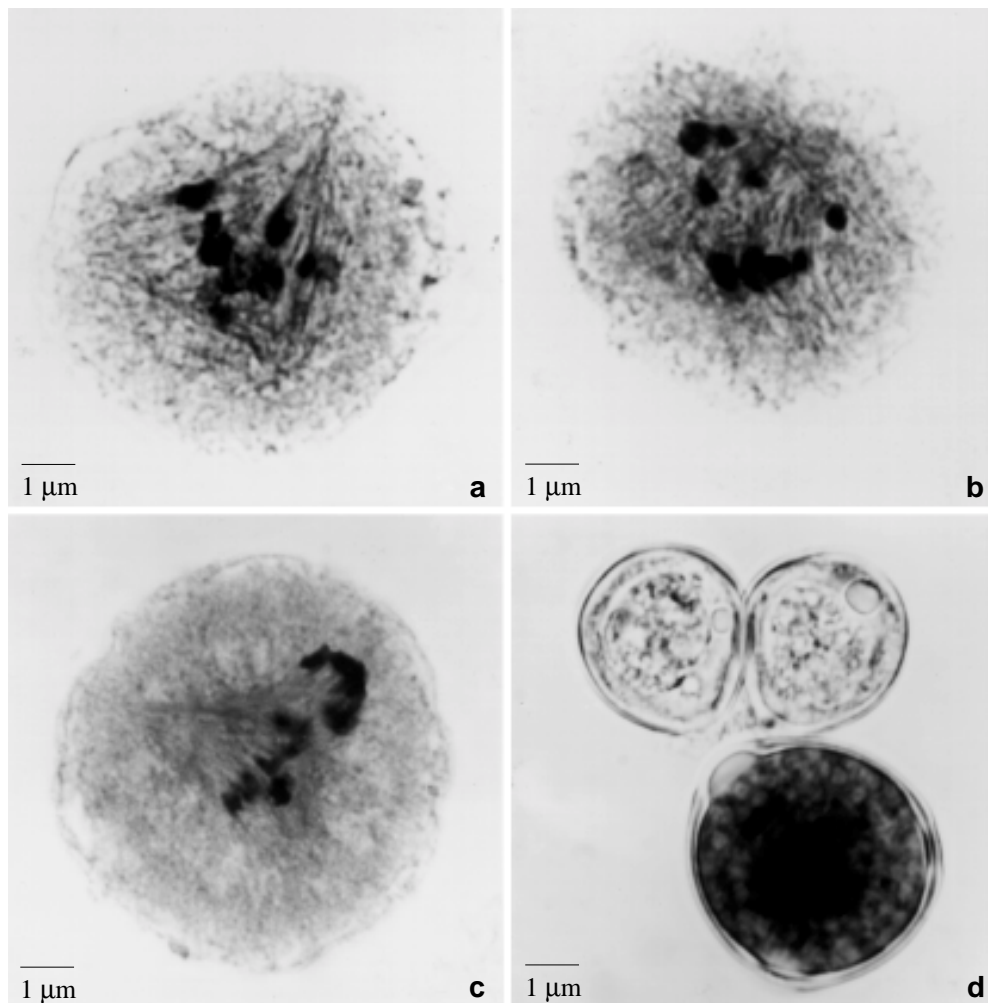


**Figure 3** - Cytoplasmic connections among cells and their consequences. **a**) Cytoplasmic channel (arrow) between pollen mother cells (PMCs) in metaphase I. **b**) Cytomixis (arrow) between PMCs. **c**) Pachytene showing some extra chromosomes (arrow). **d**) Metaphase I with extra chromosomes (arrow).

**Table II** - Percentage of pollen mother cells (PMCs) with cytoplasmic connections.

Group	Variety	No. of PMCs analyzed	PMCs with cytoplasmic connections (%)					Means*
			1	2	3	4	5	
I (Paraná State)	EMBRAPA 58	1250	21.6	32.1	3.0	33.4	8.1	19.1a
	EMBRAPA 61	1362	4.1	19.9	3.9	4.8	37.3	14.0ab
	EMBRAPA 62	1381	5.6	15.5	12.2	1.2	18.2	10.6ab
	BR-36	880	17.0	9.3	6.8	2.2	8.3	8.7ab
	BR-37	1098	3.0	4.3	19.8	12.7	3.5	8.7ab
	BR-16	1393	1.8	14.5	7.9	1.4	13.8	7.9ab
	OCEPAR 14	1288	2.1	5.5	10.2	5.9	3.7	5.5b
	EMBRAPA 48	1067	2.6	-	13.3	5.5	0.35	4.3b
II (Central Brazil)	MG/BR-48	1461	28.9	24.1	10.8	32.1	26.8	24.5a
	EMGOPA 314	1128	33.8	13.3	23.5	11.4	16.4	19.7ab
	EMGOPA 308	1006	35.3	4.4	13.0	21.1	14.3	17.6ab
	FT-Cristalina	1313	5.3	30.6	25.1	3.5	7.5	14.4abc
	BR/IAC-21	1334	15.8	12.5	10.2	25.8	6.0	14.1abc
	MT/BR-47	1472	-	1.2	2.1	15.8	17.1	7.3bc
	MT/BR-45	1196	5.7	2.5	0.4	1.9	5.2	3.2c

\*Means with the same letter were not significantly different by Duncan's test ( $\alpha = 0.05$ ).



**Figure 4** - Abnormal spindles and pollen sterility. **a,b**) Metaphases I with a tripolar spindle. **c**) Metaphase II showing a convergent spindle. **d**) Pollen grains: fertile (dark) and sterile (empty).

1981). In soybean, the percentage of cells with meiotic abnormalities was higher in metaphase I and decreased until telophase II, indicating that some chromosomes were included in the main nucleus. This seems to be normal behavior for many species (Koduru and Rao, 1981).

Sticky chromosomes were first reported in maize (Beadle, 1932) and are seen as intense chromatin clustering in the pachytene stage. The phenotypic manifestation of stickiness may vary from mild, when only a few chromosomes of the genome are involved, to intense, with the formation of pycnotic nuclei that may involve the entire genome, culminating in chromatin degeneration (for a review, see Consolaro and Pagliarini, 1996). In the soybean varieties, the stickiness was of both types. Some cells showed mild stickiness, in which case it was possible to identify the meiotic stage. In other cells, the intense phenotypic manifestation led to the formation of pycnotic nuclei.

Chromosome stickiness may be caused by genetic or environmental factors. Genetically controlled stickiness has been described in other cultivated plants such as maize (Beadle, 1932; Golubovskaya, 1989; Caetano-Pereira *et al.*, 1995), pearl millet (Rao *et al.*, 1990) and wheat (Zanella *et al.*, 1991). Several agents have been reported to cause chromosome stickiness, including X-rays (Steffensen, 1956), gamma rays (Rao and Rao, 1977; Al Achkar *et al.*, 1989), temperature (Eriksson, 1968), herbicides (Badr and Ibrahim, 1987) and some chemicals present in soil (Levan, 1945; Steffensen, 1955; Caetano-Pereira *et al.*, 1995). However, the primary cause and biochemical basis of chromosome stickiness are still unknown. Gaulden (1987) postulated that sticky chromosomes may result from the defective functioning of one or two types of specific non-histone proteins involved in chromosome organization, which are needed for chromatid separation and segregation. The altered functioning of these proteins leading to stickiness is caused by mutations in the structural genes coding for them (hereditary stickiness) or by the action of mutagens on the proteins (induced stickiness).

Cytoplasmic connections, the most common abnormality observed in soybeans, is a phenomenon widely described in angiosperms (see Heslop-Harrison, 1966; Risueño *et al.*, 1969; Whelan, 1974). The first description was made by Gates (1908), who observed delicate threads of cytoplasm connecting adjacent pollen mother cells in *Oenothera*. Gates (1911) subsequently suggested that these connections must form an important avenue of exchange between PMCs, and described the transfer of nuclear material through them from one meiocyte to another, calling the process "cytomixis". According to Heslop-Harrison (1966) and Risueño *et al.* (1969), the role of cytoplasmic channels is related to the transport of nutrients between meiocytes. Investigations in angiosperms have provided evidence that massive protoplasmic connections are formed among microsporocytes. Our study showed that the frequency of cytoplasmic connections among varieties varied from 3.2 to 24.5%. Although

cytoplasmic connections are very common in angiosperms, the movement of nuclear material through them is rare. In the soybean varieties studied here, only one case of chromosome transfer (cytomixis) among microsporocytes was observed. In general, cytomixis has been detected at a higher frequency in genetically imbalanced species such as hybrids, as well as in apomictic, haploid and polyploid species (see Yen *et al.*, 1993). Among the factors proposed to cause cytomixis are the influence of genes, fixation effects, pathological conditions, herbicides and temperature (see Caetano-Pereira and Pagliarini, 1997). Cytomixis may have serious genetic consequences by causing deviations in chromosome number and may represent an additional mechanism for the origin of aneuploidy and polyploidy (Sarvella, 1958).

The abnormal spindles observed in a few cells have also been reported for other genera (see Harlan and De Wet, 1975; Veilleux, 1985). The spindle apparatus is normally bipolar and acts as a single unit, playing a crucial role in the alignment of metaphase chromosomes and their poleward movement during anaphase. Distortion in meiotic spindles may be responsible for unreduced gamete formation. While the tripolar spindles seen in metaphase I of some cells may cause genome fractionation, convergent spindles in metaphase II rejoin the homologues segregated in meiosis I, leading to the formation of unreduced gametes. Although the formation of unreduced gametes has been investigated in studies of evolution (Harlan and De Wet, 1975) and in breeding programs (Veilleux, 1985), the frequency of convergent spindles in metaphase II in soybean was very low (0.3 to 1.4%) and not enough to be useful in breeding programs.

In normal soybean genotypes meiotic abnormalities are rare whereas they are common in meiotic mutants that cause male sterility. Chromatin bridges and micronuclei were described for the first time in interspecific hybrids of *Glycine max* x *Glycine soja* by Ahmad *et al.* (1977), who found that the extent of abnormalities was influenced by environmental conditions. The same abnormalities were reported by Ahmad *et al.* (1984), who concluded that chromosome behavior and fertility depended on the parentage of the hybrids and on environmental temperature. Their results, obtained in greenhouse and controlled environmental studies, suggest that at least three factors (genotype, temperature and genotype x temperature interaction) influence chromosome behavior and fertility.

All of the meiotic abnormalities found in the soybean varieties analyzed here have been reported to be responsible for pollen sterility. Fertility depends on the efficiency of the meiotic process. Studies on different plant species have shown that the decline in seed production is correlated with meiotic irregularities (La Fleur and Jalal, 1972; Dewald and Jalal, 1974; Moraes-Fernandes, 1982; Smith and Murphy, 1986; Pagliarini and Pereira, 1992; Pagliarini *et al.*, 1993; Consolaro *et al.*, 1996; Khazanehdari and Jones, 1997). In most of the soybean varieties, pollen

fertility showed a close relationship with meiotic abnormalities. Most of the varieties had few meiotic abnormalities and, as a consequence, a high pollen fertility. Soybean is an autogamous, diploid and genetically stable species that produces a low number (300 to 800) of pollen grains per anther (Palmer *et al.*, 1978). For this reason high meiotic stability is required in order to guarantee seed production. From our study, we suggest that the differential seed production observed among varieties is due to genetic control and not only to meiotic abnormality.

Soybean has not been considered a model system for cytological studies. According to Singh and Hymowitz (1991b), this may explain why soybean cytogenetics has lagged behind genetic studies of maize, barley and tomato. Our experience with soybean cytogenetics confirms this conception. Squash preparations of PMCs routinely employed for other species did not give good results. Some small modifications in the smear and stain in relation to the standard methods were necessary to obtain satisfactory results. The fact that the plants were cultivated in fields probably affected the analysis since, according to Palmer and Kilen (1987), greenhouse-grown plants yield a higher percentage of acceptable preparations, whereas plants grown under hot and dry conditions give very poor results. Despite the difficulties, we conclude that it is possible to conduct cytogenetic studies on soybean.

## RESUMO

Quinze variedades de soja adaptadas ao cultivo para duas regiões do país foram citogeneticamente analisadas para melhor compreender a citologia desta cultura e aprimorar a técnica para estudos meióticos. A análise mostrou uma baixa frequência de anormalidades meióticas e as variedades não foram igualmente afetadas. Segregação cromossômica irregular, aderências cromossômicas, conexões citoplasmáticas entre células, citomixia e fusos irregulares foram as anormalidades observadas entre quatorze variedades, nenhuma das quais foi descrita previamente em soja. Todas as anormalidades observadas podem afetar a fertilidade do pólen. A fertilidade do pólen foi alta e, exceto para algumas variedades, esteve correlacionada com as anormalidades meióticas. Embora soja não seja um sistema modelo para estudos citológicos, nosso primeiro contato com esta espécie mostrou que é possível a condução de estudos citogenéticos. Algumas modificações no método padrão para estudos meióticos foram necessárias para se obterem resultados satisfatórios.

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