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Meiotic behavior in tetraploid populations of *Pfaffia tuberosa* (Amaranthaceae)

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Four populations of *Pfaffia tuberosa,* the Brazilian ginseng, collected in the central region of the state of Rio Grande do Sul (Brazil) were cytologically analyzed for meiotic behavior. All were tetraploid (2n = 4x = 68) and this condition could be observed through multiple chromosome pairing in pachytene and quadrivalent formation at diakinesis. Irregular chromosome segregation in both divisions led to the formation of a wide diversity of meiotic products. Chromosome stickiness was also frequently found among microsporocytes. Pollen grains of different sizes were observed, while pollen sterility ranged from 49.56 to 64.20% among analyzed populations. This is the first cytological report involving chromosome number determination and meiotic behavior for the species.

Key words: *Pfaffia tuberosa*, chromosome, polyploidy, meiosis, pollen, sterility

Introduction

Pfaffia tuberosa is a native species from the state of Rio Grande do Sul, southern Brazil, with wide occurrence in the Santa Maria municipality. It is an herbaceous plant with a subterranean ligneous system primarily composed of roots. Roots show alternate tuberose and non-tuberose zones. Root tuberosity is caused by the growth in the number of parenchymatic cells between the conducting elements in the center of the organ and by the fibro-vascular groups (SIQUEIRA et al. 1993).

Due to its wide use, *Pfaffia tuberosa*, like *P. glomerata*, is also known as the »*Brazilian ginseng*«. In folk medicine, it is used in the treatment of rheumatic and vascular diseases and as an aphrodisiac tonic (TERÀN 1990, SIQUEIRA et al. 1993, TANIGUCHI et al. 1997). ARENAS and AZORERO (1977) reported the use of this species in the treatment of female sterility. According to NISHIMOTO et al. (1986), the ecdysterone content in *P. tuberosa* is lower than that in *P. iresinoides.*

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Despite its importance for medicinal purposes, few cytological studies have been devoted to *P. tuberosa* or the genus *Pfaffia* (TASCHETTO and PAGLIARINI 2003a, b). The current cytological study reports, for the first time, the meiotic behavior of four Brazilian populations of *P. tuberosa.*

Materials and methods

Cytogenetic studies were carried out on four populations of *P. tuberosa* collected in the central region of the state of Rio Grande do Sul: the Santa Maria, São Vicente do Sul, Jaguari and Santiago municipalities where they widespread (Fig. 1).

For meiotic studies, floral buds in the proper stage were collected and fixed in acetic ethanol (3:1) fixative for 24 hr, transferred to 70% ethanol, and stored under refrigeration until use. Microsporocytes were prepared by squashing in 0.5% propionic carmine. More

Fig. 1. Morphology of *Pfaffia tuberosa* (Scale bar denotes 30 cm)

than 5.000 cells, including microsporocytes in meiosis I and II, and meiotic products, were analyzed in each population. Pollen fertility was evaluated also by using 0.5% propionic carmine. More than 3.200 pollen grains were analyzed in each population. Photomicrographs were taken with Kodak Imagelink-HQ, ISO 25, black and white film.

Results and discussion

The four populations of *P. tuberosa* collected in the state of Rio Grande do Sul presented 2n = 68 chromosomes and reported tetraploid in relation to other species of the genus, *P. glomerata*, previously analyzed by the same authors (TASCHETTO and PAGLIARINI 2003 a),

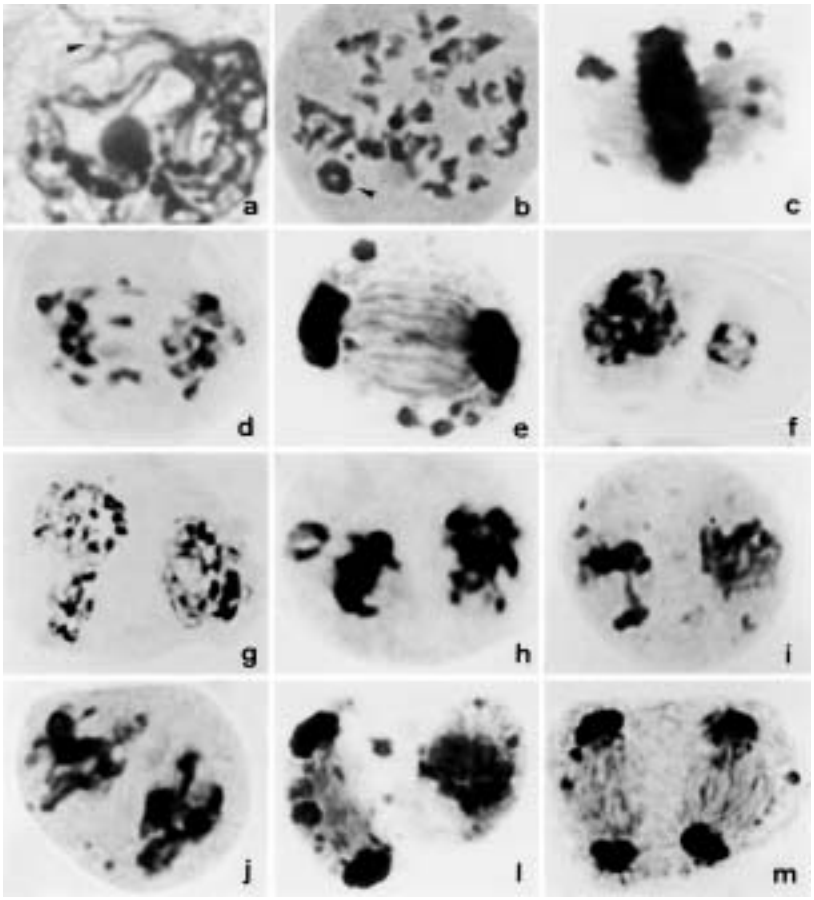

Fig. 2. Microsporogenesis in *Pfaffia tuberosa*. a – microsporocyte in pachytene showing multiple chromosome association. b – diakinesis with a tetravalent. c – metaphase I with precocious chromosome migration to the poles. d – anaphase I with laggards. e – telophase I with several micronuclei. f – telophase I with two nuclei of different sizes. g – telophase I with three nuclei of different sizes. h, i – metaphases II with a big micronucleous (h) and precocious chromosome migration to the poles (i). j – anaphase II showing stickiness. l – asynchronous division metaphase II / telophase II. m – telophase II with some micronuclei.

with chromosome number scoring 2n = 34 in nine populations. The polyploid condition of the species under analysis could be inferred also by the pattern of multiple chromosome pairing visualized at pachytene (Fig. 2a), and multivalent chromosome association at diakinesis (Fig. 2b). Quadrivalents were frequently observed.

Consequently to polyploid condition, many abnormalities related to irregular chromosome segregation were observed from the metaphase I to the tetrad stage. The percentage of meiotic abnormalities for each meiotic phase was similar among populations (Tab. 1). Precocious chromosome migration to the poles at metaphase I and II (Figs. 2c and i), laggard chromosomes at anaphase I and II (Figs. 2d and l) and micronuclei at telophase I and II (Figs. 2e and m) were observed. Micronuclei gave rise to microcytes at tetrad stage (Figs. 3a to h) which, in turn, gave rise to small and sterile pollen grains (Fig. 3i). Pollen sterility among populations ranged from 49.56 to 64.20%.

Meiotic abnormalities related to irregular chromosome segregation leading to pollen sterility are a common feature in polyploids. However, chromosomes with precocious migration to the poles or laggard chromosomes generally remain isolated or in small groups so that small micronuclei are formed. Besides this typical behavior, a distinctive feature has also been reported in the four polyploid populations of *P. tuberosa*. Sometimes the amount of chromosomes irregularly segregated to the poles was so different that two telophase nuclei of extremely different sizes were observed (Fig. 2f). This behavior led to the formation of a tetrad with two big microspores and two small ones (Fig. 3g). In other cases, chromosomes formed two telophase nuclei of different sizes at the same pole (Fig. 2g), giving rise to hexads with microspores of three different sizes. The amount of different kinds of segregational abnormalities was so great that an infinite diversity of meiotic products could be observed. Figure 3 illustrates only a small parcel of this diversity. In spite of representative aspects of irregular meiosis shown in Figure 2, certain meiotic products, such as those represented in Fig. 3a, 3b and 3d, and many others not given in this plate, could not be explained merely by irregular chromosome segregation. Monads (Fig. 3a), dyads (Fig. 3b), triads with three microspores of different sizes (Fig. 3d) suggest that restitutional nuclei may also occur. Another cause of triad formation could be asynchrony in the second division, as observed in Figure 3l, where one cell is in metaphase and the other is entering telophase. If the former fails to enter the cycle, in due time a triad with one 2n microspore and two n microspores will be formed. The causes and consequences of 2n and jumbo pollen formation in *P. tuberosa* and *P. glomerata* are better discussed by TASCHETTO and PAGLIARINI (2003 b).

Another segregational meiotic abnormality observed in a small percentage of cells in all populations was related to the inability of some bivalents to reach the metaphase plate, and thus remaining non-congressed. Non-oriented bivalents are very common among plants. They were reported in *Chlorophytum comosum* (PAGLIARINI et al. 1993), in some *Paspalum* species (PAGLIARINI et al. 2001) and in soybean (BIONE et al. 2000). The inability of chromosomes to congregate on the equatorial plate may be related to the kinetochore. NICKLAS and WARD (1994) enumerated some factors that might impair the attachment of kinetochores to the spindle fibers.

Chromosome stickiness, frequently reported among microsporocytes of all populations, was another important cytological feature of *P. tuberosa*. Sticky chromosomes are seen as intense chromatin clustering affecting chromosome segregation. The phenomenon

Tab. 1. Number of analyzed cells and percentage of meiotic abnormalities in tetraploid populations of *P. tuberosa* and pollen sterility.

Phases	Abnormalities	No of cells analyzed and percentage of abnormalities/populations							
		Santa Maria		São Vicente do Sul		Jaguari		Santiago	
Metaphase I	Precocious migration to poles	(1545)	23.30	(1525)	19.15	(1691)	10.88	(1515)	18.28
	Non-congressed bivalents		5.70		3.02		1.01		3.49
	Chromosome stickiness		27.90		19.15		20.05		37.82
Anaphase I	Laggard chromosomes	(201)	7.46	(198)	6.06	(167)	10.18	(212)	4.72
	Chromosome stickiness		25.87		18.69		17.56		21.23
Telophase I	Chromosome bridges	(810)	6.92	(795)	7.04	(760)	2.50	(855)	1.52
	Micronuclei		39.14		23.43		25.66		27.37
	Trinucleate cells		2.60		1.00		0.0		0.94
Prophase II	Micronuclei	(425)	17.65	(830)	2.89	(388)	9.02	(440)	8.18
	Chromosome bridges		6.82		0.72		1.29		1.81
	Trinucleate cells		2.82		0.48		1.55		1.59
Metaphase II	Precocious migration to poles	(68)	67.64	(152)	27.73	(162)	28.57	(185)	30.28
Anaphase II	Laggard chromosomes	(126)	16.66	(136)	16.24	(131)	20.18	(128)	26.74
Telophase II	Micronuclei	(935)	3.42	(893)	2.64	(901)	1.87	(907)	2.75
	Polynucleate cells		2.24		2.27		2.77		2.20
Tetrad	Microcytes	(1095)	41.83	(920)	41.42	(1024)	30.08	1034)	40.62
	Polyads		7.30		4.35		2.73		3.48
Pollen sterility		3390	(58.84)	3420	(49.56)	3280	(61.28)	3380	(64.20)

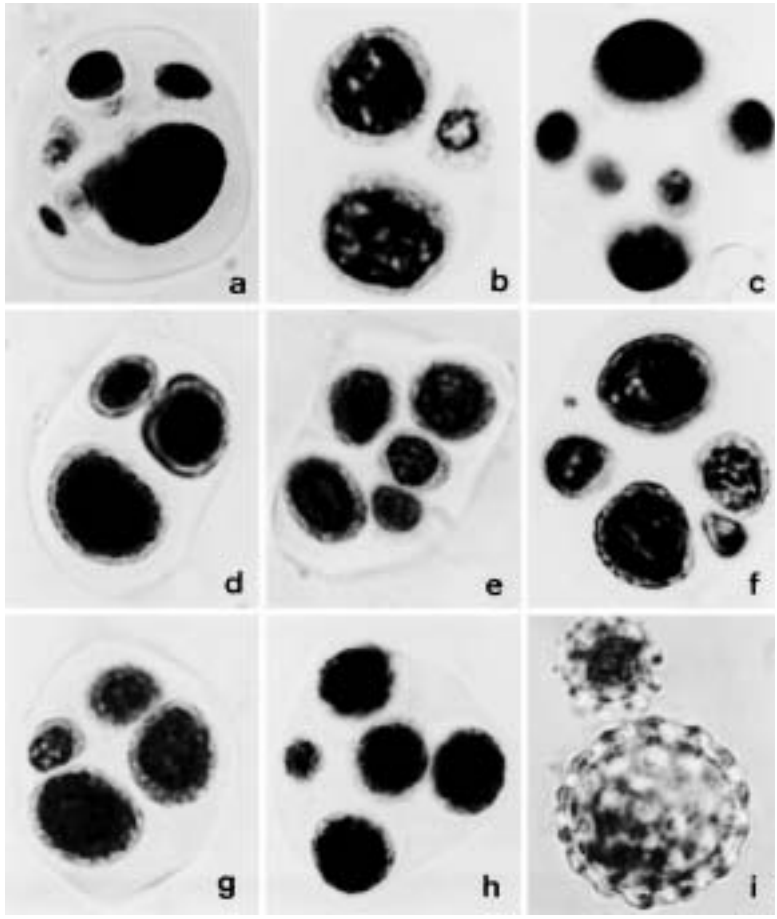

Fig. 3. Meiotic products in *P. tuberosa*. a – monad with several microcytes. b – dyad with a microcyte. c – dyad with several microcytes. d – triad with microspores of different sizes. e – pentad. f, g, h – tetrad with microspores of different sizes and microcytes in f and h. i – pollen grains of different sizes. The small one resulted from microcyte.

was first described in maize (BEADLE 1932) and has been widely reported in the last few years (SOUZA and PAGLIARINI 1996, CONSOLARO and PAGLIARINI 1996, MENDES-BONATO et al. 2001). Although many studies have pinpointed the occurrence of chromosome stickiness, the primary cause and biochemical basis of the phenomenon still remain unknown. Genetic and environmental factors are reported to cause chromosome stickiness (see BAPTISTA-GIACOMELLI et al. 2000).

At least 50% of the world's plants consist of polyploid taxa (STEBBINS 1971). The evolutionary success of polyploids is explained by their fixation of heterozygosity and greater recombination potential. Tetraploids may differ in fitness from their diploid progenitors for several reasons associated with chromosome doubling (BURTON and HUSBAND 2000). Fitness differences may arise due to increased cell size (STEBBINS 1971), increased rate of cell

division (GOTTSCHALK 1985), irregular pairing during meiosis (STEBBINS 1950), gene dosage effects (LEITCH and BENNETT 1997), increased masking of deleterious mutations (HUSBAND and SCHEMSKE 1997), or differential gene expression (GALITSKI et al. 1999). Each factor may affect the performance of tetraploids at a range of life stages as well as influence their response to selection subsequent to the doubling event (MACIEIRA et al. 1993). Polyploids occur more frequently among perennial plants (STEBBINS 1980). When annuals are polyploid, such as *P. tuberosa*, they are frequently inbreeders, because self-fertilization of a founding polyploid individual among diploids is their only means of producing fertile offspring. The reproductive mode in *P. tuberosa* has not yet been studied, but we cannot rule out the possibility of vegetative reproduction. This is due to the fact that, after flowering, the vegetative part of the plant disappears and quickly emerges again from pre-existent roots totally covering the soil in the following season. The amount of meiotic abnormalities leading to a high frequency of pollen sterility in the analyzed populations reinforces the assumption that the vegetative mode is the reproductive strategy of *P. tuberosa*.

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