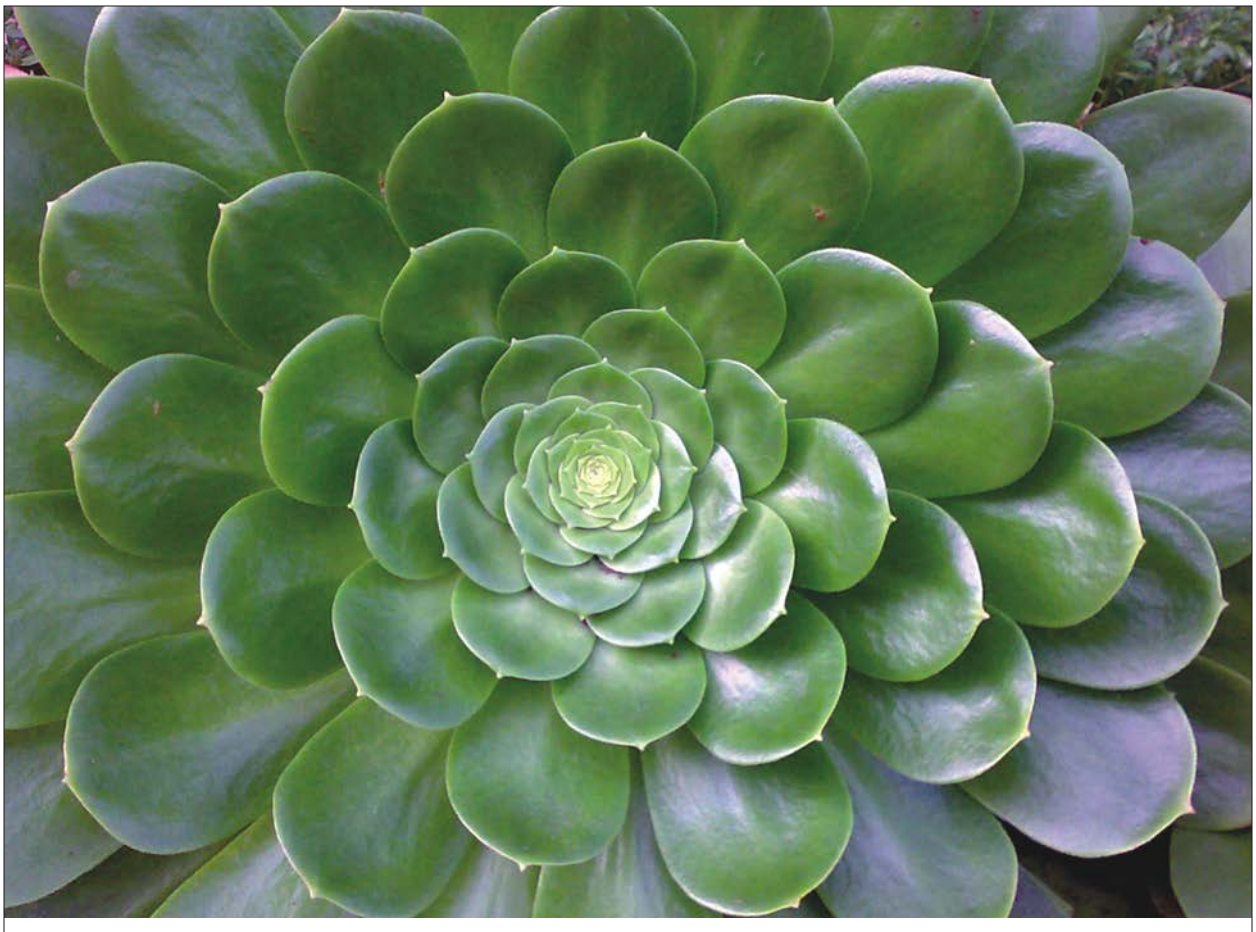

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Microsporogenesis in Somaclones of *Cereus peruvianus* Mill. (Cactaceae)

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Abstract: Current study investigates the microsporogenesis in three somaclones of *Cereus peruvianus* since information on the meiotic characteristics of somaclones is important to indicate somaclones and/or their descendents for the species's genetic breeding programs. Whereas meiotic irregularities were recorded in the two meiotic divisions, in pachytene, small asynaptic regions were observed in some bivalents. One terminal chiasma predominated in bivalents in diakinesis and one to three pairs of univalent were recorded in this phase. Precocious chromosome migration to the poles, laggards and micronuclei were also observed, mainly in the first division. Partial or total chromosome transfer between microsporocytes (cytomixis) was recorded in the first division. Two types of spindle arrangement were observed in the second division, generating isobilateral and tetrahedral tetrads. Since abnormalities recorded in the S3, S9 and S71 somaclones were also detected in control, pollen of somaclones or progenies of somaclones may contribute towards the broadening of the species's genetic basis.

Key words: *Cereus peruvianus*, cytomixis, genetic breeding, genetic diversity, meiotic splindle, microsporogenesis, somaclones

Introduction

Genetic variability is pivotal for the success in plant breeding programs. In the 1970s, plant cell culture was acknowledged to be a new and exciting option for obtaining increased genetic variability without sophisticated technology. Historically, culture cycles were essentially seen as a method of cloning a particular genotype. However, phenotypic variants were frequently found amongst regenerated plants (Larkin and Scowcroft, 1981).

Atypical shoot morphologies and new isozyme were reported in *in vitro*-regenerated *Cereus peruvianus* from somatic cell culture (somaclones) (Mangolin *et al*., 1997, 1999; Machado *et al*., 2000). *C. peruvianus*, a common ornamental cactus species found in gardens of tropical and subtropical countries, has been domesticated in Israel as a fruit crop (Nerd *et al*., 2002). Gutman *et al*. (2001) have shown that *C. peruvianus* has only a limited genetic base and that further improvement of this crop may require the introduction of additional germplasm during breeding programs.

Among different factors reported as possible causes of somaclonal variation, chromosomal instability is considered one of its main sources. Many techniques have been employed to study chromosomal variation in cultured cells and regenerated plants (Lee and Phillips, 1988).

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Analysis of meiotic cells had made an important contribution to the understanding of the cytological variation associated with tissue culture. Actually meiotic analysis has the advantage of evaluating pairing relationship among homologous and chromosome behavior through several stages of meiosis.

Current study investigates the microsporogenesis in somaclones of *C. peruvianus* since information on the meiotic characteristics of somaclones is important to indicate somaclones and/or their descendents for the species's genetic breeding programs.

Materials and Methods

Somaclones of *C. peruvianus* obtained from callus culture (Oliveira *et al*., 1995) have been planted since 1997 at the Experimental Botanic Gardens of State University of Maringá (Maringá PR Brazil; altitude 554.9m; 23° 25´S; 51° 25´W). Flower buds from three somaclones (S3, S9 and S71) and from one plant control (P7) obtained from seed and naturally maintained for 15 years in the Garden of Medicinal Plants of the State University of Maringá, were collected and fixed in a mixture of 95% ethanol and acetic acid (3:1) for 24 h, transferred to 70% ethanol and stored under refrigeration until use. Studies were conducted during the flowering period during the spring/summer 2004-2005 (from October 2004 to February 2005). Microsporocytes (PMCs: Pollen Mother cells) were prepared by squashing and stained with 0.5% propionic carmine. Meiotic phases from pachytene to tetrad were evaluated under light microscopy.

Results and Discussion

Despite the large number of buds collected per plant, meiotic analysis was difficult to accomplish because meiosis occurs very early and the hundred of anthers inside the bud are generally synchronized in the same meiotic stage. Thus, the number of buds analyzed per plant was small.

Cytological analysis in six buds scored among the four plants, including the somaclones and the control, showed 2n = 22 chromosome and some abnormalities during meiosis (Table 1). In the two types of plants, pachytene configuration was very clear and revealed chromosome pairing and dense chromomeres patterns along the bivalents (Fig. 1a). Small asynaptic regions were detected along the bivalent in some cells (Fig. 1b). In diakinesis, up to three pairs of univalent chromosomes were found

Table 1: Number of meiocytes analyzed per plant and percentage of abnormal cells in somaclones of *C. peruvianus*.

		Phases of meiosis								
Plant	Bud	Diak	MI	AI	TI	PII	MII	AII	TII	Tetrad
Control	1	-	85	21	5	170	305	86	227	151
			(18.82)	(9.52)	(0.0)	(0.0)	(0.0)	(0.0)	(2.89)	(3.21)
	2	25	136	18	106	131	155	72	182	166
		(52.00)	(23.52)	(0.0)	(0.0)	(0.0)	(0.0)	(8.33)	(8.33)	(0.0)
S3	1	24	267	279	206	264	195	32	174	123
		(16.66)	(4.49)	(23.65)	(12.62)	(3.40)	(0.0)	(0.0)	(1.15)	(2.0)
S9	1	39	182	19	145	280	290	12	275	210
		(25.64)	(14.83)	(10.52)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
	2	7	167	124	195	240	145	67	260	56
		(98.54)	(2.99)	(3.22)	(0.0)	(0.0)	(0.0)	(10.44)	(0.0)	(0.0)
S71	1	33	265	6	105	177	81	6	5	82
		(48.48)	(33.58)	(0.0)	(10.90)	(1.12)	(2.40)	(0.0)	(0.0)	(0.0)

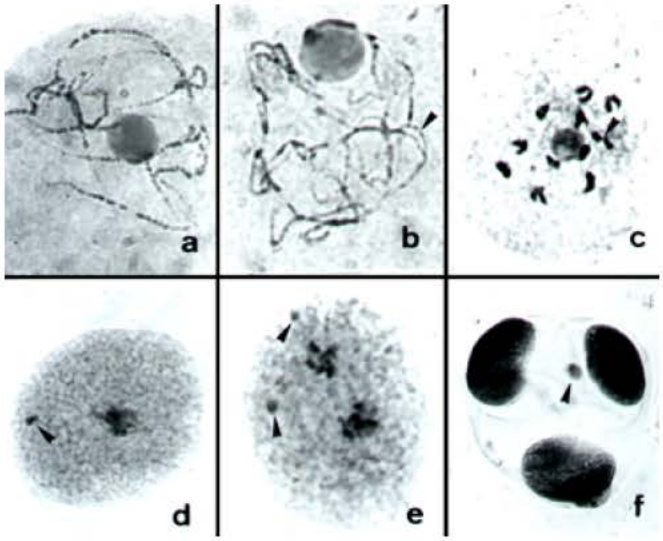

Fig. 1: Some aspects of meiotic behavior
a: Normal pachytene showing dense chromomeres along the bivalents
b: Pachytene showing a small asynaptic region (arrowhead)
c: Diakinesis with a pair of univalent chromosomes (arrowhead)
d: Metaphase I with a chromosome migrating precociously to the pole (arrowhead)
e: Telophase I with two micronuclei (arrowhead)
f: Tetrad with a microcyte (arrowhead)

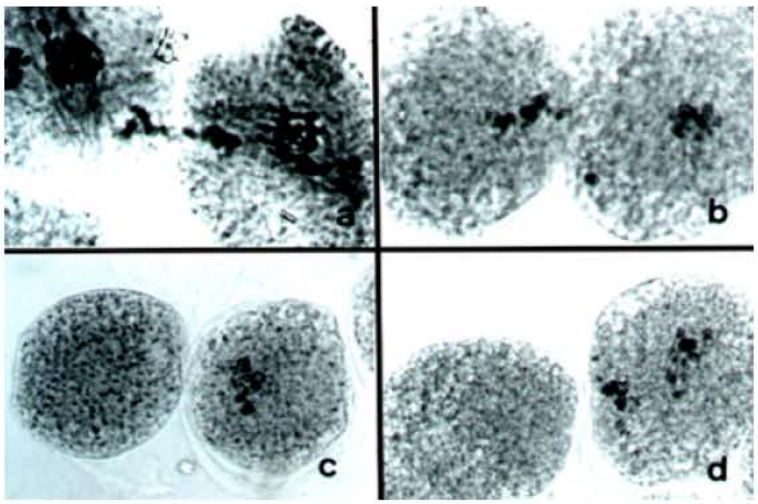

Fig. 2: Cytomixis among meiocytes
a and b: Partial transference of genome between two cells
c and d: Total transference of genome; observe that one cell is anucleate

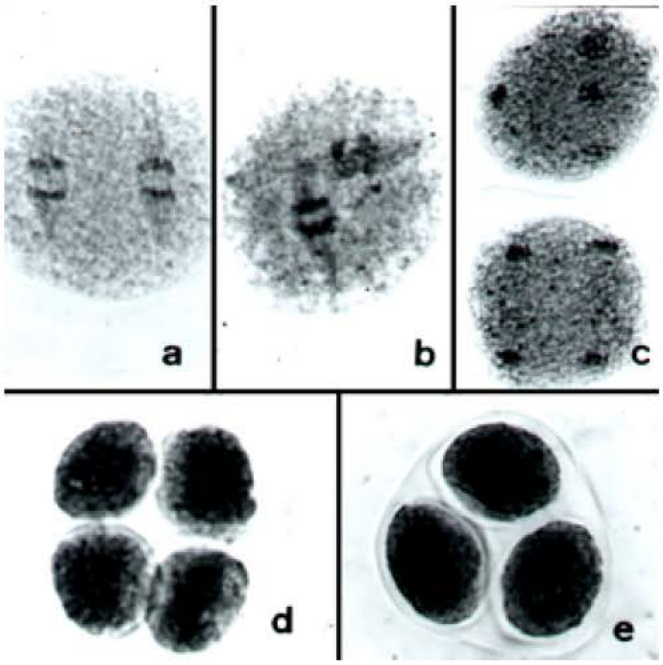

Fig. 3: Aspects of spindle arrangement in tje second division
a to c: Diffrent spindle dispositions inside the cell
d: Isobilateral tetrad
e: Tetrahedral tetrad; one microspore is occult and the arrangement seems to be a triad

in some cells (Fig. 1c). Bivalents rarely showed two chiasmata; most of them presented one terminal chiasma. In the next stages, abnormalities due to univalents, such as precocious chromosome migration to the poles in metaphases (Fig. 1d), laggards in anaphases and micronuclei in telophases (Fig. 1e) were recorded. Consequently microcytes were only found in rare tetrads (Fig. 1f). The percentage of abnormal cells was higher in the first division and varied among plants and buds.

Abnormalities recorded among meiocytes in somaclones of *C. peruvianus* are very common in higher plants and have been reported in several species (Pagliarini, 2000). They are generally due to absence of chiasma or precocious chiasma terminalization. The number of chiasmata and chiasma terminalization is under genetic control (Golubovskaya, 1979; Bascom-Slack *et al*., 1997). Results showed that the *C. peruvianus* is characterized by terminal chiasmata, some of whose would terminalize precociously and give rise to univalents. Asynaptic genes constitute another factor that would originate univalents (Koduru and Rao, 1981). Only partial asynapsis was found among meiocytes and in small regions of the bivalent, without any possible impairing in crossing-over occurrence. Normal chromosome number and meiotic pairing behavior in variant tobacco somaclones were also reported by Burk and Chaplin (1980).

Chromosome transfer among cells (cytomixis) has also been recorded in some meiocytes (Fig. 2). The phenomenon was recorded during the first division and involves only a part (Fig. 2a and b) or the

whole genome (Fig. 2c and d). In the latter, anucleate cells were formed. Cytomixis is also a very common cytological phenomenon among higher plants (Consolaro and Pagliarini, 1995; Souza and Pagliarini, 1997; Utsunomyia *et al*., 2004). Gottschalk (1970) proposed that cytomixis is a process limited to genetically unbalanced genomes such as polyploids, hybrids and apomictics. Cytomixis has never been reported in somaclones. The role of cytomixis in evolution is speculative and the process is considered to be a source of aneuploid and polyploidy gametes.

Another interesting aspect of microsporogenesis in *C. peruvianus* was the spindle arrangement in the second division (Fig. 3). In dicotyledons, the two nuclear divisions take place in rapid succession and the four resulting nuclei separate themselves and take up positions as far away from each other as possible. A tetrahedral arrangement results (Maheshwari, 1978). As a rule, each species presents a typical and unique arrangement for microspore tetrad, suggesting a genetic control for the characteristic. In the species under analysis, there was a predominance of parallel spindle (Fig. 3a) that gave rise to isobilateral tetrads (Fig. 3d). However, another arrangement was found (Fig. 3b) which originated tetrahedral tetrads (Fig. 3e). Different spindle arrangements have been reported in some species normally cultivated (Rudall *et al*., 1997; Silva *et al*., 2001).

Until recently it was usually assumed that variants amongst somaclones were due to gross karyotype changes. According to Singh (2003), most morphological variants among the regenerated plants are due to numerical and structural chromosome changes induced by the culture. However, many variant somaclones fail to show gross karyotype changes. Lee and Phillips (1988) reported many other factors involved and originating from the chromosomal basis of somaclonal variation. All the abnormal meiotic aspects recorded in S3, S9 and S71 somaclones of *C. peruvianus* are frequently reported in higher plants. In our research this evidence is of paramount importance since the pollen of somaclones or progenies of somaclones may contribute towards the broadening of the species's genetic basis.

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