

Cytological and embryological studies in *Setaria cordobensis* Herrmann and *Setaria leiantha* Hackel (Poaceae)

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SUMMARY — Cytogenetics and embryology of *Setaria cordobensis* Herrmann and *S. leiantha* Hackel were investigated in four populations collected from different environments in Argentina. A somatic chromosome number of $2n = 36$ was confirmed for *S. leiantha* and reported for the first time for *S. cordobensis*. Bivalent pairing was regular in pollen mother cell meiosis, but abnormalities such as dyads, triads and tetrads with micronuclei or non-conventional arrangement were described with different frequencies. A high degree of similarity between the two species was found investigating megasporangium, megasporogenesis and megagametogenesis. There were major differences in ovule length and width at anthesis. Both species follow a typical *Polygonum* development and no indicator of apomictic processes was noted.

Key words: chromosome number, embryology, meiotic analysis, *Setaria cordobensis*, *Setaria leiantha*.

INTRODUCTION

The genus *Setaria* belongs to the tribe Paniceae of the family Poaceae (*Gramineae*) and includes approximately 125 species widely distributed in both hemispheres. In Argentina grow about 25 species that are considered good native pasture grasses (CORREA 1978). Among them, *Setaria cordobensis* Herrmann and *Setaria leiantha* Hackel grow preferentially at xerophytic elevations in the Central, North-eastern and Pampean regions. *S. leiantha* is found more frequently in Mesopotamia (NICORA and RÚGOLO 1987). Within the genus, foxtail millet [*S. italica* (L.) Beauv.] has been the most widely studied species for its genetic, breeding and evolutive aspects, due to its use as a good domesticated grass (NARAYANASWAMI 1956; CROULLEBOIS and SILJAK-YAKOVLEV 1989; CHENNAVECRAIAH and HIREMATH 1991). The embryology, cytogenetics and evolution of other *Setaria* species have been investigated (EMERY 1957; ROST 1973; FREITAS-SACCHET *et al.* 1984; WARWICK *et al.* 1986; BHANWRA 1988), but to date there is no detailed report on the cytological and embryological characteristics of *S. leiantha* and *S. cordobensis*. Therefore, the aim of this study

was to investigate the cytological and reproductive aspects of the two species, to search for differences and similarities useful for understanding the phylogenetic relationships within the genus and to collect data of interest for future breeding programs.

MATERIALS AND METHODS

The material studied included four natural populations from the district of Córdoba, Argentina, collected in 1992 at the sites Dean Funes and Totoral for *Setaria leiantha* and Dean Funes and Quilino for *S. cordobensis*.

Chromosome numbers were counted on metaphase plates from root-tips pretreated with α -bromonaphthalene for four hours, fixed in Carnoy (1:3 glacial acetic acid: ethanol v/v) for 24-48 hours and hydrolyzed in 1 N HCl at 60° C for six minutes. Root-tips were stained according to the Feulgen method and squashes were performed in 1% acetic orcein.

For meiotic studies on microsporogenesis, panicles at different developmental stages were fixed in Carnoy. Dissected anthers were stained in squashes with 1% aceto-carmin.

For embryological analyses, inflorescences were collected at different stages from 7 and 8 plants of *S. cordobensis* and *S. leiantha*, respectively. For *S. cordobensis*, plants from the two populations were used, while for *S. leiantha*, plants from the population Totoral grown in the original environment and in Córdoba were used. About 50 flowers per plant grown in 1991 and 1992 were fixed in Carnoy, dehydrated, cleared in an ethanol-xytol series and embedded in Paraplast. Single florets were sectioned at 7-12 μ m with a rotative microtome and stained in a safranin-fast green combination.

RESULTS AND DISCUSSION

Table 1 reports the geographic and rainfall data for the three collection sites and for Córdoba. There were little differences in average annual rainfall at the three sites. The four locations must be considered as semi-arid environments (PROHASKA 1976).

Figs. 1A-G. — Mitotic and microsporogenesis studies in *Setaria cordobensis*. (A) Metaphase plate of 36 chromosomes; (B) chromosome pairing in diakinesis of meiotic prophase I; (C) absence of spindle formation in one cell of a dyad leading to the formation of a triad; (D-E-F-G) changes in the position of cells during the second meiotic division leading to the formation of triads and tetrads with non-conventional arrangement. Bars = 10 μ m

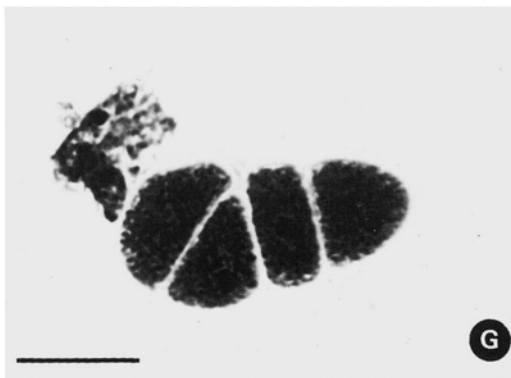
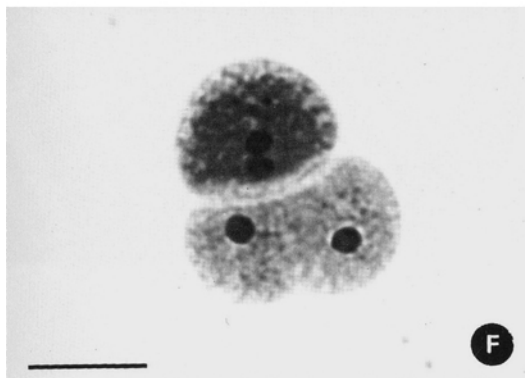
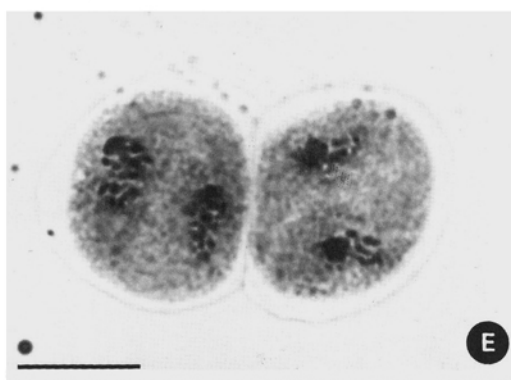
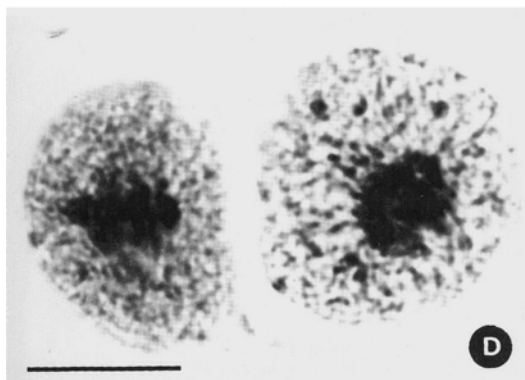
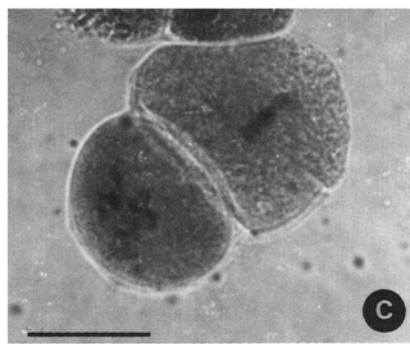
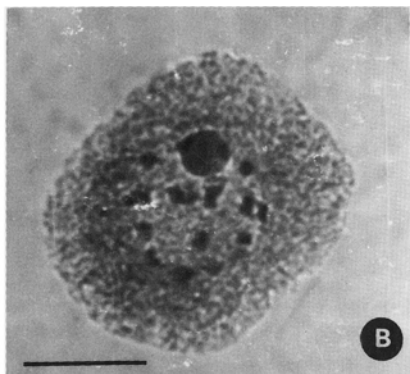


TABLE 1 - Geographic and rainfall characteristics for the collection sites and Córdoba.

Location	Altitude (m a.s.l.)	South latitude	West longitude	Rainfall (mm year ⁻¹)
Dean Funes	889	30.25	84.22	480
Quilino	391	30.15	84.29	450
Totoral	550	30.42	84.05	490
Córdoba	425	31.34	84.11	650

Mitotic study.

The analysis of somatic metaphases in *S. leiantha* confirmed the chromosome number to be $2n = 36$ as previously reported by MANERO DE ZUMELZÙ and OCHOA DE SUÁREZ (1991). In *S. cordobensis*, where the chromosome number was not previously reported, the same number was found (Fig. 1A). The determined chromosome numbers show that the two species have to be considered as tetraploid, being that $x = 9$, as reported for *Setaria* by EMERY (1957) and MEHRA and SHARMA (1975).

Differences in the number of satellites were found between the two species, being two for *S. leiantha* and four for *S. cordobensis*.

Microsporogenesis study.

In both species meiotic analyses of pollen mother cells indicated a bivalent pairing of 18 chromosomes in diakinesis of prophase I (Fig. 1B). In *S. leiantha* microsporogenesis was quite normal and only in the population Dean Funes was evident the presence of univalents at metaphase I with a frequency ranging from 2 to 8% (data not shown). Absence of pairing gave rise to tetrads with micronuclei.

In *S. cordobensis* different abnormalities were found in both populations studied (Table 2). An average percentage of dyads of 5.8% was found. Dyad formation was due to the absence of meiotic spindles in both cells after meiosis

Figs. 2A-F. — Megasporogenesis and megagametogenesis in *Setaria leiantha* (A-B-C-D) and *Setaria cordobensis* (D-F). (A) Young ovule with the archesporial cell in the micropylar position; (B) functional megaspore; (C) functional megaspore with remnants of the three degenerating megaspores; (D) two-nucleated embryo sac; (E-F) eight-nucleated embryo sac. (ch, chalaza; d, distal region; do, dorsal ovary wall; e, egg cell; fm, functional megaspore; ii, inner integument; mic, micropylar end; nu, nucellus; oi, outer integument; p, proximal region; pn, polar nuclei; vo, ventral ovary wall). Bars = 40 μ m

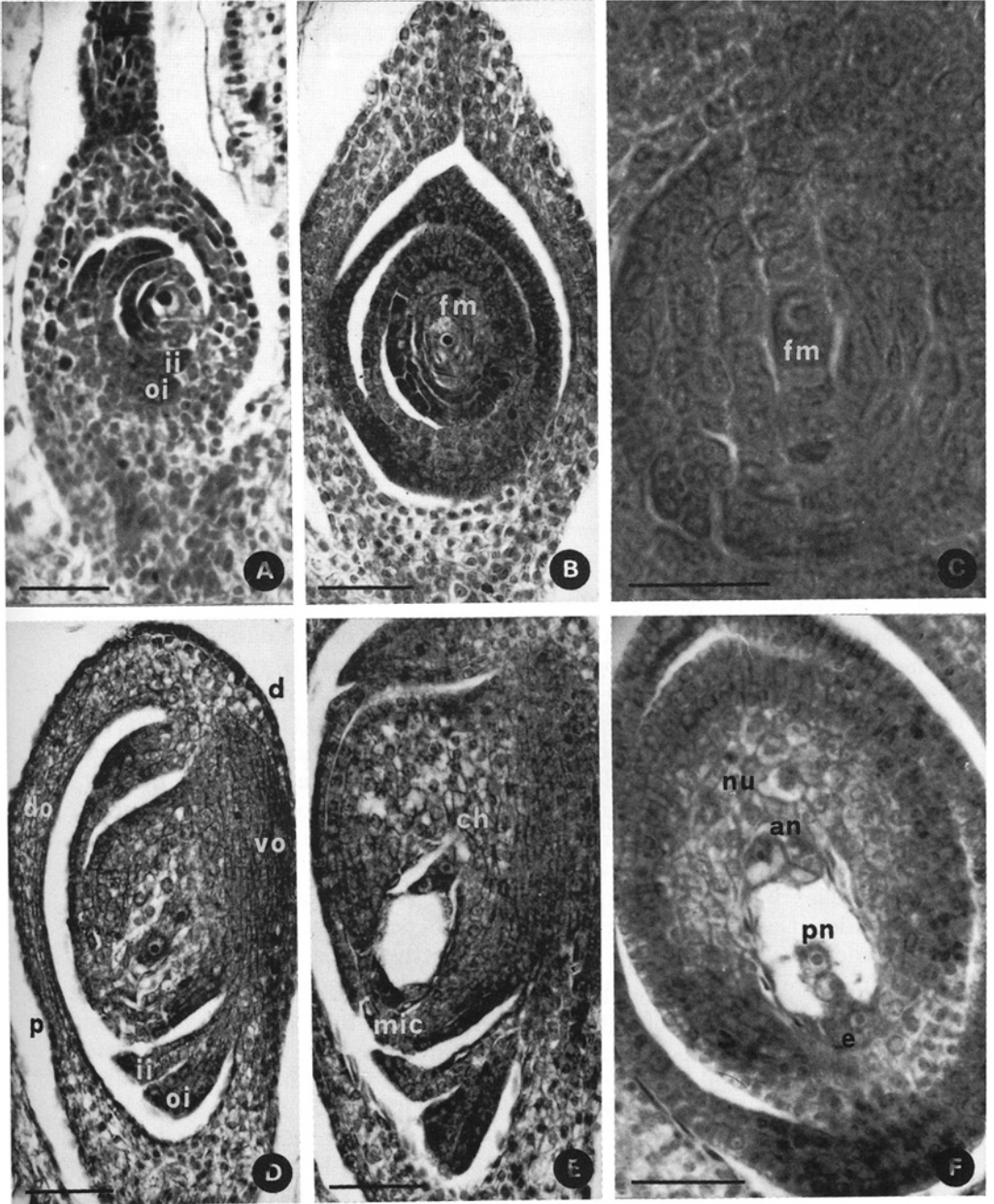


TABLE 2 - Analysis of microsporogenesis in *Setaria cordobensis*: percentages of normal tetrads, dyads, triads and tetrads with non-conventional arrangement of microspores.

Population	Plant code	Number of observed cells	Normal tetrads	Dyads	Triads	Tetrads with non-conventional arrangement
Dean Funes	P2	286	93.3	1.4	1.1	4.2
	P3	147	82.3	6.8	4.7	6.1
	P4	328	85.1	6.7	2.7	5.5
	P5	159	85.5	4.4	6.9	3.1
	P6	119	89.1	4.2	3.4	3.4
Quilino	P1	306	88.6	7.8	2.9	0.6
	P2	274	88.0	7.6	2.2	2.2
	P3	591	85.3	8.0	3.8	2.9
	P6	145	90.3	5.5	2.1	2.1

I at metaphase II. Absence of spindle in just one of the cells led to the formation of triads (Fig. 1C). Changes in the position of cells during the second meiotic division led to the formation of triads and tetrads with non-conventional arrangement (Figs. 1D-G). This behaviour is in accordance with that reported for *S. texana* and *S. leucopila* by EMERY (1957) and for *S. verticillata* by SINGH and GUPTA (1977). The frequency of dyads reported for *S. cordobensis* (5.8%) was higher than that determined in other grasses (e.g. 2.7% in *Dactylis glomerata* L., FALISTOCCO *et al.* 1994). The percentages of microsporogenesis aberrations showed a higher frequency of tetrads with non-conventional arrangement in the population Dean Funes in comparison with Quilino (Table 2). The frequency of other aberrations was more variable between plants in the population Dean Funes, where the range in percentage of dyad and triad formation was 1.4-6.8% and 1.1-6.9%, respectively. Plants in the population Quilino were less variable in this respect, having a generally high and low frequency of dyads and triads, respectively (Table 2).

Megasporangium, megasporogenesis and megagametogenesis.

In both species the gynoecium is normally composed of the ovary and two styles with plumed stigmas. The ovary is ovoid as typical of the subfamily Panicoideae (BHANWRA 1988) and contains a single ovule inserted in the

TABLE 3 - Comparison of embryological features between *Setaria leiantha* and *Setaria cordobensis*.

Measured trait	<i>Setaria leiantha</i>	<i>Setaria cordobensis</i>
1 - Embryo sac length (μm)	84.4	87.5
2 - Ratio between chalaza and ovule length at anthesis	37.8%	46.5%
3 - Ovule length at anthesis (μm)	236.1	276
4 - Ovule width at anthesis (μm)	127.2	173
5 - Number of layers of parenchyma cells in the distal region of the ovary wall	12-14	13-15
6 - Number of parietal layers in the vicinity of the micropyle	2-3	3
7 - Ovary shape	ovoid	ovoid
8 - Number of layers of the inner integument	2	2
9 - Number of layers of the outer integument		
a - Distal region	3	3
b - Proximal region	4	5-6

posterior ovary wall. The premeiotic ovule is hemitrope with the micropyle oriented laterally (Fig. 2A). During macrosporogenesis and embryo sac growth, due to the greater growth of the nucellus in the apical region, the ovule bends downward becoming hemicampitropous (Figs. 2D-E). Morphological observations regarding ovule and ovary dimensions are reported in Table 3. The two species had a high degree of similarity in all the traits measured, with the exception of ovule length and width at anthesis. In both species the inner integument consists of two cell layers. The outer integument is composed of three cell layers in the distal region and of four and five-six layers in the proximal region in *S. leiantha* and *S. cordobensis*, respectively (Table 3).

A similar reproductive development of the female organs has been observed in the two species, corresponding to the normal pathway reported for grass species belonging to the Panicoideae sub-family (SNYDER *et al.* 1955; EMERY 1957). In the young nucellus, as a rule, a single megasporocyte differentiates in a micropylar position (Fig. 2A) giving rise, after meiosis, to a linear tetrad of megasporocytes. While the megasporocyte is elongating, the inner integument enlarges, enclosing the ovule. Usually the chalazal megasporocyte is functional, the others soon degenerate (Figs. 2B-C). During megasporogenesis two micropylar lip cells become evident, differentiated by their large size with respect to the surrounding nucellus. The functional megasporocyte undergoes three

mitotic divisions, forming a two-nucleated (Fig. 2D), four-nucleated and finally eight-nucleated, seven-celled, '*Polygonum* type' embryo sac (Figs. 2E-F). The mature embryo sac is oriented parallel to the longitudinal axis of the ovule with a slight curvature in the chalazal part.

The mature embryo sac has three polarized cells in the chalazal part and three in the micropylar with two polar nuclei in the central cell. The egg apparatus consists of three triangular cells (the egg cell and two synergids). The egg cell has a large vacuolus in the chalazal part and the nucleus on the opposite side. Synergid nuclei differently were usually observed in chalazal position. Soon after fertilization, the synergids degenerate and the egg cell becomes globular. Polar nuclei, from an initial polar position, migrate in the embryo sac to occupy a position in close proximity to the egg apparatus (Fig. 2F). The three antipodals in both species usually proliferate producing a cluster of 3-20 cells. Antipodals are usually uninucleate and do not change position after fertilization.

The mature embryo sac takes up to 35.5% and 31.7% of the ovule length in *S. leiantha* and *S. cordobensis*, respectively.

In *S. leiantha* Totoral, 15% of the megasporocytes observed in plants grown in the collection site were degenerated. In this accession a fraction of the harvested seed was actually empty (data not shown). Heavy seed sterility has been correlated with meiotic abnormalities such as asynesis of chromosomes, precocious and lagging chromosomes and degeneration of the megasporocyte in early prophase I in other *Setaria* species (EMERY 1957).

No evidence of apospory was observed in the two species studied. *S. leucopila* and *S. villosissima* therefore remain the only taxa with apomictic reproduction in the genus *Setaria* (EMERY 1957).

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