

Embryo sac development in some representatives of the tribe Cynodonteae (Poaceae)

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ABSTRACT

Chloris virgata Sw., *Cynodon dactylon* (L.) Pers., *Harpochloa falx* (L. f.) Kuntze, and *Tragus berteronianus* Schult. have a *Polygonum* type of embryo sac development. Unreduced embryo sacs were found in *Eustachys paspaloides* (Vahl) Lanza & Mattei, *Harpochloa falx*, and *Rendlia altera* (Rendle) Chiov. Both facultative and obligate apomixis were observed. The *Hieracium* type of embryo sac development was observed in the aposporic specimens.

UITTREKSEL

Die *Polygonum*-tipe kiemsak-ontwikkeling, is by *Chloris virgata* Sw., *Cynodon dactylon* (L.) Pers., *Harpochloa falx* (L. f.) Kuntze en *Tragus berteronianus* Schult. waargeneem. Ongereduseerde kiemsakke is by *Eustachys paspaloides* (Vahl) Lanza & Mattei, *Harpochloa falx* en *Rendlia altera* (Rendle) Chiov. aangetref. Beide fakultatiewe en obligate apomiksie het voorgekom. *Hieracium*-tipe kiemsakontwikkeling is by aposporiese eksemplare waargeneem.

INTRODUCTION

The subfamily Chloridoideae Rouy is divided into several tribes. All the species included in this study belong to the tribe Cynodonteae Dumort. The chloridoid grasses that originated in Gondwanaland (Jacobs 1987), are predominantly species of arid and semi-arid areas, where their photosynthetic systems and associated anatomical and morphological adaptations appear to give them competitive advantages (Jacobs 1987). The Cynodonteae comprises annual and perennial grasses that provide good grazing for domestic and wild animals. In addition, they play an important role in erosion control, and their presence gives an indication of the kind of management maintained (Van Oudtshoorn 1991).

Cultivation and selection of the better adapted cultivars of these grasses may increase their grazing potential. Studies of their mode of reproduction can increase our knowledge and enhance their improvement. The type of reproduction, asexual or sexual, can be established through a study of embryo sac development. Different types of embryo sac development occur in different species. Diploid to tetraploid species usually reproduce sexually, whereas apomixis occurs at tetraploid and higher polyploid levels (Stebbins 1972). Facultative apomixis, where apomixis and sexual reproduction co-exist, occurs in most apomictic grasses. Species that reproduce sexually have theoretically a wider variability and a better chance of survival. In contrast, obligate apomicts have a limited variability which can only be improved by mutation.

The aim of the study was to determine how these grasses reproduce, whether sexually or apomictically, by estab-

lishing the types of embryo sacs that occur in the Cynodonteae.

Unlike Mogie (1992) we used the following definitions for the purpose of this study: reduced embryo sacs (embryo sacs in which meiosis occurs) imply sexual reproduction, whereas unreduced embryo sacs imply asexual (or apomictic) reproduction. Sexual reproduction is the result of double fertilization, where the offspring contain genetic material from both parents. Asexual reproduction may include single fertilization (pseudogamous fertilization), where the pollen donor does not contribute to the genetic composition of the offspring. With these stricter definitions of sexual and asexual reproduction, the double fertilization of unreduced embryo sacs, resulting in polyploid lineages (Harlan & De Wet 1975; Lewis 1980), is excluded. We believe that it is justifiable to exclude these specific cases since no such lineages have been observed in any of the species studied (Strydom & Spies 1994).

MATERIAL AND METHODS

Voucher specimens are housed in the Geo Potts Herbarium, Department of Botany and Genetics, University of the Orange Free State, Bloemfontein (BLFU) or the National Herbarium, Pretoria (PRE).

Inflorescences at different stages of development were collected and fixed in the field in Carnoy's fixative. Ovules were dissected from the inflorescences in 70% ethanol, serially dehydrated in ethanol and tertiary butanol and embedded in a synthetic wax. Serial longitudinal sections (7 µm) of the ovules of all the species, except *Harpochloa falx* and *Rendlia altera* were obtained by using conventional methods. The inflorescences of the last-mentioned species were sectioned transversely. Sections were stained in safranin and fast green according to the technique used by Spies & Du Plessis (1986) and

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mounted in Eukitt. At least 20 embryo sacs at various developmental stages were studied per specimen.

SPECIMENS EXAMINED

Chloris virgata

ORANGE FREE STATE.—2827 (Senekal): 6 km from Clocolan to Peka bridge, (–DC), *Spies 4799*. 2926 (Bloemfontein): 26 km from Dewetsdorp to Hobhouse, (–DB), *Spies 4783*.

Cynodon dactylon

CAPE.—3318 (Cape Town): on top of Bothmaskloof, (–BD), *Spies 4424*. 3420 (Bredasdorp): De Hoop Nature Reserve, (–DC), *Spies 4626*.

Eustachys paspaloides

TRANSVAAL.—2530 (Lydenburg): 10 km from Boshhoek to Buffelsvlei, (–AC), *Spies 1521*.

Harpochloa falx

TRANSVAAL.—2530 (Lydenburg): 11 km from Dullstroom to Lydenburg via Frischgewaagd, (–AC), *Spies 5118*; 16 km from Dullstroom to Lydenburg via Frischgewaagd, (–AC), *Spies 5125*; 5 km from Belfast to Dullstroom, (–CA), *Spies 5113*.

ORANGE FREE STATE.—2827 (Senekal): 6 km from Nebo to Fouriesburg via Generaalsnek, (–DB), *Spies 4827*. 2729 (Volksrust): 53 km from Harrismith to Newcastle via Normandien Pass, (–DC), *Spies 5063*; 92 km from Harrismith to Normandien, (–DC), *Spies 5065*; 97 km from Harrismith to Normandien, (–DC), *Spies 5078*.

CAPE.—3027 (Lady Grey): 52 km from Rhodes via Lundeansnek, (–DD), *Spies 4729*. 3028 (Matatiele): 65 km from Rhodes to Maclear, (–CC), *Spies 4695*. 3128 (Umtata): 38 km from Maclear to Elliot, (–AC), *Spies 4712*.

Rendlia altera

TRANSVAAL.—2530 (Lydenburg): Nederhorst turnoff on the Lydenburg-Roosenekal road, (–AA), *Spies 5129*.

ORANGE FREE STATE.—2729 (Volksrust): 93 km from Harrismith to Normandien, (–DC), *Spies 5072*; 97 km from Harrismith to Normandien, (–DC), *Spies 5077*.

CAPE.—3128 (Umtata): 38 km from Maclear to Elliot, (–AC), *Spies 4713*.

Tragus berteronianus

ORANGE FREE STATE.—2827 (Senekal): 6 km from Clocolan to Peka bridge, (–DC), *Spies 4803*.

RESULTS AND DISCUSSION

The Cynodonteae ovule consists of the nucellus, integuments (Figure 1A), chalaza, raphe, and funicle. The nucellus is enveloped by two integuments. They grow beyond the nucellus and arch over its apex forming the micropyle. Both *Harpochloa falx* and *Rendlia altera* have ovules that are amphitropous, whereas the other species studied have anatropous ovules.

The female gametophyte develops from the archesporium formed in the nucellar tissue of the ovule (Figure 1A). The archesporium divides meiotically, to form a linear tetrad of macrospores, of which only the chalazal one is functional. This macrospore enlarges and after three mitotic divisions (Figure 1B–F) a small coenocyte differentiates. After cytokinesis the macrogametophyte consists of an egg cell, two synergids, the central cell with two polar nuclei, and three antipodal cells (Figure 1G–J). Therefore, up to this stage, embryo sac development conforms to the *Polygonum* type. The egg cell and the two synergids show

a triangular arrangement. The largest portion of the embryo sac is occupied by the central cell with the two polar nuclei. Usually, the antipodal cells are the smallest cells of the embryo sac. However, the antipodal cells of the Cynodonteae often undergo numerous divisions, which result in the formation of a mass of antipodal cells or an antipodal complex (Figure 1K, L) in the mature embryo sac. This phenomenon was also observed in other grasses (Willemse & Van Went 1984).

The specimens of *Chloris virgata* had reduced embryo sacs, and antipodal complexes were present (Table 1; Figure 1B, D, K). The reduced embryo sacs of the specimens of *Cynodon dactylon* had no antipodal complexes (Table 1). Some specimens of *Harpochloa falx* had reduced embryo sacs with the egg cell, the polar cells, and two synergids clearly visible in most of the embryo sacs (Figure 1A, C, E, G–J). Antipodal complexes were present in some of these embryo sacs (Table 1; Figure 1L). The embryo sacs of *Tragus berteronianus* were reduced (Table 1).

The specimens of *Eustachys paspaloides*, *Harpochloa falx* and *Rendlia altera* had unreduced embryo sacs (Table 1; Figure 2A–E). Apomixis means asexual (agamic) reproduction by seed, that is, agamospermy (Nogler 1984). There are two kinds of apomicts, namely, obligate apomicts, which have a completely closed recombination system and heterozygotic genotypes which are preserved at the cost of evolutionary flexibility, and facultative apomicts, where unreduced and reduced modes of reproduction coexist (Mogie 1992). Apomixis leads to maternal offspring which are normally genetically exact copies of the mother plant (Nogler 1984).

Eustachys paspaloides and *Harpochloa falx* were facultative apomicts with both reduced and unreduced embryo sacs. In most cases the reduced embryo sacs degenerate eventually. *Rendlia altera* was obligate apomictic because all reduced embryo sacs degenerated at an early stage. The embryo sacs of these species were aposporous. There are two types of aposporous embryo sacs, the *Hieracium* type, a bipolar, eight-nucleate embryo sac, and the *Panicum* type, a monopolar, four-nucleate embryo sac (Nogler 1984). The specimens of *Eustachys paspaloides*,

TABLE 1.—Type of embryo sac development in some representatives of the tribe Cynodonteae (chromosome numbers from Strydom & Spies 1994)

Species	Embryo sac		Antipodal complex
	Reduced <i>Polygonum</i>	Unreduced <i>Hieracium</i>	
<i>Chloris virgata</i> (2n = 2x = 20)	100%		present
<i>Cynodon dactylon</i> (2n = 4x = 36)	100%		absent
<i>Eustachys paspaloides</i> (2n = 4x = 40)	40%	60%	absent
<i>Harpochloa falx</i> (2n = 4–6x = 40–60)	80%	20%	present
<i>Rendlia altera</i> (2n = 4x = 40)		100%	absent
<i>Tragus berteronianus</i> (2n = 2x = 20)	100%		absent

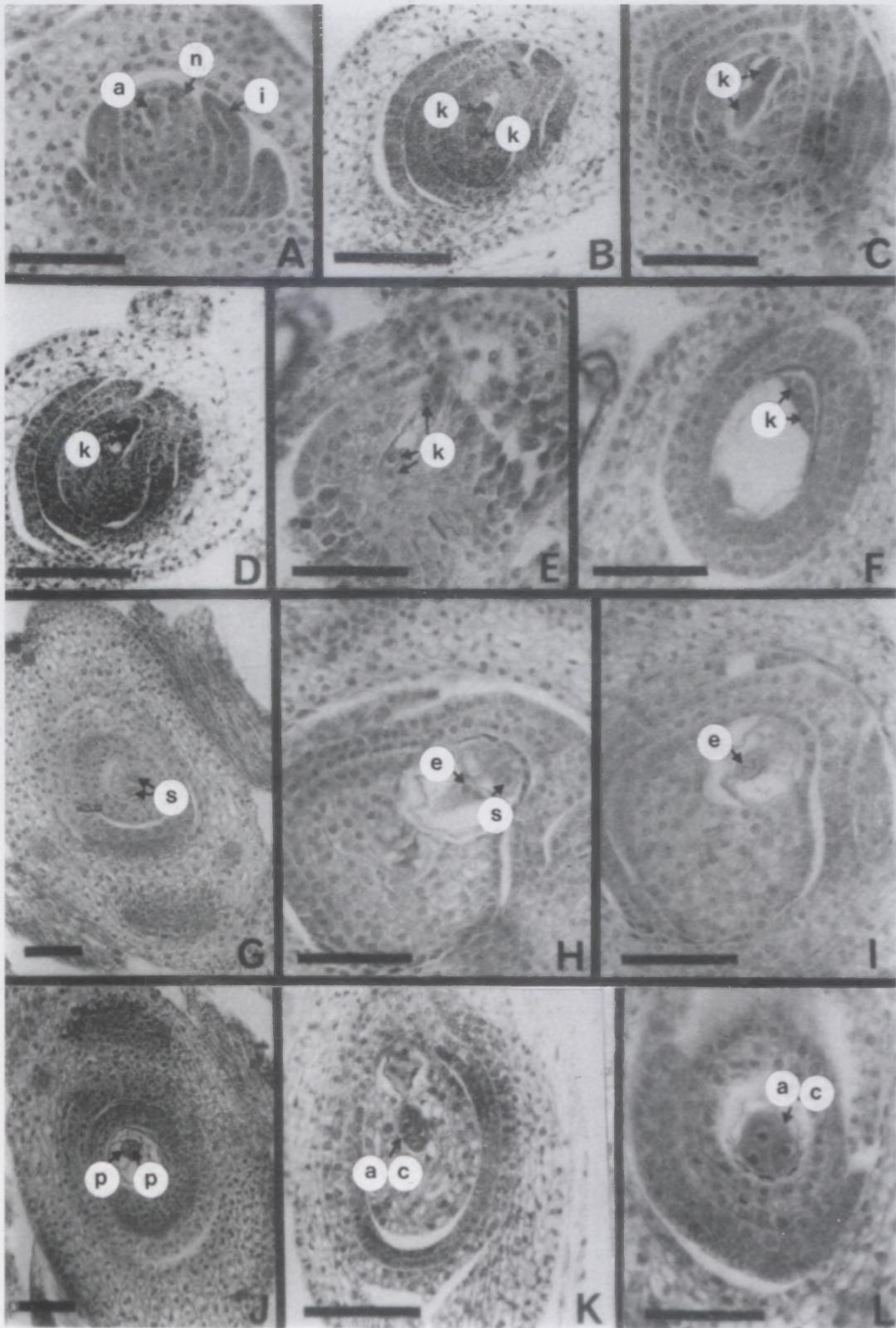


FIGURE 1.—Photomicrographs of reduced embryo sac development in Cynodonteae. A, archesporium in *Harpochloa falx*, Spies 4729; B, binucleate embryo sac in *Chloris virgata*, Spies 4783; C, binucleate embryo sac in *H. falx*, Spies 5078; D, tetranucleate embryo sac in *C. virgata* with only three nuclei visible in section, Spies 4783; E, tetranucleate embryo sac in *H. falx* with only three nuclei visible, Spies 5125; F, tetranucleate embryo sac of *Eustachys paspaloides*, Spies 1521, with only two nuclei visible; G, two synergids in *H. falx*, Spies 4827; H, I, egg cell and synergids of *H. falx*, Spies 5113; J, two polar nuclei in *H. falx*, Spies 4827; K, antipodal complex with only three antipodal cells visible in this section of *C. virgata*, Spies 4799; L, antipodal complex with only three antipodal cells visible in this section of *H. falx*, Spies 5118. a, archesporium; ac, antipodal complex; e, egg cell; i, integument; k, nucleus; n, nucellus; p, polar nucleus; s, synergid. Scale bar: 80 μ m.

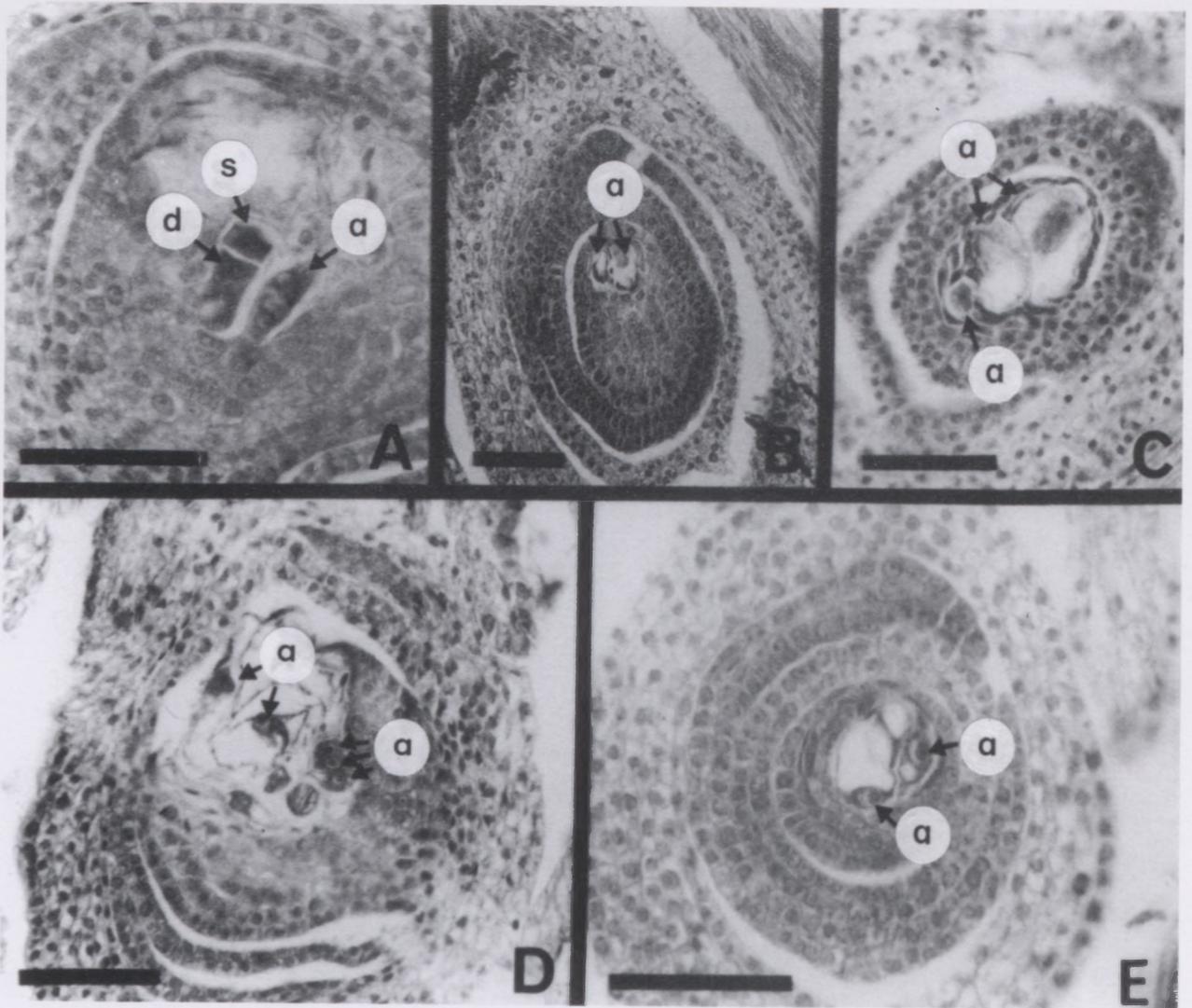


FIGURE 2.—Photomicrographs of un-reduced embryo sac development in Cynodonteae. A, reduced and un-reduced embryo sacs in *Rendlia altera*, Spies 5129; a degenerating macrospore can be seen next to a reduced one; B, two developing un-reduced embryo sacs in *Harpochloa falx*, Spies 4695; C, three un-reduced embryo sacs in *R. altera*, Spies 5072, two with one nucleus and one with two nuclei; D, five un-reduced embryo sacs in *R. altera*, Spies 5129, each with one nucleus; E, two un-reduced embryo sacs in *Eustachys paspaloides*, Spies 1521, each with one nucleus. a, un-reduced embryo sac; d, degenerating embryo sac; s, reduced embryo sac. Scale bar: 80 μ m.

Harpochloa falx, and *Rendlia altera* had bipolar embryo sacs, therefore the *Hieracium* type. Antipodal complexes were absent in *Eustachys paspaloides* and *Rendlia altera*. The ovules of *Eustachys paspaloides* contained reduced embryo sacs with the synergids or the egg cell visible, whereas other ovules had up to two or three different embryo sacs each (Figure 2E). One specimen of *Harpochloa falx* had two different embryo sacs (Figure 2B). The ovules of *Rendlia altera* had up to five different embryo sacs per ovule (Figure 2A, C, D).

In conclusion, the grasses of Cynodonteae reproduce both sexually and apomictically. Reduced embryo sacs of the *Polygonum* type occur. The apomicts have un-reduced bipolar *Hieracium* type embryo sacs. Sexual reproduction occurs at different polyploid levels as seen in *Harpochloa falx* and *Rendlia altera*. Some species have reduced embryo sacs at the tetraploid and higher polyploid levels (*Harpochloa falx*), whereas others have un-reduced embryo sacs at these polyploid levels (*Rendlia altera*) (Strydom & Spies 1994). *Eustachys paspaloides* and *Harpochloa falx* were both facultative apomicts and *Rendlia*

altera was an obligate apomict. The types of ovules differ as seen in *Harpochloa falx* and *Rendlia altera*. Both have amphitropous ovules, whereas the other species have anatropous ovules.

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REFERENCES

- HARLAN, J.R. & DE WET, J.M.J. 1975. On O. Winge and a prayer: the origins of polyploidy. *Botanical Review* 41: 361–390.
 JACOBS, S.W.L. 1987. Systematics of the Chloridoid grasses. In T.R. Soderstrom, *Grass systematics and evolution*: 277–286. International Grass Symposium.
 LEWIS, W.H. (ed.) 1980. *Polyploidy: biological relevance*. Plenum Press, New York.
 MOGIE, M. 1992. *The evolution of asexual reproduction in plants*. Chapman & Hall, London.

- NOGLER, G.A. 1984. Gametophytic apomixis. In B.M. Johri, *Embryology of angiosperms*: 475-510. Springer-Verlag, Berlin.
- SPIES, J.J. & DU PLESSIS, H. 1986. The genus *Rubus* in South Africa III. The occurrence of apomixis and sexuality. *South African Journal of Botany* 52: 226-232.
- STEBBINS, G.L. 1972. *Chromosomal evolution in higher plants*. Edward Arnold, London.
- STRYDOM, A. & SPIES, J.J. 1994. A cytotoxic study of some representatives of the tribe Cynodonteae (Chloridoideae, Poaceae). *Bothalia* 24: 92-96.
- VAN OUDTSHOORN, F.P. 1991. *Gids tot grasse van Suid-Afrika*. Briza Publikasies.
- WILLEMSE, M.T.M. & VAN WENT, J.L. 1984. The female gametophyte. In B.M. Johri, *Embryology of angiosperms*: 159-191. Springer-Verlag, Berlin.