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Miscellaneous notes

POACEAE

APOMICTIC EMBRYO SAC DEVELOPMENT IN *CENCHRUS CILIARIS* (PANICOIDEAE)

Apomixis is distributed throughout the plant kingdom and is known in over 300 species of at least 35 different families (Hanna & Bashaw 1987). Research on apomixis in grasses began in the early 1930's with *Poa pratensis* L. (Müntzing 1933). Apomixis is especially prevalent among perennial forage grasses and has been reported in more than 125 species representing most of the tribes. Well-known apomictic grasses include *Paspalum dilatatum* Poir. (Snyder 1957), *P notatum* Fluegge (Snyder 1957), *Setaria* P.Beauv. (Emery 1957) and *Cenchrus ciliaris* L. (Fisher *et al.* 1954; Snyder *et al.* 1955). Apomixis in the angiosperms means asexual reproduction by seeds (Nogler 1984). In this study reduced embryo sacs (embryo sacs in which meiosis occurs) imply sexual reproduction, whereas unreduced embryo sacs imply asexual or apomictic reproduction.

The aim of this study is to determine whether reduced embryo sacs are formed in *C. ciliaris* specimens included in this study. The type of embryo sac will give an indication of the sexual or apomictic nature of the species and will suggest to what extent cross-fertilisation contributed to the genetic variation in this species.

MATERIAL AND METHODS

The plant material used in this study was collected in the field (Table 1). A selection from the material, collected for meiotic analysis, was used to study embryo sac development. Florets in various developmental stages were used. Inflorescences were dehydrated with ethanol and tertiary buthanol before being embedded in pastulated Paraffin wax. Sections (6 µm) were cut with a rotary microtome, mounted and stained with a modified (Spies & du Plessis 1986) safranin (Johansen 1940) and fast green (Sass 1951) procedure. A minimum of twenty embryo sacs per developmental stage were studied in each specimen. A Nikon photomicroscope and Ilford Pan-F film (ASA 50) were used for the photomicrographs.

RESULTS

Embryo sac development was studied in 27 specimens, representing four different ploidy levels (Table 1) The nucellus was multiseriate and protected by various

TABLE 1.—List of *Cenchrus ciliaris* specimens with *Panicum* type of embryo sacs, their localities and their chromosome number (B-chromosomes are abbreviated as B)

2n =	Locality	Voucher
34	2627 (Potchefstroom): in Potchefstroom, on route to Orkney, (–CA)	*Spies 5883*
36	2624 (Vryburg): near Vryburg, on route to Kuruman, (–DC)	*Spies 5529*
	2723 (Kuruman): 16 km from Kuruman to Vryburg, (–BC)	*Spies 5525*
	2724 (Taung): 101 km from Kuruman to Vryburg, (–AB)	*Spies 5527*
	2725 (Bloemhof): 2 km from Britten to Christiana, (–CB)	*Spies 5542, 5543*
	2822 (Glen Lyon): 7 km from Smidtsdrift to Postmasburg, (–DA)	*Spies 5521*
	2822 (Glen Lyon): 25 km from Bloemfontein to Brandfort, (–CD)	*Spies 5577*
	2925 (Jagersfontein): 56 km from Petrusburg to Kimberley, (–AA)	*Spies 5509*
	3222 (Beaufort West): 5 km from Beaufort West, (–BC)	*Spies 5487*
	3224 (Graaff-Reinet): 58 km from Jansenville to Graaff-Reinet, (–BC)	*Spies 5240*
	3224 (Graaff-Reinet): 131 km from Uitenhage to Graaff-Reinet, (–DC)	*Spies 5236*
	3224 (Graaff-Reinet): 145 km from Uitenhage to Graaff-Reinet, (–DC)	*Spies 5237*
	3324 (Steytlerville): 102 km from Uitenhage to Graaff-Reinet, (–BD)	*Spies 5232*
36+0–2B	2624 (Vryburg): near Vryburg, on route to Kuruman, (–DC)	*Spies 5531*
	2925 (Jagersfontein): 60 km from Petrusburg to Kimberley, (–AA)	*Spies 5512*
	3125 (Steynsburg): 10 km from Steynsburg to Hofmeyer, (–BC)	*Spies 5584*
	3222 (Beaufort West): 5 km from Beaufort West, (–BC)	*Spies 5488*
	3324 (Steytlerville): 68 km from Uitenhage to Graaff-Reinet, (–DA)	*Spies 5231*
	3325 (Port Elizabeth): 30 km from Uitenhage to Graaff-Reinet, (–CD)	*Spies 5229*
45	2522 (Sanie): in the riverbed at Watersend, (–DB)	*Spies 5497*
	3024 (Colesberg): 27 km from Verwoerd Dam to Venterstad, (–DA)	*Spies 5581, 5583*
	3224 (Graaff-Reinet): 39 km from Jansenville to Graaff-Reinet, (–DA)	*Spies 5239*
	3224 (Graaff-Reinet): 15 km from Jansenville to Graaf-Reinet, (–DC)	*Spies 5238*
54	2824 (Kimberley): 1 km from Kimberley to Griekwastad, (–DA)	*Spies 5514*
54+0–1B	2824 (Kimberley): 1 km from Kimberley to Griekwastad, (–DA)	*Spies 5517*

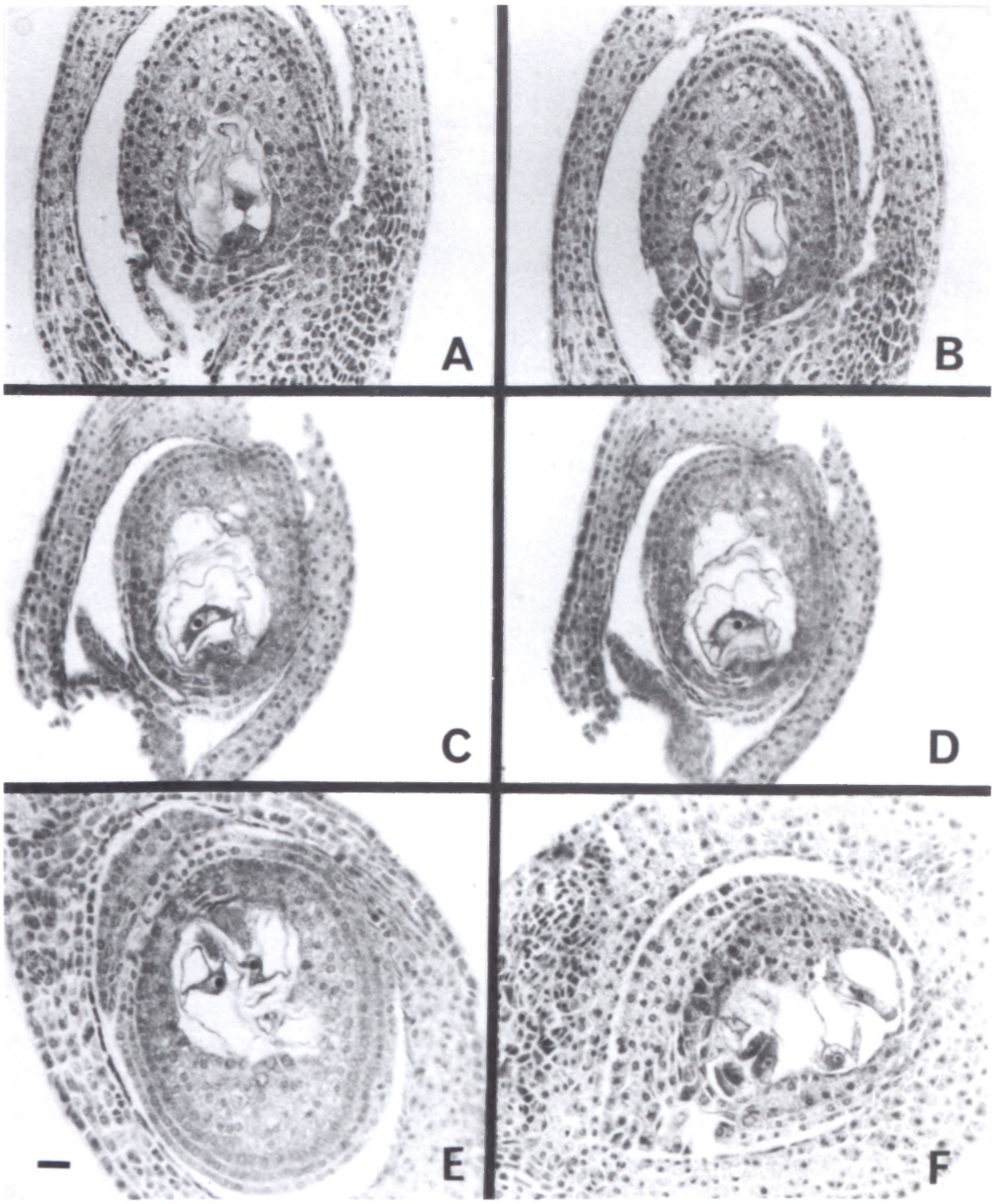

FIGURE 1.—Photomicrographs of unreduced embryo sac development of the *Panicum* type in ovules of *Cenchrus ciliaris*. A, B, *Spies 5240*: A, three embryo sacs in ovule with only two visible in this section; two nuclei in micropylar region of one embryo sac resembling either an egg nucleus and a synergid or two synergids, with one chalazal polar nucleus; B, next section of ovule in A, three embryo sacs visible; third nucleus of embryo sac in A in micropylar region, representing either an egg or a synergid. C–E, *Spies 5232*: C, two embryo sacs per ovule; two nuclei in micropylar region, resembling an egg nucleus and a single synergid, with one chalazal polar nucleus; D, second section of ovule in C with a second polar nucleus in chalazal region of embryo sac; E, at least four unreduced embryo sacs in mature ovule. F, *Spies 5488*, at least five unreduced embryo sacs in mature ovule. Scale bar: 10µm.

multicellular integuments. Some of the integuments completed their development at a relatively late stage. The archesporial cell functioned directly as the primary megasporocyte. One or more somatic cells with prominent nuclei, lying in the centre of the nucellus, also enlarged. These cells soon obscured all traces of the degenerated gametophyte. These cells had dense cytoplasms and were usually adjacent to the sporogenous material. The nuclei of the aposporous cells underwent one to several divisions and formed a well-defined dyad.

The aposporic embryo sacs are extremely vacuolated and mature embryo sacs usually contained four nuclei. Some of the nuclei resembled the egg and polar cells of a reduced embryo sac. These embryo sacs usually included one polar nucleus, an egg nucleus and two synergid cells (Figure 1A, B). Some embryo sacs included an egg nucleus, one synergid cell and two polar nuclei (Figure 1C, D). These unreduced embryo sacs were classified as four nucleated *Panicum* type aposporic embryo sacs (Figure 1A, D).

The total number of aposporic embryo sacs per ovule varied among the specimens studied (Figure 1E, F). A maximum of eight aposporic embryo sacs per ovule was observed (*Spies 5239*). These multiple embryo sacs were usually concentrated in the central part of the ovule and, at maturity, occupied most of the former region of the nucellus (Figure 1E, F).

DISCUSSION

The mechanism of apospory in *C. ciliaris* involves the development of the embryo from an unreduced nucleus, in an embryo sac derived from a somatic cell in the ovary. These aposporous embryo sacs may develop in the nucellus of the ovule or in some species in the integuments and ovary wall (Bashaw & Hanna 1990). Apospory is the apomictic mechanism most common in the grasses, particulary in the tribe Paniceae, which accounts for more than 95% of known apomictic species (Bashaw & Hanna 1990). The origin of aposporous cells in the grass ovule is quite different from the normal pattern of sexual megasporogenesis (Bashaw 1980). Early development of the megaspore mother cell is usually identical in aposporous and sexual ovules. In both cases, the megaspore mother cell differentiates in the hypodermal layer of the nucellus in the micropylar region, during the enlargement stage of the young ovule. Meiosis in both aposporous and sexual ovules generally results in a linear tetrad. The similarity between apospory and sexual embryo sac development ends at this point (Bashaw & Hanna 1990).

Aposporic development in *C. ciliaris* is initiated by the unusual enlargement of one or more somatic (nucellus) cells. These cells, in comparison to the normal nucellar cells, usually have prominent nuclei and dense cytoplasms. The nucleus of the aposporous cell initially undergoes one to several mitotic divisions. The degree of differentiation of aposporous embryo sacs in *C. ciliaris* varies. In some grass species, one or more of the aposporous sacs may develop to the extent that they closely resemble the typical sexual embryo sac (Bashaw & Hanna 1990). This is not the case in *C. ciliaris*, for a four-nucleated embryo sac was most often observed (Figure 1A–D). This included a polar nucleus, an egg nucleus and two synergid cells (Figure 1A, B) or one egg, one synergid and two polar nuclei (Figure 1C, D). Antipodal cells were completely lacking. The embryo sacs observed were, therefore, classified as unreduced aposporic embryo sacs, of the *Panicum* type. This finding corresponds with previous reports by various authors (Bashaw & Holt 1958; Brown & Emery 1958; Bashaw 1962) and is typical of the Panicoideae (Bashaw & Hanna 1990; Mogie 1992). The number of embryo sacs of nucellar origin varied from two to eight in *Spies 5239*. Occasionally observed, was a cluster of unreduced embryo sacs which could not be accurately counted.

Various authors have reported a number of ovules in *C. ciliaris* containing a single aposporous embryo sac in the nucellus (Fisher *et al.* 1954; Bashaw & Hanna 1990). According to their reports, these sacs were centrally located, in the micropylar region of the ovule, and occupied more or less the same location and total area as the sexual gametophyte in sexual plants of this species. This type of embryo sac development was not observed in the current study, in which the mimimum number of embryo sacs present in the ovule, was two.

The variation in the arrangement of structures of the aposporous embryo sacs (Figure 1A–D) is characteristic of *C. ciliaris*. This embryological variation corresponds with the variation observed on chromosome level (Visser *et al.* 1998a, b, c). This species is morphologically (De Lisle 1963) and cytogenetically highly polymorphic and complex. Chromosome abnormalities, observed during meiosis, and varying polyploid levels were common (Visser *et al.* 1998a, b, c). The various polyploid natures (varying from autosegmental-alloploidy to pure alloploidy) indicated the presence of hybridization (Visser *et al.* 1998a, b, c). However, during this study, no correlation was found between ploidy level and embryo sac development or cell morphology during embryo sac development. The absence of any suggestion of sexual reproduction in the specimens studied, indicates either that the genetic variation originated before these plants became obligate apomicts, or that the frequency of sexuality is extremely low. The latter possibility is contradicted by the genetic variation observed in these specimens.

When Taliaferro & Bashaw (1966) discovered a sexual plant, they investigated the inheritance of apomixis in *C. ciliaris*. The plant was an obligate sexually reproducing specimen, but heterozygous for the method of reproduction. The progeny, after selfing, was either obligate sexual or obligate apomictic, indicating the heterozygous nature of this specimen. Based on their data, Bashaw & Hanna (1990) suggested that the mode of reproduction is controlled by two different genes, with epistasis favouring dominant expression of the gene for sexuality. They proposed that the genotype of the sexual plant was AaBb and that of the two apomictic cultivars were Aabb. Their hypothesis assumed that dominant gene B conditions sexual reproduction and is epistatic to dominant gene A, which conditions apospory. Due to the absence of dominant gene A, a double recessive aabb was expected to reproduce sexually. Gene A, therefore, controls all of the processes resulting in development of unreduced nucellar embryo sacs and abortion of the normal sexual sporogenous tissue (Bashaw & Hanna 1990).

The genetic inheritance of apomixis suggests that a small percentage of sexual plants representing *C. ciliaris* may still prevail in nature. It is accepted that sexual or partially sexual plants probably exist in most apomictic species. Based on this fact, facultative apomicts are claimed to precede obligate apomicts in the development of the agamic complex (Bashaw & Hanna 1990). Clausen (1954) described facultative apomixis as an evolutionary equilibrium system in which the apomictic process is in balance with an almost dormant sexual process, which can be invoked and can release a part of the stored sexual variability for a certain period. In the geographic distribution of *C. ciliaris*, completely sexual, facultative and obligate plants may still be present. Sexually reproducing plants serve as foundation for the considerable genetic variation found among, and in, populations representing this species. Facultative apomictic

plants are, therefore, a seemingly dormant but effective source of variation, for heterozygous genotypes are produced each time when sexual and apomictic plants hybridize (Bashaw *et al.* 1970).

Although no reduced embryo sacs (suggesting sexual reproduction) were observed during the current study, chromomosomal and morphological differences were observed in plants representing a specific region. This indicates that a small percentage of specimens of *C. ciliaris* have to be facultative apomicts, for the offspring were not exact replicas of the maternal plants. It is concluded that both facultative and obligate apomixis are present in *C. ciliaris*. This conclusion is based on the presence of more genetic variation than can be accounted for by mutations alone. There may also be sexual specimens in nature, but they were not found and sampled during this study.

CONCLUSIONS

Apospory as a mode of asexual reproduction is common in the Poaceae. It involves the development of an embryo from an unreduced nucleus, in an embryo sac derived from a somatic cell. Aposporic development in *C. ciliaris* varies, for this type of development is initiated in various somatic cells simultaneously, and leads to the maturing of various numbers of embryo sacs in the ovule.

Embryological variations regarding embryo sac development were observed in this species. These aposporous embryo sacs were characterized as unreduced four-nucleated embryo sacs of the *Panicum* type. Although the presence of cytogenetic and morphological variation indicates that this species may be characterized as a facultative apomictic species, all ploidy levels appear to be obligate apomicts. This suggests that the morphological and/or genetic variation originated before obligate apomixis occurred.

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HYACINTHACEAE

CHROMOSOME STUDIES ON AFRICAN PLANTS. 13. *LACHENALIA MUTABILIS*, *L. PUSTULATA* AND *L. UNICOLOR*

The genus *Lachenalia* Jacq.f. ex Murray consists of small bulbous geophytes and shows a great potential for use as pot plants (Niederwieser *et al.* 1997). Various chromosome numbers, and even different basic chromosome numbers, have been reported for this genus (Moffett 1936; De Wet 1957; Riley 1962; Mogford 1978; Ornduff & Watters 1978; Nordenstam 1982; Crosby 1986; Hancke & Liebenberg 1990; Hancke 1991; Johnson & Brandham 1997; Kleynhans 1997; Hancke & Liebenberg 1998; Kleynhans & Spies 1999).

Lachenalia mutabilis Sweet belongs to the *L. orchioides* group (Crosby 1986) and the chromosome numbers reported for this species vary from 2n = 10