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Embryo sac development in some South African cultivars of *Lantana camara*

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ABSTRACT

Twenty embryo sacs from each of 20 different *Lantana camara* L. cultivars naturalized in South Africa were examined. The normal sexual embryo sacs were monosporic 8-nucleated embryo sacs of the polygonum type and were encountered in 55% of the material examined. Several deviations from this pattern were recorded. Occasionally one of the nuclei failed to develop into a synergid, resulting in three polar nuclei.

Contrary to published information, the antipodal cells did not increase in size, nor was there an increase in the number of nuclei per cell. Although the occurrence of sexuality is confirmed, no definite evidence exists for the occurrence of apomixis. The occurrence of two embryo sacs per locule might be the result of either apospory or of sexuality whereby two embryo sacs were formed from two megaspores.

INTRODUCTION

Lantana camara L. is rated as one of the world's ten worst weeds (Holm & Herberger, 1969). This South American species is today recognized as a polyploid aggregate species (Stirton, 1977) and has recently been confirmed as such in South Africa (Spies & Stirton, 1982a).

Although some genetic studies of *L. camara* have suggested the occurrence of apomixis (Raghavan & Arora, 1960; Khoshoo & Mahal, 1967), this phenomenon has not been reported in any of the embryological studies done so far (Junell, 1934; Patermann, 1935; Tatachar, 1940; Padmanabhan, 1959; Kahleel & Nalini, 1972). We decided to investigate whether apomixis plays any role in the reproductive cycle of this successful weed in South Africa, because we have encountered over 40 different cultivars of *L. camara* with variable seedset.

MATERIALS AND METHODS

The plants used in this study are naturalized cultivars collected from throughout South Africa and transplanted in the Pretoria National Botanical Garden. This material has been studied cytogenetically (Spies & Stirton, 1982b) and taxonomically (Stirton & Spies, 1982). The research material has consequently been chosen carefully within each polyploid level and is representative of plants with normal and abnormal meiosis.

Young inflorescences were collected and fixed at 4°C for at least 24 hours in Navashin fixative (Stockholm modification, Maheshwari, 1939). The material was then washed in running tap water and serially dehydrated in ethyl alcohol and tertiary butyl alcohol. Tissue Prep (T565) was used as an embedding agent. The 9–12 micron sections were stained in Safranin and Fast Green (Johansen, 1940 — with minor modifications).

Twenty different embryo sacs from each plant were examined to determine the percentage of normal sexual embryo sacs.

RESULTS

L. camara has a bilocular ovary with a single anatropous ovule in each locule. The nucellus is one cell layer thick and is surrounded by a single massive integument. The archesporium is hypodermal and functions directly as megaspore mother cell (Fig. 1).

Meiotic cell divisions result in a linear tetrad of megaspores, of which only the chalazal one is usually functional (Fig. 2). Normally the development of the megaspore into an embryo sac causes degeneration of the other three megaspores. The non-functional megaspore of *L. camara* are mostly persistent (Fig. 10). The functional megaspore undergoes mitosis producing two daughter-nuclei (Fig. 3) that are subsequently separated to opposite poles by the formation of a central vacuole (Fig. 4). Each nucleus divides twice, resulting in an eight-nucleated embryo sac (Fig. 5 and 6).

The mature embryo sac is broader in the micropylar region and tapers towards the chalazal pole. The egg apparatus consists of two synergids and an egg. In the centre of the embryo sac there are two adjacent polar nuclei with three antipodal cells at the chalazal pole. This pattern was found in 54,75% of the embryo sacs studied (Table 1).

The most common deviation from this generalized pattern was the occurrence of two embryo sacs in each locule. In these cases the embryo sacs are always located at the polar ends of the locule (Fig. 7), with only a slight overlap occurring in the equatorial region. This pattern was found in 42,5% of the embryo sacs studied.

In the remaining 2,75% of embryo sacs examined, there was a maldistribution of the nuclei. In ten embryo sacs one of the synergids moved with the polar nucleus towards the equatorial region. In these cases, the egg apparatus consisted of an egg and only a single synergid (Fig. 8). Finally, we found a single embryo sac with twenty antipodal nuclei (Fig. 9).

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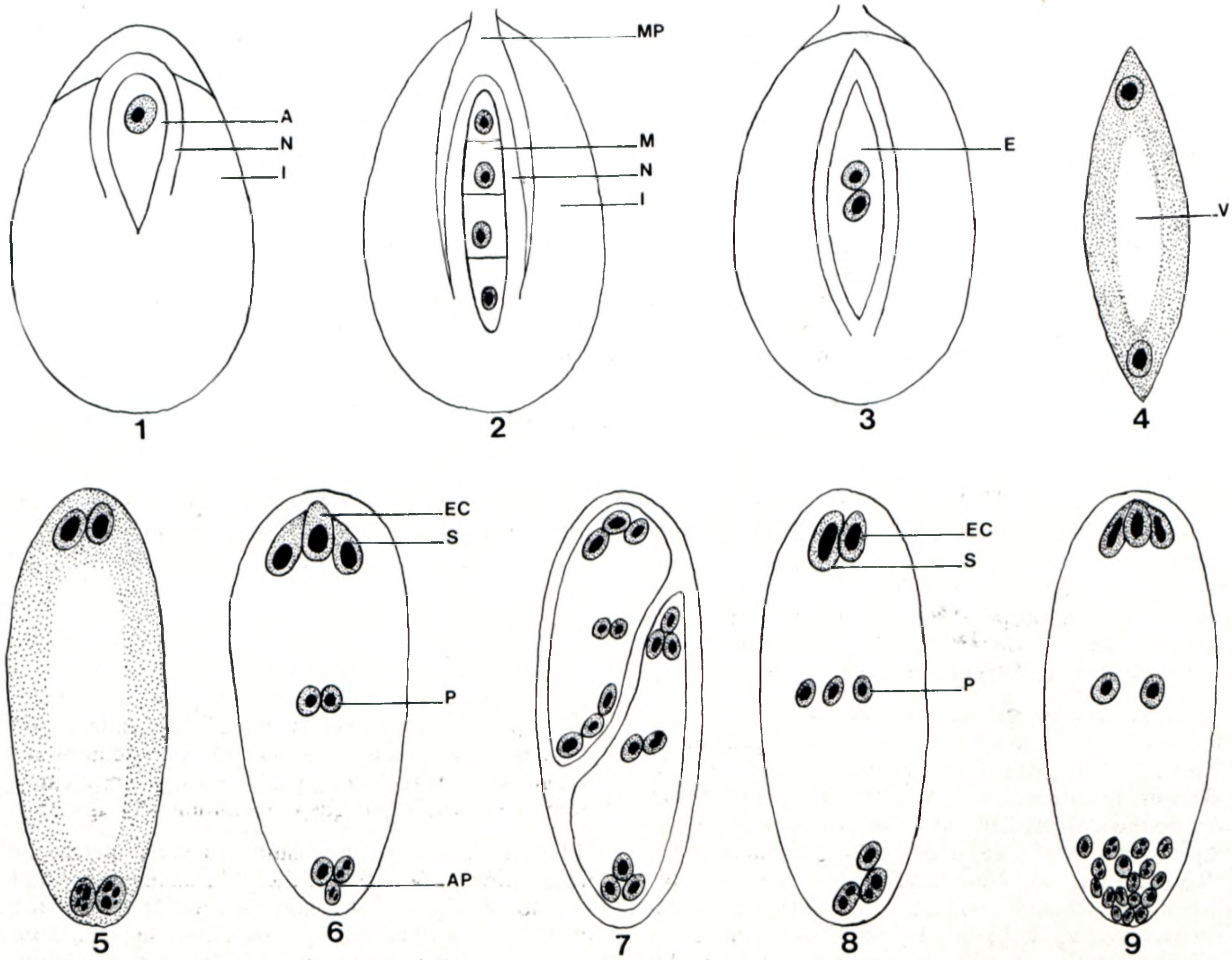

FIGS 1–9.—Schematic representation of embryo sac development in *Lantana camara*. 1, archesporium; 2, tetrad; 3 & 4, two-nucleated embryo sac; 5, four-nucleated embryo sac; 6, mature sexual embryo sac; 7, two embryo sacs per locule; 8, three polar nuclei; 8, conglomeration of antipodal nuclei. (A—archesporium; AP—antipodal nuclei; E—embryo sac; EC—egg cell; I—integument; M—megaspore; MP—micropylar region; N—nucellus; P—polar nuclei; S—synergid; V—vacuole.)

DISCUSSION AND CONCLUSIONS

The reproductive studies of Raghaven & Arora (1960) and Khoshoo & Mahal (1967) on *L. camara* have shown that there is a tendency among different open-pollinated cultivars to produce matroclinous progeny when growing together.

Raghavan *et al.* (1960), who studied diploid, triploid and tetraploid cultivars, concluded their study with the observation that reproduction was by obligate apomixis only. Contrary to these findings Khoshoo *et al.* (1967) who studied a wider selection of cultivars, including those of the previous workers, were unable to find a single case of obligate apomixis. Their results ranged from a case of total sexuality in a diploid cultivar to facultative apomixis with 66,7% sexuality or semi-sexuality in a pentaploid plant. In both papers there is speculation about the reproductive mechanisms in *L. camara* but no evidence is led about the mechanisms themselves. It is also clear that the same material gave different results, but it is not clear whether this was due to climatological differences or not.

The tendency to produce matroclinous progeny clearly indicates a high degree of self-fertilization or some kind of asexual reproduction. That emasculated bagged flowers did not produce any seed implies that reproduction was either sexual or apomictic, accompanied by pseudogamy (Khoshoo *et al.*, 1967). Tatachar (1940) has reported the occurrence of double fertilization.

These conflicting indications of apomictic reproduction pointed to the need for the current investigation to determine what mechanism was involved in the reproduction of *L. camara*.

a) *Occurrence of sexual embryo sacs*

54,7% of the ovules investigated by us produced a normal sexual monosporic eight-nucleated embryo sac of the polygonum type (nomenclature according to Maheshwari, 1950) and were found in diploid (2n = 22), triploid (2n = 33) and tetraploid (2n = 44) plants, but not in pentaploids (2n = 55) and hexaploids (2n = 66).

The percentage occurrence of these normal sexual embryo sacs ranged from the extremes of 0% in one plant to 95% in another. Diploid plants produced an average of 69,2% sexual embryo sacs,

TABLE 1.—Embryo sac analysis of 20 cultivars of *Lantana camara* in South Africa. Twenty embryo sacs were counted in each cultivar

Plant no. (Stirton)	Chromosome no.	Number of unpaired chromosomes	Embryo sacs			
			Normal	2 sacs	3 polar nuclei	Many antipodal nuclei
6882	22	—	10	10	—	—
7066	22	—	13	4	2	1
7311	22	4,4	19	0	1	—
7314	22	4,3	17	2	1	—
7361	22	4,1	14	6	—	—
7374	22	—	10	10	—	—
6878	33	3,5	11	9	—	—
6883	33	2,5	17	3	—	—
7348	33	3,2	13	7	—	—
7381	33	2,8	18	—	2	—
7383	33	6	18	2	—	—
7384	33	6,4	13	6	1	—
7387	33	2,8	15	4	1	—
7393	33	3	2	18	—	—
7394	33	5,2	7	13	—	—
7087	44	4,3	5	13	2	—
7230	44	0,9	14	6	—	—
7351	44	0,7	3	17	—	—
5287	55	3,1	—	20	—	—
7058	66	5,4	—	20	—	—
		Average	10,95	8,5	0,5	0,05

ranging between 50% and 95% among plants. Triploids ranged from 10% to 90% with an average of 63,3% sexual embryo sacs. Tetraploids produced an average of 36,7% sexual embryo sacs and showed a range of 15% to 70%.

It is clear from these results that there are large differences within each polyploid group in the percentage of normal sexual embryo sacs produced and as the polyploid level increases, the number of sexual embryo sacs produced drops off rapidly until none are produced in the pentaploid and hexaploid plants. Although we cannot yet furnish any cytogenetic evidence to explain these phenomena, we can conclude that the occurrence of a polygonum type embryo sac proves that *L. camara* has at least the potential to reproduce sexually.

Perhaps the most surprising find of this investigation is the high average percentage (63,3%) of sexual embryo sacs that were found within the triploid series. There are, however, indications that cytogenetic irregularities during the development of the embryo sac occur. These irregularities can be seen in the form of chromosome laggards during anaphases (Fig. 10).

b) *Occurrence of abnormal embryo sacs*

Junell (1934) and Khaleel *et al.* (1972) have described the occurrence of more than one archesporium cell during the early stages of embryo sac development, but we were unable to find any in our material.

It has also been reported in the literature that all four megaspores can undergo development. This has been seen only rarely by Tatachar (1940) in *L. indica* Roxb., but appears to be a frequent occurrence in *L. camara* L. and *L. involucrata* L. (Junell, 1934) and in *L. aculeata* L. (Khaleel *et al.*, 1972). We found a low rate of degeneration of the nonfunctional megaspores. In one ovule two megaspores persisted with a 4-nucleated embryo sac. No evidence for the development of an additional megaspore into an embryo sac could be found.

In all cases where two embryo sacs per locule (42,5%) were found, these embryo sacs were positioned in the polar regions. We did not encounter any cases in which the two embryo sacs were positioned together in the equatorial region. This might mean that both the embryo sacs were sexual and derivatives of two megaspores. However, the polar positions of the two embryo sacs do not totally exclude the possibility of apospory. If apomixis occurs in the form of apospory, this polar position may be accounted for by the constraints of development being such that the space available only allows for a linear development between the poles.

In 2,5% of the embryo sacs examined, a third polar nucleus was encountered. In all such cases the egg apparatus consisted of one synergid and an egg cell. This observation is normal, but the frequency is much higher than one might expect (Maheshwari, 1963). Since these embryo sacs are sexual, their frequency could be added to those of the normal sexual embryo sacs to give a total of 57,25% embryo sacs for that class.

Tatachar (1940) and Padmanabhan (1959) observed an increase in the size of the three antipodal cells even before the egg apparatus was completed. This increase in cell size is accompanied by mitotic divisions resulting in 4–8-nucleated antipodal cells in the mature embryo sacs of *L. camara* and *L. indica*. This phenomenon was not observed by Khaleel *et al.* (1972) in *L. aculeata*. We have

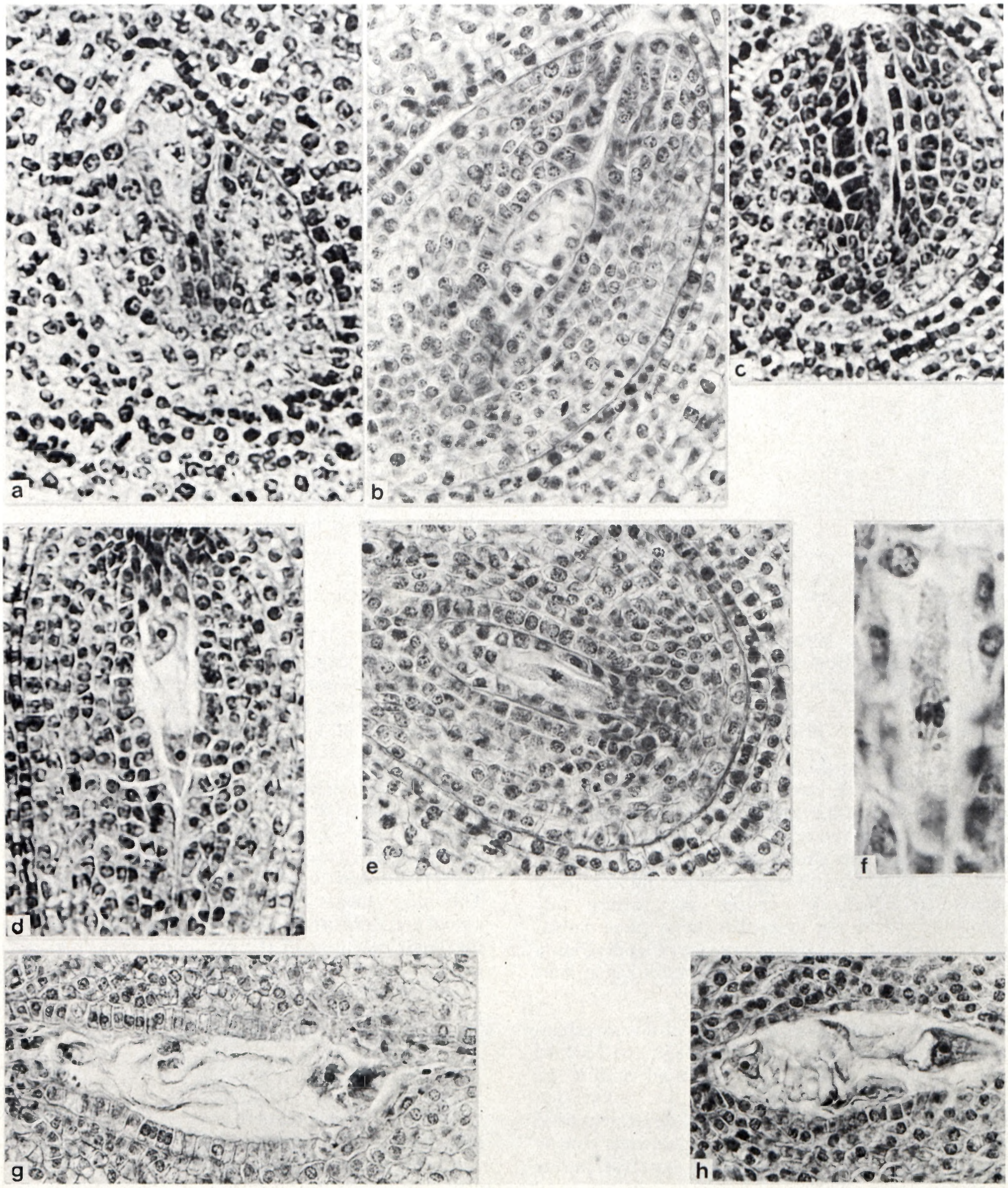

FIG. 10.—Photomicrographs of embryo sac development in *Lantana camara*. a, archesporium; b megaspore; c & d two-nucleated embryo sac; e & f cell division during development of the embryo sac (note fragments of chromosomes not participating in metaphase in f—2n=33); g & h, formation of two embryo sacs per locule, (f ± ×2880; rest ± ×1800.)

encountered a single embryo sac (in *Stirton* 7066, PRE) in which there was a conglomeration of about 20 nuclei, but no separate antipodal cells could be distinguished. So with only one out of 400 ovules studied resembling this phenomenon, we can state that we observed a significant increase neither in cell size nor in the number of nuclei in the antipodal cells of our material.

c) *Seed-setting and microsporogenesis*

Differences in seed-setting by different cultivars of *L. camara* have been reported by Tandon & Bali (1955), Natarajan & Ahuja (1957) and Khoshoo & Mahal (1967). Natarajan *et al.* (1957) did not find any correlation between irregularities in microsporogenesis and seed-setting. Our own data support their findings, as we did not find a correlation (r = -0,0456) between any irregularities in microsporogenesis and the development of abnormal embryo sacs.

In conclusion, it is clear that, although we have shown the occurrence of sexuality in *L. camara* in the form of polygonum type embryo sacs, we have not yet obtained definite evidence for the occurrence of apomixis. The occurrence of two embryo sacs per locule might be attributable to apospory or it might be a result of sexuality where two embryos were formed from two megaspores.

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UITTREKSEL

Twintig kiemsakke van elk van twintig verskillende Lantana camara *L. kultivars wat in Suid-Afrika aangepas is, is ondersoek. Die normale geslagtelike kiemsakke was monosporiese agtkernige kiemsakke van die polygonum tipe en het in 55% van die materiaal voorgekom. Verskeie afwykings van hierdie toestand het egter voorgekom. Soms kon een kern nie in 'n sinergiede verander nie en in dié gevalle kom daar drie poolkerne voor.*

In teenstelling met gepubliseerde inligting het die antipodale selle nie vergroot of meerkernig geraak nie. Die bestaan van geslagtelikheid word dus bewys maar geen bepalende bewys vir die voorkoms van apomiksie is gevind nie. Die voorkoms van twee kiemsakke per vrughok mag die resultaat wees van aposporie of geslagtelikheid waar twee kiemsakke uit twee megaspore ontwikkel.

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