

Breeding systems in some representatives of the genus *Lycium* (Solanaceae)

L. MINNE*, J.J. SPIES*†, H.J.T. VENTER* and A.M. VENTER*

ABSTRACT

The development of the ovule and the embryo sac of five of the 17 species of *Lycium* and of one hybrid, recorded for southern Africa, was investigated. All specimens of four of the species and the hybrid (between a hermaphroditic and a functionally dioecious species) were found to be functionally dioecious: they express only one sex, although both male and female organs are present in the same flower. One species was hermaphroditic. The embryo sacs of all species, and of the hybrid, were of the normal eight-nucleate *Polygonum* type. The structure of the ovary and the development of the embryo sac are similar to those of *L. europaeum* L. The absence of unreduced embryo sacs indicates that apomixis does not occur at any ploidy level in the species studied.

UITTREKSEL

Die ontwikkeling van die saadknop en die embriosak van vyf van die 17 *Lycium*-spesies en van een baster wat in suidelike Afrika aangeteken is, is ondersoek. Daar is bevind dat alle eksemplare van vier van die spesies en die baster (tussen 'n hermafroditiese en 'n funksioneel tweehuisige spesie) funksioneel tweehuisig is: hulle gee uitdrukking aan slegs een geslag, hoewel manlike en vroulike organe albei in dieselfde blom teenwoordig is. Een spesie was hermafrodities. Die embriosakke van al die spesies, en van die baster, was van die normale agt-kernige *Polygonum*-tipe. Die bou van die vrugbeginsel en die ontwikkeling van die embriosak is soortgelyk aan dié van *L. europaeum* L. Die afwesigheid van ongereduseerde embriosakke dui daarop dat apomiksie nie by enige ploëidievlak by die spesies wat ondersoek is, voorkom nie.

INTRODUCTION

The genus *Lycium* (Solanaceae) consists of small to large shrubs with a wide distribution in arid to subarid, temperate to subtropical regions of the world (Joubert 1981; Bernardello 1982). The only exceptions, which are more treelike, are *L. afrum* L. and *L. horridum* Thunb. (= *L. austrinum* Miers) (Palmer & Pitman 1973). Seventeen *Lycium* species are known in southern Africa, whereas 50–60 species are found in the western hemisphere (Joubert 1981).

Polyploidy occurs in the genus *Lycium* (Minne 1992; Spies *et al.* 1993). As polyploidy and apomixis are often associated (Stebbins 1971), the aim of this research was to determine whether apomixis occurs in the polyploid specimens. The mode of development of the ovule and the type of embryo sac present in *L. afrum*, *L. arenicolum*, *L. horridum* and *L. tetrandrum* was therefore studied.

MATERIALS AND METHODS

The material used during this study was collected and fixed in the field. Voucher specimens are housed in the Geo Potts Herbarium, Bloemfontein (BLFU).

Specimens examined [haploid chromosome numbers from Minne (1992) and/or Spies *et al.* (1993)]

L. afrum n = 12

CAPE.—3318 (Cape Town): Bokbaai, (–CB), A. Venter 346, 348; Melkbosstrand, R27 road, (–CD), A. Venter 353; Melkbosstrand, Otto du Plessis Ave., 50 m north of Dutch Reformed Church on road shoulder, (–CD), A. Venter 371, 373.

L. afrum × *L. ferocissimum* n = 12

CAPE.—3318 (Cape Town): Melkbosstrand, R27 road, (–CD), A. Venter 352, 355; Melkbosstrand, Otto du Plessis Ave., 50 m north of Dutch Reformed Church on road shoulder, (–CD), A. Venter 372.

L. arenicolum n = 36

CAPE.—2824 (Kimberley): on Barkly West road, 6 km from the Windsorton turnoff to Riverton, (–DA), A. Venter 361; Kimberley Dist., Maidavale, on Nootgedacht road, (–DB), Joubert 339–343.

ORANGE FREE STATE.—2825 (Boshof): Modder River bridge on Petrusburg-Kimberley road, (–CD), Joubert 324, 325; Flamink Pan, 5.5 km west of Modder River bridge on Petrusburg-Kimberley road, (–CD), Joubert 329, 330, 2926 (Bloemfontein): Bloemfontein, University of the Orange Free State Campus, (–AA), Spies 5173.

L. horridum n = 24

CAPE.—2824 (Kimberley): Maidavale, Kimberley Dist. on Nootgedacht road, (–DB), Joubert 338; A. Venter 384, 3119 (Calvinia): 17 km outside Calvinia on the way to Williston (R27) at the R354 turnoff to Middelpos and Sutherland, (–BD), A. Venter 384, 3123 (Victoria West): 7.2 km from Richmond on N1 to Beaufort West, (–BD), A. Venter 366; 13.7 km from Richmond on N1 to Beaufort West, (–BD), A. Venter 368, 3220 (Sutherland): 25 km north of Sutherland, (–BC), Joubert 333, 334, 3224 (Graaff-Reinet): 102 km from Uitenhage to Graaff-Reinet, (–DC), Spies 5234.

ORANGE FREE STATE.—2825 (Boshof): 1 km east of Modder River bridge on Petrusburg-Kimberley road, (–CD), H. Venter 9232, 2925 (Jagersfontein): 36 km from Petrusburg to Kimberley, (–AB), H. Venter 9230, 9231.

L. tetrandrum n = 36

CAPE.—3218 (Clanwilliam): Lambertsbaai beach, (–AB), Spies 5001, 5002, 3318 (Cape Town): just outside Yzerfontein on R315 road shoulder, (–AC), A. Venter 376; in Yzerfontein on vacant lot, (–AC), A. Venter 377; Bokbaai vicinity, (–CB), A. Venter 347; Ganzekraal, (–CB), A. Venter 350, 351, 3420 (Bredasdorp): 53 km from Bredasdorp to Swellendam, (–CA), Spies 5055.

L. villosum n = 24

CAPE.—2824 (Kimberley): 24.7 km to Barkly West from the Kimberley-Grekwastad turnoff, (–DA), Joubert 312, 313; at the Nootgedacht turnoff on the Kimberley-Riverton road, (–DA), A. Venter 388, 389; 6.5

* Department of Botany and Genetics, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300.

† Author to whom correspondence should be addressed.

MS. received 1992-07-13.

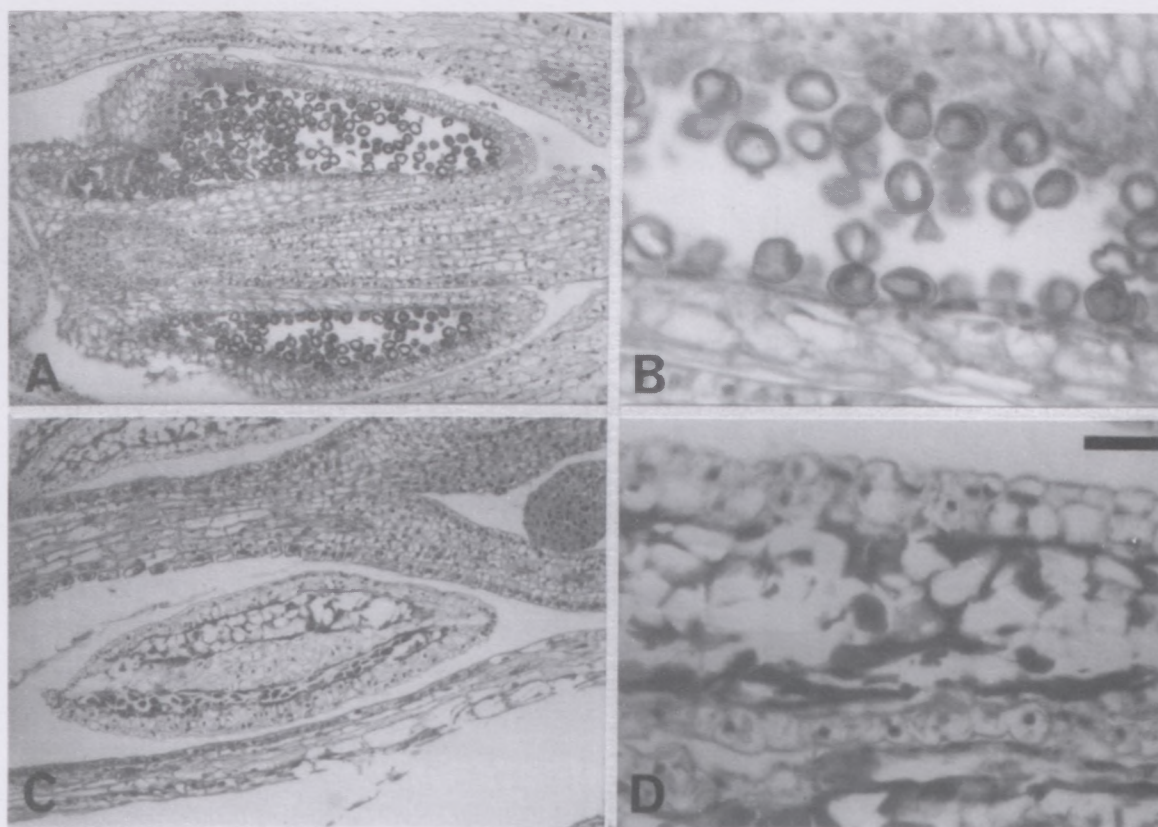


FIGURE 1.—Longitudinal sections through anthers and ovaries of functionally male and female specimens of *Lycium*. A, B, *L. arenicolum*, Joubert 340, pollen in the functionally male plant; C, D, *L. tetrandrum*, Spies 5001, pollen in the functionally female plant. Scale bar: A, C, 136 μ m; B, D, 34 μ m.

km past the Nooitgedacht turnoff on the Kimberley-Barkly West road, (—DA), A. Venter 357; 7 km past the Nooitgedacht turnoff on the Kimberley-Barkly West road, (—DA), A. Venter 358; 8 km from the turnoff to Riverton on the Kimberley-Windsorton road, (—DA), A. Venter 362, 363.

The ovules and anthers of flowers of varying ages were fixed in Carnoy's fixative in the field. The fixative was replaced after 24–48 hours by 70% ethanol. Dehydration was done in ethanol and tertiary butanol after which the flowers were embedded in pastulated synthetic paraffin wax, sectioned at 5–7 mm and stained with Safranin and Fast Green as described by Spies & Du Plessis (1986). At least twenty embryo sacs were studied per specimen.

For the scanning electron microscope (SEM) study, flowers were dissected in order to expose the stamens, ovaries and pistils of the specimens. The dissected flowers were mounted on aluminium stubs with the aid of a fast setting glue. The mounted material was then covered with carbon gold (Spurr 1969) for 135 seconds in the Bio Rad SEM coating system. The structure of the pistil, stamen, and pollen of both sexes of the dioecious species were studied with a Jeol Winsem JSM-6400 scanning electron microscope.

RESULTS AND DISCUSSION

A study of a large number of specimens of *Lycium* in southern African herbaria, Kew (K), British Museum (BM), Paris (P) and a few other herbaria revealed that all specimens of *L. arenicolum*, *L. horridum*, *L. tetrandrum*

and *L. villosum* are functionally dioecious*. Such plants express only one sex, although both male and female organs are present in the same flower. In *Lycium* the term functionally dioecious means the following:

- 1, functionally male plants have fully developed anthers with seemingly fertile pollen (Figures 1A, B; 2B). Usually two of the stamens of the functionally male plants are included in the corolla tube, whereas two or three are a little exerted. These plants also carry female reproductive organs, but the pistil is underdeveloped and not functional (Figure 2A). The ovule will experience difficulties in receiving pollen, since only a rudimentary style without a stigma may occur on the ovary, or the style and stigma are totally absent (Figure 2A). Occasionally fruit can be found on functionally male plants, but this condition is rare.

- 2, in functionally female plants the ovary, style and stigma are normal in structure and slightly exerted from the corolla tube (Figure 2C), whereas the anthers of these flowers are underdeveloped and produce no pollen (Figures 1C, D; 2D). Fruit formation is normal. In the hermaphroditic *Lycium* species every flower bears normal male and female organs.

The ovaries of the *Lycium* specimens are subsessile, glabrous and have two fused carpels with axile placentation. The base of the ovary is enclosed by a greenish, red, orange or yellow-brown nectar gland. The ovules of both functionally male and functionally female plants are unitegmic, tenuinucellate (Figure 3A, B) and anatropous to hemi-campylotropous. These findings correspond to the

* Studied by A.M. Venter.

results obtained by Chiang-Cabrera (1981) on *L. europaeum* L.

During early stages of development, one cell, lying directly below the nucellar epidermis, differentiates into a primary archesporial cell that is conspicuous by its larger size (Figure 3B), denser-cytoplasmic contents and more prominent nucleus. This archesporial cell gives rise to the primary sporogenous cell, that again forms the macrospore mother cell, which divides meiotically. Usually, a linear tetrad of macrospores is formed in *Lycium*. The chalazal macrospore develops into a *Polygonum* type embryo sac that contains an egg cell (Figure 3F), two synergids (Figure 3C, G), two polar nuclei (Figure 3C, D) and three antipodal cells (Figure 3H, I). These findings correspond to the results obtained by Chiang-Cabrera (1981) on *L. europaeum*.

In *Lycium* the ovules of both the functionally male and functionally female plants have a monosporic eight-nucleate *Polygonum* type embryo sac. In the functionally male plants the embryo sac is fully developed, although no fertilization takes place because there is no stigma, only an ovary with, at most, a rudimentary style.

Lycium horridum is functionally dioecious, whereas *L. ferocissimum* is hermaphroditic. All the *L. horridum* × *L.*

ferocissimum hybrid specimens examined were functionally dioecious. Therefore, the gene(s) causing functional dioecy is/are dominant over the gene(s) for hermaphroditism.

The embryo sacs of *L. afrum* and the *L. afrum* × *L. ferocissimum* hybrid are of the normal eight-nucleate *Polygonum* type. All the nuclei in the embryo sacs of the studied species and hybrid, are of similar size. In the dioecious *L. horridum* the nuclei of the embryo sac differ in size. In the functionally male plant, all the nuclei are of the same size, but in the functionally female plants, the polar nuclei are much larger than any of the other nuclei. Both the functionally male and functionally female plants of *L. arenicolum* and *L. tetrandrum* have larger polar nuclei than the functionally female plants of *L. horridum*, although these larger nuclei only occurred in one of the two functionally male specimens of *L. arenicolum* examined.

Cytogenetic studies showed that *L. arenicolum* and *L. tetrandrum* display more meiotic abnormalities, such as univalents and bivalents during metaphase, as well as an anaphase bridge in *L. tetrandrum*, than *L. afrum* and *L. horridum* (Minne 1992; Spies *et al.* 1993). Although polyploidy and meiotic abnormalities occur during microsporogenesis in the anthers of the functionally male plants,



FIGURE 2.—Anthers and ovaries of functionally male and female specimens of *Lycium*. A, *L. villosum*, A. Venter 362, ovary of functionally male plant; B, *L. villosum*, A. Venter 362, anthers of functionally male plant; C, *L. villosum*, A. Venter 358, pistil of functionally female plant; D, *L. villosum*, A. Venter 358, anthers of functionally female plant. Scale bars: A, B, D, 100 µm; C, 1 mm.

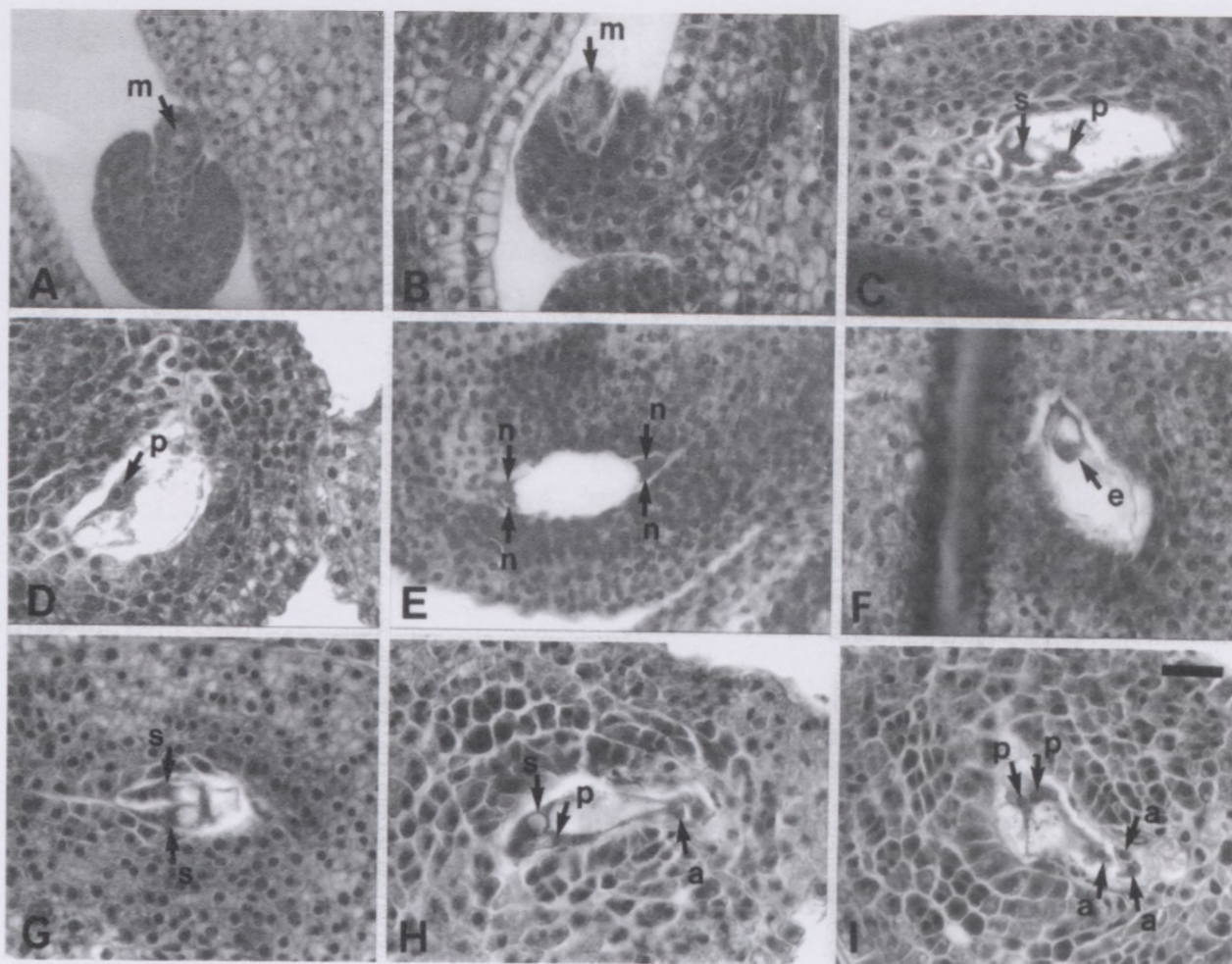


FIGURE 3.—Embryo sac development in some *Lycium* specimens. A, E, F, *L. afrum*: A, A. Venter 352; E, F, Joubert 346. B, *L. arenicolum*, Joubert 340. C, *L. horridum*, Joubert 334. D, G–I, *L. tetrandrum*: D, Spies 5001; G, A. Venter 376; H, I, Spies 5055; H, central cell including only one polar nucleus. a, antipodal cell; e, egg cell; m, macrospore mother cell; n, nucleus of four nucleate embryo sac; p, polar nucleus; s, synergid. Scale bar: 34 μ m.

all the functionally female plants have normal embryo sacs (Minne 1992). The pollen of the functionally male plants was fully developed, whereas the pollen in the functionally female plants degenerated.

CONCLUSIONS

Functionally dioecious species are present in *Lycium*. All species studied, the hermaphroditic as well as the dioecious ones, have an eight-nucleate *Polygonum* type of embryo sac. The nuclei of the synergids, egg cell, central cell and antipodal cells were the same size in the hermaphroditic *L. afrum* and the functionally male plants of *L. horridum*. In the functionally female plants of *L. horridum*, the nuclei of the central cell, i.e. the polar nuclei, were larger than the other nuclei of the embryo sac. However, in *L. arenicolum* and *L. tetrandrum*, the polar nuclei of both sexes were larger than the other nuclei of the embryo sac. At all polyploid levels *Polygonum* type embryo sacs were formed, irrespective of the nature and number of meiotic abnormalities in the anthers. The absence of unreduced embryo sacs indicates that apomixis does not occur at any ploidy level in the species studied. A conspicuous difference in pollen development was also evident. Seemingly viable pollen was found in the functionally male plants, but none in the functionally female plants.

ACKNOWLEDGEMENTS

We thank the University of the Orange Free State for facilities provided during this study and the Foundation for Research and Development for financial support.

REFERENCES

- BERNARDELLO, L.M. 1982. Estudios en *Lycium* (Solanaceae). II. Recuentos cromosomicos en entidades Argentinas. *Hickenia* 1: 321–328.
- CHIANG-CABRERA, F. 1981. A taxonomic study of the North American species of *Lycium* (Solanaceae). Ph.D. thesis, Department of Botany, University of Texas, Austin.
- JOUBERT, A.M. 1981. 'n Taksonomies-morfologiese studie van *Lycium* in suider-Afrika. M.Sc. thesis, Department of Botany, University of the Orange Free State, Bloemfontein.
- MINNE, L. 1992. A biosystematic study of the genus *Lycium* section *Lycium* (Solanaceae). M.Sc. thesis, Department of Botany and Genetics, University of the Orange Free State, Bloemfontein.
- PALMER, E. & PITMAN, N. 1973. *Trees of southern Africa*. Balkema, Cape Town.
- 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.
- SPIES, J.J., MINNE, L., VENTER, H.J.T. & VENTER, A.M. 1993. A cytogenetic study of the functionally dioecious species in the genus *Lycium* (Solanaceae). *South African Journal of Botany* 59: 535–540.
- SPURR, A.R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructural Research* 26: 31–34.
- STEBBINS, G.L. 1971. *Chromosomal evolution in higher plants*. Edward Arnold, London.