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Apomixis and Polyembryony in the Guggul Plant, *Commiphora wightii*

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The present report is a part of our study on the reproductive biology of a traditional Indian medicinal plant, *Commiphora wightii*, a source of guggul steroids. Field examination showed a predominantly large number of isolated and groups of female individuals. Only one andromonoecious and two exclusively male plants were recorded. Female plants set seed irrespective of the presence or absence of pollen. Hand-pollination experiments and embryological studies have confirmed the occurrence of non-pseudogamous apomixis, nucellar polyembryony and autonomous endosperm formation for the first time in this plant, which is presently threatened by over-exploitation.

Key words: *Commiphora wightii*, autonomous endosperm, guggul, medicinal plant, non-pseudogamous apomixis, nucellar polyembryony.

INTRODUCTION

Commiphora wightii (Arnott) Bhandari of the family Burseraceae is a slow growing, highly branched, shrubby plant that grows wild in the arid rocky tracts of Rajasthan, Gujarat, Madhya Pradesh and Karnataka states of India, and in the Sind and Baluchistan states of Pakistan. It yields guggul, an important oleo-gumresin used as incense, fixative in perfumery and in Ayurvedic medicine. Its antiarthritic, hypocholesterolaemic and hypolipidaemic properties have been established (Satyavati, 1990) and a commercial product 'guglip' has been marketed in India since 1988. Owing to the enormous demand for the drug, the plant is subjected to crude and destructive tapping procedures. There is an important need to conserve its germplasm and ensure a sustained supply of the valuable raw material.

The anatomy of the oleo-gumresin ducts has been reported (Setia, Parthasarathy and Shah, 1977). The ultrastructural details of secretion, seasons of production and methods of enhancing the yield have been reported (Bhatt, Nair and Mohan Ram, 1990). However, there is no critical information on the reproductive biology, natural means of seed dispersal and seedling establishment of this plant. The embryological account of *Commiphora* is sketchy and ambiguous (Wiger, 1934; Mauritzon, 1935; Shukla, 1954). It is uncommon to see seedlings in the vicinity of wild adult plants. Propagation through cuttings is a commercial practice.

In this study we report on the reproductive characteristics of *C. wightii* and present evidence for the occurrence of apomixis, polyembryony and autonomous endosperm development.

MATERIAL AND METHODS

This investigation is based on plants raised in plantations and those from natural habitats (Table 1). Phenological details and sexual fidelity of marked plants were studied through regular field visits during 1992–95.

To determine the breeding system, female flower buds were bagged and the pistils were observed until fruit development. In the only andromonoecious individual identified at the Central Arid Zone Research Institute (CAZRI), Jodhpur, 50 bisexual flower buds were emasculated 1 d prior to anthesis and bagged, and another 50 buds were bagged without emasculation to assess self-pollination. Stigmas from a large number of open flowers were examined under a microscope for the presence of pollen grains on day 0 and up to 4 d after anthesis (DAA).

Hand-pollinations were also made *in vivo* and on excised pistils implanted in petri dishes containing agar (0·8%) medium and maintained under laboratory conditions. The pistils were fixed 6–48 h after pollination in Carnoy's fixative. Fixed pistils were cleared overnight in 4 N NaOH and mounted in decolourized aniline blue with one drop of 50% glycerine (Martin, 1959). The preparations were observed under fluorescence microscope (Nikon Optiphot) for pollen germination and pollen tube growth.

For embryological studies flower buds and ovaries at various stages of development were fixed in FAA (formalin: acetic acid: 90% ethanol, 1:1:9), dehydrated through an ethanol series and embedded in paraffin. Serial sections were cut at a thickness of 8–12 μm and stained with iron-hematoxylin-erythrosin or Harris hematoxylin-erythrosin combination. Some representative stages of flower buds and fruits were also fixed in acrolein, dehydrated in ethanol series and embedded in glycol methacrylate monomer mixture (Feder and O'Brien, 1968). Thin sections (2–3 μm) were cut with glass knives and stained with toluidine blue O.

Some of the sections of ovules and young seeds were also stained with decolourized aniline blue and observed under the fluorescence microscope for localization of callose surrounding embryo initials in the nucellus (Wakana and Uemoto, 1988).

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TABLE 1. *Details of the age, number of plants used at various sites and their sex expression*

Sites	Garden, Delhi University Botany Department, Delhi	Central Arid Zone Research Institute, Rajasthan	Indroka village Jodhpur, Rajasthan	Guggul Herbal Farm, Ajmer, Rajasthan	Vasad, Gujarat
Nature of plantation	Irrigated	Irrigated	Natural	Irrigated	Natural
Total no. of plants	2	58	75	1000	50
Age of plants (years)	7	8–25	> 20	> 25	> 25
No. of male plants	0	2	0	0	0
No. of female plants	2	55	75	1000	50
No. of andromonoecious plants	0	1	0	0	0

To study ovule development, pistils dissected from flower buds and flowers at various intervals were fixed in FPA (formalin : propionic acid : 50 % ethanol, 5 : 5 : 90) and cleared using a clearing-squash technique (Herr, 1971). Aceto-carmine squash preparations (Bradley, 1948) were used to trace the development of embryo sacs. Fully mature and dry fruits were collected in different seasons and were dissected to estimate seed set and to determine the presence of embryos.

RESULTS

Individual plants exhibiting a particular flower sex (Table 1) in the first year of observations maintained the same sex in the subsequent years.

In spite of the absence of male plants or presence of only two male plants in the population (Table 1), all females showed seed bearing fruits. Seeded fruits were harvested from the bagged female flower buds and also from emasculated and bagged bisexual flowers.

Microscopic examination of stigmas of 456 flowers collected from female plants growing at different sites showed no pollen grains. The cleared pistil preparations of 315 hand-pollinated flowers disclosed germination of a limited number of pollen grains (Fig. 1A). However, the pollen tubes were mostly confined to the stigma surface; one or two pollen tubes were observed only in five pistils in the distal one third portion of the style. Pollen tubes never entered the ovary. They showed thick callose deposition and were often branched (Fig. 1B).

The ovule is anatropous, bitegmic and crassinucellate (Fig. 3A). A *Polygonum* type of embryo sac (Fig. 2A–C) develops deep in the nucellus. Frequently two embryo sacs were observed (in 27 out of 100 ovules analysed) (Fig. 3A). However, at anthesis partial or complete degeneration of embryo sac(s) was noted (Fig. 3C). In the former condition, the egg apparatus and the antipodal cells were found to have been resorbed whereas the polar nuclei were intact (Figs 2D and 3C). The polar nuclei fused and gave rise to a free nuclear endosperm. There was not a single instance of parthenogenesis.

The nucellar cells situated above the embryo sac were richly cytoplasmic and contained a large nucleus each (Fig. 3B), whereas the peripheral nucellar cells had large vacuoles and sparse cytoplasm. Sections of young seeds (15 DAA) occasionally showed degenerated embryo sacs with hypertrophied and highly vacuolated nucellar cells bordering them (Fig. 3E).

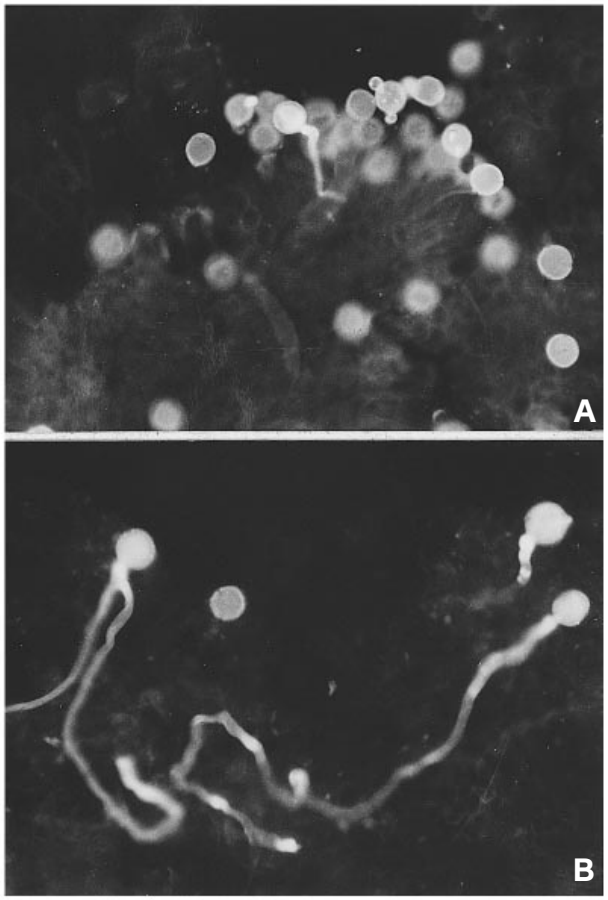

FIG. 1. Pollen germination and pollen tube growth in hand-pollinated pistils. Whole mounts of cleared stigmas stained with decolourized aniline blue. A, Only one pollen grain out of many has germinated (× 113). B, Portion of stigma with elongated pollen tubes on the surface. One pollen tube has branched (× 113).

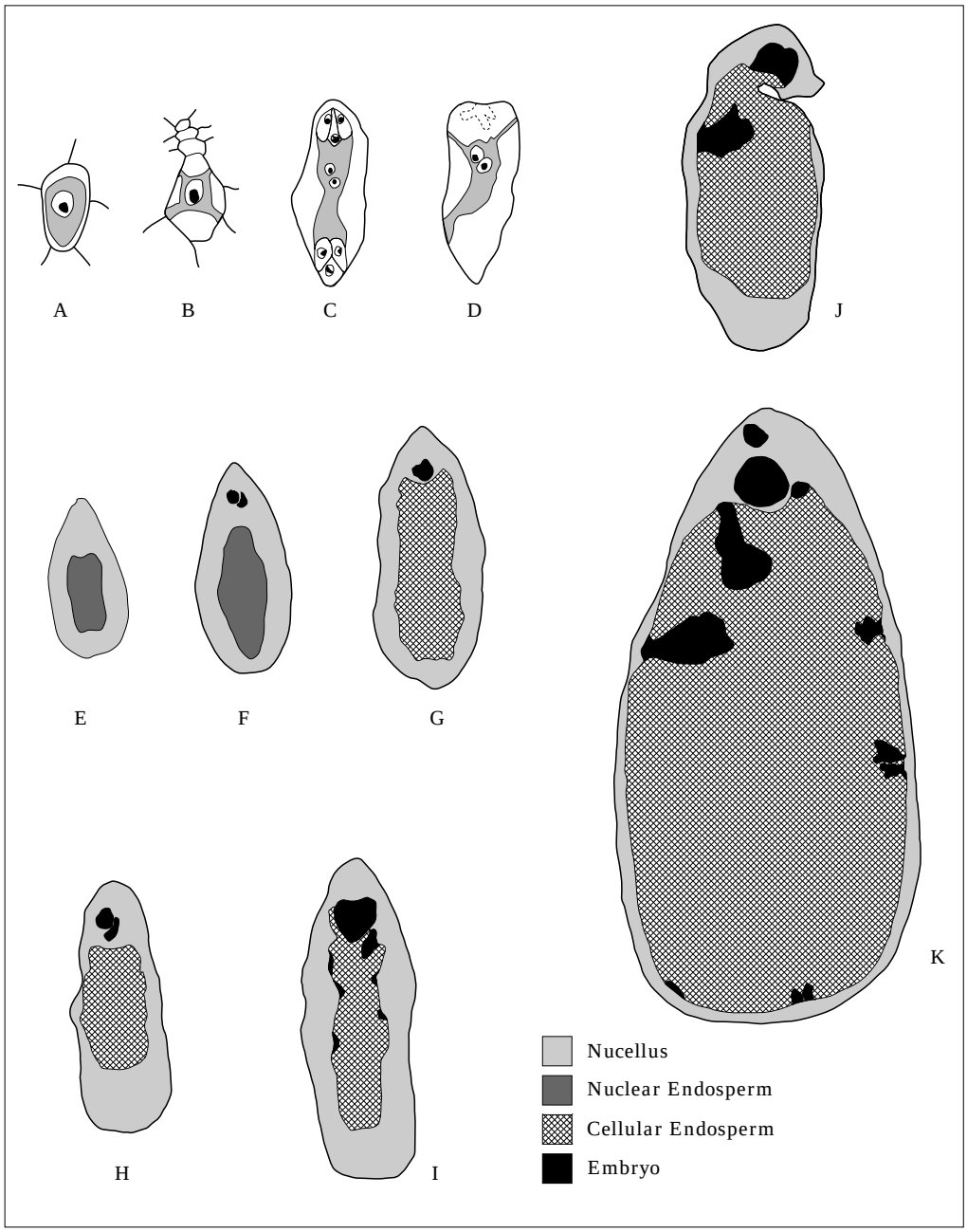

FIG. 2. Diagrammatic representation of the development of embryo sac, endosperm and nucellar embryos (based on dissection and serial sections). A, Megaspore mother cell. B, A megaspore tetrad with a functional chalazal megaspore and three degenerating micropylar megaspores. C, Organised *Polygonum* type of embryo sac. D, Embryo sac at a later stage showing juxtaposed polar nuclei and remnants of degenerated egg apparatus. The antipodals have been resorbed. E, Free nuclear endosperm (20 DAA). F, Note two young embryos in the micropylar part of the nucellus (25 DAA). G–K, Later stages reconstructed from serial sections to show cellular endosperm and differentiation and development of numerous nucellar embryos, especially towards the micropyle.

Sections of slightly older seeds (25–40 DAA) contained several nucellar embryos at various stages of development in the micropylar region of the nucellus (Fig. 2F–H). Incipient embryos were frequently present just beneath the nucellar epidermis. The embryonal initials as well as young embryos were isolated by a distinct callose wall (Fig. 3D, H). In older embryos, the surrounding callose wall was discontinuous (Fig. 3H).

Even in the absence of double fertilization, a large number of ovules showed free nuclear endosperm (Fig. 2E, F) which eventually became completely cellular (Fig. 3F). The details of development of nucellar embryos and endosperm are depicted in Fig. 2 (E–K). The number of nucellar embryos that were initiated varied from 2 to 11 (Fig. 2K). Two to six of them reached the globular or older stages as seen in dissected young fruits. In later stages of seed development (40–60 DAA), the embryos were found embedded in the deeper layers of the endosperm tissue (Fig.

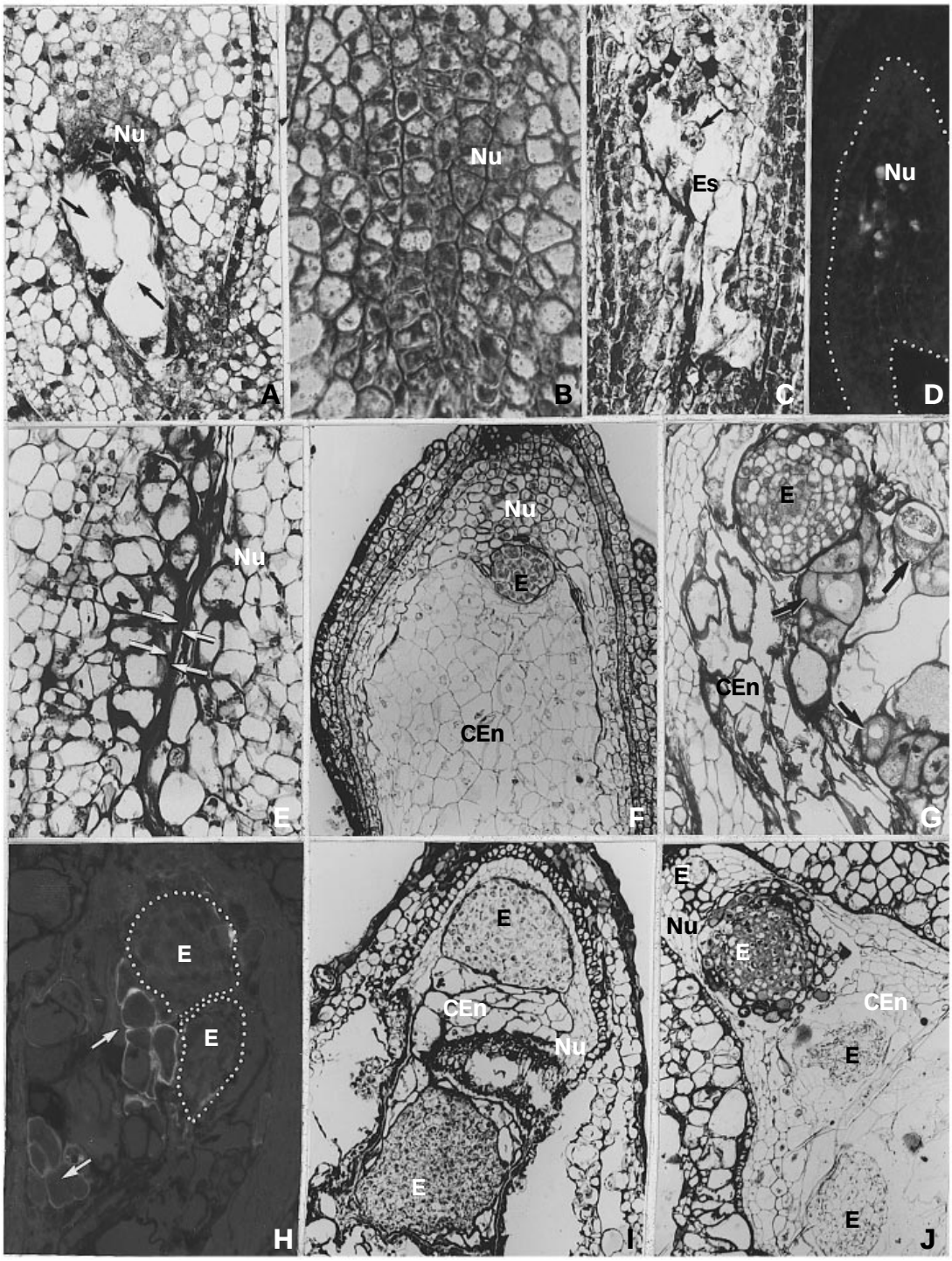

FIG. 3. Photomicrographs of longisections of ovules and young seeds showing the origin and development of nucellar embryos and endosperm. The micropylar pole is pointed up in all the photographs. A, Ovule showing crassinucellate condition and twin embryo sacs (arrows). (× 185). B, Micropylar part of nucellus enlarged to show the richly cytoplasmic cells of the nucellus. (× 271). C, Portion of an ovule showing degenerating twin embryo sacs. The embryo sac on the left shows degenerated egg apparatus and two appressed polar nuclei (arrow). (× 176). D, Fluorescent micrograph of an ovule (8 DAA) stained with decolourized aniline blue. Note callose deposition around initials of nucellar embryos. Outline of nucellar tissue is indicated by dotted line. (× 130). E, Portion of an ovule (15 DAA) showing hypertrophied nucellar cells which have obliterated the embryo sac. Arrows indicate the position of embryo sac. Such ovules degenerate at an early stage. (× 226). F, Micropylar part of seed (30 DAA). A globular nucellar embryo is seen partly embedded in the micropylar part of cellular endosperm. (× 136). G, A part of seed (40 DAA). An early heart-shaped embryo is prominently seen. Other nucellar embryo initials (arrows) are distinct by their deep staining. Endosperm cells show marked vacuolation. (× 108). H, Another section of the same seed shown at G stained with decolourized aniline blue. The callose is not continuous round the two older embryos. The nucellar embryo initials are encased by a callose wall (arrows). (× 125). I, Micropylar part of an

3G–J). In about 50% of the seeds, the endosperm cells became hypertrophied and vacuolated (Fig. 3G, I) indicating the onset of disintegration. Seeds with atrophied endosperm probably abort later. During seed maturation the nucellus and endosperm are consumed and the embryos fill up the entire seed. The cotyledons (usually two and occasionally three) are massive and contorted, and show the presence of resin canals lined by an epithelial layer.

The fruits are red when fully ripe and typically two chambered drupes (occasionally three or four chambered). Only about 50% of the fruits examined contained mature seeds, the remaining were either empty or contained aborted or shrivelled seeds. Dissections of seeds from mature fruits generally showed the presence of twin embryos at the same stage of development.

DISCUSSION

The present report documents for the first time in *C. wightii* (*a*) formation of seeded fruits from female flowers with no access to pollen or even after emasculation and bagging, (*b*) failure of pollen tubes to enter the ovule following hand pollination, (*c*) occurrence of nucellar polyembryony and (*d*) autonomous development of endosperm. These events clearly indicate that *C. wightii* is a non-pseudogamous apomict.

Apomixis has been observed in more than 300 species of flowering plants belonging to 35 families (Hanna and Bashaw, 1987). Of these, adventive embryony is recorded in over 250 species (Naumova, 1989). Nucellar polyembryony is reported in 16 out of 172 crassinucellate families of angiosperms (Rangaswamy, 1981). Integumentary polyembryony is noticed in tenuinucellate ovules of certain plants but none of these embryos reaches maturity. *Garcinia mangostana* represents the only example of obligate apomixis in a woody perennial in which a single adventitious embryo develops from a cell in the inner integument (Horn, 1940). In this plant, the nucellus and the embryo sac become non-functional (Lim, 1984). According to Lakshamanan and Ambegaokar (1984), no polyembryonic seed bearing integumentary or endothelial embryos at maturity has been recorded. Several dipterocarps (*Shorea ovalis*, *S. agamis*, *Hopea subalata*) (Kaur *et al*., 1978) and Clusiaceae (*Clusia rosea*, *Garcinia parvifolia*) (Maguire, 1976; Ha *et al*., 1988) are also reported to be apomictic (the authors do not specify clearly whether they are obligate or facultative or both). Kaur *et al*. (1986) suggested that apomixis may have a significant role in the speciation of tropical trees. These authors believe that apomicts may be favoured by natural selection if the population densities are low and distances between individual trees are greater than the permissible cross-pollination range, or when the species occupy narrow niches. Apomixis should be a product of natural selection although asexual reproduction should certainly put a brake on natural selection.

In vivo initiation of adventive embryos without the stimulation of pollination and fertilization appears to be a characteristic feature of habitual apomictic plants (Wakana and Uemoto, 1987). However, most recorded cases of apomictic plants require endosperm (generated by pseudogamy) for their development (Hanna, 1991; Asker and Jerling, 1992; Koltunow, 1993). In *C. wightii*, endosperm development is not pseudogamous as there is no pollen tube entry into the ovule. Moreover amphimictic endosperm formation is a widespread phenomenon but the occurrence of autonomous endosperm development is rather rare (Naumova, 1989). The origin of the endosperm in *C. wightii* is being investigated further to obtain a clearer understanding of the mechanism involved. Nevertheless, the development of endosperm seems to be necessary for continued growth of nucellar embryos. Nearly all ovules in *C. wightii* show initiation of nucellar embryos. However, embryonate seeds are seen in only 50% of the mature fruits. It is likely that failure of nucellar embryos to attain maturity is associated with the lack of endosperm development or its early breakdown. This aspect is being studied.

Autonomous apomixis and polyembryony in *C. wightii*, a native of semi-arid regions of India could be a strategy for reproduction and survival in the absence of male plants. Nevertheless, the presence of two males and one andromonoecious individuals in one of the populations indicates that in other populations/conditions, sexual reproduction could occur. More populations are being screened to identify sexual plants.

Adventitious embryony is important for seed production in plants in which double fertilization is limited (Naumova, 1981). Also in dioecious forms, sexual reproduction might be looked upon as an extravagant allocation of resources, and in contrast it may be visualised that apomictic plants have a reproductive superiority twice that of sexually reproducing ones (Asker, 1979; Yahava, 1990). A 'clonal seed' comprising multiple embryos has certain advantages over the monoembryonic sexual seed, the significance of which is being examined.

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LITERATURE CITED

Asker S. 1979. Progress in apomixis research. *Hereditas* **91**: 231–240.
Asker S, Jerling L. 1992. *Apomixis in plants*. Boca Raton: CRC Press.

obliquely sectioned seed (50 DAA). Two embryos of advanced stage are embedded in the endosperm. The part of the endosperm below the upper embryo has become vacuolated. The darkly stained tissue between the two embryos represents the seed coat. (×130). J, A part of seed (60 DAA) with four embryos. Three of the embryos are faintly stained. This section is from the seed depicted in Fig. 2K. (×119). CEn, Cellular endosperm; E, embryo; Es, embryo sac; Nu, nucellus.

Bhatt JR, Nair MNB, Mohan Ram HY. 1990. Enhancement of oleo-gumresin production in *Commiphora wightii* by improved tapping technique. *Current Science* **58**: 349–357.

Bradley MV. 1948. An aceto-carmine squash technic for mature embryo sacs. *Stain Technology* **23**: 29–40.

Feder N, O'Brien TP. 1968. Plant microtechnique: some principles and new methods. *American Journal of Botany* **55**: 123–142.

Ha CO, Sands VE, Soepadmo E, Jong K. 1988. Reproductive patterns of selected understory trees in Malaysian rain forest: the apomictic species. *Botanical Journal of Linnean Society* **97**: 317–332.

Hanna WW. 1991. Apomixis in crop plants—cytogenetic basis and role in plant breeding. In: Gupta PK, Tsuchiya T, eds. *Chromosome engineering in plants: genetics, breeding, evolution*, Part A. Amsterdam: Elsevier, 229–242.

Hanna WW, Bashaw EC. 1987. Apomixis: its identification and use in plant breeding. *Crop Science* **27**: 1136–1139.

Herr Jr JM. 1971. A new clearing squash technique for the study of ovule development in angiosperms. *American Journal of Botany* **58**: 785–790.

Horn CL. 1940. Existence of only one variety of cultivated mangosteen explained by asexually formed 'seed'. *Science* **92**: 237–238.

Kaur A, Ha CO, Sands VE, Chan HT, Soepadmo E, Ashton PS. 1978. Apomixis may be widespread among trees of the climax rain forest. *Nature* **271**: 440–442.

Kaur A, Jong K, Sands VE, Soepadmo E. 1986. Cytoembryology of some Malaysian dipterocarps, with some evidence of apomixis. *Botanical Journal of Linnean Society* **92**: 78–88.

Koltunow AM. 1993. Apomixis: embryo sacs and embryos formed without meiosis or fertilization in ovules. *The Plant Cell* **5**: 1425–1437.

Lakshamanan KK, Ambegaokar KB. 1984. Polyembryony. In: Johri BM, ed. *Embryology of angiosperms*. Berlin: Springer Verlag, 445–474.

Lim AK. 1984. The embryology of *Garcinia mangostana* L. (Clusiaceae). *The Gardens' Bulletin, Singapore* **37**: 93–103.

Maguire B. 1976. Apomixis in the genus *Clusia* (Clusiaceae)—a preliminary report. *Taxon* **25**: 241–244.

Martin FW. 1959. Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technology* **34**: 125–128.

Mauritzon VJ. 1935. Kritic von J. Wigers Abhandlung 'Embryological studies on the families Buxaceae, Meliaceae, Simarubiaceae and Burseraceae'. *Botaniska Notiser*: 490–502.

Naumova TN. 1981. Nucellar and integumentary embryony in angiosperms. *Acta Societatis Botanicorum Poloniae* **50**: 213–216.

Naumova TN. 1989. Apomixis and amphimixis in angiosperms: classification. *Apomixis Newsletter* **2**: 33–38.

Rangaswamy NS. 1981. Nucellus as an experimental system in basic and applied tissue culture research. In: Rao AN, ed. *Proceeding of the COSTED symposium on 'Tissue culture of economically important plants'*, Singapore, 269–289.

Satyavati GV. 1990. Use of plant drugs in Indian traditional systems of medicine and their relevance to primary health care. In: Wagner H, Farnsworth NR, eds. *Economic and medicinal plant research*, vol 4. London: Academic Press, 39–56.

Setia RC, Parthasarathy MV, Shah JJ. 1977. Development, histochemistry and ultrastructure of gum-resin ducts in *Commiphora mukul* Engl. *Annals of Botany* **41**: 999–1004.

Shukla RD. 1954. Gametophytes in *Balsamodendron mukul* Hook. *Current Science* **23**: 333.

Wakana A, Uemoto S. 1987. Adventive embryogenesis in *Citrus* I. The occurrence of adventive embryos without pollination or fertilization. *American Journal of Botany* **74**: 517–530.

Wakana A, Uemoto S. 1988. Adventive embryogenesis in *Citrus* (Rutaceae) II. Post fertilization development. *American Journal of Botany* **75**: 1033–1047.

Wiger J. 1934. *Embryological studies on the families Buxaceae, Meliaceae, Simarubiaceae and Burseraceae*. Diss. Lund.

Yahava I. 1990. Evolution of agamospermous races in *Boehmeria* and *Eupatorium*. *Plant Species Biology* **5**: 183–196.