Proc. Indian natn. Sci. Acad. **B47** No. 4 pp. 519–526 (1981)

Floral Morphology, Development of Sporangia and Sporogenesis in *Dendrocalamus hamiltonii*

B HARI GOPAL and MANASI RAM*
Department of Botany, University of Delhi, Delhi 110007

(Received 4 April 1981)

The anther is tetrasporangiate, and the development of its wall is of the monocotyledonous type described by Davis. The mature anther wall shows spinulate cuticle and radial thickenings in the epidermis and the endothecium. Successive cytokinesis in the microspore mother cells results in the formation of isobilateral tetrads. The pollen grains are shed at the 3-celled stage. The ovules are hemianatropous, bitegmic and pseudocrassinucellate. The integuments do not cover the nucellus completely. The embryo sac development is of the polygonum type. The number of antipodal cells ranges from 40 to 60.

Key Words: *Dendrocalamus*, **Angiosperm embryology, Floral morphology, Sporogenesis**

Introduction

The tribe Bambuseae comprising both herbaceous and woody bamboos has been considered as the most primitive among the grasses (Yakovlev 1950). Although the value of studies dealing with the ontogeny of reproductive organs in the Bambuseae has been realized, the existing information is fragmentary, on account of the fact that a large majority of the bamboos are monocarpic and show gregarious flowering at intervals ranging from 1 to 120 years (Kawamura 1927, Mc Clure 1966). Sporadic flowering, however, occurs in some species. A survey of the embryological literature shows that there are only a few accounts dealing with *Bambusa* (Yamura 1933, Philip 1972, Philip & Haccius 1976), *Arundinaria chino* (Yoshida 1963) and *Melocanna bambusoides* (Yakovlev 1970, Yakovlev & Kamelina 1973). These works also largely deal with embryo development and not with the early embryological events. Reeder (1957, 1962) has discussed the systematic importance of the structure of the mature bambusoid embryo.

The present report deals with floral morphology and structure and development of micro- and megasporangia and sporogenesis in *Dendrocalamus hamiltonii*.

*Department of Botany, Miranda House, University of Delhi, Delhi 110007

Material and Methods

According to Gamble (1896) this bamboo flowers sporadically and that clumps in flower may always be found in some region or the other. The flowering material of different developmental stages was collected from the forests near Burnihat (Assam) in June 1978, and fixed in FAA (formalin : acetic acid : 70% ethyl alcohol :: 5 : 5 : 90) for 24 hr and stored in 70% ethyl alcohol. Some material was also fixed in a mixture of lactic acid and glycerine (1 : 1) as it acts as a good preservative and clearing agent (Calderon & Soderstrom 1973). After dehydrating the material in alcohol : xylene series, infiltration and embedding were done in paraffin wax. Microtome sections were cut at a thickness of 8–10μ for microsporogenesis and at 12–14μ for megasporogenesis. The sections were stained with haematoxylin and erythrosin.

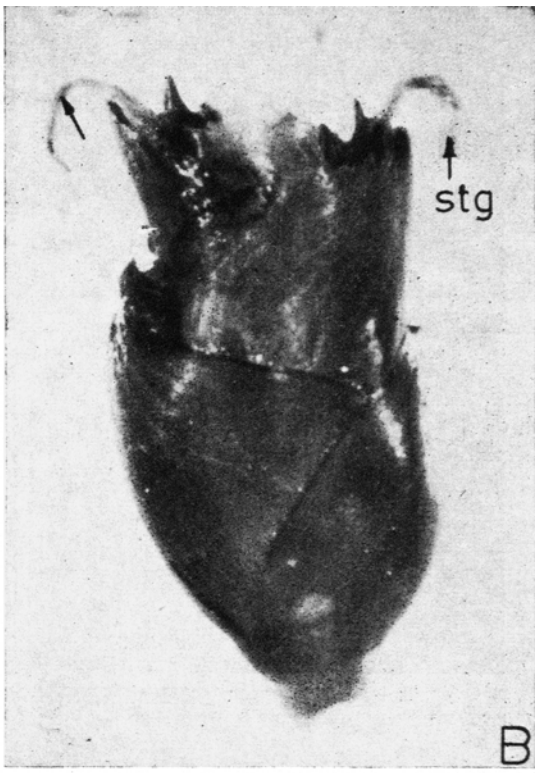
Observations

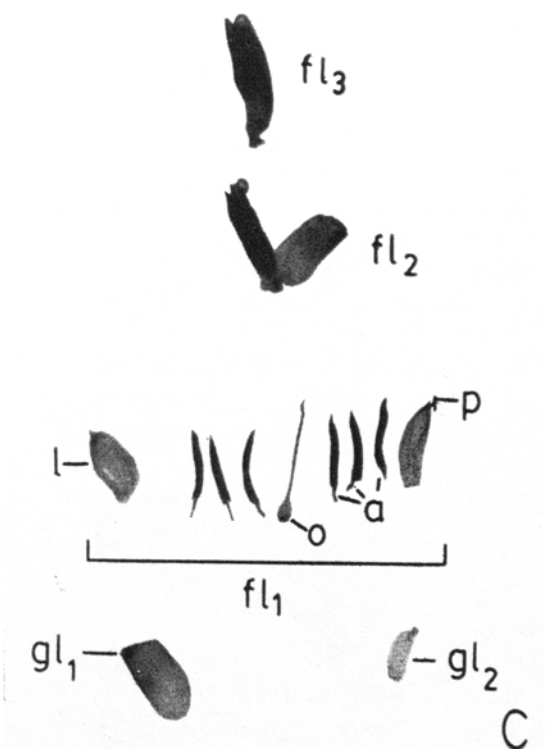
External morphology: Gamble (1896) has given an excellent description of this plant and a brief account is given below: It is a large bamboo (12–14 M high) with a strongly segmented cauline axis and a well-developed rhizome. The inflorescence is a huge, much-branched panicle with many whorls of branchlets, bearing semi-globular heads of purple spikelets supported by rounded scarious bracts (figure 1A). Heads vary from 1.2 cm to 3.5 cm in diameter and bear fertile spikelets. Each spikelet has 2–4 florets (figure 1B, C) and each floret bears six stamens arranged in a single whorl and devoid of staminodes and lodicules (figure 1D). Stamens are 1.5 to 2 cm long and pendulous, and the anthers are tetrasporangiate and purple. In a mature floret the ovary is suborbicular and hairy, with a long hairy style and a plumose stigma. Gamble has described the stigma as trifid, but our observation shows that it is simple (figure 1B, C).

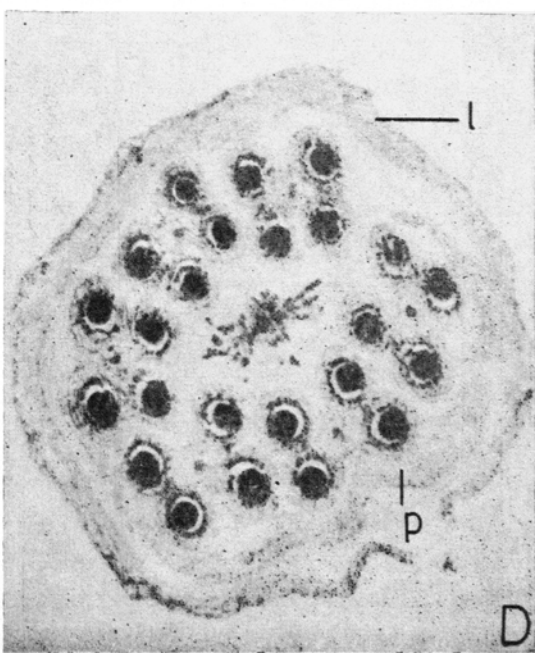
Microsporangium and microsporogenesis: The archesporium is hypodermal in origin and in transverse section is represented by 2 or 3 large, darkly stained cells (figure 2A, arrow). These cells divide periclinally and produce a primary parietal layer and a primary sporogeneous layer. The former divides and produces two secondary parietal layers (figure 2B, arrow). Of these the outer layer directly becomes the endothecium while the inner layer divides periclinally to produce a middle layer and a tapetum (figure 2C arrow). Thus the anther wall has four cell layers—epidermis, endothecium, one middle layer and tapetum (figure 2D). The endothecial cells are gorged with compound starch grains (figure 2E, arrow). The cells of the sporogenous tissue become separated from one another, assume a spherical outline and enter into meiotic divisions. It is interesting to note that the divisions begin at the tip of the anther and proceed downwards. Thus in the same anther various divisional stages can be observed. After meiosis I, a cell plate is laid down at the centre of the microspore mother cell and it extends centrifugally on both the sides, dividing it into two equal halves. Cytokinesis is successive (figure 2E, F). The microspores are always arranged in isobilateral tetrads (figure 2F). A well-developed callose wall

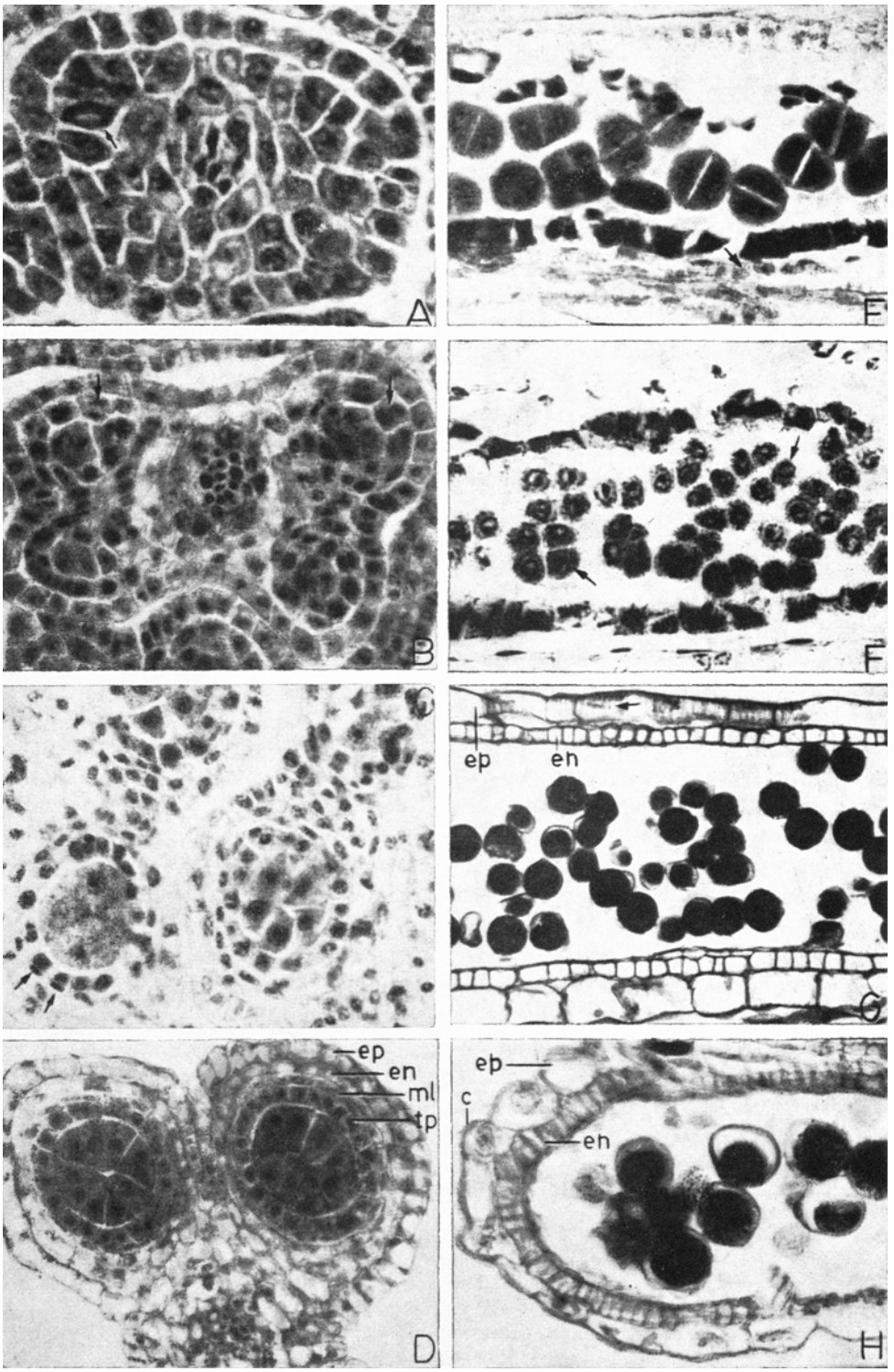
Figure 1 A–D Floral morphology (**a**, stamens; **fl**, floret; **gl**, glume; **l**, lemma; **o**, ovary; **p**, palea; **stg**, stigma). *A*, a portion of the inflorescence containing heads of spikelets (×1). Note the projecting stamens; *B*, one spikelet enlarged to show the protruding stigma (×9); *C*, a spikelet dissected out to show two empty glumes (gl_1 and gl_2) and three fertile florets (×2). The parts of the lowermost floret (fl_1) have been dissected and displayed. Note the six stamens and the absence of lodicules; *D*, transection of the apical part of a floret showing lemma, palea, six stamens and style (in the centre) (×25)

A


stg
B


fl3
fl2
l
p
a
o
fl1
gl1
gl2
C


l
p
D


A
E
B
F
ep
en
G
ep
en
ml
tp
D
ep
c
en
H

around the microspore mother cells is absent (figure 2E, F). No chromosomal abnormalities have been observed during meiosis. The mature pollen grains are spherical, smooth and monoporate, and three-celled at the time of shedding. They contain abundant starch as reserve material.

At the mature pollen grain stage, the wall of the anther shows epidermal cells which are elongated (in the longitudinal direction) with radial thickenings (figure 1G) and prominent spinulate cuticle (figure 1H). The endothecium consists of small thick-walled cells in which the starch grains show gradual disintegration. The tapetum is of the secretory type and its cells are binucleate. The radial thickenings of the endodermal cells can be seen clearly in the transection of the anther (figure 1H).

The lemma and the palea do not become separated in the spikelet; the anthers and stigma emerge through the apical pore between lemma and palea (figure 1B), the stigma coming out first. The anther dehisces by a longitudinal slit.

Ovule and megasporogenesis: The single ovule primordium arises as a small bulge from the side of the ovary (figure 3A). The integuments differentiate from the base of the bulge (figure 3B), the inner appearing first; each integument is two cells thick. The outer integument is shorter than the inner. Neither of the integuments covers the tip of the nucellus. Thus, there is no true micropyle in this plant (figure 3F, G).

The hypodermal archesporial cell (figure 3B) directly functions as the megaspore mother cell and divides to produce a linear tetrad of megaspores (figure 3D), of which the three towards the micropylar end degenerate and the fourth becomes the functional megaspore. In some cases the divisions in the dyad cells are asynchronous (figure 2C). The functional megaspore enlarges and becomes vacuolated and its nucleus divides to produce a two-nucleate embryo sac followed by a four-nucleate condition (figure 3E). The four nuclei divide once to form the eight-nucleate embryo sac. Wall formation in the micropylar region occurs earlier than in the antipodal pole (figure 3F). The synergids are hooked (figure 3G) and hyaline and the egg contains an abundant quantity of compound starch grains. The antipodal nuclei become encased by individual walls and by repeated divisions give rise to 40–60 antipodal cells (figure 3G, only a few are seen in this section). These cells remain uninucleate, become densely stained, and persist until the 2–4 celled stage of the embryo. The endosperm is free nuclear.

Discussion

The occurrence of staminodes in the florets

Figure 2 A–H Microsporangium and microsporogenesis (**c**, cuticle; **en**, endothecium; **ep**, epidermis; **ml**, middle layer; **tp**, tapetum). *A*, transection of a young anther showing hypodermal archesporial cells (see arrow) (×400); *B*, transection of a developing anther showing periclinal divisions in the primary parietal layer (see arrows) (×400); *C*, transection of anther showing divisions in the inner secondary parietal layer to form the middle layer and tapetum (see arrows) (×400); *D*, part of a mature anther showing a four layered anther wall (×400); *E*, part of longisection of an anther at the dyad stage (×200). Note the absence of well-developed callose wall. Arrow indicates compound starch grains in the endothecial cells; *F*, same as figure 2 E, showing isobilateral tetrads (see arrow) (×200); *G*, longisection of part of an anther at maturity (×200). Note the formation of radial thickenings in the epidermis (see arrow) and complete degeneration of the tapetal layer; *H*, part of transection of an anther about to dehisce (×250). Note the spinulate cuticle of the enlarged epidermal cells and radial endothecial thickenings

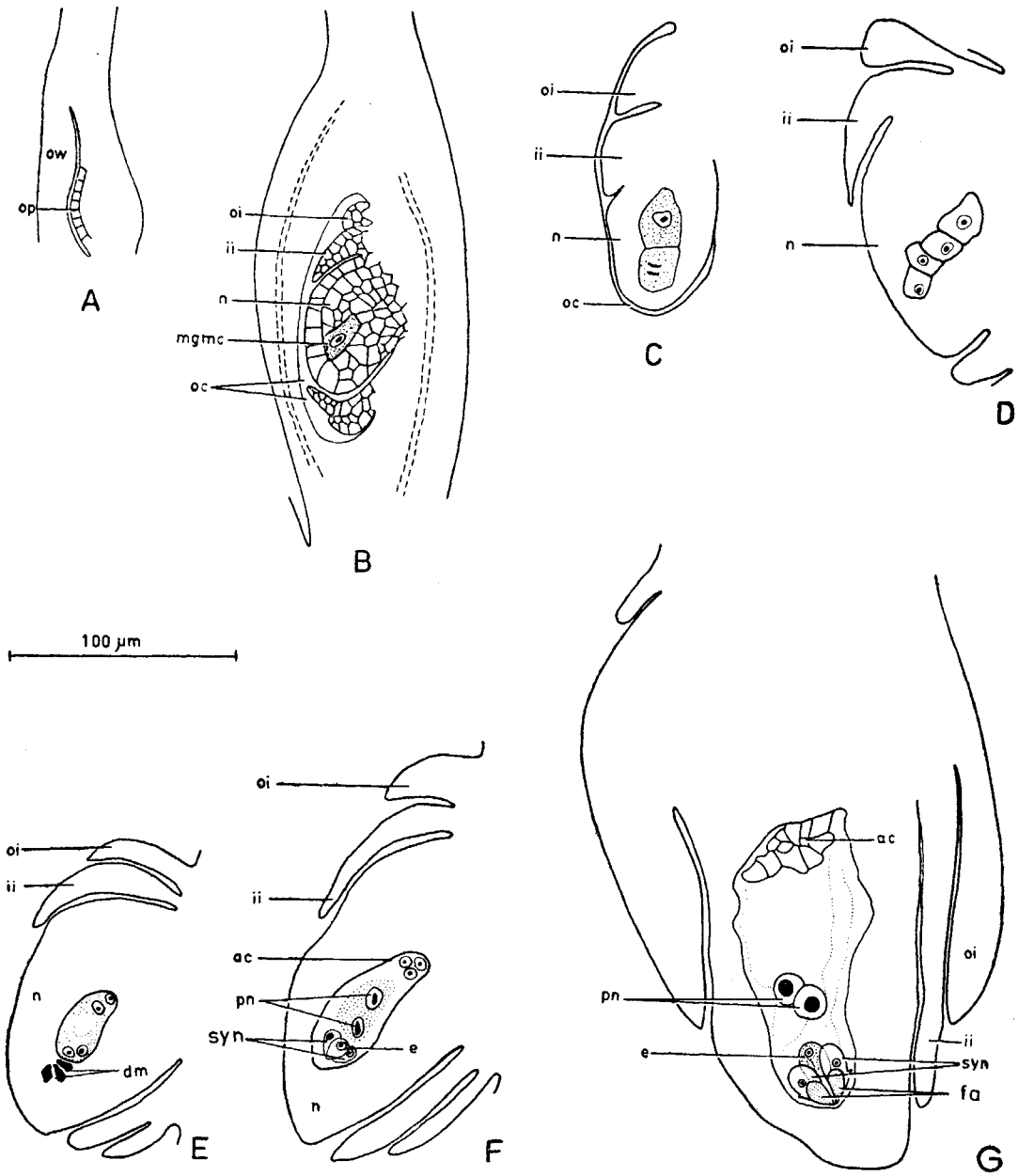


Figure 3 A–G Stages in ovule and embryo sac development (**ac**, antipodal cells; **dm**, degenerated megaspores; **e**, egg; **fa**, filiform apparatus; **ii**, inner integument; **mgmc**, megaspore mother cell; **n**, nucellus; **oc**, ovarian cavity, **oi**, outer integument; **op**, ovule primordium; **ow**, ovary wall; **pn**, polar nuclei; **syn**, synergids). *A*, longisection of part of ovary showing the ovule primordium; *B*, longisection of ovary showing an ovule at the megaspore mother cell stage. Note the developing integuments; *C*, longisection of an ovule showing a dyad, meiosis II in this ovule is asynchronous; *D*, a linear tetrad of megaspores; *E*, ovule in longisection at the four-nucleate embryo sac stage. Note the remnants of the three degenerated micropylar megaspores; *F*, an organized embryo sac; *G*, longisection of an ovule showing mature embryo sac containing the egg apparatus, two polar nuclei and numerous antipodal cells. The integuments have failed to cover the nucellar tip .

is a common feature among the bamboos (Holttum 1958, Calderon & Soderstrom 1973). In the present investigation neither the staminodes nor the lodicules were observed. The lodicules, when present in bamboos are reported to assist in the separation of lemma and palea during anthesis. However, Calderon and Soderstrom (1973) have noted that the lodicules have no role in the separation of lemma and palea in *Maclurolyra tecta.*

In the Gramineae the ovule is generally campylotropous (*see* Davis 1966 and the references cited therein; Chikkannaiah & Mahalingappa 1976a, Deshpande & Raju 1979). However, amphianatropous (Cooper 1937), anatropous (Narayanaswami 1954, Chandra 1976) and anacampylotropous (Calderon & Soderstrom 1973) conditions have been reported. In *D. hamiltonii* the ovule is attached to the ventral side of the ovary wall, and becomes hemianatropous due to unequal growth of its upper and lower sides.

The ovules in the Gramineae have been described as crassinucellate (Narayanaswami 1955a, b, 1956) or tenuinucellate (Narayanaswami 1955c, Chandra 1976, Chikkannaiah & Mahalingappa 1976a, b). Davis (1966) has introduced the term pseudocrassinucellate to account for the periclinal divisions in the nucellar epidermis. In the present investigation this term has been found most suitable to explain the condition of the ovule. The embryo sac development is of the Polygonum type as also reported in *Melocanna bambusoides* by Yakovlev (1970).

Considerable variation in the size and role of the integuments in the formation of the micropyle has been noted in the Gramineae. Generally the outer integument is shorter than the inner and the micropyle is formed by the inner integument (Narayanaswami 1954, 1955c, Calderon & Soderstrom 1973, Philipson 1978, Deshpande & Raju 1979). However, there are also a few reports of the participation of the outer integument in the formation of the micropyle (Krishnaswami & Ayyangar 1937, Khosla 1946, Chandra 1976). Whereas a micropyle is reported to be absent in *Pennisetum typhoideum* (Narayanaswami 1953), in *Melocanna bambusoides* both the integuments are represented by small primordia (Petrova 1965, Yakovlev 1970). In *Dendrocalamus hamiltonii* (present work) both the integuments remain short and fail to cover the nucellus and thus there is no true micropyle.

Much variation has been noted among the grasses in the number of antipodal cells and the number of nuclei in them (*see* Davis 1966, and references cited therein). In *Melocanna bambusoides*, Yakovlev (1970) has reported as many as 40 antipodals and in *Sasa paniculata* over 300 antipodal cells have been recorded (Yamura 1933). In the present study 40–60 antipodal cells have been observed.

The post-fertilization events in the bamboos have not been worked out in detail and the authors have undertaken this task and intend to present the findings subsequently.

Acknowledgements

The authors express their sincere thanks to Professor H Y Mohan Ram for going through the manuscript and giving valuable suggestions. One of us (B H G) acknowledges the financial assistance from the Indian Council of Agricultural Research, New Delhi.

References

Calderon C E and Soderstrom T R 1973 Morphological and anatomical considerations of the grass subfamily Bambusoideae based on the new genus *Maclurolyra*; *Smithson. Contr. Bot.* **11** 1–55.

Chandra S 1976 Post-pollination development of wheat Caryopsis; in *Embryology of Crop Plants*; Indo-Soviet Symposium pp 46–47 (New Delhi: Indian National Science Academy)

Chikkannaiah P S and Mahalingappa M S 1976a Embryological studies in *Eleusine*. 7; *Embryology of Crop Plants*; Indo-Soviet Symposium (New Delhi: Indian National Science Academy)

—— 1976b The female gametophyte and activities of antipodals in *Cynodon dactylon* (L.) Pers.; in *Physiology of Sexual Reproduction*; International Somposium (Ludhiana: Punjab Agricultural University)

Cooper D C 1937 Macrosporogenesis and embryo sac development in *Euchlaena mexicana* and *Zea mays*; *J agric. Res.* **55** 539–551

Davis G L 1966 *Systematic Embryology of Angiosperms* (New York: John Wiley & Sons) 528 pp

Deshpande P K and Raju P S G 1979 Development of caryopsis and localization of DNA and proteins in *Triticum* species; *Phytomorphology* **29** 100–111

Gamble J S 1896 The Bambuseae of British India; *Ann. R. bot. gdn*, Calcutta

Holttum R E 1958 The bamboos of the Malay peninsula; *Gdns' Bull.*, Singapore **16** 1–135

Kawamura S 1927 On the periodical flowering of the bamooos; *Jap. J. Bot.* **3** 335–349

Khosla S 1946 Developmental morphology in some Indian millets; *Proc. Indian Acad. Sci.* **B24** 207–222

Krishnaswamy N and Ayyangar G N R 1937 Cytological studies in *Eleusine coracana* Geartn; *Beih. bot. Zbl.* **57** 297–318

Mc Clure F A 1966 *The Bamboos—A Fresh Perspective* (Massachusetts: Harvard University Press) 347 pp

Narayanaswami S 1953 The structure and development of caryopsis in some Indian millets. I. *Pennisetum typhoideum* Rich.; *Phytomorphology* **3** 98–112

—— 1954 The structure and development of caryopsis in some Indian millets. II. *Paspalum scrobiculatum* L.; *Bull. Torrey bot. Club.* **81** 288–299

—— 1955a The structure and development of caryopsis in some Indian mllets. III. *Panicum millare* Lamk. and *Panicum miliaceum* Lamk.; *Lloydia* **18** 61–73

—— 1955b The structure and development of caryopsis in some Indian millets. IV. *Echinochloa frumentacea* Link.; *Phytomorphology* **5** 161–171

—— 1955c The structure and development of caryopsis in some Indian millets. V. *Eleusine coracana*; *Michigan Acad. Sci. Arts Lett.* **40** 33–45

—— 1956 The structure and development of caryopsis in some Indian millets. VI. *Setaria italica*; *Bot. Gaz.* **118** 112–122

Petrova L R 1965 The morphology of the reproductive organs of the bamboo *Melocanna bambusoides* Trin.; *Bot. Zhurn.* **9** 1288–1304

Philip V J 1972 On the embryogenesis in *Bambusa arundinacea* Willd; *Curr. Sci.* **40** (4) 153

—— and Haccius B 1976 On the embryogenesis in *Bambusa arundinacea* Willd. and structure of the mature embryo; *Beitr. Biol. Pfl.* **52** 83–100

Philipson M N 1978 The Nucellar degeneration in *Cortadaria* (Gramineae); *Protoplasma* **95** 361–369

Reeder J R 1957 The embryo in grass systematics; *Am. J. Bot.* **44** 756–768

—— 1962 The bambusoid embryo: a reappraisal; *Am. J. Bot.* **49** 539–641

Yakovlev M S 1950 The structure of the endosperm and embryo in cereals as a systematic feature; *Ann. Bot. Inst. Acad. Sci.* USSR **7** 121–219

—— 1970 On the embryology of *Melocanna bambusoides*. *Ann. Bogor.* **5** (2) 109–115

—— and Kamelina O P 1973 *Melocanna bambusoides* Trin. embryo development; *Bot Zhurn.* **58** 248–263

Yamura A 1963 Karyologische und embryologische studien uber einiger *Bambusa* Arten (Vorläufige Mitteilung); *Bot. Mag., Tokyo* **47** 551–555

Yoshida O 1963 Embryologische studien von *Arundinaria chino* Maknino; *J. Coll. Arts Sci. Chiba Univ.* **4** 33–43