

density polythene bags for ease in handling inoculant during storage and transit also resulted in the rapid loss of moisture from inoculants. Low density polythene permits high gas exchange and moisture transmission from inoculants. Approximately 45–50% decline in the moisture content of polythene-packed inoculant was found during storage<sup>4</sup> at 26°C.

In India, inoculants are generally prepared either in charcoal-soil<sup>5</sup> (3:1) or in lignite<sup>6</sup> carrier which possesses low water-holding capacity in comparison with standard peat. Besides, low density polythene bags which are used for packing inoculants, allow rapid loss of moisture leading to heavy reduction in viable rhizobial population in inoculants.

The present paper deals with improving the water-retaining capacity of charcoal-soil carrier material for longer survival of rhizobia. The above carrier is suitably amended with soymeal and gelatin which are known for their water-retaining capacity and the effect of such amendment on the survival of rhizobia in carrier was studied. The results of the study are reported here.

The charcoal-soil (3:1) mixture was sun-dried and powdered to pass through 100 mesh sieve. It was then mixed with finely powdered calcium carbonate (0.2%) and  $K_2HPO_4$  (0.5%). This mixture was amended separately with 1 and 2% of gelatin and 1, 2, 5 and 10% of soymeal. To each of these samples 10% water was added before autoclaving at  $1\text{ kg/cm}^2$  for 4 hr. An efficient strain of red gram *Rhizobium* (cowpea sp.) ARS-83 was grown in yeast-extract-mannitol (YEM) broth medium<sup>7</sup> by shaking it continuously for 4 days at 28–30°C on a rotary shaker having 120 rpm. The broth culture of *Rhizobium* sp. ARS-83 containing  $18 \times 10^9$  cells per ml of broth was added to the carrier samples and the moisture contents were brought to 30% of their water-holding capacity, inclusive of the water carried by the broth culture. After inoculation, 200 g of each sample was packed in a polythene bag which was then incubated at 28–30°C in a B.O.D. incubator. The rhizobial population in carrier was enumerated at regular intervals of storage by dilution-plate count method using YEMA medium containing congo red<sup>7</sup>. The moisture contents of the samples were also determined at these intervals of storage.

It was observed that the addition of gelatin (1–2%) or soymeal (1–10%) in the carrier material helped in maintaining higher moisture contents and rhizobial population in carrier for longer duration (table 1). The moisture-retaining capacity was found to increase with the quantity of soymeal or gelatin added to the carrier. Gelatin was better than

soymeal in this regard as its 2% quantity was enough to retain 16% moisture in carrier till the end of 24th week of storage whereas soymeal added at the same rate could retain only 10.4% moisture. The rhizobial population was also found to increase with the quantity of soymeal in the carrier. Gelatin, however, showed a marginal increase in rhizobial population with increase in its quantity in carrier. This confirms the earlier findings<sup>8</sup> that addition of gelatin and casein gave no response to the multiplication of rhizobia in pellets, but increased the water-retaining capacity of the inoculant carrier.

7 May 1987

1. Bhatnagar, R. S., Jauhri, K. S. and Iswaran, V., *Curr. Sci.*, 1982, **51**, 430.
2. Vincent, J. M., In: *Nutrition of legumes*, Proceedings of the University of Nottingham 5th Easter School of Agricultural Sciences, (ed.) E. G. Hallsworth, Butterworths, London, 1958.
3. Steinborn, J. and Roughley, R. J., *J. Appl. Bacteriol.*, 1974, **37**, 93.
4. Thompson, J. A., *ICRISAT Inf. Bull. No. 17*, 1984, p. 1.
5. Jauhri, K. S., Bhatnagar, R. S. and Iswaran, V., *Curr. Sci.*, 1979, **48**, 170.
6. Kandaswamy, R. and Prasad, N. N., *Curr. Sci.*, 1971, **40**, 495.
7. Vincent, J. M., *Manual for the practical study of root-nodule bacteria*, IBP Handbook No. 15, Blackwell, Oxford, 1970.
8. Bergersen, F. J., Brockwell, J. and Thompson, J. A., *J. Aust. Inst. Agric. Sci.*, 1958, **24**, 158.

## EMBRYOLOGY OF *EUPHORBIA MADDENI* AND *EUPHORBIA NIVULIA*

R. K. BHANWRA

Department of Botany, Panjab University,  
Chandigarh 160 014, India.

*EUPHORBIA* Linn. is a large genus consisting of about 2,000 species<sup>1</sup>. About 52 species have been recorded from India<sup>2</sup>. The genus includes herbs, shrubs and trees of widely diverse habitats. The present studies which deal with the embryology of *E. maddenii* Boiss. and *E. nivulia* (Buch.) Ham. were undertaken mainly with a view to finding if there are any habitual differences in the reproductive features of the herbaceous and dendroid forms.

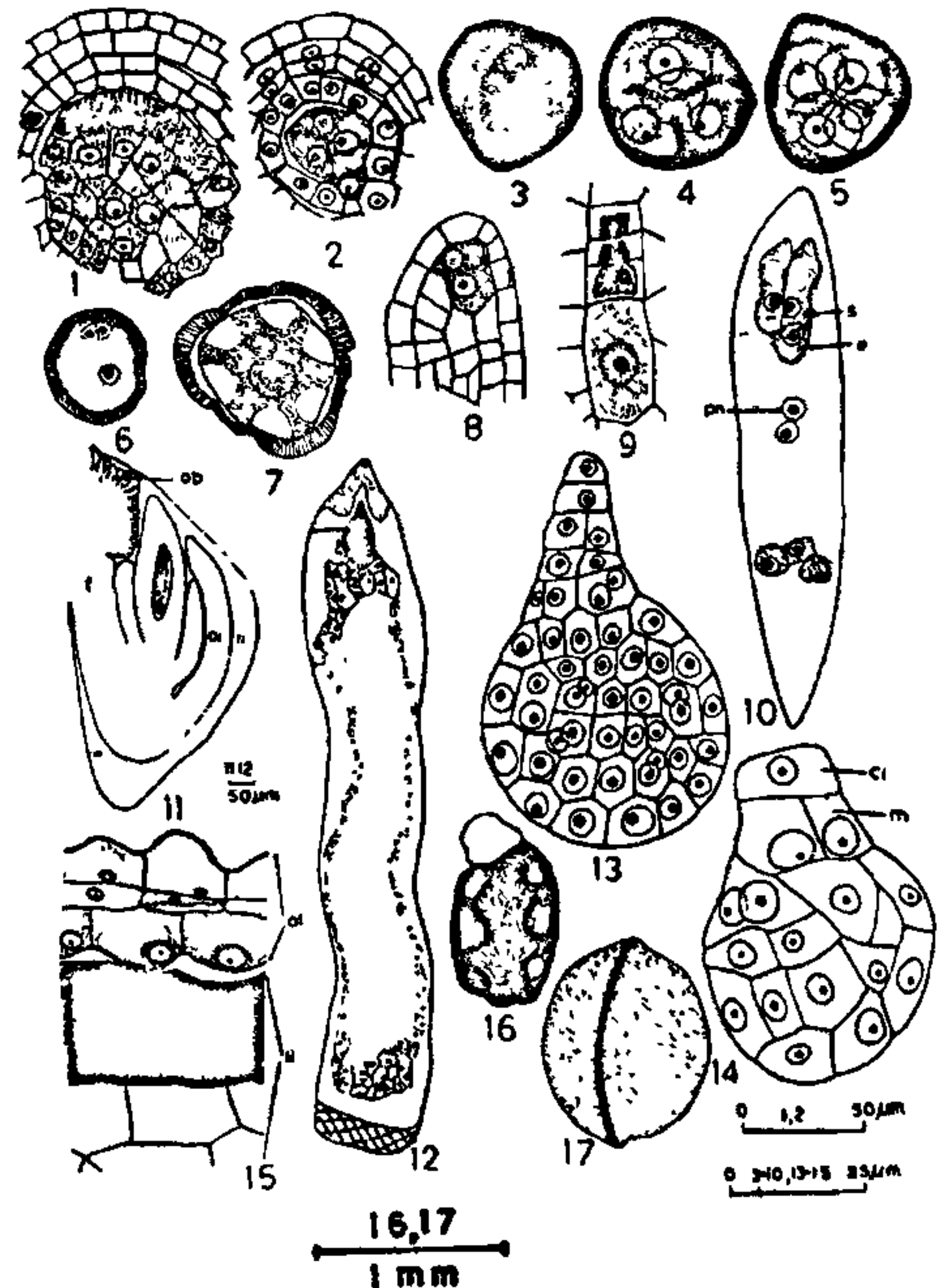
The material of *E. maddeni*, a small to medium-sized herb was collected from Srinagar (Kashmir) in September while that of *E. nivulia*, a dendroid taxon, was procured from plants cultivated at the Panjab University Botanical Garden and from the natural population growing at Ajmer (Rajasthan) during April.

The anthers are tetrasporangiate. The development of the anther wall follows the basic pattern in *E. nivulia* but it is of the Dicotyledonous type in *E. maddeni*. The sporogenous tissue is enclosed by an epidermis, endothelial layer, two middle layers and the tapetal layer in *E. nivulia* (figure 1). There is only one middle layer in *E. maddeni* (figure 2). The middle layer/s is ephemeral. The tapetum is of the glandular type and its cells become radially elongated and binucleate during meiosis in microspore mother cells. The tapetal cells may contain three or four nuclei in *E. nivulia*. Simultaneous cytokinesis follows meiosis in microspore mother cells resulting in isobilateral tetrads of microspores (figure 3). Both tetrahedral and isobilateral tetrads are formed in *E. nivulia* (figures 4,5). The development of the male gametophyte occurs as in other Euphorbiaceae and pollen is shed at three-celled stage in *E. maddeni* (figure 6) but at two-celled state in *E. nivulia* (figure 7). The pollen is tricolpate with a thick striated exine and thin smooth intine. According to Brewbaker<sup>3</sup>, *Euphorbia* is one of the ten genera out of all angiosperms which have both binucleate and trinucleate pollen grains. Trinucleate condition has been derived from the binucleate during evolution of angiosperms.

The pendulous ovules are bitegmic, crassinucellate and anatropous as in other species of the genus *Euphorbia*. The micropyle is usually formed by the outer integument. The nucellus quite often projects in the form of a tongue-like process (figure 11), a feature not only common to *Euphorbia*<sup>8,9</sup> but has also been recorded in some other genera like *Acalypha*<sup>10</sup> and *Chrozophora*<sup>11</sup>. Obturator is of placental origin in both the species studied here and is composed of loose cells forming a canopy-like structure over the micropyle. Recent studies have shown the cells of obturator to differentiate into transfer cells<sup>12</sup>.

There is a hypodermal archesporial cell which divides periclinally to form the outer, primary parietal cell and inner, primary sporogenous cell (figure 8). The primary parietal cell forms a parietal tissue five or six layers thick. The primary sporogenous cell increases in size and acts as megaspore mother cell. It divides meiotically resulting in a

linear tetrad of megaspores (figure 9). The chalazal megaspore functions and develops into a Polygonum type of embryo sac (figure 10). The egg is globular and projects beyond the two synergids which are somewhat pear-shaped. The polar nuclei occupy a position nearer to the egg apparatus. The three antipodals are ephemeral and degenerate soon after fertilization. The monosporic Polygonum type of embryo sac is the most common in the genus *Euphorbia* (being found in 42 species of the 52 that



**Figures 1-17.** Microsporogenesis, megasporogenesis, male and female gametophytes, endosperm, embryo and seed coat. 1,4,5,7,8,11,14,17 *E. nivulia*; 2,3,6,9,10,12,13,15,16 *E. maddeni*. 1,2. Sporogenous cells enclosed by wall layers. 3. Telophase-II. 4, 5. Tetrahedral and isobilateral tetrads of microspore. 6, 7. 3-celled and 2-celled pollen respectively. 8. Ovule primordium with primary parietal and primary sporogenous cells. 9. Linear tetrad of megaspores. 10. Polygonum type of embryo sac. 11. Mature ovule. 12. Globular proembryo and initiation of walls in the endosperm. 13, 14. Globular proembryos. 15. V.S. part of seed-coat. 16, 17. Mature seed. (e. egg; f. funiculus; ii. inner integument; ob. obturator; oi. outer integument; ci. m. tiers of proembryo; s. synergid).

have been worked out so far). A few species show bisporic or tetrasporic type of embryo sac development, while there are some species where the type of embryo sac development is controversial<sup>4-7</sup>.

The endosperm is of the 'Nuclear' type. Wall formation is initiated at globular stage of the pro-embryo (figure 12).

In *E. maddeni*, the cells of the outer integument proliferate at the micropylar end and form the caruncle. This structure is not distinguished in *E. nivulia*. The cells of the nucellus get completely consumed by the developing endosperm on the lateral sides. A few nucellar cells remain at the micropylar end and form the nucellar cap (figure 12). At the chalazal end some of the nucellar cells become thick-walled with dense cytoplasm and organize into a saucer-shaped hypostase.

Embryogeny of both *E. maddeni* and *E. nivulia* conforms to the Onagrad type of Johansen<sup>13</sup>. In *E. maddeni* there is a suspensor of 3-4 cells arranged in a linear row (figure 13) which is lacking in *E. nivulia* (figure 14). The embryogeny is in the species much resembles that of *Sebastiania*<sup>14</sup>. Various other variations of Onagrad type have been reported in other species of *Euphorbia*<sup>14-17</sup>. Solanad type of embryogeny has been reported in *E. heterophylla*<sup>19</sup> while Piperad type occurs in *E. preslii*<sup>20</sup>. Adventive embryony has been reported in *E. dulcis*<sup>7</sup>.

The outer epidermal cells of the inner integument become radially elongated at the cordate stage of the embryo. In mature seed these cells differentiate into macro-sclereids which form stony layer of the seed-coat (figure 15). In *E. maddeni*, these sclereids are obliquely-oriented in relation to the surface of the seed. Thus the seed-coat is derived from the outer epidermis of the inner integument. These cells differentiate into sclereids as reported in *E. hirta*<sup>14</sup>, *E. geniculata*<sup>21</sup> and *Acalypha indica*<sup>22</sup>. A mature seed in *E. maddeni* has a warty brown covering with a well-developed caruncle (figure 16). The seeds show ridges and furrows. The mature seeds in *E. nivulia* are smooth and there is a well-developed raphe but the caruncle is not conspicuous (figure 17).

The herbaceous *E. maddeni* shows certain differences from the dendroid *E. nivulia*. They are: (i). The anther wall is four-layered in *E. maddeni* but is five-layered thick in *E. nivulia*. (ii). The pollen is shed at three-celled stage in *E. maddeni* but it is shed at two-celled stage in *E. nivulia*. (iii). The development of embryo is regular in *E. maddeni* and the globular embryo has a suspensor comprising 3 or 4 vertical rows of cells. In *E. nivulia*, the development of the embryo is somewhat irregular and a

suspensor is absent. (iv). The caruncle is well-developed in *E. maddeni* but it is inconspicuous in *E. nivulia*. (v). The macrosclereids are obliquely-oriented in relation to the surface of the seed in *E. maddeni* but they are almost at right angles to the surface of the seed. (vi). The seed has ridges and furrows in *E. maddeni* but it is smooth in texture in *E. nivulia*. Thus it seems that the herbaceous and dendroid forms studied here exhibit a mixture of features regarded as primitive and advanced.

30 October 1986; Revised 28 April 1987

1. Airyshaw, H. J., *A dictionary of the flowering plants and fern*, Revised 8th edition of the late J. C. Willis, 1973.
2. Hooker, J. D., *The flora of British India*, London, 1885.
3. Brewbaker, J. L., *Am. J. Bot.*, 1967, 54, 1069.
4. Donati, G., *C. R. Acad. Lincei, Roma*, 1912, 21, 512.
5. Ventura, M., *Ann. Bot. (Roma)*, 1933, 20, 267.
6. Sanchez, S. T., *Bull. Unn. Philipp. Net. Appl. Sci.*, 1938, 6, 59.
7. Kapil, R. N., *Phytomorphology*, 1961, 11, 24.
8. Mukherjee, P. K., *Bull. Bot. Soc. Univ. Saugar*, 1957, 9, 7.
9. Mukherjee, P. K., *Proc. Indian Acad. Sci.*, 1961, B53, 217.
10. Kapil, R. N., *Phytomorphology*, 1960, 10, 174.
11. Kapil, R. N., *Phytomorphology*, 1956, 6, 278.
12. Johri, B. M., *Embryology of Angiosperms*, Springer-Verlag, Berlin, 1984.
13. Johansen, D. A., *Plant Embryology*, Waltham Massachusetts, 1950.
14. Thathachar, T. J., *Mysore Univ. J.*, 1953, B13, 43.
15. Modilewski, J., *Ber. Dtsch. Bot. Ges.*, 1909, 28, 413.
16. Soueges, R., *C. R. Acad. Sci. Paris*, 1924, 179, 989.
17. Kajale, L. B., *Proc. Nat. Inst. Sci. India*, 1964, 20, 353.
18. Venkateswarlu, J. and Rao, P. N., *J. Indian Bot. Soc.*, 1973, 52, 313.
19. Singh, R. P., *Phytomorphology*, 1954, 4, 118.
20. Weniger, L. C., *Bot. Gaz.*, 1917, 63, 266.
21. Singh, R. P., *J. Indian Bot. Soc.*, 1959, 38, 103.
22. Johri, B. M. and Kapil, R. N., *Phytomorphology*, 1953, 3, 137.