

# Contributions of Panchanan Maheshwari's school to angiosperm embryology through an integrative approach

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**P. Maheshwari who served as Professor and Head of the Department of Botany, University of Delhi from 1950 to 1966 built a flourishing school of embryology which became internationally recognized. His colleagues and students have contributed significantly to all areas of embryology through integrative approaches. In memory of his birth centenary year, we have prepared this article that summarizes the work done by his students and traces the phenomenal advances made in some areas in the post-Maheshwari era.**

**Keywords:** Angiosperm embryology, experimental embryology, integrative approach, Panchanan Maheshwari.

AFTER joining the Department of Botany, University of Delhi in 1950, Panchanan Maheshwari started developing a school of embryology of flowering plants which attained world status by the mid-1960s. In his seminal book *An Introduction to the Embryology of Angiosperms*<sup>1</sup>, Maheshwari classified embryology into descriptive, phylogenetic and experimental aspects. He defined experimental embryology as that branch 'concerned with the imitation and a modification of embryological processes with a view to understanding the physics and chemistry of various events so as to bring them under human control'<sup>1</sup>. In his book Maheshwari articulated the progress of experimental embryology until 1950 and outlined its future course of events. He also emphasized the importance of integration of techniques from other disciplines for its further progress. Fortuitously histochemistry, electron microscopy and plant cell and tissue culture had just emerged and these were put to good use for embryological investigations<sup>2</sup>. Maheshwari and his students have advanced our knowledge in several areas of embryology. The purpose of this review is to recapitulate the salient features of research in integrative embryology by Maheshwari's school and also focus on the significant advances made in the post-Maheshwari era.

Some of the important areas initiated by the Delhi school of embryology during 1950s and early 1960s are

control of fertilization, *in vitro* culture of ovary and ovule, isolated nucellus tissue, embryo, endosperm, seed and anther culture, and induction of somatic embryogenesis. These pursuits generated new knowledge including many firsts to merit a statement by Preston<sup>3</sup> in the preface to his edited work *Advances in Botanical Research*: 'One of the most remarkable developments of our time in plant sciences has been the way in which hitherto purely observational regions are progressively becoming experimental or even mathematical. One case of the former...is the remarkable developments in embryology at the hands of Professor Maheshwari and his colleagues and students....'

## Control of fertilization

Fertilization in flowering plants is a complex process. Whereas in the cryptogams and to a large extent in the gymnosperms the male and female gametes come in direct contact with each other, in the angiosperms the pollen grain (male gametophyte that carries the male gametes or their progenitor cell, the generative cell), does not have direct access to the embryo sac (female gametophyte) that contains the egg. The pollen grain lands on the stigma, following effective pollination through autogamy or biotic/abiotic agents. The pollen grain germinates on the stigma and the resulting pollen tube carries the male gametes through the tissues of the stigma and style, enters the ovule and eventually the embryo sac, and discharges the two male gametes to effect double fertilization.

During the initial decades after the discovery of double-fertilization by Nawaschin<sup>4</sup> in 1898 embryologists concentrated on events such as the development of the pollen grain, embryo sac, embryo and endosperm in a large number of families of flowering plants and documented the variations in detail. A major outcome of these efforts was the understanding that a more or less uniform mode of fertilization occurs in the flowering plants. These reports were based on simple techniques of serial sectioning of wax embedded flowers and/or reproductive organs, staining of sections and light microscopy. There were very few studies on pollen–pistil interaction. However, from 1960s onwards interest on events of pollen–pistil interaction increased

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steadily and there has been a considerable progress on the structural and functional aspects of pollen–pistil interaction in Delhi and in several laboratories around the world.

### *Structure of the pistil and pollen–pistil interaction*

Pollen–pistil interaction deals with a series of sequential events in the pistil from the time pollen grain lands on the stigma until the pollen tube enters the ovules. Pollen–pistil interaction is unique to flowering plants and is a prerequisite for effective fertilization and subsequent fruit and seed set.

Studies made by Vasil and Johri<sup>5</sup> and Johri<sup>6,7</sup> are among the first detailed accounts on the structure of the stigma and style in several members of the Liliaceae and Amaryllidaceae. The Liliaceae are characterized by hollow style and the Amaryllidaceae present much variation from typically hollow to partially hollow and totally solid conditions. Based on these reports, Johri<sup>6,7</sup> proposed the evolution of the solid style from the hollow style in the Amaryllidaceae.

Subsequently Konar of the University of Delhi who went to the laboratory of Linskens in Nijmegen, The Netherlands, made pioneering studies on scanning and transmission electron microscopic details of the stigma and style of *Petunia*. He showed that the stigmatic exudate originates from the epidermis and the subjacent cell layers of the stigma<sup>8</sup>. Konar and Linskens<sup>9</sup> also identified the stigmatic exudates as principally lipoidal in nature. Recent studies by Wolters-Arts *et al.*<sup>10</sup> have confirmed the lipoidal nature of the stigma exudate and have further shown that the stigmatic lipids establish and maintain a moisture gradient from the stigmatic cells to the outer surface of the exudate and assist in guiding the pollen tubes to enter the stigmatic tissue.

Shivanna, an alumnus of the Delhi school, carried out investigations on the structural and functional aspects of the pistil during 1970s in association with J. and Y. Heslop-Harrison at Kew and subsequently continued at the University of Delhi with his team until recently. Heslop-Harrison and Shivanna<sup>11</sup> screened the stigmas of nearly 1000 species belonging to about 900 genera belonging to some 250 families under light and scanning electron microscope and documented a wide spectrum of variability. They classified stigmas into different groups based on the presence or absence of the exudate/papillae and the nature of the papillae. The categories of stigmas established by them are being followed by botanists throughout the world. These were the first comprehensive studies on the morphology of the stigma and demonstrated significant correlations between the characteristics of the stigma surface and the physiological details of pollen–pistil interaction. For example, sporophytic self-incompatibility systems are invariably associated with dry papillate stigmas and most gametophytic systems with wet stigmas. Dry stigmas are generally associated with trinucleate pollen plants and

wet stigmas tend to have binucleate pollen. However, many species which possess binucleate condition of pollen at the stage of shedding also have a dry stigma. These studies<sup>11</sup> also confirmed that the presence of extracellular proteins on the stigma surface, a feature which had been reported earlier by Mattsson *et al.*<sup>12</sup>, is a general feature of the stigma, irrespective of stigma morphology.

The work done by Shivanna's group at Delhi on the pistil structure and pollen–pistil interaction was directed to important crop plants such as *Linum* spp<sup>13</sup>, *Nicotiana*<sup>14</sup>, *Cicer* spp<sup>15</sup>, *Crotalaria* sp<sup>16</sup>, *Vigna* and *Cajanus* spp<sup>17</sup> and *Arachis hypogaea*<sup>18</sup>. These studies also yielded information on reproductive biology and breeding systems. More recently studies on pistil and pollen–pistil interaction have been extended in collaboration with Mohan Ram to a number of important Indian tree species (*Dalbergia sissoo*<sup>19</sup>, *Commiphora wightii*<sup>20,21</sup>, *Elaeis guineensis*, the oil palm<sup>22</sup>, *Acacia senegal*<sup>23</sup>, *Butea monosperma*<sup>24</sup>, *Sterculia urens*<sup>25</sup> and *Boswellia serrata*<sup>26</sup>). Apart from documenting the histochemical structural details of the pistil the researchers have shown that the presence of intercellular spaces filled with extracellular proteins in the path of the pollen tube growth in the style is a general feature in both solid and hollow styled species. The structure–function studies carried out in Delhi have brought out several novel features, a few of which are mentioned below:

(i) In a dimorphic species, *Linum grandiflorum*, Ghosh and Shivanna<sup>13</sup> showed for the first time differences in the stigma type of the two morphs; stigma of the pin morph is of the dry type and that of the thrum morph is of the wet type. These differences play an important role in pollen–pistil interaction and incompatibility.

(ii) Even in species with wet stigma type, the stigma surface in younger pistils is similar to the dry stigma type with a pellicle-cuticle layer outside the cell wall<sup>14</sup>. The stigmatic exudate appears below the cuticle and eventually disrupts the pellicle–cuticle layer and spreads on the surface of the stigma. These details have turned out to be a general features in all wet stigma types.

(iii) In papilionaceous legumes, the style just below the stigma is solid for varying lengths, whereas it is hollow further down. The distal hollow part of the style is formed as a result of disintegration of the cells of the transmitting tissue. These features initially documented by Shivanna's group, turned out to be the characteristic features in papilionaceous legumes by later studies<sup>27,28</sup>. Another important feature in some papilionaceous legumes is the series of outgrowths of the canal cells as papillae into the lower part of the stylar canal to loosely fill the canal. These appear to play an important role in controlling pollen tube growth.

(iv) All the six populations of *Commiphora wightii* collected from Delhi and Rajasthan in India are obligate apomicts and do not produce sexual seeds at all<sup>20</sup>. The egg degenerates and the embryos are produced as a result

of nucellar adventive polyembryony. Other interesting features are that apomixis is independent of pollination stimulus (i.e. non-pseudogamous type) and endosperm formation occurs without fertilization. Thus seed-raised plants of the species represent a clonal progeny. *Commiphora* provides a good system for studies on the molecular basis of apomixis. Further, in this species the stigma allows pollen germination but the style does not support pollen tube growth<sup>21</sup>. These features have been traced to a changed orientation of the cells of the transmitting tissue and absence of proteins in the intercellular spaces of the transmitting tissue<sup>21</sup>. It may be recalled that the presence of extracellular proteins in the transmitting tissue is a general feature noted in all other species that support normal pollen tube growth.

(v) In all the taxa investigated with the exception of *Sterculia urens*, the transmitting tissue of the mature style (before pollination) is characterized by the presence of intercellular spaces filled with an extracellular matrix. In *S. urens*, the above features are manifested only after pollination<sup>25</sup>. Thus the development of intercellular spaces filled with extracellular matrix in *S. urens* is a new report of pollination-induced changes recorded so far (see Shivanna<sup>29</sup>).

(vi) In *Elaeis guineensis* (oil palm), pollination stimulus induces additional secretion in the stylar canal<sup>22</sup>. In post-pollination secretion, the pectinaceous caps of the canal cells lining the stylar canal are disintegrated to release nutrients into the canal to facilitate pollen tube growth.

Other works on the structure of the pistil from Delhi school are on *Triticum*<sup>30</sup>, *Zephyranthes*<sup>31</sup> and *Cheiranthus*<sup>32</sup>.

### Self-incompatibility

Shivanna's group has also carried out extensive studies on the breeding system, particularly on several self-incompatible species, both in homomorphic (*Petunia hybrida* and *Nicotiana glauca*<sup>33-35</sup>, species of Poaceae<sup>36,37</sup>, *Dalbergia sissoo*<sup>19</sup>, *Acacia senegal*<sup>23</sup>, *Sterculia urens*<sup>25</sup> and *Boswellia serrata*<sup>26</sup>) and heteromorphic (*Linum* spp.<sup>38,39</sup> and *Primula vulgaris*<sup>40-42</sup>) systems. In *Petunia* and *Nicotiana*, Sharma and Shivanna<sup>33</sup> were able to standardize an *in vitro* assay in which inhibition of self-pollen grains was significantly higher than that of the cross-pollen. Their studies<sup>34,35</sup> also provided evidence for the involvement of lectin-like components of the pollen and the sugar moiety of the pistil in pollen recognition. However, this has not been substantiated by subsequent studies using molecular techniques<sup>43</sup>.

In several self-incompatible species of Brassicaceae such as *Raphanus* and *Iberis*, quantitative details of pollen germination, pollen tube entry into the stigmatic papillae and callose deposition in the stigmatic papillae were investigated<sup>44</sup>. These studies clearly indicated that in the flower

buds which permitted self-seed set, the stigma surface components concerned with self-incompatibility response were not yet present in quantities sufficient to inhibit self-pollen germination and pollen tube growth. However, surface components involved in promoting pollen germination and tube growth were already present. Enzymatic digestion of pellicle proteins reduced germination of both compatible and incompatible pollen and totally prevented pollen tube entry into the stigmatic papillae to show that proteinaceous pellicle was required for pollen tube entry.

Painstaking studies on pollen tube growth in self- and cross-pollinations in two self-incompatible taxa, *Acacia senegal*<sup>23</sup> and *Sterculia urens*<sup>25</sup> have shown delayed inhibition of self pollen tube after pollen tube entry into the embryo sac.

In two members of the Poaceae, *Saccharum bengalense*<sup>36</sup> and *Alopecurus pratensis*<sup>37</sup>, individual plants show variation in the degree of their self-incompatibility response. In certain individual plants, the zone of inhibition resides on the stigma (inhibits pollen germination or entry of pollen tube into the stigma); in others, pollen tubes enter the stigma but their further growth is inhibited; in still others, pollen tubes grow to a considerable length in the transmitting tract of the pistil and then stop. These features indicate that the area of inhibition in gametophytic self-incompatible systems may not be specific but varies among individuals/species.

Several methods have been applied to overcome self-incompatibility in homomorphic systems such as use of mentor pollen in *Sesamum*<sup>45</sup>, *Petunia*, *Raphanus* and *Brassica*<sup>46</sup> treatment of stigma with lectins and sugars in *Petunia* and *Eruca*<sup>47</sup>, treatment of stigma with aqueous extracts of compatible pistils in *Petunia*<sup>48</sup> and placental pollination<sup>49</sup>.

Contrary to the earlier concept that inhibition of self pollen is a passive event as a result of mismatch of osmotic potential of the pollen and the stigma of the two morphs in *Linum grandiflorum*<sup>50</sup>, a dimorphic self-incompatible species, studies made by Ghosh and Shivanna<sup>39,51</sup> clearly showed that differences in osmotic potential have no role to play in the inhibition of self-pollen. These results were later confirmed by Murray<sup>52</sup> and Dulberger<sup>53</sup>. Ghosh and Shivanna<sup>39</sup> also showed that self-incompatibility in *Linum* is a combination of both passive and active inhibition. Passive inhibition is the result of mismatch of the stigma and pollen features in the two morphs whereas active inhibition results from pollen recognition. In *L. grandiflorum* intramorph incompatibility operates at least at three levels: pollen adhesion, pollen hydration and pollen tube growth in the stigma; the first two are passive due to lack of morphological and/or physiological complementation and the third is active as a result of pollen recognition. Active inhibition of pollen tube requires protein synthesis in the pistil. Investigations made on several heteromorphic species of *Linum* by Ghosh and Shivanna<sup>51</sup> also showed that intermorph compatibility and intramorph incompatibility

transgress species boundaries. Based on these studies the authors have suggested that S gene is involved in both intra- and interspecific-incompatibility in *Linum*.

In another dimorphic self-incompatible system, *Primula vulgaris*, Shivanna *et al.*<sup>41,42</sup> showed that self-incompatibility inhibition may occur on the stigma surface, inside the stigma and in the transmitting tract of the style. Thus inhibition of self-pollen tubes in this species is cumulative at different sites. The authors also reported an *in vitro* assay that showed differential effects on pollen of the two morphs. Extracts from thrum pistil inhibited germination of thrum pollen to a greater extent than that of pin pollen. Similarly, extract of pin pistil inhibited pin pollen more markedly than that of thrum pollen.

### *Recent advances through the use of modern techniques*

Several other laboratories outside India have taken up work on pollen biology, pollen–pistil interaction and self-incompatibility. An additional feature of the studies in recent years is the integration of the techniques particularly of cell biology, molecular biology and genetics in solving problems of pollen–pistil interaction and fertilization. Due to limitation of space, these aspects are not discussed in detail. The following are a few of the salient features of recent advances.

(i) Demonstration of the male germ unit (composed of the vegetative nucleus and the two sperm cells in physical association) in pollen grains and/or pollen tubes, dimorphism of the two male gametes in the same pollen grain, and preferential fertilization (plastid-rich male gamete fusing with the egg and mitochondria-rich one fusing with the secondary nucleus in *Plumbago zeylanica*<sup>54</sup>).

(ii) Elaboration of the role of cytoskeleton elements (microtubules and microfilaments) in pollen germination and pollen tube growth (see Cai *et al.*<sup>55</sup>, this issue).

(ii) Characterization of pollen surface components and extracellular matrix present in the transmitting tissue of the pistil in pollen germination and pollen tube growth (see Lord and Russell<sup>56</sup>).

(iv) Identification of the components/cells involved in pollen tube guidance on the stigma surface and in the ovary (lipids on the surface of stigma/pollen in directing pollen tubes into the stigma; embryo sac, particularly the synergids in facilitating pollen tube into the micropyle of the ovule)<sup>10,57–62</sup>.

(v) Identification of genes associated with initiation of floral organs, embryological processes such as sporogenesis, gametogenesis and embryogenesis, and those expressed exclusively or predominantly in embryological systems such as pollen grains, tapetum, transmitting tissue of the pistil, embryo sac, zygote, embryo and endosperm<sup>63–65</sup>.

(vi) Identification and characterization of genes and their products involved in homomorphic self-incompatibility, and understanding of the mechanism of inhibition of self-pollen<sup>43,66–68</sup>.

These landmarks are so far confined to a few model systems but have provided important stimuli for further investigations.

### **Manipulation of pollen biology and fertilization**

Another dimension to the study on fertilization is the manipulation of fertilization, especially in overcoming barriers to fertilization in hybridization programmes. The success of interspecific hybridization has been limited due to the absence of any control on the barriers imposed by the stigma and style. A major approach followed by plant breeders prior to 1950s was to carry out pollinations in large numbers and to pray for the development of at least a few hybrid seeds in the ovary. Apart from the barriers imposed by the stigma and style, the two parent species selected for hybridization do not often flower simultaneously and may also be growing in distant places. Pollen storage has been one of the effective techniques to overcome such problems. While highlighting the importance of pollen storage in plant hybridization programmes, Maheshwari<sup>1</sup> wrote that pollen storage 'had the advantage of enabling the breeder to cross two varieties which are separated from each other not only in time but also in space'. He promptly initiated studies on pollen biology related to pollen viability and storage at Delhi in the late 1950s.

### *Pollen viability and storage*

Vasil was the first to initiate studies on anther and pollen culture, and pollen storage at Delhi<sup>69–71</sup>. His pioneering work on anther culture was largely aimed at understanding the physiology of meiosis. He showed that meiosis proceeds normally in cultured anthers excised at the zygotene or later stages<sup>69</sup> and results in one-celled microspores. He was successful in inducing the division of the microspore nucleus in the presence of a mixture of four nucleotides applied in the culture medium<sup>69</sup>. Following the report of induction of pollen embryos in cultured anthers by Guha and Maheshwari<sup>72,73</sup>, attention on anther and microspore culture both at Delhi and elsewhere in the world was diverted from using the technology of anther culture for understanding pollen development to induction of androgenic haploids. This is an example of changing trends which have occurred several times in the history of science.

When Vasil initiated studies on pollen storage in the mid-1950s, it was already clear that 2-celled pollen grains could be stored for many months/years under low relative humidity. However, pollen grains of economically important members of the Poaceae which are shed at the 3-celled stage are difficult to store in spite of the best efforts. For example pollen grains of maize, sorghum and sugarcane could be stored only for a few days, and that too under highly humid conditions<sup>74</sup>. The pollen grains of *Pennisetum* are exceptional as they can be stored under 0% relative humidity for up to 186 days<sup>75</sup>. This was the longest period

of storage of any cereal pollen at that time. These studies were later taken up in Delhi by Chaudhury and Shivanna<sup>76,77</sup> who reconfirmed that the pollen grains of *Pennisetum typhoideum* are indeed an exception to the general feature of cereal pollen as these can withstand desiccation and can be stored for up to one year under low temperature and relative humidity. Taking this lead, Hoekstra *et al.*<sup>78</sup> carried out biochemical studies on pollen of *Pennisetum* and *Zea mays* and showed that the differences in the desiccation tolerance and storability of pollen in these two species depended on their sucrose level; the amount of sucrose in *Zea* was only 4% while that in *Pennisetum* it was as high as 14%.

Storage of pollen grains in organic solvents was one of the simple techniques reported by the Japanese workers in *Petunia*<sup>79</sup>. Workers at Delhi tested this method and were able to successfully store the pollen grains of several legumes<sup>80-83</sup> in organic solvents. Jain and Shivanna<sup>81,82</sup> showed that non-polar solvents such as hexane and cyclohexane are favourable for pollen storage whereas polar solvents are unsuitable. Jain and Shivanna<sup>84</sup> were also successful in storing pollen grains by following another novel and simple method of storing them in olive oil, soybean oil and liquid paraffin for several months. Presently, long-term storage of pollen grains of several horticultural plants is being practised at the Indian Institute of Horticultural Research (IIHR), Hesaraghatta, near Bangalore through cryopreservation in liquid nitrogen<sup>85</sup>. Pollen biologists at IIHR have been able to store successfully pollen grains of several horticultural species such as papaya for up to 8 years in liquid nitrogen (S. Shashi Kumar and S. Ganeshan, pers. commun.).

Presently it has become possible to store pollen grains of even cereals such as maize and wheat in liquid nitrogen for over 10 years<sup>86</sup>. The technology of pollen storage has advanced to such an extent that by using one of the available methods, pollen grains of any species can be kept viable for at least one year, which is the maximum time required for overcoming the barriers imposed by temporal/spatial separation of the parent species in any hybridization programme.

In their quest to understand the basic principles of pollen viability, Shivanna and Heslop-Harrison<sup>87</sup> demonstrated that the plasma membrane plays a very important role in maintaining pollen viability. Their studies clearly indicated that irreversible loss of membrane integrity is the primary cause for the loss of pollen viability. Jain and Shivanna<sup>83</sup> estimated the membrane phospholipids in pollen grains stored for varying periods and showed a positive and significant correlation between the loss of viability, breakdown of membrane integrity and reduction in total and individual membrane phospholipids, particularly phosphatidyl choline. These studies put at rest the earlier suggestions such as deficiency of respiratory substrates, inactivation of enzymes and/or auxins to account for the loss of pollen viability<sup>74</sup>.

By conducting a series of experiments on pollen grains of *Crotalaria*, *Nicotiana*, *Agave*, *Iris* and *Tradescantia*

exposed to high temperature and/or humidity stress as well as storage stress, Shivanna and co-workers<sup>83,88-90</sup> have shown that these stresses reduce pollen vigour (assessed on the basis of time taken for pollen germination and/or rate of pollen tube growth) before affecting pollen viability (ability to germinate). As the ability of vigorous pollen is apparently better than non-vigorous pollen in achieving fertilization in hybridization programmes, the above studies have highlighted, for the first time, the need to assess the quality of pollen, particularly of stored pollen not only on the basis of viability but also vigour. In seeds the distinction between viability and vigour is clearly recognized. In fact, the quality of seeds is being assessed not on their ability to germinate but on vigour, i.e. the time taken for germination (Association of Official Seed Analysts, Seed Vigour Committee 1983).

#### *Attempts to induce male sterility through the application of growth regulators*

To exploit hybrid vigour and to overcome the laborious process of emasculation, hand pollination and bagging, scientists had been toying with the idea of using chemical compounds that can selectively induce male sterility without affecting seed fertility or seed quality. A detailed study carried out by Mohan Ram and his students<sup>91,92</sup> using Mendok (sodium 2,3-dichloroisobutyrate), Dalapon (sodium 2,2-dichloropropionate) and morphactin (fluorene-9-carboxylic acid) on wheat and linseed indicated that a temporary period of developmental or functional male sterility can be obtained, but none of the compounds tested is reliable as a specific male sterilizing agent for commercial application.

#### *In vitro pollination and fertilization*

A major limitation in understanding fertilization and manipulating it in a desirable way is the location of the embryo sac – deep inside the ovule and the ovary. One of the approaches has been to eliminate pollen–pistil interaction altogether and bring the pollen grains in direct contact with the ovules. A more effective approach would be to bring the male and female gametes together under the microscope to achieve *in vitro* fertilization. This is being done routinely in a number of animals and has added substantial knowledge on fertilization and its application. In flowering plants *in vitro* fertilization would provide an effective means of overcoming crossability barriers imposed by the stigma and style.

The Delhi embryologists led by Maheshwari took a lead in this area in early 1960s. The first attempt to eliminate the barriers of stigma and style and bring pollen grains in direct contact with ovules was to carry out intraovarian pollination in members of the Papaveraceae. The technique of intra-ovarian pollination is perhaps the outcome of the earlier reports of intracarpellary pollen grains by

Johri<sup>93</sup> in *Butomopsis lanceolata*. Maheshwari<sup>1</sup> described in detail this phenomenon in *B. lanceolata* and drew attention to the instance of a carpel in which pollen grains had germinated on the surface of the ovule. The impact of this discovery on subsequent quest for intraovarian pollination is obvious from his statement that 'another method of overcoming the difficulty caused by an extremely slow growth of the pollen tube would be a direct introduction of the pollen grains into the ovary'.

Intraovarian pollination<sup>94,95</sup> involved injection of pollen grains suspended in a suitable liquid nutrient medium into the ovary and achieving pollen germination, pollen tube entry into ovule and fertilization. Viable seeds following intraovarian pollination were obtained in several species of Papaveraceae – *Papaver somniferum*, *P. rhoeas*, *Argemone mexicana* and *A. ochroleuca*. This technique was also effectively used to overcome interspecific incompatibility between *A. mexicana* and *A. ochroleuca*<sup>95</sup>. Although the technique of intraovarian pollination is very simple and effective in eliminating the stigma and the style, it has not been used extensively. One possible reason for lack of its application is that there is insufficient space in many angiosperms between the ovules and the ovary wall to accommodate a sufficient quantity of pollen suspension. The other reason could be the high rate of success of *in vitro* pollination.

As an extension of the plant tissue culture exercises begun in his laboratory in the late 1950s, Maheshwari suggested culturing groups of ovules and pollen grains on the semi-solid nutrient medium to attain large scale fertilization and seed production. The methods of *in vitro* pollination of ovules were first standardized for *Papaver somniferum*<sup>96,97</sup> and essentially consisted of culturing isolated groups of ovules and pollen grains together on a nutrient medium. Pollen germination, pollen tube entry into the ovules and double fertilization proceeded normally and fertilized ovules developed into viable seeds. The technique was then extended to *Argemone mexicana*, *Eschscholtzia californica*, *Nicotiana tabacum*<sup>97</sup> and *Dicranostigma franchetianum*<sup>98</sup>. Subsequently Rangaswamy and Shivanna<sup>49</sup> refined this technique to minimize injury to the ovules and used this modified technique termed placental pollination to overcome self-incompatibility in *Petunia*. Subsequently the technique of placental pollination has been used over the years by other workers particularly Zenkteler from Poland to overcome interspecific and intergeneric crossability barriers<sup>99,100</sup>.

Rapid advances in protoplast technology during the 1970s and 1980s gave a new impetus to the studies on *in vitro* fertilization. Effective techniques were standardized to isolate male gametes from the pollen grains and egg cells from the embryo sac<sup>101</sup>. There was no significant contribution by workers from the Delhi school on these lines except for a paper on isolation of sperm cells from 2-celled pollen grains in *Rhododendron macgregoriae* and *Gladiolus gandavensis*<sup>102</sup>. This is the first report on

isolation of sperm cells from 2-celled pollen. Using this technique<sup>102</sup> sperm cells have been isolated in another 2-celled pollen, *Nicotiana tabacum* and used in a number of experiments involving fertilization<sup>102a,102b</sup>. By 1990, protocols were available to isolate male and female gametes in viable condition in several plants and the stage was set to make serious attempts to achieve *in vitro* fertilization. The pioneering success of *in vitro* fertilization was achieved in maize by Kranz and his associates<sup>103,104</sup>. The male and female gametes were aligned under the microscope in microdroplets of fusion medium covered with mineral oil and fusion was accomplished by giving one or two pulses of direct current. The zygotes formed *in vitro* were cultured on a semipermeable membrane placed on fast growing morphogenetic cell suspension cultures derived from maize embryos or microspores. In these nurse cultures the zygotes established polarity and divided mitotically to give rise to globular structures, proembryos and transition phase embryos (comparable to seed embryos) that developed into seedlings which on transplantation to soil developed into fertile plants.

Subsequently *in vitro* fusion was achieved in maize between isolated sperm and the central cell<sup>105</sup>. More importantly, the resulting primary endosperm cell developed into a characteristic tissue similar to the endosperm. Thus after 100 years of the discovery of double fertilization in flowering plants, it became possible to perform the two fertilization processes *in vitro*. Apart from providing an efficient method to overcome crossability barriers, this development provides an excellent experimental procedure for investigations on fundamental and applied areas of double fertilization and early development of the embryo and endosperm (see Okamoto and Kranz<sup>65</sup>, this issue). The technique of *in vitro* fertilization and development of *in vitro* formed zygote into embryo also makes it feasible to use the egg or zygote for genetic transformation<sup>106</sup>.

### Embryo culture and production of wide hybrids

As early as 1950 Maheshwari<sup>1</sup> had anticipated that the technique of embryo culture would not only enhance our understanding of the nutritional requirements but would also enable us to obtain successful hybrids in difficult crosses. He wrote that embryo culture 'promises to be of great economic value as a means of achieving a much wider range of hybrid combinations'. Rangaswamy<sup>107</sup> was successful in culturing globular embryo of *Citrus microcarpa* measuring 15–30 µm in diameter in modified White's medium containing casein hydrolysate and obtained plants. Subsequently, polyembryony was induced in cultured embryos of a parasitic flowering plant, *Dendrophthoe falcata*<sup>108,109</sup> by the budding of embryonal cells. Although not much work was conducted subsequently on fundamental aspects of embryo culture at the Delhi school, it was taken up by other investigators and

significant advances were made in using the technique in understanding embryogenesis. These aspects have been discussed in detail by Raghavan<sup>110</sup>.

Since 1960s embryo culture technique has been effectively used in a large number of species to obtain hybrids by researches at Delhi and elsewhere<sup>111,112</sup>. The first attempt in this direction from the Delhi school was made by Joshi and Pundir<sup>113,113a</sup> to rescue hybrid embryos of cotton. The cross between the Old World diploid *Gossypium arboreum* × the New World tetraploid *G. hirsutum* is desirable but attempts made to obtain the hybrid were unsuccessful. A thorough investigation on the experimental embryology of these interspecific and reciprocal crosses tracing the time-relation studies on pollination, pollen tube growth, fertilization, development of seed and seed fibres was published by Pundir<sup>113</sup>. Joshi and Pundir<sup>113a</sup> dissected heart-shaped embryos resulting from the cross and grew them *in vitro* on a medium containing casein hydrolysate, gibberellic acid and kinetin. The cultured embryos showed expansion of cotyledons, elongation of the hypocotyls and radicle and produced triploid seedlings. However, these seedlings could not be established in the soil. Understandably our ability to harden *in vitro* raised plants was inadequate at that time and glasshouse facilities were also lacking.

Shivanna and his students initiated studies on wide hybridization in *Brassica* (between cultivars and wild species) during 1980s to increase genetic variability in the cultivated species, particularly with the aim of tapping traits (from the wild species) which would impart tolerance/resistance to biotic and abiotic stresses. His group used all the cultivated Brassicas (*B. campestris*, *B. juncea*, *B. carinata* and *B. napus*) and several wild species such as species of *Diplotaxis*, *Enarthrocarpus*, *Erucastrum* and *Sinapis* as parents for raising distant hybrids. The crosses between wild and cultivated species are strongly incompatible. By devising a technique termed sequential culture (culturing of ovaries followed by ovules from cultured ovaries and in some crosses embryos from cultured ovules) for rescuing the embryo, the researchers have been able to raise over 10 interspecific and 40 intergeneric hybrids in the Brassicaceae<sup>114-128</sup>. It is difficult to think of any other laboratory in which such a large number of wide hybrids have been raised. These hybrids have generated extensive genetic variability in crop brassicas. Through cytoplasmic substitution by repeatedly backcrossing the hybrid with the cultivated species, these researchers were able to develop six new cytoplasmic male sterile lines<sup>120,126-128</sup>.

### Ovary and ovule culture

Initial studies at Delhi using the methods of tissue culture were aimed at growing isolated ovaries and ovules of several species<sup>129</sup> primarily to investigate their nutritive requirements in relation to embryo, endosperm and fruit

development. It may be recalled that the first report of aseptic culture technique from India was made as early as 1956 by Rau<sup>130</sup>. He cultured pollinated ovaries of *Phlox drummondii* on Nitsch's medium and obtained normal development of the embryo and endosperm. Subsequently pollinated ovaries of *Tropaeolum majus*<sup>131</sup>, *Linaria macrocarpa*<sup>132</sup>, *Iberis amara*<sup>133</sup>, *Althaea rosea*<sup>134</sup> and *Allium cepa*<sup>135</sup> were cultured on semi-solid medium with various adjuvants. Although ovaries grown *in vitro* developed into mature fruits on a simple nutrient medium such as Nitsch's or White's, addition of growth substances, individually or in combination improved fruit growth considerably. Another important outcome of these studies was that retention of calyx/perianth with the ovaries, particularly when they were cultured at an early stage, greatly promoted fruit growth. The role of various growth substances on the cultured ovules has been carried out using *Papaver somniferum*<sup>136</sup>, *Zephyranthes*<sup>137</sup>, *Abelmoschus esculentus*<sup>138</sup>, *Gynandropsis gynandra*<sup>139</sup> and *Gossypium hirsutum*<sup>140</sup>. These studies showed that whereas isolated ovaries could be grown *in vitro* into mature fruits with viable seeds on a simple nutrient medium, cultured ovules (excised at the zygote stage) required addition of growth substances in the medium.

As the entire cotton used for textiles is derived from epidermal hairs from mature ovules, the physiology of fibre elongation received attention by Beasley and coworkers<sup>141</sup>. The ovules of *Gossypium hirsutum* excised and cultured on the day of flower opening or one day later, failed to initiate fibres or developed only a limited number. However, two days after anthesis, most of the ovules showed profuse growth of fibres. Those ovules that were floating on the liquid medium produced greater number of fibres than those that were submerged<sup>141</sup>.

Another response reported in cultured ovaries of several species of Apiaceae: *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*<sup>142</sup>, *Ammi majus*<sup>143</sup> and *Coriandrum sativum*<sup>144</sup> is the induction of embryonal budding. The ovaries cultured at the zygote and free-nuclear endosperm stage showed embryo proliferation and produced embryo-like structures which could be isolated and grown into plantlets.

### Embryogenesis in cultured tissues and organs

Induction of embryo-like structures in cultured tissues and organs has been an important research contribution from Delhi. As early as 1950s, Rangaswamy<sup>107</sup> initiated studies on induction of embryos in the nucellus from pollinated ovary cultures of *Citrus microcarpa*, a naturally polyembryonic species. The nucellus proliferated into a callus and differentiated into embryo-like structures, which were termed pseudobulbils at that time. The pseudobulbils eventually gave rise to plantlets. Sabharwal<sup>145</sup> obtained similar results in *Citrus reticulata*. Subsequently Rangan from

Delhi joined Murashige's laboratory at the University of California, USA and worked on the induction of nucellar embryos in several other species of *Citrus*<sup>129,146,147</sup>, using excised nucellar cultures. These investigators were able to induce nucellar embryos in many species of *Citrus* such as *C. grandis*, *C. limon*, *C. temple*, *C. maxima*, *C. latifolia* and *C. reticulata* × *C. sinensis*; these species represented polyembryonate, monoembryonate as well as seedless varieties. The response of the nucellus in different species depended on the developmental stage of the ovule at the time of excision. An interesting outcome of these studies has been that *in vitro* obtained nucellar seedlings were free from viruses even when their mother plants were infected. Subsequently nucellar embryos have been induced from cultures of unfertilized ovules of *Citrus*<sup>148,149</sup>. Under a coordinated programme sponsored by the Department of Biotechnology, Government of India, five research groups attempted to culture the nucellus tissue for inducing somatic embryogenesis with the aim of obtaining true to type plants. Although somatic embryogenesis could be induced, conversion to rooted plantlets and their establishment in pots with garden soil met with no success, with the exception of one report<sup>149a</sup>.

Several attempts have been made to induce nucellar embryony in species which are not naturally polyembryonic. Although nucellus cultures of *Luffa cylindrica* and *Trichosanthes anguina*<sup>150,151</sup>, and cotton<sup>152,153</sup> were established, no organogenesis or embryogenesis could be induced. Detailed investigations<sup>151-153</sup> have provided strong evidences to indicate that cucurbitacins present in the nucellus of cucurbits and gossypol in the nucellus of cotton could be inhibiting organogenesis/embryogenesis in the cultured nucellus tissues.

Induction of somatic embryos in cultured flower buds of *Ranunculus sceleratus*<sup>154,155</sup> has been one of the spectacular stories from the Delhi school. Cultured floral buds containing primordia of various floral organs produced friable callus which differentiated into numerous embryoids capable of developing into plantlets. Another interesting feature observed in *R. sceleratus* is the differentiation of a large number of embryoids originating from the epidermal cells of *in vitro* raised plantlets all along the stem<sup>155</sup>. Although at present somatic embryogenesis has been induced in practically all parts of the plant body of a large number of species, during 1960s the phenomenon was demonstrated in only a limited number of species. Somatic embryogenesis in *R. sceleratus* reported from Delhi has been a frequently cited work. *Ranunculus* represented one of the most responsive systems for somatic embryogenesis next only to carrot at that time. In an attempt to clarify the phenomenon in which various embryo-like structures are formed and given different names, Rangaswamy<sup>156</sup> proposed the following definition: Somatic embryogenesis is the development of embryos from somatic tissues as well as from situations which do not involve directly the gametes, haploid cells and gametophytes of whatever ori-

gin; an embryo is *ab initio* a polarized entity bounded by cuticle<sup>156</sup>.

### Endosperm culture

Although both embryo and endosperm are the products of double fertilization, they follow entirely different morphogenetic pathways *in vivo*. The embryo differentiates into a new sporophyte with a root-shoot axis that perpetuates the continuity of the species, the endosperm lacks the capacity for organ formation but accumulates reserve materials to support the nutritional needs of the embryo. Whether these distinct fates are determined by the ploidy level or result from physiological interactions between the embryo and endosperm were not clear. Induction of organogenesis/embryogenesis in cultured endosperm had been a challenge to experimental embryologists since long. Earlier attempts to culture mature endosperm tissue did not meet with success although there were reports of continuously proliferating tissues from immature endosperm (see Bhojwani and Bhatnagar<sup>157</sup>). A serendipitous finding stimulated endosperm culture work at Delhi. When decoated castor seeds (*Ricinus communis*) were soaked in 2,4-D and allowed to germinate, the germination of the embryo was suppressed but the mature endosperm cells proliferated to produce a callus<sup>158</sup>. This work although not adequately acknowledged by later investigators, triggered a good deal of further research in endosperm culture.

Endosperm cultures of *Santalum album*<sup>159</sup> and *Ricinus communis*<sup>160</sup> failed to differentiate organs or embryos. Subsequent attempts by Johri and his students using endosperm tissues of a number of semi-parasitic flowering plants such as *Exocarpus cupressiformis*<sup>161</sup>, *Scurrula pulverulenta*<sup>162</sup>, *Dendrophthoe falcata*, *Taxillus* spp and *Lep-tomeria acida*<sup>163-165</sup> were successful in inducing shoot buds from cultured endosperm. These were the first reports on the demonstration of morphogenetic potentiality of endosperm cultures.

Subsequently researches of the Delhi school<sup>166</sup> demonstrated this potential in autotrophic angiosperms (*Jatropha panduraefolia* and *Putranjiva roxburghii*). Lakshmi Sita *et al.*<sup>167</sup> working in Bangalore reported plantlet regeneration from the cultured endosperm tissue in *Santalum album*. Rao and Raghava Ram<sup>168</sup> also demonstrated triploid plantlets arising from the callus of endosperm tissue in *Santalum album*. The list of plants in which protocols have been developed to obtain triploid plants developed from endosperm callus is growing (*Emblica officinalis*<sup>169</sup>, *Mallotus philipensis*<sup>170</sup>, *Acacia nilotica*<sup>171</sup>, *Diospyros kaki*<sup>172</sup>, *Morus alba*<sup>173</sup> and *Azadirachta indica*<sup>174</sup>).

### Whole seed cultures

Considerable work has been carried out on seed cultures of several species of unusual interest. In many species of root parasites such as *Sopubia delphinifolia*, *Orobanch*

*aegyptiaca*, *Cistanche tubulosa* and *Striga asiatica*, seeds require a stimulus present in the root exudate of the host plant for germination and seedling establishment. It has been possible to induce seed germination *in vitro* in these species in the absence of host stimulus<sup>175,176</sup>. In *S. delphinipholia*, the haustoria which enable the parasite to absorb nutrients from the host, could be induced *in vitro* in the presence of host root exudates<sup>177</sup>. Another interesting feature in *O. aegyptiaca* and *C. tubulosa* is that the embryo lacks primary meristems and organs; it is merely a block meristem<sup>176</sup>. In *C. tubulosa*<sup>178</sup> germination *in vitro* occurs only in the presence of coconut water in the nutrient medium. Germination is characterized by the activity of only the radicular pole of the embryo which emerges through the micropyle and eventually gives rise to the shoot buds. The plumular pole remains *in situ* and does not take part in organ formation. In *O. aegyptiaca*<sup>176,179</sup> the activity of the radicular and plumular poles in the formation of the seedling depends on the composition of the medium. When the medium is supplemented with yeast extract/casein hydrolysate/coconut water, the entire seedling is formed by the activity of the radicular pole of the embryo as in *C. tubulosa*. In the presence of gibberellic acid or kinetin, however, the radicular pole produces several roots and the plumular pole the shoot; thus both the poles become activated in the formation of the root-shoot axis.

Rangaswamy<sup>176</sup> has compiled a list in which 19 families of angiosperms have been reported to have taxa in which seeds contain embryos totally devoid of organs. The work dealing with *O. aegyptiaca* and *C. tubulosa* has been referred to above. The case of genus *Utricularia* which has over 275 species of submerged or terrestrial rootless herbs (the insectivorous bladderworts) is unusual as all of them have embryos that lack organs. Although several attempts have been made by early botanists to follow germination and name the parts of the shoot system<sup>180,181</sup>, in *Utricularia* the distinction among cotyledons (cotylenoids), primary leaves and shoots is not at all clear-cut. In *Utricularia inflexa* the mature embryo looks like a bun and puts out a whorl of 5–8 cotyledonoids from one of the poles. A leafy shoot bearing bladders arises in the axil of one of the cotyledonoids and it is this shoot which develops the whole plant, inflorescence and fruits<sup>181</sup>.

In the strange family of aquatic flowering plants, the Podostemaceae growing attached to rocks and boulders, in rivers and cataracts by means of rhizoids and haptera, the flattened plant body resembles an alga, lichen or a bryophyte. The podostemads display such an enormous phenotypic diversity and fuzzy morphology that the conventional distinction into root and shoot is obscure. The mature plant body has been interpreted as a root, stem or a combined shoot<sup>182</sup>.

The principal reason for the poor understanding of the development of the plant is due to difficulties in tracing germination of the minute seeds in flooded rivers. The seed

has an embryo with two well-developed cotyledons but no distinct plumule or radicle. A method for germinating the seed up to vegetative stage of the plant was developed by Vidyashankari and Mohan Ram<sup>183</sup>. This consists of using polystyrene (thermocole) cubes (*ca* 1 cc) to support the seeds on the surface of the liquid medium such that they are partially exposed to air. This simple, reproducible technique has paved the way to understand the developmental morphology in several Indian Podostemaceae.

The recent investigation of seedling histology of *Hydrobryopsis sessilis*, a highly crustose and endedmic member of Podostemoideae<sup>184</sup>, has shown that a diminutive shoot apical meristem (SAM) is formed endogenously at the junction of the cotyledons. This meristem forms an apical determinate primary axis and a lateral indeterminate thallus primordium. The radicular pole does not give rise to a root. Further, the thallus in *Hydrobryopsis* can be interpreted as a flattened stem because it has a tunica corpus-like organization at its apex<sup>184</sup>. In earlier papers the thallus was being referred to as a lateral outgrowth of the hypocotyle. It is only gradually that it has become possible to trace the origin of the thallus to the SAM. This calls for a re-investigation of the early histology of seedlings of other taxa of Podostemoideae<sup>184</sup>.

### Anther, microspore and pollen culture

Haploid plants (sporophytes with gametophytic chromosome number) are of immense importance in plant breeding programmes. Some of the major applications of haploids are in the development of homozygous diploids, mutation research and genetic transformation. Since the discovery of the first natural haploid in *Datura* in 1921, plant breeders have been attempting not only to isolate natural haploids but also to induce them through delayed pollination, distant hybridization, hormone or chemical treatments, temperature shocks, irradiation with X-rays or UV radiation<sup>2</sup>. However, their number was so low and the success was so unpredictable, that the haploids remained an academic curiosity and thus their potential in fundamental and applied research could not be exploited. The situation changed dramatically following the reports of induction of haploids in cultured anthers of *Datura innoxia* by Guha and Maheshwari<sup>72,73</sup> at Delhi. Induction of androgenic embryos through anther and microspore cultures has since become a topic of great interest throughout the world and the progress has been phenomenal. Androgenic haploidy has been reported in over 250 species belonging to 88 genera and 34 families<sup>185</sup>. Using pollen-derived haploid pathway, a number of new agronomically useful varieties have been developed and released for commercial cultivation. There is no universal recipe for haploid induction. In fact in some plants years of persistent efforts have yielded only very poor or negative results. Thus much of our knowledge about haploids has come from plants that

respond *in vitro*<sup>186</sup>. One of the most promising uses of microspore culture has been in developing populations of Indian mustard *Brassica juncea*, containing zero erucic acid and low glucosinolate content (unpublished work of D. Pental *et al.*). Recent exciting developments on androgenic haploids have been covered by Datta in this special section.

### Gynogenic haploids

There are distinct advantages in the production of androgenic haploids over gynogenic haploids. There are thousands of haploid cells (each pollen grain is a gametophyte containing 2 or 3 haploid cells, out of which 2 constitute the male gametes) in a single anther, which are accessible as compared to the limited number of cells/nuclei (4–16) in the embryo sac, deeply embedded in the ovule. This aspect has not been sufficiently emphasized in the literature<sup>186</sup>.

Nevertheless, there are several crop plants in which the yield of androgenic haploids is very low or zero. A case in point is mulberry, *Morus alba*. Although, in the Chinese genotypes, the induction percentage is reported to range from 0 to 13.6, in the elite Indian germplasm the percentage of embryonic cultures is as low as 0.23 in RFS 35 and 1.82 in RFS 175. In the Japanese genotype<sup>187</sup> (Goshoerami) it is 0.39.

Mulberry is the only feeding material for the mori silk larvae (*Bombyx mori*) and is consequently indispensable for the sericulture industry. Mulberry is also a multipurpose tree in great demand. There have been persistent attempts to obtain haploids for possible use in mulberry breeding. Lakshmi Sita and her associates<sup>188</sup> were the first to report gynogenic haploids in mulberry. Out of the gynogenic plants obtained, 50% were haploid ( $2n = x = 14$ ) and the remaining diploid. As these scientists had used individual ovaries separated from the composite fruit without preventing pollination, it is quite likely that fertilization might have occurred.

To overcome this problem, Bhojwani and co-workers<sup>189,190</sup> cultured segments of *in vitro* raised inflorescences (starting with nodal cuttings) on MS basal medium + BAP (4.5  $\mu$ M) + 2,4-D (4.5  $\mu$ M). After three weeks, the ovaries were isolated and transferred to MS + glycine (500 mg/l) + proline (200 mg/l) + 2,4-D (4.5  $\mu$ M). Gynogenic plants were formed by direct embryogenesis and the resulting plants were haploids, with a few aneuploid cells in the root tips<sup>189,190</sup>.

It may be recalled that gynogenesis had been recorded in barley by Noeum<sup>191</sup> and subsequently in about 20 species in ovary/ovule culture (see Bhojwani and Bhatnagar<sup>157</sup>). The production of dihaploids in the inter-specific crosses of tetraploids of *Hordeum vulgare* and *Hordeum bulbosum* in which there is a selective elimination of *bulbosum* chromosomes has become a classical study and has inspired a good deal of subsequent work<sup>192</sup>.

### Some unique discoveries from Maheshwari's school

Several unique reports were made by members of the Maheshwari school in developmental embryology during 1950s and 60s. It is worthwhile to mention a few as they provide ideal experimental systems for further investigations. A significant finding is embryo sac development in the Lorantheaceae<sup>193</sup>. Members of this family are characterized by the absence of true ovules, and development of several embryo sacs from the base of the stylar canal in the ovary. In many taxa the embryo sacs at the micropylar end extend into the stylar canal and reach various lengths. In some, such as *Helixanthera ligustrina* they reach up to the level of stigmatic papillae<sup>194</sup>. In *Mouquiniella rubra*<sup>195</sup> the embryo sacs extend in the style as much as 48 mm and reach the base of the stigma; interestingly they even curve downwards after touching the stigma and continue for another 2–4 mm. The embryo sacs of this species are the longest reported for any angiosperm. Further, the primary endosperm nucleus of each embryo sac descends from the style to the lower part of the ovary and the endosperms of several embryo sacs develop into a composite mass. Members of Santalaceae also present several novel features. An unusual instance is the development of embryo sac-like structures from the sporogenous cells in *Leptomeria billardieri*<sup>196</sup>. These are fascinating observations from the point of view of developmental embryology.

Another interesting family from the point of view of fertilization is the Podostemaceae. This family shows several remarkable features not known in such combination in any other angiosperm family. Among the interesting embryological features brought out by botanists of Mysore<sup>197,198</sup> and Delhi<sup>199,200</sup>, are the presence of pseudo-embryo sac, lack of antipodals, triple fusion and endosperm, prevalence of single fertilization and the absence of plumule and radicle in the mature embryo and presence of suspensor haustoria. These characteristics not only make the Podostemaceae markedly distinct from other angiosperms but also evolutionarily enigmatic.

It is a tribute to the dedication of these investigators to discover these unusual features using limited techniques of microtomy and light microscopy available at that time. Unfortunately, these interesting embryological systems have so far been ignored by experimental embryologists. With the use of modern integrated techniques such as electron microscopy, tissue clearing, cell biology, aseptic culture technology, and molecular biology and genetics, these systems would undoubtedly provide immense opportunities to investigate a number of fundamental problems of embryology.

### Concluding remarks

The above account briefly highlights a few pioneering contributions of Maheshwari's students on a wide spectrum of dynamic features in the embryology of flowering

plants. Probably no other laboratory had pioneered studies on such a wide range of problems in the subject. Many of the classical studies from his school have stimulated intensive researches leading to significant progress not only in understanding the fundamentals of embryological processes and their controlling factors, but also in manipulating them for practical gains. The reports of *in vitro* pollination of ovules and induction of androgenic embryos have turned out to be trendsetters and have led to the development of new vistas of research and useful applications. Many of the predictions of Maheshwari have become realities. For example, his statements 'it will be a major landmark in plant science when the zygote of an angiosperm is removed from the embryo sac and reared in a test tube to give rise to a normal embryo. The next step would be to grow the unfertilized egg and to induce artificial fertilization'<sup>201</sup>. These landmarks have now been achieved and attempts are being made to use these techniques to pursue several problems associated with fertilization and embryogenesis. Cultured zygotes are also being used to achieve genetic transformation. The progress on various facets of embryology through integrative techniques particularly of cell biology, molecular biology and genetics is expected to be more rapid in the coming years and would enable a comprehensive understanding of embryological events.

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