Pollination biology: Contributions to fundamental and applied aspects

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We review here our work on the physiological and biochemical basis of pollen grain germination, pollen–pistil interactions, incompatibility, pollen competition and sex expression in plants. We discuss our studies on the floral cues involved in attracting pollinators and their implications to plant–pollinator coevolution.

We also review our efforts in extending the basic studies to address specific problems in the genetic improvement of crop plants such as overcoming the intra- and inter-specific crossing barriers, development of cytoplasmic male sterile lines in Brassica, production of true seed potato and developing gametic selection techniques.

In a broad sense pollination biology includes: (a) pre-pollination pollen biology, (b) pollen transfer from the anther to the stigma, (c) pollen–pistil interaction leading to fertilization, and (d) fruit and seed development, seed germination and seedling establishment. Information on all these aspects is required not only for a comprehensive understanding of the efficiency of breeding system of a species and its evolutionary success but also for effective optimization of yield, conservation and rational genetic improvement. This review summarizes our work on these aspects.

Pre-pollination pollen biology

Generally, there is a time lag between shedding of pollen and their transfer to the stigma through biotic and abiotic means. During this period pollen grains are exposed to a range of environmental stresses, particularly of temperature and humidity, which may affect the quality of pollen. Any effect on pollen quality would affect their competitive ability in the pistil or even their ability to sire vigorous progeny.

Pollen viability

The quality of pollen is assessed on the basis of viability and vigour. Viability refers to the ability of the pollen to deliver functional sperm cells to the embryo sac following compatible pollination[1]. Assessment of pollen viability on the basis of its function is cumbersome, time-consuming and not always feasible[2]. Therefore, many short-cut methods which would reflect the functional ability of the pollen have been devised[3]. Of these, *in vitro* germination test and fluorescein diacetate (FDA) test are important. The former cannot be used for a large number of species in which it has not been possible to achieve satisfactory *in vitro* germination. FDA test assesses the integrity of the plasma membrane of the pollen and the activity of esterases in the pollen cytoplasm[4]. It is rapid, simple, convenient and can be used even in species which do not show *in vitro* germination[5]. Our extensive studies on a range of systems have shown a close correlation between FDA test and *in vitro* germination[2,6] as well as *in vivo* seed set[7,8]. FDA test is presently considered most dependable of all the indirect tests and is being used routinely for assessing pollen viability[9].

The period over which pollen grains retain viability after shedding is highly variable. Pollen grains of most species remain viable for a few days or weeks under laboratory conditions. However, pollen of some species, particularly cereals, are very sensitive to desiccation and lose viability within one hour under dry conditions. Pollen grains of *Pennisetum typhoides* are an exception; they can withstand desiccation, and storage under low humidity for up to 200 days[10,11].

The causes for the loss of viability are not clearly understood. Based on circumstantial evidences, deficiency of respiratory substrates and inactivation of enzymes have been suggested as possible factors for the loss of viability[3]. Analysis of the integrity of plasma membrane and its components in the pollen stored for different periods of time[7,12,13,14] have indicated the importance of the membrane in maintaining pollen viability. Irrespective of the storage conditions, a positive and significant correlation between the loss of viability and reduction in the total phospholipid content and amounts of individual phospholipids have been observed[13]. A similar correlation was observed in pollen samples stored in different organic solvents[12]. Pollen grains stored in non-polar organic solvents such as hexane, cyclohexane and diethyl ether retained viability and showed very little leaching of phospholipids, sugars and amino acids into the solvents. Nevertheless, pollen grains stored in polar solvents such as isopropanol and methanol lost viability and showed extensive leaching of these substances. These studies clearly show that the

depletion of membrane constitutes the primary cause for the loss of pollen viability.

A series of experiments to study the effects of temperature and humidity stress on pollen quality have been carried out. The extent of desiccation which pollen grains can withstand varies with the species[6]. For example the pollen of *Cytisus* did not show reduction in viability when desiccated for up to 24 h. Pollen grains of many other species such as *Iris*, *Lonicera* and *Plantago* registered a significant reduction in viability following desiccation for 1–24 h. However, viability in desiccated pollen was restored when they were exposed to controlled hydration before testing for viability. These studies indicate that in many systems desiccation results in disruption of membrane integrity and controlled hydration provides favourable conditions for its restoration. However, in cereals such as rye, pollen grains are highly sensitive to desiccation resulting in irreversible loss of membrane integrity[6].

Pollen vigour

Pollen vigour refers to the speed of germination of pollen grains and the rate of pollen tube growth. Pollen vigour plays a significant role in pollen competition and pollen selection during pollen-pistil interaction[15]. Although extensive studies have been carried out on pollen viability, particularly in relation to storage, information on pollen vigour is scanty. The ultimate aim of pollen storage has been the establishment of 'pollen banks' through which required pollen can be procured at any time and at any place. To make pollen banks a reality, it is necessary to standardize storage conditions not only for maintenance of viability (which has been the major consideration of pollen biologists so far) but also of vigour. It is important to recognize that in seed storage programmes seed vigour is routinely checked in assessing their quality[16].

During the last five years a number of studies have been carried out in our laboratory to obtain information on the relationship between pollen viability and pollen vigour. Two tests have been used for assessing pollen vigour:

i) *In vitro germination test.* This test has been used extensively to assess pollen viability by scoring the germination percentage of a pollen sample after a fixed time, generally much longer than the time required for germination of vigorous pollen. *In vitro* germination can also be used for assessing pollen vigour by monitoring the rate of germination over a period of time (as is being followed in seeds) or the length of pollen tubes. The rate of germination and/or pollen tube length of treated sample can be compared with that of fresh pollen to determine the loss of vigour in the treated sample.

ii) *Semi-vivo technique.* In this technique, the stigma is pollinated with the pollen sample; pollen grains are allowed to germinate on the stigma and grow into the style for some length. The style is then cut in front of the growing pollen tubes and the cut end is implanted in agar or liquid medium[5]. Pollen tubes would emerge from the cut end of the style and grow into the medium. Pollen vigour is assessed on the basis of the time taken for pollen tube emergence and the number of pollen tubes that grow into the medium.

The effects of stresses such as high humidity, high temperature and storage have been studied on pollen viability and pollen vigour. Exposure of pollen grains of *Nicotiana tabacum* to high humidity (90% RH, at laboratory temperature) or high temperature (up to 45° C under dry condition) for 4 h did not affect pollen viability or vigour[1,9,17].

Alternating cycles of 1 h of high RH and 1 h of drying at laboratory temperature (22° ± 2° C) did not affect pollen viability and vigour for up to two cycles; beyond two cycles pollen vigour was reduced but not viability. Similarly, exposure of pollen to high RH at 38° C significantly reduced vigour without affecting pollen viability. Whereas in fresh pollen, maximum germination was achieved in 2–4 h; in stressed pollen it took 8 h to attain maximum germination scores.

High RH treatment even at 45° C did not affect pollen viability but such pollen grains failed to germinate *in vitro*. However, they germinated on the stigma, although they took a much longer time than fresh pollen. Apparently some factor(s) required for germination which becomes limiting in stressed pollen is/are made good by the stigma. The results of semi-vivo technique on pollen vigour were similar to those of *in vitro* germination test. Pollen grains subjected to high RH at 45° C showed many abnormalities in the pistil and their tubes reached the ovary 40 h later than those from fresh pollen[17].

The major ultrastructural effects of high RH and temperature stress was on the rough endoplasmic reticulum (RER). Stacks of RER, characteristically present in fresh pollen, were dissociated in stressed pollen[18].

Pollen grains of *Brassica* and *Petunia* are capable of withstanding high temperature[8]. Exposure of pollen samples to 45/60° C for up to 12 h did not affect pollen viability but reduced pollen vigour. However, pollination with these pollen samples did result in normal fruit and seed set. Storage stress also affects pollen vigour before affecting viability[1,13,19].

Pollen transfer

Details of pollen transfer have been studied in *Lantana camara*[20–22], *Solanum tuberosum*[23], *Dalbergia sissoo*[24–26] and a few aquatic flowering plants[27].

L. camara is a highly successful exotic weed and is considered one of the ten worst weeds of the world. Our investigations[20,22] have given an insight into the interaction between the pollinators and flowers, and the reproductive success of this weed. A wide spectrum of flower colour is seen in *Lantana* sp. The colour variant used in our studies produces yellow flowers (rich in β-carotene) at anthesis which subsequently change to orange, scarlet and magenta, due to synthesis of delphinidin monoglucoside. The flowers are bisexual and self-compatible but require insects for effective pollination. Two members of the Thysanoptera — *Thrips hawaiiensis* and *Haplothrips tenuipennis* — have been identified as regular pollinators. The flower structure is such that it does not permit the thrips to reach the nectar; they feed on the stigmatic exudate and pollen. They visit only yellow flowers and avoid flowers of other colours. The colour change is triggered by pollination. Interestingly the presence of even one pollen on the stigma is sufficient to cause the colour change. An extract of *Lantana* pollen is also effective in inducing the colour changes. Preference of yellow flowers by thrips enables the partitioning of pollinators and thus helps in conserving pollinator's energy and enhancing pollination efficiency. Although butterflies visit *Lantana* in two seasons (June–October and February–March) they do not seem to play an important role in seed production; seed set is not markedly reduced even when butterflies are excluded from flowers. There is structural evidence that the proboscis of a butterfly can descend and extract the nectar without coming into contact with pollen and stigma. The presence of thrips, floral abundance and high fruit set over several months, and bird dissemination of seeds significantly contribute to the spread of this weed.

The pollination biology of aquatic angiosperms is a fascinating field of study. The details of pollination biology have been studied in *Utricularia inflexa* and two species of *Ceratophyllum* under *in vitro* conditions. The *in vitro* method has special advantage for studies on pollination biology of aquatic plants as precise observations can be made under controlled conditions using a small volume of the liquid medium/water. In *U. inflexa* var. *stellaris*, the inflorescence is held above the level of water by a circlet of floats. In cultures that respond to photoperiodic induction, the stamens in an open flower are seen closely appressed to the funnel-shaped stigma. The pollen grains germinate inside the anthers and are deposited *en masse* by the inward bending of the stamens and dehiscence of anthers.[28] This ensures good seed set even in the absence of cross-pollination[28].

In *Ceratophyllum*[27,29] a monoecious plant, the male flowers bear a large number of spirally arranged stamens, each with an apical float. At maturity the stamens abscise and rise to the surface of water by the buoyancy of the floats. A few pollen grains germinate *in situ* within the anthers of intact stamen. Following abscission of the stamens more pollen grains germinate *in situ*. The stamens dehisce after floating on the surface of the medium for a day, and liberate both germinated and ungerminated pollen grains. The pollen grains gradually sink and come in contact with the stigma of submerged female flowers. The production of pollen is so copious, that the bottom of the culture vessel shows a thick pollen mass. The details of pollination in *Ceratophyllum* growing *in vivo* are similar to those observed *in vitro*.

Detailed studies on sex-expression, breeding system and pollen biology of *Ricinocarpos pinifolius*[30] have shown that the species produces male and hermaphrodite (with staminate and pistillate flowers) plants. Pollen grains from male and hermaphrodite plants are equally viable and are able to effect fertilization and seed-set. Geitonogamous selfing is largely prevented by temporal separation of male and female flowers within the plants.

Phenology, breeding system and pollination ecology have been studied[24–26] in *Dalbergia sissoo*, a species of immense rural, commercial and ecological importance. Flowering in this tree is confined to a short period of 6–10 weeks. An interesting outcome of these studies has been the demonstration that the species is predominantly an outbreeder and exhibits self-incompatibility. *Apis dorsata* is the main pollinator. The bee operates the flower in a highly specialized manner using an olfactory attractant and nectar as the predominant reward. The plants ensure efficient pollination by presenting floral resource for a short while in a concentrated manner, whereas the bee achieves maximum dietary supply with minimum energy expenditure.

We have recently initiated studies on pollination biology of some of the tree species, which yield gums and gum resins, and members of Podostemaceae[31].

True seed potato (TPS)

Potato is traditionally propagated vegetatively by seed tubers. This has certain advantages such as (a) it ensures maintenance of quality, (b) it is too easy to establish, (c) leads to vigorous growth of seedlings, and (d) requires no transplantation. Nevertheless, in tropical countries potato cultivation is beset with major problems such as: (i) high cost of tubers accounting for nearly 50 per cent of the cost of production, (ii) bulkiness of tubers requiring large storage space, (iii) high cost of transportation, (iv) rapid deterioration of tubers at room temperature, (v) inadequate cold storage facilities, (vi) high energy requirement for cold storage, (vii) transmission of virus diseases through tubers, and (viii) drain on food reserves[23].

Use of true seeds has been suggested as a means of overcoming many of these problems. True seeds are small, easy to store and convenient to handle and transport. A packet of seeds weighing 100 g is sufficient for planting one hectare of land as against two tonnes of seed tubers, thus substantially reducing the initial cost of planting and cultivation. Further being dry, they are less susceptible to pests and storage diseases and do not transmit virus diseases.

The success of raising potatoes from true seed will depend on the technology of production of true seed. A thorough knowledge of the reproductive biology of potato is a pre-requisite for producing consistently good seeds on a larger scale. Extensive studies on sexual reproduction in potato, *Solanum tuberosum* sub sp. *tuberosum* var. TPS 3 (F_1 hybrid of Ekishiraju × Kathahdin) have been carried out[23]. Scions of potato were grafted on to stocks of tomato seedlings to ensure flowering and fruit set under Delhi conditions. Comparative studies were also made on plants growing under natural conditions near Manali (2521 M high in Himachal Pradesh). The parameters examined included floral ontogeny, floral biology, pollination, fruit and seed development, and seed germination. This variety is characterized by self-pollination by the pollen released through apical pores. No insect visitors are involved in pollination, although gravity and wind vibrations may have some role in pollen transfer.

Open pollination results in 27.25 per cent seed set, whereas one, two and three hand pollinations of emasculated and bagged flowers resulted in 3.3, 7.75 and 41.26 per cent seed set respectively. Maximum fruit weight was recorded in those resulting from three hand pollinations. These studies clearly show that insufficient pollination is a major limitation for seed set under natural conditions[32,33].

Seed produced as a result of open pollination showed only 32 per cent germination while those produced by three hand pollinations registered 80 per cent germination. Thus treatments involving three hand pollinations of emasculated and bagged flowers seem to be most effective in producing seeds in potatoes.

Pollen–pistil interaction

To obtain a clear understanding of pollen–pistil interaction, it is necessary to study the structural organization of the pistil. An initial survey of the stigma of over 1000 species of about 900 genera and 250 families[34] not only documented a range of morphological diversity but brought out many correlations between the morphology of the pistil and of pollen–pistil interaction.

A subsequent inquiry addressed itself to the structural details of the pistil in *Saccharum*[35], *Petunia*, *Nicotiana*, *Crinum* and *Amaryllis*[36] over 25 papilionoid legumes such as *Vigna*, *Cajanus*, *Cicer* and *Arachis*[36–42], *Zephranthes*[43], *Linum*[44–46], *Lantana*[21], *Brassica*[47], *Solanum*[23], *Dalbergia*[24] and *Hypericum*[48,49]. Stigma receptivity, pollination, pollen germination and pollen tube growth were also investigated in many of these taxa.

In all the members studied, the receptive surface of the stigma invariably was found to have extracellular proteins either as a part of the exudate (in wet stigma) or as a thin extracellular lining, the pellicle (in dry stigmas). At early developmental stages, the wet stigmas lack the exudate but contain the pellicle as in dry stigmas. The pellicle becomes disrupted during the secretion of the exudate. The stigmatic exudate does not seem to be necessary for pollen germination in species such as *Petunia* and *Nicotiana* whereas in others, such as *Amaryllis* and *Crinum*, the exudate is essential[36]. Extracellular proteins are also present in the path of the pollen tubes in both solid and hollow styles. Extracellular proteins, present on the stigma and in the style, come in direct contact with pollen grains and pollen tubes and play an important role in pollen–pistil interaction.

In all the papilionoid legumes studied, the pistil is of the hollow type. However, unlike hollow styles of the monocotyledons, those of the legumes in the upper part of the style originate due to lysogeny of the transmitting tissue. In many species the stylar cavity remains dry and in some, such as *Crotalaria*, it becomes filled with the secretion fluid. An analysis of the organization of the stigma in different legumes shows a close relationship between the structure of the stigma and breeding system of the species. Variations in the distribution of the receptive region, thickness of the cuticle, and the nature of non-receptive hairs around the stigma play an important role in preventing self-pollination[42].

Some aspects of floral biology of *Cassia fistula* have been studied[49a]. The golden yellow flowers bear 10 stamens in two whorls of five each, in three sizes; 3 long, 4 medium and 3 short. In some trees the three short stamens are sterile. Although the Fabaceae are reported to be characterized by starchless pollen grains[50] both starchy (27.5–47.0%) and lipid-rich (53.0 to 72.5%) pollen have been identified in the same flower. Starchy pollen are abundant in the anthers of the inner whorl and lipid-rich pollen occur in the stamens of the outer whorl.

Of the 0.65 million pollen produced on an average by a flower, only about 250–350 pollen are used for pollination. The pollen were detected in the hollow apical part of the style which acts as the stigma and not on the outer surface of the stylar tip. It is quite likely that pollination in *Cassia fistula* is affected by some large-bodied insects which push the pollen into the

hollow stigma with some force; this however requires confirmation.

Pollen competition and selection

One of the recent developments in pollination biology is the demonstration of the existence of pollen competition during pollen–pistil interaction (resulting in non-random fertilization) and the potential of pollen selection as a breeding tool to achieve preferential transmission of adaptive genes to the progeny[15,51]. Results of *in vitro* studies on pollen germination and pollen tube growth in the presence of pistil leachates in *Crotalaria retusa* have provided evidence for the operation of selection pressure during pollen–pistil interaction[39]. Leachates from the pistil stimulate the growth of a limited number of pollen tubes giving them an advantage in effecting fertilization over others.

Application of pollen selection as a tool in plant breeding is the outcome of recent studies documenting the expression of genes imparting resistance to biotic and abiotic stresses (such as pathotoxins, salinity, temperature and herbicide) in both the sporophyte and the pollen. This has opened up the potential of using pollen not only for screening plants for the presence of desirable genes but also for application of selection pressure on pollen to increase the frequency of plants in the progeny with desirable genes. This aspect of pollen biotechnology has received considerable attention because the use of pollen as a selection unit is rapid, convenient, efficient and far less expensive than the use of whole plants.

For an effective application of pollen selection, a primary requirement is the demonstration of the expression of adaptive genes in the pollen. A convenient method used for these studies is to correlate the responses of the pollen and of the plant to a given stress. We have carried out studies on these lines for a toxin (destruxin B) of *Alternaria brassicae* which causes a serious disease of oilseed *Brassica*[52]. Effects of the [illegible] tudied on *in vitro* pollen germination and [illegible] growth of many host species susceptible to [illegible] and compared them with the [illegible] to the toxin. The degree of [illegible] species was commensurate with [illegible] rains, indicating that the genes imparting susceptibility/resistance to the toxin are also expressed in the pollen. A method of incorporating the toxin to pollen grains through excised inflorescence axis has also been standardized. Such a method is necessary for applying selection pressure on pollen because *Brassica* pollen treated with the toxin solution lose their ability to germinate on the stigma or their pollen tubes fail to penetrate the stigma.

Following the results of studies on the effect of high temperature and humidity stress on pollen viability and vigour[1,9,17], we explored the possibility of using temperature and humidity stress as selection pressure on pollen. Pollen grains were subjected to high temperature (38° C and 45° C) and/or high RH (95%) for 4 h and then used for pollination[53]. Seeds obtained from pollen samples subjected to 38° C + high RH and 45° C (under dry condition) showed early germination and their seedlings were significantly more vigorous (when compared with those derived from untreated pollen) as manifested by fresh and dry weight measurements. These interesting findings need to be extended.

Incompatibility

Incompatibility has been a major field of research of the senior author and several aspects of both self-incompatibility and inter-specific incompatibility have been investigated during the past two decades.

Heteromorphic self-incompatibility

Heteromorphic self-incompatibility has been examined in several species of *Linum*. In *Linum grandiflorum*, the proteins of the stigma as well as of pollen of pin and thrum morphs show quantitative and qualitative differences. The stigma of the pin morph is of the dry type while that of the thrum morph is of the wet type. These studies have explained the differences observed in the responses of intra- and inter-morph pollinations. Such differences in the stigmas of the two morphs have subsequently been confirmed in a few other heteromorphic species by other investigators.

In *Linum*, intra-morph incompatibility was shown to be a combination of structural mismatch between the pollen and the stigma and active inhibition of the pollen tubes. The former was a passive inhibition and the latter resulted from positive recognition of the pollen. This interpretation was contrary to the classical elucidation of self-incompatibility in heteromorphic systems based on mismatch in osmotic potential of the pollen and the stigma[54]. Subsequent studies by other investigators have supported our conclusions[55].

Some physiological aspects of pollen and extent of pollen inhibition following intra-morph pollinations have been investigated in *Primula absconica*. Pollen grains from pin and thrum morphs differ markedly in their water economy[56] which appears to have key functions in self-incompatibility responses. Inhibition of intra-morph pollen may occur either at the stigma surface or in the tissues of the stigma after pollen tube entry or in the transmitting tissue of the style. Thus inhibition of intra-morph pollen is a result of cumulative screening at different levels[57]. These studies

also provided evidence for the presence of factors that influence pollen tube growth in the intracellular secretions of the transmitting tract.

Homomorphic self-incompatibility

Homomorphic self-incompatibility has been investigated in *Saccharum*[35], *Zephyranthes*[43] *Petunia* and *Nicotiana*[58-60].

An *in vitro* bioassay in which self-pollen grains are selectively recognized and inhibited was established in both *Petunia* and *Nicotiana*[61]. These studies clearly established that incompatibility factors in the pistil are synthesized before pollination, and inhibition of incompatible pollen does not require transcription/translation in the pistil. Using the bio-assay, evidences were obtained for the involvement of lectin-like components of the pollen and specific sugar moiety of the pistil in self-incompatibility recognition[59-64]. Recently, a lectin has been isolated from pollen grains of *Petunia* and partially characterized[65]. Our results on the involvement of pollen lectins in self-incompatibility have been supported by studies made by Speranza and Calzoni[66].

Self-incompatibility is an impediment in genetic studies of a species and also in plant breeding programmes[3]. The following techniques have been used to overcome self-incompatibility: (i) bud pollination[67], (ii) use of mentor pollen[68], (iii) treatment of stigma with lectins or pollen with sugars before pollination[59,60], (iv) treatment of stigma with an extract of compatible stigma[63] and (v) placental pollination[69-71].

Placental pollination is the modification of an earlier technique of *in vitro* fertilization developed by Kanta *et al.*[72]. Placental pollination essentially involves culturing of the entire mass of ovules attached to the placenta (together with a short length of pedicel) and sprinkling the ovules with pollen grains. Following placental pollination the pollen grain germinates and pollen tubes enter the ovules, leading to normal fertilization and seed development. The technique was effective in overcoming self-incompatibility. As placental pollination eliminates the zone of pollen inhibition (stigma and style), it is regarded as the most effective means of overcoming both self- and inter-specific incompatibility. The technique has been used by other scientists to achieve inter-specific and inter-generic hybridization[73].

Inter-specific incompatibility

Introgression of genes imparting resistance to biotic and abiotic stresses into the cultivars has been the main objective of modern plant breeding programmes. As these adaptive genes have been exhausted in most of the crop species, breeders have to explore wild and weedy germplasm to tap them. Also, many of the adaptive traits are polygenic and are not readily amenable to recombinant DNA technology. Hence wide hybridization has been considered as a priority area of research in several crop species.

With this in view, extensive studies on wide hybridization in *Brassica* have been carried out at the University of Delhi. Existing cultivars of oilseed brassicas (*B. juncea*, *B. campestris* and *B. napus*) are highly susceptible to *Alternaria* blight, white rust and aphids. The genes imparting resistance to these diseases and pests are not available in the genomes of the cultivated species, but are present in many wild species. Crosses between cultivated species and wild species are highly incompatible. Shivanna and coworkers have been able to produce over 25 inter-specific/inter-generic hybrids through embryo rescue. Some of the wild and related species used are: many wild species of *Brassica*[74-76], many species of *Diplotaxis*[77], *Eruca sativa*[78], *Enarthrocarpus*[79], *Raphanobrassica*[80] and *Sinapis*[81]. As the hybrids are invariably sterile, colchiploidy has been induced in many of the hybrids to restore fertility and backcrosses have been shown promise for breeding improved varieties of the cultivars.

Wide hybrids have also been produced in some legumes, for example *Cicer arietinum* × *C. reticulatum*[40] and *Vigna umbellata* × *V. minima*[82].

Development of new cytoplasmic male sterile lines

Exploitation of hybrid vigour for augmenting crop yields has been a major strategy in agriculture in the 20th century. Procedures involving emasculation and hand-pollination are time-consuming and labour intensive resulting in a high cost of hybrid seed production. Use of cytoplasmic and genetic male sterile lines in breeding programmes have been largely responsible for overcoming these disadvantages.

Employing wide hybridization it has been possible to develop cytoplasmic male sterile (CMS) lines through the substitution of the cytoplasm of the cultivars with that of the wild species (alloplasmic lines). This approach essentially involves hybridization using the wild species as the female parent and cultivar as the male parent and repeated backcrossing of the hybrid with pollen of the cultivar. Although some CMS lines are already available in *Brassica*, it has not yet been possible to exploit hybrid vigour because of one or more limitations in the existing CMS lines. Development of new CMS lines is important not only for the commercial production of hybrid seeds, but also as a safeguard against vulnerability to diseases associated with certain cytoplasms.

The wide hybridization programmes in *Brassica* taken up in the senior author's laboratory includes development of new CMS lines. Two new CMS lines in

B. juncea, one in the cytoplasmic background of *Diplotaxis siifolia*[8], and another in the cytoplasmic background of *Erucastrum gallicum* (Rao *et al.* unpublished) have already been developed. They show good female fertility and nectary development. Seeds of the CMS line with the cytoplasmic background of *D. siifolia* have been distributed to breeders for trials. A new CMS line has also been developed in *B. campestris* in the cytoplasmic background of *Enarthrocarpus lyratus*[81]. Many more alloplasmic lines are in different backcross generations. Efforts are being made to restore fertility in these new CMS systems.

Suitable genetic male sterile systems are not available in many crop species. With the increasing application of plant growth regulators (PGRs) in crop production, the prospects of using selective male gametocidal agents as an alternative to genetic male sterility has been drawing the attention of physiologists. Mohan Ram and Rustagi[83] reviewed the literature on phytogametocidal compounds. These authors subsequently carried out detailed studies on the effects of two herbicidal compounds with reported gametocidal properties, namely Mendok (sodium 2,3-dichloroisobutyrate) and Dalapon (sodium 2,2 dichloro-propionate) on linseed (*Linum usitatissimum* L. var. N.P. (R.R.) 9) and wheat[84].

Linseed plants were sprayed with aqueous solutions of these PGRs at 250, 500 and 1000 ppm, with a view to inducing male sterility. Both compounds produced more or less similar responses except that Mendok was slightly more effective than Dalapon. Gamete fertility was reduced by almost all treatments. Female sterility was caused by decrease in size and abnormal morphology of pistils, and non-separation of styles and stigmas. Male sterility was induced in two forms: developmental and functional. The former was characterized by fully or partially barren anthers and non-viable pollen. Rhythms of pollen non-viability interspersed with periods of restoration of viability were noted. Functional male sterility resulted from lack of anthesis, fusion and non-dehiscence of fertile anthers and agglutination of pollen.

The tapetal cell in the anthers of undersized flowers was highly vacuolate, radially stertched and it persisted up to the mature pollen grain stage. Another feature of morphological interest was the differentiation of microsporangia on petals. It is concluded that neither Mendok nor Dalapon can be used as a specific male sterilizing agent in linseed.

Concluding remarks

Although India is endowed with a rich flora distributed over a wide range of habitats, the number of species in which pollination biology has been studied is rather limited and the data collected are fragmentary. The majority of our tree species, especially of the tropical forests, endangered species, and aquatic species have remained particularly untouched.

Pollination biology can be studied at different levels from careful field observations and gross morphology to molecular biology. Studies on basic pollination biology do not require expensive infrastructure, rather it needs techniques that are simple and straightforward. Thus, studies on pollination biology can be undertaken at any educational institution located close to the natural habitat of species and cultivated fields. This is of particular advantage as pollination biology has to be studied at the population level rather than at the level of individuals or species. Studies need to be taken up by a large number of research workers spread throughout the country.

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