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Incompatibility in Angiosperms: Significance in Crop Improvement

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I. INTRODUCTION

There is a continuous need for modifying crop plants to suit changing human needs in existing environments and to fit the crops into new environments. Most often such modifications are achieved by hybridization. The objective for modification, such as alteration of a character or introduction of a new character into a cultivar, dictates the choice of parents in any breeding program. Most often the parents are close to each other taxonomically and usually belong to the same species. However, there are instances when the parents are only distantly related and may also be reproductively isolated. Such situations are growing in number, for desired characters are not (and need not be) always available in closely related taxa. In such cases the choice of parents may be limited and is governed primarily by the availability of character(s) in a taxon; but the taxon in which the character is avail-

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able may be distantly related, and the hybrid may not be produced at all, and even if produced it may not be viable or fertile.

In this article an account of the problems usually encountered in such situations and the methods to circumvent them are discussed. Incompatibility in angiosperms has been known for about 200 years. The very existence of these barriers between taxa has been used as a criterion for taxonomic delimitations, but has been the cause of frustration to plant breeders interested in transfer of character(s) from one taxon to another, as well as to evolutionary biologists interested in the phylogeny of a group of taxa. The solution to this problem has often come from geneticists, physiologists, and cytologists who have repeatedly attacked this problem. Commendable progress has been made, as is evident by two full-length discussions on the subject by the Royal Society, London—"Incompatibility in Angiosperms" in 1975 and "Manipulations of Genetic Systems in Plants" (Rees *et al.*, 1981)—in addition to a 300-page monograph by Professor de Nettancourt (1977) and a large number of research and review papers on the topic.

II. INCOMPATIBILITY

Incompatibility is defined as the inability of the functional male and female gametes to fuse with each other to form a viable zygote and a hybrid (Arasu, 1968). Incompatibility is used here to refer to failure of seed set after either self- or cross-pollinations. Temporal and/or geographic separation (or isolation) of two taxa to be hybridized sometimes occur, but incompatibility should not be assumed in these cases. Such problems have been solved by low-temperature storage of pollen until required or by transporting pollen to overcome geographical separation. There are instances when certain genetic changes may lead to incompatibility between two taxa. Incompatibility between taxa, referred to as interspecific incompatibility (or cross-incompatibility) in the literature, prevents promiscuous hybridization, whereas incompatibility within a taxon, referred to as intraspecific (or self-) incompatibility, is an evolutionary strategy to promote outcrossing.

For convenience, therefore, incompatibility can be discussed under two broad titles: intraspecific and interspecific. In the context of crop improvement, however, incompatibility between taxa is of greater concern as it prevents the desired transfer of genes. But investigations on several aspects of self-incompatibility, and some on interspecific incompatibility, have revealed that inhibition of pollen germination and pollen tube growth are similar in both. There may also be a common genetic control; for instance, in *Nicotiana*, Pandey (1976) observed that alleles governing self-incompatibility are effective in interspecific incompatibility also.

A. SELF-INCOMPATIBILITY (INTRASPECIFIC)

About half of the flowering plant species investigated so far have been found to be self-incompatible (de Nettancourt, 1977). Self-incompatibility is the rejection by a plant of its own pollen, or pollen from the same genotype, before or after it has germinated on the stigma, but mostly before fertilization. It is believed to be the result of an interaction between the male gametophyte (pollen grain) and the sporophytic tissue of the pistil. Geneticists have recognized taxa with either a sporophytic or a gametophytic type of self-incompatibility depending on whether self-incompatibility is controlled by the genotype of the sporophyte (pollen parent) or that of the gametophyte (pollen grain), respectively, Brewbaker (1957, 1967) found that in taxa with the sporophytic type of self-incompatibility, the pollen grain is usually three celled at anthesis and is inhibited on the stigma, whereas in taxa with gametophytic self-incompatibility, pollen grains are two celled at anthesis and it is the pollen tubes that are inhibited in the style. This seems to be the general trend, but there are a few exceptions (Brewbaker, 1967).

During the last two decades there has been a great interest in structural and functional aspects of the incompatibility reaction. In spite of concerted efforts by physiologists and biochemists, a precise interactive model is still to be defined.

1. *Sporophytic self-incompatibility*

Brassica campestris, Brassica oleracea, Raphanus sativus, Eruca sativa, Iberis amara (Brassicaceae), *Cosmos bipinnatus, Helianthus annuus* (Asteraceae), and *Ipomoea* spp. (Convolvulaceae) are well-known examples of the sporophytic system of self-incompatibility, and in these cases incompatible pollen is invariably inhibited on the stigma. A phenomenon correlated with this is the characteristic synthesis and accumulation of callose in the form of lenticular deposits in the stigma cells in direct contact with the pollen grain (Dickinson and Lewis, 1973; Heslop-Harrison and Heslop-Harrison, 1975), and this has been suggested as a bioassay. This phenomenon is strongly suggestive of the fact that the pollen and the pistil do communicate with each other. Cytochemical investigations have revealed that there are certain proteinaceous substances on the surface of the pollen grains (Heslop-Harrison *et al.*, 1973, 1974; Dickinson and Lewis, 1973; Howlett *et al.*, 1975) as well as on stigma cells (Mattsson *et al.*, 1974; Heslop-Harrison *et al.*, 1975; Knox *et al.*, 1976; Heslop-Harrison and Shivanna, 1977; Heslop-Harrison, 1981). The pollen wall proteins are labile and diffuse within minutes on the moist substratum of the stigma or on agar gel (Heslop-Harrison *et al*., 1974). These diffusates from incompatible pollen are potentially capable of inducing callose synthesis in the stigma papillae (Dickinson and Lewis, 1973; Heslop-Harrison *et al.*, 1973, 1974). On the other hand, incubation of the stigma in a protein-digesting enzyme (Heslop-Harrison and Heslop-Harrison, 1975; Heslop-Harrison and Shivanna, 1977) or coating the stigma with concanavalin (a lectin) (Heslop-Harrison, 1976; Knox *et al.*, 1976) has been found to disturb the behavior of even compatible pollen grains, i.e., preventing the entry of pollen tubes into the stigmatic tissue. Serological and electrophoretic investigations on *B. oleracea* stigma proteins have led to the identification of the self-incompatibility allele (*S* allele) specific proteins (Nasrallah and Wallace, 1967; Nasrallah *et al.*, 1970; Sedgley, 1974; Nishio and Hinata, 1977, 1978, 1980). The most likely source of these proteins is the stigma surface, as shown for *Brassica* (Heslop-Harrison *et al.*, 1975). Furthermore, it has also been reported that *Brassica* stigmas have a factor that inhibits self-incompatible pollen *in vitro* (Ferrari and Wallace, 1975, 1976).

The nature of the pollen grain in contact with the stigma papillae determines the direction of the events leading to either pollen acceptance or rejection. The first event, viz., adhesion of self-incompatible pollen, is slower than that of the compatible pollen grains in *B. oleracea* (Roggen, 1975; Stead *et al.*, 1979; Roberts *et al.*, 1980). This is followed by the diffusion of the pollen wall proteins onto the stigma accompanied by imbibition by the pollen grains of moisture from stigma. Stead *et al.* (1979, 1980) and Roberts *et al.* (1980) have proposed that hydration of compatible pollen is different from that of incompatible pollen. They have also suggested that there is a protein fraction responsible for pollen grain adhesion, and Ferrari *et al.* (1981a) have shown that a hydrophilic stigmatic factor is involved in pollen hydration. The next discernible change is the germination of the pollen grain and the growth of the pollen tubes, which are different in compatible and incompatible pollinations (see reviews by Heslop-Harrison, 1975a,b, 1978a,b).

2. *Gametophytic self-incompatibility*

In taxa with gametophytic self-incompatibility, the genotype of the pollen (gametophyte) is responsible for the incompatibility (see de Nettancourt, 1977). The first observation of this kind of incompatibility was in *Nicotiana* (East and Mangelsdorf, 1925). Subsequently, other taxa, such as *Petunia hybrida, Lilium longiflorum, Trifolium pratense,* and *Oenothera organensis*, have also been found to have gametophytic self-incompatibility. In these taxa the site of inhibition is usually the style, and the stigma is usually covered with a copious exudate at the time when pollination normally takes place; these are thus referred to as wet-type stigmas. Lipids, sugars, phenols, proteins, and water have been identified in the stigmatic exudate of some taxa, and a role has been proposed for each of these components in stigma receptivity and pollen germination. In comparing the self-incompatible taxa having dry-type stigmas, proteins in the stigmatic exudate of the taxa with wet stigmas have not been attributed with specific roles in pollen recognition and pollen germination. But proteins on the stigma surface have been identified; these are extracellular and are present on the stigma papillae during early stages of development. The fact that the inhibition of incompatible pollen tubes in these taxa is in the style led East (1934) to suggest that the inhibition is in some way analogous to the antigen–antibody reaction found in animals. This assumption has prompted several investigators to propose hypotheses on incompatibility assuming that proteins are indeed the interacting molecules involved in rejection or acceptance of the pollen tubes (see discussion in Ferrari and Wallace, 1977; de Nettancourt, 1977; Heslop-Harrison, 1978a,b; Ferrari *et al.*, 1981b). Whatever the mechanism, it has been amply clarified that pollination triggers a reaction characteristic of the nature of the pollination. This is evident from structural, ultrastructural, physiological, and biochemical comparisons of the compatibly and incompatibly pollinated pistils.

In *P. hybrida* no apparent distinctions have been found between the behavior of the compatible and incompatible pollen grains on the stigma, or even of the pollen tubes within it. The differences are apparent only when the pollen tubes have come in contact with the stylar tissue (Sastri and Shivanna, 1980a; Shivanna and Sastri, 1981; Herrero and Dickinson, 1980b). In incompatible pollinations there may be a reduction in the number of pollen tubes deeper in the styles, slower rates of growth of incompatible pollen tubes and heavy callose deposits along the pollen tube lengths, and abnormalities at the tube tips such as swelling, bursting, or branching of the pollen tubes. Incompatible pollen tube walls are much thicker than those of compatible pollen tubes (vander Pluijm and Linskens, 1966). Differences have been found in the pistil also. In *P. hybrida*, for instance, Herrero and Dickinson (1979) observed that in a compatibly pollinated pistil, starch and lipid reserves are mobilized in the style faster than after incompatible pollination. In the incompatibly pollinated pistil of *Lycopersicon peruvianum*, it was found that self-incompatible pollen tube tips revealed a concentric organization of the rough endoplasmic reticulum (de Nettancourt *et al.*, 1973a,b, 1974; Cresti *et al.*, 1980), which is inhibitory for protein synthesis. A similar observation was also made in *P. hybrida* (Cresti *et al.*, 1979).

van der Donk (1974a,b, 1975) reported differences in protein and RNA synthesis in compatibly and incompatibly pollinated pistils. In *Nicotiana alata* there are differences in peroxidase patterns corresponding to the kind of pollination (Bredemeijer, 1974). Around 18 hr after self-pollination in *P. hybrida,* floral metabolites flow away from the flowers, whereas in compatible pollination the ovary continues to be the major sink (Linskens, 1975). Deurenberg (1976, 1977) observed that ovaries of crossed and selfed flowers revealed differences in proteins 12 hr after pollination.

B. INTERSPECIFIC INCOMPATIBILITY

During speciation and evolution, populations differentiate to such an extent that morphologically, physiologically, and/or genetically each one becomes a distinct entity warranting a unique taxonomic status. Reproductive isolation at some stage prevents gene flow among them, and the taxa are then described as incompatible with each other. Interspecific incompatibility has not been studied as extensively as intraspecific incompatibility. However, it is known that there is some similarity between the two kinds of incompatibility. Pollen tube growth may be inhibited in the style, as can be seen in a self-pollinated pistil of a taxon with the gametophytic type of self-incompatibility. In addition to the types of pollen inhibition met within self-incompatible systems, the incompatible taxa may reveal other phenomena. In spite of a normal pollen germination and pollen tube growth, fertilization between the two gametes may not occur; in the event of a normal fertilization the resulting hybrid zygote may collapse any time before it develops into an embryo or a seedling. Such a phenomenon may be due to lethality [e.g., *Gossypium davidsonii* when used as a parent in crosses with most *Gossypium* taxa (Lee, 1981)], genic disharmony, inefficient endosperm as in several cases, or the failure of the embryo. In a few cases the hybrid seeds and seedlings are formed, which then develop into plants, but these are sterile due to meiotic irregularities, do not produce gametes, and so do not form fruits and seeds.

Sometimes species can be crossed in one direction only and not in the reciprocal direction. Such observations have led to the concept of "unilateral incompatibility" as suggested by Harrison and Darby (1955). In such instances it is often found that the pistil of a self-compatible plant did not have any inhibitory effect on the pollen of the self-incompatible plant; the reciprocal cross, however, was not a successful one. Investigations on interspecific crosses in *Nicotiana* by Anderson and de Winton (1931), followed up by Pandey (1964, 1976), revealed that incompatibility in such cases was governed by a gene that also effected the self-incompatibility of the female parent. Martin (1968) concluded that unilateral incompatibility and self-incompatibility are under the same genetic control.

There is now another school of thought that considers interspecific incompatibility as a separate function with no interference by the factors controlling self-incompatibility. Hogenboom (1973), based on crosses between *L. peruvianum* × *Lycopersicon esculentum*, suggested that the inhibition of *L. esculentum* pollen tubes in *L. peruvianum* pistils was governed by loci different from those governing self-incompatibility. From the same crosses de Nettancourt *et al.* (1974) arrived at a different inference—that loci inhibiting pollen tube growth in this cross are either closely linked to or are allelic to the *S* locus.

Interspecific incompatibility is believed to be controlled by one gene or a group of genes and is often accompanied by zygotic and postzygotic inviability. Therefore, based on time and site of incompatibility, one or more of the following methods have to be critically selected for creation of new hybrids, as has been done in several cases in the past. It has to be emphasized that the determination of the cause of incompatibility is an essential prerequisite for deciding upon or developing a method for combining the two parental genomes. Some of these methods are indicated in Tables I–IV for some well-known crosses attempted in the past.

III. Circumvention of Barriers

A. The Early Methods

The early realization that the stigma or the style acted as the barrier to foreign pollen prompted certain surgical methods. These surgical methods evolved from the observation by Jost (1907) that transversely cut styles of two species, when placed end to end in the form of a graft, did permit the growth of pollen tubes. With refinements, this method was successfully applied to crosses that involved heterostylous parents. It is believed that pollen grains of long-styled plants have potentiality for longer growth (Rangaswamy, 1963). For example, pollen grains of *Nicotiana paniculata* (whose styles are 2–3 mm long) are not successful when dusted on the styles (∼10 mm) of *Nicotiana rustica*, whereas the reciprocal cross was successful (see Rangaswamy, 1963). Such incompatibility was overcome by grafting by Gardella (1950) in *Datura* and by Davies (1957) in *Lathyrus*. Elegant grafting experiments by Hecht (1960, 1964), in *O. organensis* revealed that self-incompatibility in this taxon could be overcome by grafting a stigmatic part (compatible with pollen grains) onto a stylar part (incompatibile with pollen grains). Fortunately, the flowers and pistils in *Oenothera* are large enough for such manipulations to be feasible. Similar experiments by Straub in *Petunia violacea* indicated that in a graft of compatible–incompatible stylar tissues, length of the compatible partner determined the extent of pollen tube growth in the incompatible partner (Straub, 1946, 1947).

These methods achieved a little more refinement in the experiments of Swaminathan (1955), who recommended the substitution of the natural stigma (causing incompatibility) with an agar–sucrose–gelatin medium on the cut end of the pistil. Swaminathan and Murty (1957) succeeded in making crosses in otherwise incompatible combinations in *Nicotiana* and *Solanum*. It was later realized that surgical operations are not always necessary; in *Brassica* and *Petunia* the stigma alone, or with some style, can be simply removed and self-pollen dusted on the cut ends to obtain fruits and seeds (see Maheswari, 1950; Frankel and Galun, 1977). In fact, in *B. oleracea* injury of the stigma by a steel wire brush is enough to break self-incompatibility (Roggen and van Dijk, 1972).

B. BUD POLLINATION

The idea of bud pollination probably arose from the realization that stigmatic secretion in mature flowers of some plants is inhibitory to self-pollen. The fact that in some taxa the mature stigmas are secretory and that the younger ones are not possibly prompted investigations on receptivity of immature pistils to incompatible pollen grains. One of the earliest of these was that of Yasuda (1934), who overcame self-incompatibility in *P. violacea* by self-pollinating the buds; Attia (1950) also succeeded in this way with *B. oleracea*. Linskens (1964) repeated the experiments of Yasuda with *P. hybrida* and found that the inhibition of incompatible pollen tubes was directly proportional to the age of the bud. Similar results were obtained when buds of *Petunia axillaris* were incompatibly pollinated (Shivanna and Rangaswamy, 1969). It was also found that smearing the stigmas of buds with stigmatic exudate from compatible mature flowers increased the success of bud self-pollination (Shivanna and Rangaswamy, 1969). In all these studies and in those on *Nicotiana alata* (Pandey, 1963; Bredemeijer, 1976) it must be noted that the developmental stage of the pistil is critical for optimum results. Pandey (1963) found that in *N. alata*, only buds at half the length of the mature flower responded to self-incompatible pollination; younger or older buds failed to do so. In the same species, Bredemeijer (1976) investigated pollen tube growth and pollen tube length in different stages of the pistils and found that in 3.5- to 5.5-cm-long buds the growth and length of compatible and incompatible pollen tubes were comparable. It was only in the later stages of development that the pistil was able to discriminate between the two kinds of pollen tubes. The results were similar when pollinated buds were analyzed for seed number per fruit (Bredemeijer, 1976). Investigations on *R. sativus*, *Cheiranthus cheiri*, and *Brassica* spp. led to similar observations (Haruta, 1966; Shivanna *et al.*, 1978; Shivanna and Sastri, 1981).

There have been some attempts to explain these results. Bredemeijer (1976) attributed the success of bud pollination in *N. alata* to the absence of a peroxidase isoenzyme (number 10) in the self-pollinated buds; this particular isoenzyme has been observed in self-pollinated mature flowers, suggesting that it is involved in the rejection of the incompatible pollen tubes (see also Bredemeijer and Blaas, 1975). It was suggested earlier that substances causing incompatibility are either absent or are not effective in immature pistils (Linskens, 1964). Nasrallah found that in immature stigmas of *Brassica oleracea*, proteins responsible for incompatibility either were absent or were present in very low concentrations (Nasrallah, 1974; Nasrallah and Wallace, 1967). The absence from buds of *S*-gene specific antigens being responsible for the success of bud pollination was also supported by studies of Shivanna *et al.* (1978) from their studies on *Raphanus* and *Cheiranthus*. Fractionation of stigmatic extracts by isoelectric focusing also revealed that there are indeed some fractions present in the mature stigmas that are absent from the buds of *B. oleracea* and *B. campestris* (Nishio and Hinata, 1977; Hinata and Nishio, 1978; Roberts *et al.*, 1979). Such differences were also apparent in *P. hybrida* (Sastri and Shivanna, 1980a; Herrero and Dickinson, 1980a; Sastri, 1981). Sastri and Shivanna (1980a) found that the pistils of buds showed some protein bands that were absent in the mature pistils.

Most of these studies have been on taxa that respond to bud pollination, and in all instances only self-incompatibility has been overcome. A question that emerges is whether bud pollination can also be extended to interspecific crosses. At the moment it is difficult to answer this because receptivity of buds in several taxa has yet to be investigated. In some taxa it is known that buds are incapable of accepting even compatible pollen, for example, *Sinapis alba* (Shivanna *et al.*, 1978), *L. longiflorum* (Ascher and Peloquin, 1966a), *Crinum defixum*, *Amaryllis vittata* (Shivanna and Sastri, 1981), *Saccharum bengalense* (Sastri and Shivanna, 1979), and *Arachis hypogaea* (D. C. Sastri, unpublished). In these and other taxa in which buds are not receptive or are poorly receptive, it has to be seen whether smearing the bud stigma with a medium such as exudate from mature stigmas (Shivanna and Rangaswamy, 1969), another extract (Frimmel, 1956), or a synthetic medium that is known to stimulate pollen germination can be of any help. However, Knott and Dvorak (1976) have suggested the possibility of using bud pollination in interspecific incompatible pollination.

C APPLICATION OF PLANT GROWTH REGULATORS

It is a well-recognized and accepted fact that, like other morphogenetic phenomena, the postfertilization changes leading to fruit formation are also under the influence of plant growth regulators, either in a sequence, independently, or in combination (Nitsch, 1952). Elucidation of hormonal regulation of fruit and seed development has been largely an academic interest. Also, the knowledge of these aspects is limited to such a small number of taxa that it is impossible to conceive a widely applicable hypothesis. Diversity in fruits is too great to warrant a general concept on hormonal regulation of fruit and seed development. However, a careful investigation of the postpollination events does reveal that these are under hormonal control. For example, Gilissen (1976) suggested that in *P. hybrida* differences in the floral wilting rates between compatible and incompatible pollinations are due to the style, which causes pollination-specific changes in the hormone metabolism. Sastri and Shivanna (1978) further showed that such changes in *Petunia* can be reversed by altering the kind of pollination. Self-incompatibly pollinated pistils of *P. hybrida*, when pollinated compatibly up to a certain time, can form pods and seeds (Sastri and Shivanna, 1978). Incidentally, Hall and Forsyth (1967) observed that among all the floral parts, the stigma and style released the greatest amount of ethylene, a gaseous hormone closely linked with wilting and ripening processes of flowers and fruits. It is also known that the changes in the flower due to incompatible pollinations are similar to those of senescence and abscission. In fact, in some of the early attempts, hormones were used to prolong the life of the flower, thereby effecting fertilization and preventing the floral abscission (see Rangaswamy, 1963). It is therefore necessary to find which hormones promote fruit develop-

2. *Hormones and interspecific incompatibility*

Achievement of pear × apple hybridization due to hormone application marked the first step (Crane and Marks, 1952; Brock, 1954) and stimulated a series of other investigations, many successful but some unsuccessful. β-Naphthoxyacetic acid applied to the stigma promoted successful germination of incompatible pollen in interspecific crosses in *Trifolium* (Evans and Denward, 1955). Dionne (1958) applied a drop of (2,4-dichlorophenoxy)acetic acid (3–6 ppm) to ovaries 24 hr after interspecific pollination in *Solanum* and obtained normal fruits and seeds. Incompatibility between *Phaseolus vulgaris* and *Phaseolus acutifolius* was overcome by applying a mixture of naphthalene acetamide and potassium gibberellate (Al Yasiri and Coyne, 1964). *Nicotiana repanda* was crossed with *Nicotiana tabacum* by applying a lanolin paste of IAA (Pittagelli and Stavely, 1975). Hybrid in the cross *Corchorus capsularis* × *Corchorus olitorius* was not obtained until 300 ppm of IAA was applied to the pedicels of flowers (Islam, 1964).

Hormone application was also used successfully for certain intergeneric crosses. By an application of 2,4-dimethylamine followed by an application of gibberellin, Kruse (1974) demonstrated that *Hordeum* species could be crossed with species of *Avena, Phleum, Dactylis, Alopecurus, Triticum, Lolium,* and *Festuca*. Bajaj *et al.* (1980) obtained culturable embryos in the cross *Hordeum vulgare* × *Secale cereale* by bathing pollinated spikes in a solution of a mixture of gibberellin (25 ppm) and kinetin (0.5 ppm) solution. Larter and Enns (1960) had found that gibberellic acid promoted better development of hybrid barley embryos *in vivo*. It was also found that a combination of gibberellic acid (25 ppm) and IAA (1 ppm) promoted pollen tube growth and ovary development in barley (4*x*) × rye (2*x*) crosses (Larter and Chaubey, 1965). Successful use of gibberellic acid (75 ppm) in an *H. vulgare* × *Hordeum bulbosum* cross (Subrahmanyam and Kasha, 1971) was demonstrated in a range of interspecific crosses in *Hordeum* (Subrahmanyam, 1979). Pickering (1979, 1980), however, was not successful in getting hybrids in an *H. vulgare* × *H. bulbosum* cross. Postpollination treatments of gibberellic acid (75 ppm) gave successful results in *Agropyron junceum* × *Triticum aestivum* (Alonso and Kimber, 1980), barley × wheat (Fedak, 1978; Islam *et al.*, 1976), *T. aestivum* × *Elymus giganteus* (Mujeeb-Kazi and Rodriguez, 1980), and *H. vulgare* × *T. aestivum* (Mujeeb-Kazi, 1981). Mujeeb-Kazi and Rodriguez (1982) consider that in addition to a postpollination treatment, a prepollination application of 2,4-dimethylamine as given by Kruse (1974) could help in obtaining seeds from backcrosses in *H. vulgare* × *Elymus can-*

adensis hybrids. The author's recent experience has shown that hormones, particularly gibberellin and kinetin, can be used in intersectional incompatible crosses in the genus *Arachis* (Singh *et al.*, 1980; Sastri and Moss, 1982; Sastri *et al.*, 1981, 1982). These studies, along with others (Table I), therefore indicate that hormones have

TABLE I. Use of hormones for hybridization in incompatible crosses

Cross	Hormone used	References
Agropyron × *Triticum aestivum*	Gibberellic acid	Alonso and Kimber (1980)
Arachis hypogaea × *Arachis* sp. P.I. No. 276233		Sastri and Moss (1982); Sastri *et al.* (1981)
A. hypogaea × *Arachis glabrata*	Gibberellic acid, kinetin,	Singh *et al.* (1980)
A. hypogaea × *Arachis pusilla*	1-naphthylacetic acid,	Sastri *et al.* (1982)
A. hypogaea × *Arachis* sp. Coll. No. 9649	indoleacetic acid,	
Arachis monticola × *Arachis* sp. P.I. No. 276233	1-naphthylacetic acid	
Corchorus olitorius × *Corchorus capsularis*	Indoleacetic acid	Islam (1964)
Hibiscus cannabinus × *Hibiscus sabdariffa*	Indoleacetic acid	Kuwada and Mabuchi (1976)
Hordeum × *Alopecurus*, *Hordeum* × *Avena*, *Hordeum* × *Dactylis*, *Hordeum* × *Festuca*, *Hordeum* × *Lolium*, *Hordeum* × *Phleum*	2,4-Dimethylamine	Kruse (1974)
Hordeum × *Triticum*	Gibberellic acid	Larter and Chaubey (1965)
Hordeum vulgare × *Secale cereale*	Gibberellic acid + kinetin	Bajaj *et al.* (1980)
H. vulgare × *T. aestivum*	Gibberellic acid	Fedak (1978); Islam *et al.* (1975)
Nicotiana repanda × *Nicotiana tabacum*	Indoleacetic acid	Pittagelli and Stavely (1975)
Phaseolus vulgaris × *Phaseolus acutifolius*	Naphthalene acetamide + potassium gibberellate	Al Yasiri and Coyne (1964)
Pyrus × *Malus*		Brock (1954); Crane and Marks (1952)
Solanum (interspecific)	(2,4-Dichlorophenoxy)-acetic acid	Dionne (1958)
Trifolium (interspecific)	β-Naphthoxyacetic acid	Evans and Denward (1955)

profitably been used in some interspecific and intergeneric incompatible crosses. It is not yet clear as to what is the precise role of the hormone used in such investigations. There are suggestions that in instances of retarded pollen tube growth and prefertilization abscission of the flower, hormones maintain the flower until the pollen tubes have grown long enough to discharge the male gametes in the vicinity of the female gametes; it is also suggested that hormones may stimulate the incompatible pollen tube growth in the pistils so that fertilization can take place before the flower has abscissed, but the hybrid zygote obtained this way may not develop any further or may not develop fully. In such cases embryos from immature fruits have to be excised and cultured for raising hybrid plants. Islam (1964) had to combine hormone treatment with embryo culture for interspecific hybridization in *Corchorus*. Similarly, Bajaj *et al.* (1980) had to culture embryos from a few developing ovaries on *Hordeum* spikes after they were pollinated with *Secale* and treated with hormones. Napier and Walton (1981) sprayed the spikes of *Agropyron* species with an aqueous solution of gibberellic acid (50 ppm), naphthaleneacetic acid (50 ppm), and 6-(γ,γ-dimethylallyamino)purine on alternate days until harvest and obtained less than 10% fruits from 15 interspecific crosses, and embryos from them had to be cultured to obtain the hybrid plants. In some interspecific incompatible crosses in *Arachis*, hormone treatments stimulate normal postpollination changes but only to a certain extent and not to maturity; in fact, ovules develop very slowly and from them embryos have to be cultured to obtain hybrid plants (Sastri *et al.*, 1981, 1982; Sastri and Moss, 1982).

Different methods of hormone application were used. A hormone may be applied as a spray (as an aqueous solution, with or without a wetting agent), injected, or applied in lanolin, or a solution may be applied to cotton wrapped around the ovary. More than one application may be necessary. Islam (1964) observed for *Corchorus* crosses that lanolin application was better than wrapping the pedicel with a cotton piece soaked in a hormone solution. In contrast to this Bajaj *et al.* (1980) found that wrapping spikes of *Hordeum* with hormone-wetted cotton led to fungal infection and therefore was inferior to the method of bathing the spikes in hormone solution.

Obviously, fruit and seed morphogenesis is a complex process and is under a complex regulation, and it is still too early to attribute precise roles to hormones in such a process. However, there has recently been great interest in the role of hormones in fruit development. It has long been known that certain hormones are produced in developing fruits and seeds of many species and that seeds are the major sources of these hormones (Nitsch, 1952). Cytokinins, for example (Burrows and Carr, 1970; Smith and van Staden, 1979), are suggested to stimulate both the cell division and the assimilate demand in growing embyronic tissues. In developing *Lupinus albus* seeds, the endosperm is rich in cytokinin, and this led Davey and van Staden (1979) to suggest that the embryo depends upon this cytokinin for its growth. Bennici and Cionini (1979) also suggested that there was a cytokinin requirement by young embryos of *Phaseolus coccineus*. It has also been shown that in interspecific crosses in *Phaseolus*, endosperm does not develop normally and has much lower levels of cytokinins than does endosperm from self-pollinations (Nessling and Morris, 1979). Cytokinin levels seem to be critical for a normal embryo development. However, whether an exogenous supply of cytokinin in this cross can prevent the embryo degeneration and promote its growth is a matter still to be investigated.

D TEMPERATURE AND INCOMPATIBILITY

Temperature is known to be an important factor in induction of flowering in a large number of taxa (Wareing and Phillips, 1978), but relatively little is known about its role in floral changes leading to fruit formation. High temperatures are known to reduce pollen viability (see Shivanna *et al.*, 1979; Stanley and Linskens, 1974; Johri and Vasil, 1961; Johri *et al.*, 1977), and low temperatures have been known to prolong the life of pollen grains. High or low temperatures also cause poor pollen germination and poor pollen tube growth (Savitri *et al.*, 1980; Kuo *et al.*, 1981).

In the context of incompatibility, and self-incompatibility in particular, there have been some reports in which excised flowers were pollinated and incubated at different temperatures for investigations of pollen behavior. Later, intact flowers on the plants were also subjected to temperature effects. Although there is a lack of knowledge of the mechanisms of the effect of temperature either on the pollen or on the pistil, high temperatures have been shown in a few instances to weaken or break down self-incompatibility, particularly gametophytic self-incompatibility.

In *O. organensis* and *Prunus avium*, self-incompatible tubes grew well at 15°C, but were inhibited above this temperature (Lewis, 1942). In *Oenothera rhombipetala*, however, incompatible pollen tubes were not affected by the range of temperatures investigated (10–39°C), but compatible tubes grew faster at higher temperatures (Bali and Hecht, 1965).

Oenothera organensis pistils pretreated with hot water at 50°C for 5 min failed to discriminate compatible from incompatible pollen tubes (Hecht, 1964). Bali (1963) made similar observations on *O. rhombipetala* and also found that for the inactivation of the incompatibility reaction, the pollinations had to be done immediately after treatment, otherwise the treated pistils would gradually recover the ability to discriminate between compatible and incompatible pollen tubes. Kwack (1965) showed that similar pretreatment of *O. organensis* pistils for even 3 min weakened the incompatibility reaction but pretreatment for 5 min was more effective. *Lilium longiflorum* pistils (both detached and intact) reacted similarly. With increase in temperatures, detached pistils of *L. longiflorum* supported better growth of self-incompatible pollen, so much so that above 39°C incompatible and compatible pollen tubes were indistinguishable (Ascher and Peloquin, 1966b), but incubation at 39°C did not overcome interspecific incompatibility (Ascher and Peloquin, 1970). A pretreatment for 6 min in hot water at 50°C was found to be optimum for the best growth of self-incompatible pollen tubes, and higher temperatures (even 55°C) adversely affected both the compatible and incompatible pollen tubes (Hopper *et al.*, 1967). *Trifolium hybridum* showed self-incompatibility at lower temperatures (Townsend, 1968). Self-incompatibility in *Trifolium* was also weakened at 40°C (Kendall, 1968). It was found that incompatible pollen tubes grew longer in styles of *T. pratense* flowers that were developed at 40°C than in those developed at 25°C (Kendall and Taylor, 1969).

In *Petunia* self-incompatibility was overcome by higher temperatures (Straub, 1958; Takahashi, 1973; Linskens, 1975). Furthermore, in *P. hybrida* it was shown that incompatible pollen grains that were developed at higher temperatures prior to pollination produced longer pollen tubes than those that were developed at lower temperatures (van Herpen and Linskens, 1981). Incubations of fresh anthers in petri dishes at 40°C for 60 to 90 min, or at 50 °C for 30 and 60 min, with or without a prior subzero temperature treatment (−20°C for 24 hr) were effective in breaking self-incompatibility in *Lilium longiflorum* (Matsubara, 1980). Matsubara found that treatment for a shorter duration was more effective in producing seed. Coupling high-temperature treatment with −20°C treatment for 24 hr produced a high percentage of fruits whose seeds were heavier than those formed in fruits after compatible pollinations. The temperature treatments were found to be more efficacious than application of a floral organ extract to the stigma (Matsubara, 1981).

Temperature is therefore an important factor that can alter incompatibility. For some reasons thermal inactivation of incompatibility has largely been confined to self-incompatibility in *Brassica* spp. (Visser, 1977), *Chrysanthemum* sp. (Ronald and Ascher, 1975), *Nemesia strumosa* (Campbell and Ascher, 1972), *Oenothera* spp., *Petunia* sp., *R. sativus* (Matsubara, 1980), and *Trifolium* spp. Even in these taxa, genotypes sensitive or insensitive to temperature treatments have been recognized. In some instances of interspecific incompatibility, heat treatments have been given but the results have not been encouraging. In some interspecific crosses in *Brassica*, Robbelen (1960) found 15°C to be the optimum temperature for pollen germination. But investigations on crosses between *B. campestris* and *B. oleracea* revealed that 25°C was better than 15°C not only for pollen germination, but also for growth of the pollen tubes, some of them even reaching ovules (Matsuzawa, 1977).

E RECOGNITION POLLEN AND INCOMPATIBILITY

The "recognition pollen effect," also called the mentor pollen effect, has evolved in principle from Michurin's (1950) work. A mixture of compatible and incompatible pollen on a stigma had a stimulatory effect on incompatible pollen. This phenomenon was also observed by Glendinning (1960), Wu (1955), Tsitsin (1962), Sarashima (1964), and others (see Ramulu *et al.*, 1979). A definite role of mentor pollen in incompatible crosses was clarified when Stettler (1968) produced hybrids between incompatible poplar species by mixing live incompatible pollen with γ-irradiated (killed) compatible pollen. The realization that the pollen wall is a physiologically active structure (Tsinger and Petrovskaya-Baranova, 1961) led Knox *et al.* (1972a,b) to propose a workable hypothesis for overcoming incompatibility and to illustrate this by repeating Stettler's (1968) hybridization experiments on the cross, *Populus deltoides* × *Populus alba*.

In this method, pollen grains of a compatible parent are killed and mixed with live incompatible pollen grains before pollination. The inviable pollen is called recognition (or mentor) pollen. The killing of the compatible pollen has been achieved in various ways. The pollen grains have been stored (Knox *et al.*, 1972a,b; Sastri and Shivanna, 1976a, 1980b), frozen and thawed repeatedly (Knox *et al.*, 1972b), treated with anhydrous methanol (Knox *et al.*, 1972b; Sastri and Shivanna, 1976a,b, 1980b; Taylor *et al.*, 1980), or irradiated with lethal doses of γ rays (Stettler, 1968; Knox *et al.*, 1972a; Stettler and Guries, 1976; Guries, 1978; Ramulu *et al.*, 1979; Howlett *et al.*, 1975; Stettler *et al.*, 1980).

The success of γ-irradiated pollen as mentor pollen was first demonstrated by Stettler (1968) in the interspecific cross between *P. deltoides* and *P. alba*. *Populus alba* pollen does not even germinate on the stigma of *P. deltoides*, hence the incompatibility between the two species. γ-Irradiated pollen grains of *P. deltoides* mixed with live pollen grains of *P. alba* apparently stimulate the incompatible pollen grains to germinate on the stigma, leading finally to formation of fruits and seeds in this interspecific and otherwise incompatible cross (Stettler, 1968). Knox *et al.* (1972a, 1972b) repeated this cross and obtained hybrids not only by the use of γ-irradiated compatible pollen mixed with live incompatible pollen, but also by the use of other methods to inactivate the compatible pollen. They found that storage at normal temperature, or repeatedly freezing and thawing the compatible pollen, was also an effective means of preparing mentor pollen (Knox *et al.*, 1972b). *Cosmos bipinnatus* and *R. sativus*, taxa with the sporophytic type of self-incompatibility, are examples wherein the incompatible pollen is inhibited on the stigma.

Subsequently, it was shown that gametophytic self-incompatibility could also be overcome by using methanol-treated compatible pollen as mentor pollen in *P. hybrida* (Sastri and Shivanna, 1976a, 1980b) and by using γ-irradiated pollen in *N. alata* (Ramulu *et al.*, 1979). Dayton (1974) had demonstrated that this method could be successfully adopted for overcoming gametophytic self-incompatibility in apple also. However, in apple, pear, and their crosses, mentor pollen prepared either by methanol treatment or by γ irradiation was found to be ineffective (Visser, 1981). It should be mentioned that in *P. hybrida*, self-pollination of buds produced a higher percentage of fruits with a larger number of seeds than were produced by the mentor pollen method (see Shivanna and Rangaswamy, 1969; Sastri and Shivanna, 1980b). Furthermore, in a strictly self-incompatible plant such as *P. hybrida*, mentor pollen prepared by methanol treatment was found to be ineffective, but its leachate, when applied to the stigma before self-incompatible pollination, gave a low percentage of fruits and the number of seeds set per capsule was comparable to that obtained by self-pollination of buds (Sastri and Shivanna, 1980b). In another taxon, *N. alata*, with gametophytic self-incompatibility, the number of seeds formed per fruit was much greater after bud pollination (see Bredemeijer, 1976) than was obtained by pollinating the mature stigmas with a mixture of mentor pollen and incompatible pollen (see Ramulu *et al.*, 1979).

Efficacy of this method has been examined in some interspecific incompatible crosses. In *Cucumis*, in each of the crosses investigated, about 50% of flowers pollinated with pollen mixture produced fruits. In all but one cross combination, ovules were larger, with well-formed embryo sacs and globular embyros, in contrast to untreated incompatible pollinations, which did not set any fruits (den Nijs and Oost, 1980). In *Sesamum indicum* × *Sesamum mulayanum*, recognition pollen prepared by methanol treatment of compatible pollen stimulated germination of incompatible pollen on the stigma as well as penetration into the stigmatic tissues (Sastri and Shivanna, 1976a). In this cross, however, no fruits were obtained because the incompatible pollen tubes that entered and the stylar tissues were not normal and were soon inhibited (Sastri and Shivanna, 1976a).

There are reports that mentor pollen was ineffective in overcoming self-incompatibility in *B. campestris* (Sastri and Shivanna, 1980b), *O. organensis*, and a hybrid between *L. esculentum* × *L. peruvianum* (Ramulu *et al.*, 1979) and in overcoming interspecific incompatibility in eight crosses of *Ipomoea* (Guries, 1978), *Trifolium* (Taylor *et al.*, 1980), and *Festuca arundinacea* × *Dactylis glomerata* (Matzk, 1981).

Although there are only a few cases in which different methods of preparing mentor pollen have been used in the same species, there are instances in which use of a specific method is crucial to the success in overcoming incompatibility. Self-incompatibility in *R. sativus* can be overcome by using the mentor pollen prepared by storage but not that obtained by methanol treatment (Sastri and Shivanna, 1980b). The mentor pollen prepared either by storage or by methanol treatment was not efficacious in overcoming self-incompatibility in *B. campestris* (Sastri and Shivanna, 1980b), but Roggen (1975) succeeded with a related species, *B. oleracea*, by using compatible pollen leachate on the stigma before self-pollination. Differences in the efficacy of methods for preparing recognition pollen were also evident in *P. hybrida*. In a strongly self-incompatible plant, methanol-treated mentor pollen was not effective, but the compatible pollen leachates were effective in overcoming self-incompatibility (Sastri and Shivanna, 1980b). The compatible pollen leachates were as effective as the mentor pollen prepared by storage, by repeated freezing and thawing, by γ irradiation, or by methanol treatment in overcoming interspecific incompatibility in *Populus* (Knox *et al.*, 1972a,b) and self-incompatibility in *C. bipinnatus* (Howlett *et al.*, 1975). It may be mentioned here that the incompatible pollen leachates were able to elicit rejection reaction in *Iberis* stigma papillae (in the form of callose deposits) just as the incompatible pollen grains do (Heslop-Harrison *et al.*, 1974). It is therefore suggested that for mentor pollen to be effective in overcoming incompatibility, the methods for its production have to be judiciously selected. In instances in which only one method has been tried and found unsuccessful, mentor pollen prepared by other methods should be tried.

When there has been success (Table II), it has been attributed to the early interaction between pollen and pistil (Knox *et al.*, 1972a). Stettler *et al.* (1980) reexamined the mentor pollen effects in some incompatible crosses of species belonging to three of the five sections of the genus *Populus*. They suggested that the success is due also to the fact that ovule and ovary are somehow stimulated by killed compatible pollen but not by incompatible pollen. That pollination provides a stimulus is evident from experiments of Illies (1974), who obtained haploids from pollinated pistils of *Populus* treated with toluidine blue. This dye arrested the pollen tube growth halfway through the styles and still the ovaries developed.

The strength of incompatibility and the extent of crossability of a parent that is the source of mentor pollen are other critical factors for

TABLE II Successes and failures in overcoming incompatibility using recognition pollen

Intraspecific		
Sporophytic	Gametophytic	Interspecific
Successes		
Cosmos bipinnatus (Howlett *et al.*, 1975)[a,d,e]	*Malus* (Dayton, 1974)[a]	*Populus* (Stettler, 1968; Stettler and Guries, 1976, Stettler *et al.*, 1980; Knox *et al.*, 1972a,b)[a,c,d,e]
	Petunia hybrida (Sastri and Shivanna, 1976b)[a,e]	
(Sastri and Shivanna, 1980b)[b,c]	*Nicotiana alata* (Pandey, 1975, 1977; Ramulu *et al.*, 1979)[a]	*Sesamum* (Sastri and Shivanna, 1976a)[c]
(Roggen, 1975)[c]	*Arachis* (D. C. Sastri, unpublished)[c]	*Cucumis* (den Nijs and Oost, 1980)[a]
Failures		
Brassica campestris (Sastri and Shivanna, 1980b)[b,c]	*Oenothera organensis* (Ramulu *et al.*, 1979)[a]	*Ipomoea* (Guries, 1978)[a]
	Lycopersicon (Ramulu *et al.*, 1979)[a]	*Trifolium* (Taylor *et al.*, 1980)[c]

[a]Recognition pollen prepared by γ irradiation
[b]Recognition pollen prepared by [illegible]
[c]Recognition pollen prepared by [illegible]
[illegible] by repeated freezing and thawing.
[e]Recognition pollen substituted by its leachates.

success. Pandey (1977, 1979) reported that mentor pollen had a promotive effect in individuals with weak incompatibility but not in individuals with strong incompatibility.

γ-Irradiated pollen and gene transfer

Attempting to overcome incompatibility in *N. alata* by the use of γ-irradiated pollen (100 krad Co), Pandey (1975, 1978, 1980) obtained some unusual results in addition to overcoming self-incompatibility. He observed that certain characters were transferred by mentor pollen and this process has been called a specialized form of "sexual transgenosis." Pandey (1975, 1979) suggested that a high dose of ionizing radiation transforms the generative nucleus into a number of small chromatin fragments, and this was confirmed by Grant *et al.* (1980). It was also shown that there is a lack of metaphase orientation and the failure of division of the generative nucleus during *in vitro* germination of the irradiated pollen grains. By using this method a small number of diploid progeny were obtained that resembled the female parent in a majority of characters but showed a few characters from the parent of the irradiated pollen. Jinks *et al.* (1981) have repeated Pandey's experiments in the same species and have arrived at similar conclusions, suggesting a novel method for *in vivo* transgenosis. These observations have opened a new method for incorporation of segments of paternal chromosomes into the maternal genome, thereby transforming the lat-

F. IMMUNOSUPPRESSANTS AND INCOMPATIBILITY

Bates and co-workers pioneered a novel concept in the light of possibilities of wide hybridization. Based on other reports that there are some organ-specific antigens (Wright, 1960) and on the existence of phytohemagglutinins in plants, Bates and Deyoe (1973) suggested the existence of an immune reaction analogous to that occurring in animal systems. They called this "stereospecific inhibition reaction" (SIR), but there is still no direct evidence for the existence of SIR in plants. However, they initiated wide hybridization experiments in which certain animal-effective immunosuppressants were used. These were ε-aminocaproic acid (εACA), chloramphenicol, acriflavin, salicylic acid, and gentisic acid. Success rates varied among immunosuppressants, εACA being the most effective. The results obtained have not only supported the hypothesis upon which these trials were initiated, but have also suggested new ways of breaking the interspecific crossability barriers. The crosses in which embryos were obtained were durum wheat × rye, barley × rye, barley × triticale, barley × oats, and maize × sorghum (Bates *et al.*, 1974). In the untreated controls even fertilization was not observed. Bates *et al.* also reported that progeny from barley × rye, durum wheat × barley, and bread wheat × barley have been advanced to F_2 generations.

The results (see Bates, 1974) with this novel group of chemicals did stimulate a few other workers, and a few reports published to date are encouraging. Tiara and Larter (1977a,b) observed that εACA stimulated embryo development in *Triticum turgidum* × *S. cereale* crosses. In all these experiments the immunosuppressant solution was applied to the leaf axils a few weeks before pollination.

In the interspecific cross between *Vigna radiata* and *Vigna umbellata*, εACA (100 ppm) applied as a foliar spray to the seed parent was twice as effective as the untreated controls (Asian Vegetable Research and Development Center, 1976). In the same cross Baker *et al.* (1975) found that an injection of 250 ppm of εACA into the internode of maternal plants gave optimum results. Foliar spray of εACA (100 ppm) applied for 14 days starting at, or earlier than, the premeiotic stage of flower development to two cultivars of *Vigna radiata* delayed but did not prevent embryo abortion in *V. radiata* × *V. umbellata* crosses (Chen *et al.*, 1978). Embryo abortion could also be prevented by defoliating the plants 4–6 days after pollination (Chen *et al.*, 1978), a procedure developed for *P. coccineus* × *P. vulgaris* crosses by Ibrahim and Coyne (1975). More recently, Mujeeb-Kazi (1981) has shown that in *Triticum timopheevii* × *S. cereale* crosses, εACA treatment (concentration not given) of *T. timopheevii* florets for 4 days after pollination reduced embryo recovery from 30.5 to 18.9%, but increased the number of ovaries with both embryo and endosperm formation from 11.4 to 18%. In this particular cross Mujeeb-Kazi has also shown that crossability is affected by the environment in which the female parents are grown and maintained. In *F. arundinacea* × *D. glomerata* cross, however, εACA treatment (concentration not given) was not effective (Matzk, 1981). These chemicals will probably repay the effort of testing on a larger number of taxa, with gametic incompatibility; chemicals with similar effects can be tried. A better understanding of the mode of action of these chemicals must be obtained to increase the effectiveness of their use in promoting other desirable but incompatible crosses.

G. MISCELLANEOUS METHODS

In addition to the preceding methods, each of which has been shown to be effective in more than one taxon, there are certain other methods that have been developed and applied to one taxon only (Table III).

TABLE III Miscellaneous methods in overcoming intraspecific (SI) or interspecific (ISI) incompatibility

Method	Taxon/cross	Type of incompatibility	References
Organic solvents	*Brassica oleracea*	SI	Ockenden (1978)
	Populus	ISI	Willing and Pryor (1976)
Humidity	*Brassica oleracea*	SI	Ockenden (1978)
Electric-aided polli-	*Brassica* sp	SI	Roggen *et al* (1972)
suppressants	Maize × sorghum	ISI	Bates (1974); Bates and Deyoe, (1973)
	Vigna	ISI	Bates *et al* (1974); Chen *et al.* (1978)
	Lycopersicon, *Triti-*	ISI	Kesicki (1979)
N-*m*-Tolyphthalmic acid, *P*-*m*-tolyphthalmic acid	*Brassica pekinensis* × *B. oleracea*	ISI	Honma and Hecht (1960)
CO_2	*Brassica* spp.	SI	Nakanishi *et al* (1969); Nakanishi and Hinata (1973)

Freshly opened flowers of *Brassica* spp., when exposed to 3–5% CO_2, behaved as self-compatible to a certain extent, although there were differences according to the genotype or the species investigated (Nakanishi *et al.*, 1969; Nakanishi and Hinata, 1973). In *B. oleracea*, self-incompatibility was also overcome by "electric-aided pollination" in which an electric potential difference of 100 V was applied between pollen and stigma (Roggen *et al.*, 1972). The efficacy of this method in this taxon, expressed as seed number per pollination, is comparable to that obtained by other methods, such as decapitated pistil pollination, bud pollination, chemical treatments, and temperature treatment. In interspecific crosses in *Populus*, certain organic solvents (ethyl acetate and hexane being the most effective ones) were applied to stigmas and hybrids were obtained (Willing and Pryor, 1976).

H. GENETIC AND CYTOLOGICAL MANIPULATION

Adverse pollen and stigma interactions are not the sole causes of incompatibility, and there are a number of genetic or cytological reasons for failure to produce hybrids or to achieve successful gene transfer. These will be mentioned briefly before considering *in vitro* methods, which have become important techniques for interspecific transfer.

Differences in number of chromosomes and/or ploidy differences in two species to be crossed can be strong factors, preventing hybridization between them. The taxa involved may have the same chromosome number, such as *Trifolium repens* and *Trifolium ambiguum* ($2n = 32$) (Williams, 1980), yet they cannot normally be crossed.

In many diploid × tetraploid crosses within or between species, the endosperm collapses, causing early embryo abortion (Brink and Cooper, 1947). Johnston *et al.* (1980) proposed that in such instances ploidy per se is not the problem. According to them, an abnormal endosperm is due to a deviation of maternal:paternal genome ratios from 2:1 in the endosperm. In this hypothesis the genome of each species has to be assigned a specific value for the endosperm, irrespective of the ploidy levels of the parental species. By manipulating these numbers, Johnston and Hanneman (1982) have succeeded in producing hybrids between some diploid species of *Solanum* that cannot be crossed otherwise, It appears that results from a few interspecific crosses, such as *Solanum, Gossypium, Lycopersicon, Datura,* and *Avena,* can be explained by this hypothesis (Johnston *et al.*, 1980).

Elimination of chromosomes of one of the parents is another problem often encountered in wide crosses, and this has been profitably employed in production of haploids in *Hordeum* (Subrahmanyam, 1979).

These problems have been tackled largely by strategic manipulation of chromosome numbers and ploidy level. Increase in ploidy level has often been achieved by using colchicine and certain other chemicals, whereas reduction in ploidy has been achieved by haploid parthenogenesis and/or by anther and pollen culture or by some chemical treatments (Illies, 1974).

When two taxa cannot be hybridized, a third taxon crossable with one of them has often been used as a bridge for transfer of character(s). Examples of such bridge crosses are found in *Nicotiana, Triticum, Cucurbita, and Solanum* (see reviews by Hadley and Openshaw, 1980; Stalker, 1980). The search for genetic control of crossability and chromosome pairing as found in *Triticum* should continue in other plant taxa. Crosses should be attempted with as many accessions as possible; possibly the different cultivars may show varying crossability with another species. Such differences have been observed in *N. tabacum* cultivars (Pittagelli and Stavely, 1975), *Trifolium nigrescens* (Hoven, 1962), *Tripsacum dactyloides* (Harlan and de Wet, 1977), and so on. *Triticum aestivum* genes controlling crossability have been identified as *Kr1* and

Kr2 and are located on the 5B chromosome. The dominant alleles of these genes in genotypes such as in the variety Hope interfere with the pollen tube growth in the micropyle in *T. aestivum* × *S. cereale* (Jalani, and Moss, 1980, 1981) and *T aestivum* × *H bulbosum* (Snape *et al*, 1980) crosses. The 5B chromosome of wheat also carries a gene (*Ph*) that restricts pairing. By eliminating this chromosome (Cauderon, 1979; Thomas, 1981) or by suppressing the activity of the *Ph* gene by *Aegilops speltoides* genotypes (Riley *et al.*, 1968), it has been possible to increase pairing and enhance recombination between genomes.

Details of these and other aspects of genetic and cytological manipulations have been listed and discussed often and are not presented here. The papers of Stalker (1980), Rees *et al.* (1981), Peloquin (1981), Hadley and Openshaw (1980), Thomas (1981), Riley *et al.* (1981), and Driscoll (1981) are suggested for consultation.

I THE *in Vitro* METHODS

The *in vitro* methods are increasingly being recognized as regular techniques for the plant breeder interested in interspecific hybridization and in overcoming incompatibility. Advances in *in vitro* techniques have been providing opportunities for sexual hybridization and, more recently, for parasexual hybridization by protoplast fusion or for gene transfer by plasmids, liposomes, viruses, chromosomes, or otherwise.

Sexual hybridization by these methods encompasses culture of embryos, ovules, or ovaries from incompatible crosses in which embryos or ovules do not develop fully after wide hybridization by conventional means. The first successful culture of embryos was from the cross *Linum perenne* × *Linum austriacum* (Laibach, 1929); there are now over 40 crosses in which hybrids have been obtained by culture of embryos (see Raghavan, 1977, and Table IV). In many instances, however, the embryo degenerates when it is too small to be dissected out for culture. In these instances, ovule or ovary culture facilitates hybrid production (Stewart, 1981). Takeshita *et al.* (1980) have compared the effectiveness of embryo culture, ovary culture, and ovule culture from some interspecific crosses involving species of *Brassica* and *R. sativus*. They found that in some crosses ovule culture was better than either embryo culture or ovary culture; this was particularly true when *B. oleracea* was one of the parents (Takeshita *et al.*, 1980). Ovules from interspecific crosses in *Gossypium* (Stewart and Hsu, 1978) and ovaries from interspecific crosses in *Brassica* (Inomata, 1978, 1979) have been cultured and hybrids obtained.

TABLE IV. Some interspecific hybrids by embryo culture*

Cross	References
Aegilops squarrosa × *Triticum boeoticum*	Gill *et al.* (1981)
Agropyron tsukushiense (6*x*) × *Hordeum bulbosum* (4*x*)	Shigenobu and Sakamoto (1981)
Arachis hypogaea × *Arachis* sp. P.I. No. 276233	Sastri and Moss (1982); Sastri *et al* (1981)
Elymus canadensis × *Hordeum vulgare*	Mujeeb-Kazi and Rodriguez (1982)
Festuca arundinacea × *Dactylis glomerata*	Matzk (1976)
Hibiscus asper × *Hibiscus cannabinus*	Kuwada and Mabuchi (1976)
H. asper × *Hibiscus sabdariffa*	Kuwada and Mabuchi (1976)
Hordeum jubatum × *Secale cereale*	Brink *et al.* (1944)
Impatiens hookeriana × *Impatiens campanulata*	Arisumi (1980)
Lolium perenne × *Festuca rubra*	Nitzsche and Henning (1976)
Lotus pedunculatus × *Lotus tenuis*	De La Tour *et al.* (1978)
Lycopersicon esculentum × *Lycopersicon peruvianum*	Thomas and Pratt (1981)
Ornithopus sp. × *Ornithopus compressus*	Williams and De La Tour (1980, 1981)
Solanum melongena × *Solanum khasianum*	Sharma *et al.* (1980)
Trifolium ambiguum × *Trifolium hybridum*	Williams (1980)
T. ambiguum × *Trifolium repens* and reciprocal	Williams (1978, 1980)
T. repens × *T. ambiguum*	Williams and Verry (1981)
Trifolium pratense × *Trifolium sarosiense*	Phillips *et al.* (1982)

*In addition to those listed by Raghavan (1977).

Both female and male gametophytes have been cultured together *in vitro*, so that pollination, fertilization, and postfertilization changes leading to formation of hybrid seed or seedlings are all achieved in the test tube (Rangaswamy, 1977; Zenkteler and Melchers, 1978; Zenkteler, 1980; Stewart, 1981). Of 22 intergeneric or interspecific combinations, 5 formed seeds with viable embryos, 13 with immature embryos, 2 showed only endosperm formation, and 2 only fertilization (Zenkteler, 1980). The test-tube fertilization is a refinement of the experiments of Kanta and associates on successful intraovarian pollinations in some members of Papaveraceae (Kanta, 1960; Maheshwari and Kanta, 1961).

Another approach to exploit the *in vitro* methods is to force the fusion of somatic protoplasts and provide conditions for the growth and differentiation of the heterokaryocyte, leading to somatic hybrids. Numerous attempts, encouraged by the initial success in fusing protoplasts in the sexually compatible taxa in *Petunia* (Power *et al.*, 1970) and *Nicotiana* (Carlson *et al.*, 1972), have been made to produce somatic hybrids from sexually incompatible species (see Schieder and Vasil, 1980). Significant among these is the creation of "*Arabidobrassica*" by fusing the protoplasts of *Arabidopsis* and *Brassica*, genera of two different taxonomic tribes. Equally significant is the fact that the methods, so far exploratory in nature and confined to well worked out model systems, are now being extended to hybridization and improvement of crop species (Wenzel *et al.*, 1979). In several other attempts at interspecific and intergeneric and protoplast fusion, hybrid callus lines have been obtained (see Schieder and Vasil, 1980; Gamborg *et al.*, 1981; Cocking, 1981). Krumbiegel and Schieder (1981) have observed that hybrids between *Datura innoxia* and *Atropa belladonna* can be produced only by somatic hybridization and not by other *in vitro* methods suggested by Rangaswamy (1977) and Zenkteler (1980). Hybrids between *N. taba-* [illegible]

were produced by *in vitro* sexual methods (Reed and Collins, 1978) and by somatic fusion (Evans *et al.*, 1981). Evans *et al.* (1982) compared these hybrids and observed that somatic hybrid clones showed a greater range of variability for certain morphological characters than did the sexual hybrids. A commonly observed problem in such wide somatic hybridization is the gradual loss of a part of or a full genome of one of the parents (Dudits *et al.*, 1980). Experiments of Szabados *et al.* (1981) and Griesbach *et al.* (1981) suggest chromosome uptake by protoplasts as another alternative to transfer of the full genome by somatic fusion. Uptake of chromosomes or of their segments can also be facilitated by encapsulating them in liposomes before fusing the latter with the recipient protoplasts. This has been shown by Matthews and Cress (1981), Lurquin (1981), and Giles (1983). Alternatively, the desired segments of DNA can be tagged to certain vectors such as *Agrobacterium tumefaciens* Ti plasmid or cauliflower mosaic virus DNA, which may transfer the DNA to the host cell for integration by its nuclear DNA. This has been demonstrated recently by Chilton *et al.* (1982) and Krens *et al.* (1982; see also reviews by Cocking *et al.*, 1981; Kado and Kleinhofs, 1980).

IV. Concluding Remarks

There is a growing interest in the use of wild relatives for reversing genetic erosion and for genetic improvement of crops. Wild species have always been of concern to students of biosystematics, but now they are of equal concern to plant breeders. A knowledge of evolution and speciation has helped our understanding of the reasons for failures of wide crosses and vice versa. A deeper search into these failures has provided methods for converting some of these into successes. It is hoped that these methods, with modifications and improvement as necessary, will stimulate new ideas for the creation of hybrids that have so far eluded us.

It is certainly not easy to pick one of the methods as the best one, but self-incompatibility was said to be overcome best by high-temperature treatments (Townsend, 1971). To break interspecific barriers, a range of parents have to be screened for the most crossable one, and the nature of incompatibility—whether pre- or postfertilization—has to be determined. Fluorescence microscopy has been a convenient method for determining this. This method (Martin, 1959) facilitates the observation of pollen tube growth through the pistil, which is generally not easy by light miscroscopic staining methods. Having determined the site of the barrier, a range of suitable methods has to be adopted. The most common of these methods have been discussed in this article, and with greater understanding of the phenomena involved more techniques are bound to emerge.

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