OVERCOMING INCOMPATIBILITY IN WIDE CROSSES

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Wild species of crop plants attract much attention as a valuable gene pool. A few successes in improving crop plants have resulted from crossing wild taxa with cultivated ones. Several other taxa are not crossable with their cultivated relatives and are therefore unavailable for sexual gene transfers. Methods for breaking these barriers to interspecific hybridization and hybrid production have been developed.

The literature on exotic germplasm abounds with examples of genetic introgression from wild taxa conventionally crossed with their cultivated relatives. But there is a wealth of germplasm that cannot be crossed with cultivated taxa.

The barriers to hybridization were known even before the present range of germplasm became available. During the last five decades, interest in incompatibility has increased to encompass phylogenetic-taxonomic purposes and the genetic improvement of crop plants. While emerging somatic methods show promise, sexual manipulation is still the first choice, and sexual methods have contributed significantly to improving crops.

The germplasm amenable to sexual manipulation is difficult to quantify. It may be an insignificant proportion of existing germplasm. Even in the well-worked genus Nicotiana, of which about 65 species are known, only a little more than 300 hybrids have been realized. About 90% of the crosses in this genus have not. The situation is similar for most other crops. A number of review articles and books on incompatibility and methods to break it are available (9, 15, 16, 17, 18, 41, 49, 51).

CAUSES OF SEED FAILURE IN INCOMPATIBLE CROSSES

The characteristic prefertilization barriers and postfertilization breakdown of zygote or embryo in incompatible crosses have been extensively described. Inhibition of pollen germination on stigma and pollen tube growth through the style,

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failure to fertilize, and zygote growth cessation at some stage of development are common to many failing crosses (41, 49, 56). Unfortunately, their molecular causes are little understood. Even so, the fragmentary information available has led to development of methods for breaking crossability barriers in a few taxa.

METHODS

Among the several methods used are recognition pollen, hormones, immunosuppressants, and temperature. Early surgical methods are also important (28, 37, 41). Delayed and bud pollinations have overcome both sporophytic (*Brassica* spp.) and gametophytic (*Petunia* spp.) self-incompatibilities in a few taxa, but not any interspecific incompatibility. Their success depends on the stigma's receptiveness, i.e., buds of Arachis *hypogaea* even a day before anthesis are not receptive even to compatible pollen; so bud pollination does not work in *A. hypogaea* (43).

Growth hormones

The discovery of growth hormones in plants inspired investigations on their potential in overcoming incompatibility, which is not mentioned in books and conferences on plant growth hormones. The influence of exogenous and endogenous hormones on developing fruit has been investigated in normal compatible (self) crosses but rarely in incompatible crosses. The effects of different growth hormones on free pollen grains in in vitro cultures have been extensively studied (25, 26, 51). The effects of hormones on compatible and self-incompatible pollen tube growth in pistils of *Lilium longiflorum* (14) and a few other species (9, 41) have been reported but the studies are too few for conclusions on self-incompatible taxa, let alone interspecific ones. Hormone applications have, however, been found to enhance a few incompatible fruit and seed set crosses.

Crane and Marks (7) and Brock (5) first used hormones to delay floral abscission in incompatibly pollinated flowers with short life. Interest in use of hormones in incompatible crosses subsequently grew. The latest hormone-aided hybridizations are interspecific crosses in *Arachis*. In a few cases application of gibberellic acid to the bases of incompatibly pollinated flowers stimulates the initiation and geotropic elongation of the peg, the aerial phase of *Arachis* fruit (Table 1; 43, 44, 45). Subsequent application of IAA, NAA, or kinetin increases the number of pods (42) and cultured ovules and embryos (29).

Over 20 interspecific or intergeneric hybrids have resulted from exogenous application of natural or synthetic plant growth hormones (41). We have yet to understand how hormones stimulate hybrid development from incompatible crosses. A comparison of hormonal balances in ovaries between compatibly and incompatibly pollinated flowers is called for and specific effects of exogenous hormones in successful cases need study. Nessling and Morris (32) found that endosperm from an interspecific *Phaseolus* cross does not develop normally and has much less cytokinin than

Table 1. Peg and pod produc[illegible] after gibberellin treatment in some intersectional (

Cross	Pollinations (no.)	Pegs (%)	Pods/pegs (%)
Section *Arachis*/Section *Triseminale*			
A. duranensis (2n=20)/*A. pusilla* (2n=20)	33	79	39
A. hypogaea cv. Robut 33-1/*A. pusilla* (2n=20)	78	48	0
Section *Arachis*/Section *Extranervosae*			
A. hypogaea cv Robut 33-1/*A. villosulicarpa* (2n=20)	39	59	3
A. hypogaea cv MK 374/*A. villosulicarpa* (2n=20)	9	89	11
Section *Extranervosae*/Section *Triseminale*			
A. villosulicarpa (2n=20)/*A. pusilla* (2n=20)	24	54	46
Section *Arachis* x Section *Rhizomatosae*			
A. hypogaea cv Robut 33-1/*Arachis* sp. Coll. No. 9649	82	44	6
A. hypogaea cv Robut 33-1/*Arachis* sp. Coll. No. 9797	46	57	2
A. hypogaea cv Robut 33-1/*Arachis* sp. Coll. No. 9806	26	62	0
A. hypogaea cv TMV2/*Arachis* sp. PI 276233	408	76	20
A. hypogaea cv TMV2/*Arachis* sp. Coll. No. 9649	11	73	0
A. hypogaea cv MK 374/*Arachis* sp. PI 276233	648	68	32
A. hypogaea cv MK 374/*Arachis* sp. Coll. No. 9649	26	42	16
A. hypogaea cv M 13/*Arachis* sp. PI 276233	75	56	5
A. hypogaea cv Chico/*Arachis* sp. PI 276233	58	66	9
A. hypogaea cv Chico/*Arachis* sp. Coll. No. 9649	28	73	19

that from selfed pollinations. Such studies not only help elucidate hormonal regulation of fruit morphogenesis, but also assist in formulating strategy for further work with incompatible crosses.

Recognition pollen

Since Tsinger and Petrovskaya-Bananova (62) showed "pollen grain walls [to be] physiologically active substances," interest in postpollination changes in pollen grains and pistil has grown. It is now believed that extracellular (wall) pollen proteins communicate with extracellular (surface) stigma papillae proteins: pollen grains are thereby recognized as either compatible or incompatible (16, 17, 18). The existence of pollen grain walls (16) and stigma surfaces (19, 20) with a protein component has been demonstrated in a number of taxa, and in a few, even stigmas of immature flowers are lined with proteins (52, 53, 50).

Knox et al (27) exploited this information to produce hybrids from an interspecific cross in *Populus*. Earlier Stettler (57) had achieved interspecific hybridization in *Populus* by mixing live incompatible pollen grains with gamma-irradiated (killed) compatible pollen grains called recognition or mentor pollen (27). Mentor pollen stimulated incompatible pollen grains to produce fruit and seed.

Subsequently this method was tested in other taxa and in other systems of self-incompatibility and interspecific incompatibility. Sporophytic self-incompatibility was overcome in *Cosmos bipinnatus* (21), *Brassica oleracea* (40), and *Raphanus sativus* (48), but not in *Brassica campestris* (48). Gametophytic self-incompatibility was overcome in apple (8), *Petunia hybrida*

(47, 48) and Nicotiana alata (55), but not in Oenothera organensis (55). Recognition pollen did not work in other interspecific crosses in Sesamum (46), Arachis (43), Ipomoea (13), Trifolium (58), and Cucumis (10).

Sastri and Shivanna (48) have suggested that the method of killing the compatible pollen grains determines success. The recognition pollen can be prepared by storage at normal temperature, repeated freezing and thawing, and gamma-ray irradiation until pollen grains lose their ability to germinate. In a few instances the recognition pollen function could be effected by leachates of compatible pollen grains (21, 48).

Pandey (33, 34, 35) found that in Nicotiana gene transfers could be effected with just gamma-irradiated compatible pollen grains. Jinks et al (22) have confirmed this. Recently Shuzikuda et al (54) combined this method with ovule culture to transfer an incomplete chromosome complement of Nicotiana tabaccum into N. rustica, two species which are not otherwise crossable.

Immunosuppressants

About 10 yr ago Bates and his coworkers began using in wide hybridization experiments animal immunosuppressants including E-aminocaproic acid (EACA), chloramphenicol, acriflavin, salycylic acid, and gentisic acid. The success rate varied, but EACA was the most effective: with EACA, hybrids were obtained in crosses between barley and rye, durum wheat and barley, and bread wheat and barley (3, 4). EACA also stimulated embryo development in crosses between Triticum turgidum and Secale cereale (60, 61).

In another interspecific cross, Vigna radiata/V. umbellata, a foliar spray of EACA (100 ppm) effected twice as many hybrids as those that resulted from the unsprayed controls (1). Baker et al (2) obtained optimum results in the same cross by injecting 250 ppm of EACA. Chen et al (6) observed in two cultivars that foliar EACA spray for 14 d starting no later than the premeiotic stage of flower development delayed but did not prevent embryo abortion. EACA application to florets on 4 consecutive d after pollination reduced embryo recovery from 30 to 19%, but increased the number of ovaries forming embryo and endosperm in Triticum timopheevi/Secale cereale (31). It was not effective in Festuca arundinacea/Dactylis glomerata (30).

OTHER METHODS

Embryo, ovule, and ovary cultures have yielded hybrids in more than 50 cross combinations with early abortion of embryo or endosperm, or both (see 36, 41). According to Johnston et al (24), when there are ploidy differences between the parents, an abnormal endosperm is caused by a deviation of the maternal and paternal genome ratio from 2 to 1 in the endosperm itself. By assigning a specific number (endosperm balance number) to the endosperm of each parent irrespective of its ploidy, and by manipulating the numbers, Johnston and Hanneman (23) ha succeeded in crossing some diploid Solanum species.

Ploidy manipulations are not new and a species with higher ploidy is generally better as a female parent. (Even when the parents have the same ploidy, one of them is raised to a higher level through colchicine treatment.) In other situations, a third species, itself crossable with each incompatible parent, is used as a bridge. Cultivar crossability differences indicate the need for a range of cultivars. Genes for crossability, such as Kr1 and Kr2 in wheat, should be investigated in other taxa. Most of these aspects have been reviewed by Rees et al (38), Thomas (59), Driscoll (11), and Riley et al (39).

CONCLUSIONS

Several means for hybridization between incompatible species have been found. Until the somatic methods (e.g., 12) can find wider applications, these methods, with or without modifications, must be exploited. In some instances combining methods with ovule or embryo culture is needed. For example, in a few interspecific crosses of Arachis, applying gibberellin to incompatibly pollinated flowers stimulated normal development of the pegs, and a few pods (Table 1) up to certain stages. Only ovules in the pods were found to be immature, with poorly differentiated embryos (29, 43, 44, 45). Ovules and embryos had to be cultured in vitro to yield plantlets (29, 43, 44). Similarly, in interspecific crosses in Sesamum (46) and Cucumis, (10) mentor pollen effected only part of the desired response. For further development, another method must be adopted.

Incompatibility must be better understood before it can be overcome and the wild germplasm exploited for further improvement of crops.

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