

EMBRYOLOGY AND PLANT BREEDING

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A PLANT breeder of today needs no apology for attempting a discussion on embryology, especially if we recall two important discoveries, namely, that of the pollen tube by Amici who was a mathematician and astronomer of Italy, and of the laws of inheritance by Mendel, a theologian of Austria who also taught mathematics. Apart from these facts of history, the advances in our knowledge of genetics and embryology have reduced to oblivion the gulf between these sciences and both have evolved from the narrow classical discipline of the past into the wider experimental philosophy of today. It is proposed here to discuss briefly the application of embryological studies in an important aspect of plant breeding research, namely, distant hybridization, a field which is the meeting place not only for embryologists and geneticists but, as will be seen later, also for the physiologists and biochemists.

The increase in our knowledge concerning the isolation mechanisms (Clausen, 1951) in plant species and the evolution of techniques to overcome the barriers to species crossing, have further widened the spectrum of variability available for recombination breeding. It will be proper, first, to consider the barriers to crossability between species and the techniques available for overcoming these. The application of embryological data concerning seed development in distant crosses will then be discussed briefly.

BARRIERS TO CROSSABILITY—PROBLEMS AND TECHNIQUES

Broadly these barriers can be divided into two kinds, (a) those appearing before fertilization, and (b) those occurring after fertilization. The pre-fertilization barriers include, non-synchronization of flowering time, failure of pollen of one species to germinate upon the stigma of another, lack of pollen tube growth in crosses between species having different lengths of style, and finally the failure of the fertilization process itself. As regards techniques, flowering time can be synchronized by adjusting the photoperiod and temperature, but with present-day facilities of air transport, it is even possible to send pollen from one part of the world to another or even store the pollen from one season to another, with the help of pollen-banks which now operate on a commercial scale (Antles, 1951). Our knowledge of the physiology of pollen (Johri and Vasil, 1961) and the techniques for extending the storage life of pollen are now fairly advanced and form an important

adjunct to plant breeding. Failure of pollen germination and bursting of pollen tubes has also been tackled successfully in some crosses. Blakeslee (1945) could overcome the incompatibility between certain $2n \times 4n$ *Datura* crosses by the use of periclinal chimeras where the style layers were of $4n$ and $2n$ constitution so that the $4n$ epidermis prevented bursting of the pollen tubes of the $4n$ parent. Styler amputation was successfully employed by Mangelsdorf and Reeves (1931) to effect the cross between *Zea* and *Tripsacum*. Since the cut surface of the style may not be always as good as the stigma, style-grafting techniques have been evolved by Buchholz *et al.* (1932) in *Datura*, by Yasuda (1931) in *Petunia* and by Hecht (1960) in *Oenothera*. However, such delicate operations are feasible only in plants having stout styles. A simpler method of replacing the natural stigma by an artificial medium containing sucrose-agar-gelatin was employed by Swaminathan (1955) in securing crosses between *Solanum pinnatisectum* and *S. bulbocastanum* and between the former and *S. lanciforme*, and by Swaminathan and Murty (1957) in the crosses, *N. tabacum* \times *N. rustica* and *N. tabacum* \times *N. debneyi*.

Styler incompatibility may also be overcome in the future by what is known as intraovarian pollination, a technique standardized in the poppy by Kanta (1960). An offshoot of this technique is "test-tube fertilization" brought about by culturing ovules and then landing pollen upon them or near them in the artificial medium (Kanta *et al.*, 1962). It remains to be seen how far this fascinating technique will work in distant crosses.

Coming next to the post-fertilization barriers, these may appear in the form of zygotic lethality, premature cessation of growth in the embryo and/or endosperm tissue, or sterility in the F_1 plant and so on. The precise location of the post-fertilization abnormality necessarily calls for an investigation of the process of seed development in crosses. We owe much of our existing knowledge on the mechanisms of seed failure in crosses to the excellent work of Brink and Cooper (1947) and of Blakeslee's school of *Datura* workers (*see* Avery *et al.*, 1959) and of several others. The various investigations of diplontic sterility may be considered under four categories for the sake of convenience.

(i) *Abnormal endosperm*.—Embryologists consider the endosperm tissue as an evolutionary device in angiosperms that probably compensates for the highly reduced female gametophyte as compared to a massive one in the gymnosperms, and also confers upon the endosperm the physiological superiority of hybridity. This cytogenetic equipment presumably enables the endosperm to maintain a certain aggressiveness over the maternal tissues surrounding it, so that it can serve as an efficient intermediary for the nutrition of the embryo. From this it will be evident that any malfunctioning of the endosperm would lead to the starvation and death of the embryo. Different kinds of abnormalities have been reported in distant crosses, such as dumbel-shaped and giant-

sized nuclei in the crosses *Avena strigosa* × *A. fatua* (Kihara and Nishiyama, 1932), *Hordeum jubatum* × *Secale cereale* (Cooper and Brink, 1944; see review by Brink and Cooper, 1947) and in *Gossypium arboreum* × *G. hirsutum* (Weaver, 1958). Reusch (1959) in his study of the cross *Lolium perenne* × *Festuca pratensis* noticed precocious wall formation but further divisions were suppressed due to pycnosis of nuclei and stickiness of chromosomes, a situation considered parallel to that reported by Koller (1943) in tumorous cells. Other cases of abnormal endosperm reported include those by Sachet (1948) in *Datura*, Johansen and Smith (1956) in *Arachis*, and by Greenshields (1954) and Shastry (1958) in *Melilotus* crosses.

(ii) *Abnormalities in maternal tissues.*—Since the maternal envelope forms an integral part of the nutritional mechanism in the seed, histological changes in the cells of the nucellus and seed coat can often provide useful clues regarding the nature of the incompatibility reaction. Cooper and Brink (1940) have designated as “somatoplastic sterility” the phenomenon of over-growth or hyperplasia of the endothelium lining the endosperm, in the cross *Nicotiana rustica* × *N. glutinosa*. Similar cases of somatoplasia have been reported by Sachet (1948) in *Datura*, Beaudry (1951) in *Elymus virginicus* × *Agropyron repens* and Johansen and Smith (1956) in *Arachis hypogaea* × *A. diogeni*.

Since abnormalities in endosperm may also occur coincidentally with somatoplasia, it is often difficult to establish definite relationship between cause and effect. Brink and Cooper (1947) consider the endosperm as the primary cause of seed failure in crosses since it often precedes the somatoplasia of nucellus or endothelium.

As regards the rôle of antipodals in early post-fertilization development, opinions differ. Brink and Cooper (1944) attributed seed abortion in a *Hordeum jubatum* × *Secale cereale* cross to the weak functioning of the antipodals which occupy a vital position in the nutrient stream. Maheshwari (1950) stated that antipodals enlarge and become glandular after fertilization and thus offer a close analogy to tapetum of the anthers. Beaudry (1951) also assumed that antipodals were secretory in function and their hypertrophy caused death of the endosperm. On the other hand, Thompson and Johnston (1945) claimed that antipodals were not different from normal to be of significance in the breakdown of the *Hordeum* × *Secale* cross. Brock (1955) has shown that breakdown in lily endosperm is not caused by antipodals but by chromosome abnormalities. Morrison (1955) has also shown that antipodals are not responsible for abnormalities in endosperm in the cross between *Hordeum marinum* × *H. vulgare*, and in wheat.

(iii) *Abnormal embryo.*—Greenshields (1954) found different kinds of abnormal embryogeny in *Melilotus* crosses ranging from a complete inhibition of division in the zygote to abortion at later stages. Buell (1953) also attributed the failure of the cross *Dianthus plumaris* × *D. chinensis* to the abortion of the embryo. Weaver (1957) also held the embryo responsible for seed failure in the cross *Gossypium hirsutum*

× *G. arboreum*, where the absence of zygotic division resulted in "embryoless" ovules. The imbalance between the endosperm and embryo has been ascribed to the production of an inhibitory substance by the embryo. Moav and Cameron (1961) postulate that the embryo is the site of hormonal upset leading to abnormality in maternal and endosperm tissues in the cross *Nicotiana rustica* × *N. tabacum*, and suggest that the changing of chromosome number level in favour of the embryo partially restored viability.

(iv) *Abnormalities due to different chromosome numbers in the parents of a cross.*—Several cases of seed failure have been attributed to an alteration of the 2:3:2 ratio of the embryo, endosperm and maternal tissues. In general the abnormalities resulting from such matings have been more prominent when the seed-parent had a lower chromosome number, as reported by Kihara and Nishiyama (1932) in *Avena*, Wakakuwa (1934) in *Triticum* crosses, Boyes and Thompson (1937) in a wheat × rye cross and Weaver (1957, 1958) in *Gossypium*.

THEORIES CONCERNING MECHANISM OF SEED FAILURE

Stephens (1942) used the concept of "genetic strength" of a genome to explain differences in seed-setting in crosses between autotetraploid *Gossypium arboreum* and diploid *G. arboreum* and several wild species of *Gossypium*. He concluded that differences in compatibility are associated with quantitative but not qualitative differences in cytological balance between endosperm and zygote, and that the extent of differences in "strengths" between different genomes would account for the success or failure of the cross. Howard (1947) employed a similar hypothesis to explain his results on seed size in crosses between diploid and autotetraploid *Nasturtium officinale* and allotetraploid *N. uni-seriatum*. Valentine (1956) has assigned genetic values to genomes of different *Primula* species, and the ratio (R) between the endosperm and maternal tissue which in normal intraspecific combinations is 3/2. The success or failure of a cross depends on the extent of deviation from the normal. R. Woodell (1960 *a, b*) suggests that differences in genetic values may be analogous to differences in chromosome number and the rate of growth prompted by pollen at fertilization may be dependent on genetic value, and may condition the course of seed development.

From a detailed study of fertilization and seed development in crosses between the two jute species Iyer *et al.* (1961) have suggested a nuclear-cytoplasmic disharmony as being responsible for seed abortion. The incompatibility in this cross was overcome by the method of hormone application by Islam and Rashid (1960) and by the use of reciprocally grafted parents and irradiated pollen by Swaminathan *et al.* (1961).

With regard to other techniques employed to overcome post-fertilization barriers mention must be made of embryo culture, which is now a well established field by itself and an important aid in plant breeding.

Several workers have used embryo culture in obtaining distant crosses which normally fail due to premature abortion of the hybrid embryo. Important among these are the work of Skirm (1942) who obtained several interspecific crosses in *Prunus* and *Lilium*, Keim (1953) in *Trifolium*, Davies (1960) in *Hordeum*, Brink *et al.* (1944) in the cross *Hordeum* × *Secale*, and Farquharson (1957) in *Tripsacum* × *Zea* cross.

Embryo transplantation is another interesting method tried successfully by Hall (1954, 1956) in crossing wheat with rye. The crossability of wheat with rye was enhanced by raising the wheat plants from embryos planted on rye endosperm.

CONCLUDING REMARKS

From the above review, which by no means is comprehensive, it is sufficiently clear that the detailed studies on the process of seed-development made by the embryologists and plant breeders in collaboration, have immensely enriched our knowledge concerning mechanisms of seed abortion. Success in this field of investigation largely depends on the standardization of techniques, and the skill of the worker. Though the customary methods in embryological studies are lengthy and time-taking, the availability of shorter and less cumbersome techniques such as the dissection squash technique (Morrison, 1955) and feulgen staining prior to embedding and sectioning (Newcomer, 1959), will prove extremely useful for the plant breeder for whom it would otherwise be hard to fit into his programme the lengthy processes of embryological methodology. Besides, there are more elegant techniques also available such as the radioactive tracer-method used by Linskens (1955) in *Petunia*, by Tupy' (1961 *a, b*) in studying changes in carbohydrate and amino acid levels in styles and ovaries of *Nicotiana* after compatible and incompatible pollinations, and by Polyakov *et al.* (1957) also in *Nicotiana* crosses.

As regards the mechanism of seed abortion, no single theory can explain all the different facts observed at the histological level. With increasing work on the biochemistry of the seed and on the changes following distant crossing, it may be possible to obtain deeper insight into the observed processes in the seed. The fact that the plant breeder is now fully aware of the problems in distant hybridization in all its various aspects has served to alter the earlier belief of the embryologist that plant breeders "merely put the pollen upon the stigma and pray for results in the ovary" (Maheshwari, 1962).

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