SPECIAL ARTICLES

THE ENDOSPERM AS A BARRIER TO INTERSPECIFIC HYBRIDIZATION IN FLOWERING PLANTS[1]

FOLLOWING fertilization between species, development of the seed is frequently arrested short of a condition which permits germination. The line of descent embodied in the hybrid embryo is thus summarily terminated. The question whether collapse is due to defects in the embryo itself or to abnormal behavior of the accessory tissues of the seed is of considerable theoretical and practical interest.

Genetically speaking, the angiosperm seed is a chimera, comprising embryo, endosperm and maternal tissues differing from each other in hereditary organization. The embryo develops from the fertilized egg. The endosperm, which is the product of an independent fertilization, is normally triploid, its primary nucleus receiving two sets of chromosomes from the macrogametophyte. These two structures are enclosed in maternal tissue usually consisting initially of a nucellus and either one or two integuments. Several investigators have considered that a disturbed developmental relationship between these genetically diverse components is responsible for failure in hybrid seeds. The evidence brought forward, however, has not been clear regarding the tissues involved or the character and sequence of events leading to collapse. Formulation of a satisfactory working hypothesis concerning the cause of failure, therefore, was not possible.

Recent investigations in this laboratory indicate that the primary cause of hybrid seed collapse is weak growth of the endosperm. The effect, however, is indirect. The subnormal development, which may be detected soon after fertilization, induces modifications in the associated maternal tissues, which in turn react unfavorably upon the endosperm. The latter becomes starved, the cells break down and the seed collapses.

The embryo, although often retarded, may continue to grow while these changes are in progress. Critical evidence to exclude it is lacking, but there are no positive indications that the embryo itself is a factor contributing to failure. It is still very small, sometimes only just beyond the zygote stage, when the transformations occur in the young seed which appear to determine largely the future course of development. On the other hand, the endosperm at this time is regularly more advanced, which strongly suggests that it, rather than the embryo, is the controlling tissue. Later, of course, the embryo may become the dominant structure of the seed, but failure of the type under consideration, even though it may be delayed, rather clearly has its origin in early post-fertilization behavior.

Since the discovery of double fertilization at the close of the last century and the interpretation of xenia in maize in the light of it, it has been apparent that the endosperm possesses some measure of genetic autonomy. The evidence from xenia, however, relates to the mature endosperm in one of the relatively few families of angiosperms in which the tissue characteristically persists. It casts no light on the extent to which early endosperm behavior is genetically controlled in the far larger proportion of species in which the tissue is present in the young seed, but later disappears. Furthermore, embryological studies have rather consistently underestimated the importance of the endosperm as a factor in early seed development.

Three facts are now established which appear important for an understanding of the type of seed failure in question. (1) In addition to the trophic properties commonly attributed to it, the endosperm plays a basic rôle in the morphogenetic changes involved in transformation of the ovule into a seed.[2] This is clearly shown in *Nicotiana rustica*. Following normal self-fertilization the funicular cells between the apex of the vascular bundle and the chalazal pocket become differentiated into conducting cells. The histological evidence strongly suggests that this important step is a response of the contiguous maternal tissue to the endosperm which is developing very rapidly at the time. The differentiation does not occur if *N. rustica* ovules are fertilized by *N. glutinosa* or *N. tabacum.* (2) Behavior of the endosperm during early post-fertilization stages is significantly influenced by the genetic make-up of the tissue. This fact was established quantitatively in *Medicago sativa,* in which a significant difference in rate of free nuclear division was observed in inbred and crossbred endosperms.[3] It was confirmed in *Nicotiana rustica,* in which the normal endosperm develops much more rapidly than do *N. rustica* × *N. glutinosa* and *N. rustica* × *N. tabacum* endosperms.[4] (3) In seeds which fail, the collapse follows changes in the maternal tissues which arise as a result of hypofunction of the endosperm. Associated with the weakly growing endosperms in self-fertilized *Medicago sativa*[3] and in the above-mentioned Nicotiana interspecific hybrids[4] there is overgrowth of the adjacent maternal tissues which eventually induces signs of starvation in the endosperm itself and precipitates collapse of the seed. It was found that

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[2] D. C. Cooper and R. A. Brink, *Genetics,* 25: 593–617, 1940.

[3] R. A. Brink and D. C. Cooper, *Bot. Gaz.,* 102: 1–25, 1940.

[4] R. A. Brink and D. C. Cooper, *Genetics,* 26: 487–505, 1941.

in *N. rustica* × *N. tabacum* seeds in which collapse is delayed so that a germinable condition may be reached, even though very rarely, the endosperm-nucellus volume ratio at early post-fertilization is higher than in *N. rustica* × *N. glutinosa* seeds, which all fail early.[4] Since the pistillate parent is the same in both crosses, it is evident that the varying degree of abnormal development of the maternal tissue in the two respective classes of seed is a function of the endosperm genotype.

The endosperm, therefore, may act as a barrier to interspecific hybridization in flowering plants. The tissue forms the basis of an isolating mechanism, to use Dobzhansky's[5] term, which tends, like numerous other physiological devices, to hinder the interchange of genes between specific groups.

It is a point of considerable practical interest that failure of the seed due to weak endosperm development does not necessarily imply that the associated embryo is inviable. The lethal mechanism is not in the line of descent but is parallel to and impinges upon a short but critical segment of it. Freed of its adverse environment in the seed before breakdown occurs, the embryo may be capable of growing into a plant. Numerous cases are on record of vegetatively vigorous interspecific hybrids having been reared from badly shrunken seeds which may have barely passed the limits of germinability in their development. If the present analysis is well founded, it is a fair inference that below this threshold there are embryos of many more hybrids the realization of whose potentialities only awaits the application of suitable technics of artificial cultivation. The work of Laibach,[6] Tukey,[7] van Overbeek *et al.*[8] demonstrates the possibilities of rearing incompletely developed embryos on synthetic media. The limits within which plant breeders may be able to explore the possibilities of interspecific hybridization now appear, therefore, to be significantly broader than earlier could have been foreseen.

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ON THE LOCALIZATION OF ENZYMES IN NERVE FIBERS[1]

CHOLINE esterase is highly concentrated at or near the neuronal surface. This was shown recently in experiments on the giant fiber of squids, where practically the whole enzyme activity was found to be in the sheath, only a negligible amount of enzyme being present in the axoplasm.[2] These observations, together with the parallelism, within certain limits, found between number of electric plates per cm, E.M.F. per cm and concentration of choline esterase in electric organs, suggest that the electrical changes observed during nerve activity may be intrinsically connected with acetylcholine metabolism.[3,4] It is necessary to know how specific is the concentration of choline esterase at the neuronal surface compared with other enzymes important for nerve activity. Studies have been started in order to determine the activity of different enzymes separately in sheath and axoplasm of the giant fiber.

Nerve activity is connected with heat production and extra oxygen uptake. Determinations of the total respiration may not yield optimal values if the cell is no longer intact. It appears safer to determine important intermediate steps. Succinic dehydrogenase is widely believed to be essential in respiratory enzyme systems. Its activity has been determined with the manometric method with ferricyanide.[5] Table 1 gives the results of an experiment showing that about 90 per cent. of the total enzyme amount is present in the axoplasm. The concentration is about 50 per cent. lower in the sheath, although the absolute amount metabolized there is so small that the values are near to the limits of sensitivity of the method. Although the greatest part of the sheath is connective tissue, the results give no indication of a higher concentration of succinic dehydrogenase at or near the surface. The contrast to the distribution of choline esterase is striking. In the whole giant fiber the concentration is intermediate between that of sheath and axoplasm, as could be expected. In the head ganglion the concentration of succinic dehydrogenase is about 10 times as high as in the fiber, whereas its concentration of

TABLE 1

SUCCINIC DEHYDROGENASE. K = CONTROL, E = WITH SUCCINATE. TIME 235 MIN. CONCENTRATION OF SUCCINATE 0.15 MOL. t = 23° C.

		Mg fresh tissue	Cmm CO_2 Observ.	Cmm CO_2 Calc. for 100 mg	μg succinic acid Absol.	μg succinic acid In per cent. of total	μg succinic acid Per 100 mg
Sheath	K	6.7	1.96	29.2			
	E	5.5	4.20	76.7	6.7	11	126
Axoplasm	K	27.5	4.5	16.3			
	E	24.9	24.6	99.0	53.5	89	216

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