THE INTERRELATION OF PLASMAGENES AND CHROMOGENES IN POLLEN PRODUCTION IN MAIZE

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AN INHERITED condition of pollen abortion is frequently found in many species of plants. In maize 20 genes for male sterility are listed by EMERSON, BEADLE and FRASER (1935). Male sterile-1 reported by SINGLETON and JONES (1930) is on chromosome 6 closely linked with the *Yy* endosperm locus. All of the gene steriles described are independent recessives, each capable of bringing about either complete or a high degree of sterility in the male inflorescence. Meiosis occurs normally and the pollen grains are formed but before anthesis these grains abort. The anthers remain enclosed in the glumes. The tassel branches are thin and characteristically limp. In some cases a few anthers are extruded several days after anthers are exposed on normal sib plants, and rarely a small amount of pollen is released. This pollen is usually non-functional.

A similar type of pollen sterility in maize and other plants has been found to be conditioned by something outside the nucleus. RHOADES (1933) described the first case in maize. He found that it was transmitted only through the ovules, never through the pollen, and that in transmission it was independent of the chromosomes. All of the offspring of a sterile plant were likewise pollen sterile irrespective of the normal maize plants used to produce seed. RHOADES concluded that this condition was brought about by something in the cytoplasm.

Similar cases of cytoplasmic pollen sterility in maize have been found in other unrelated varieties and in five other genera: Linum, Nicotiana, Allium, Beta and Daucus.

In all types of cytoplasmic sterility pollen production varies widely from complete sterility to the production of functional pollen in varying amounts. GABELMAN (1949) has studied this type of sterility further and finds that it is dependent upon a discrete particle that is transmitted regularly from cell to cell during mitosis, but may be reduced in numbers but never eliminated entirely in some of the egg cells. These particles are never transmitted by the pollen and therefore are eliminated during meiosis from some of the sporocytes. Apparently one particle can prevent the functioning of a male germ cell. Since the particles are increased during mitosis but not during meiosis they have something in common with chromatin, dividing regularly when the chromosomes divide and not dividing when the chromosomes fail to divide at the reduction division.

Since there exists something, both within and without the nucleus, that is transmitted from generation to generation, each having similar results, it is necessary to distinguish between these inherited determiners. Genes in the chromosomes can logically be called chromogenes. The determiners in the cytoplasm have been referred to as cytogenes or plasmagenes. Both terms carry the implication that the nuclear and extra nuclear factors are similar. Since there is evidence that they are similar in some respects the term plasmagene is used here.

Both the plasmagene and the chromogene for pollen abortion have been combined in the same plants. By crossing and backcrossing a white-seeded, cytoplasmic-sterile plant by using normal pollen from a heterozygous gene-sterile plant, also heterozygous for yellow-white endosperm color, it was possible to obtain on a cytoplasmic-sterile plant yellow seeds that were heterozygous gene-fertile and white seeds that were homozygous gene-sterile. These two lots of seed were grown separately.

The heterozygous gene-fertile, cyto-sterile plants showed a few extruded anthers and produced a small amount of pollen. The same amount of pollen production was shown by other crosses of these cytoplasmic-sterile plants where the gene for sterile pollen was not involved. These plants averaged 79.2 inches in height compared to 76.3 inches for the gene-sterile, cyto-sterile plants in an adjoining row. This small difference in height could be due to the slightly greater heterozygosity of the yellow seeded plants. The gene-sterile, cyto-sterile plants showed no anthers and no pollen was released. The two different types of genes when present together in the same plant seem to be without effect on each other. Each gene had the same effect on pollen production as it has when acting alone.

Variation in the production of normally functioning pollen grains is characteristic of all the cytoplasmic pollen-sterile plants so far studied. Material from three different sources has been grown and two additional sources have been reported. In the process of converting standard inbred lines used in the production of commercial hybrid seed corn, it has been found that some lines are relatively easy to sterilize and others are difficult. The usual procedure is to cross the fertile inbred line on to selected, completely pollen-sterile plants of approximately the same maturity. By repeated backcrossing with the same fertile inbred on to sterile plants that were selected as the nearest in type and time of flowering to the inbred used as the pollinator, it was possible to convert a fertile line into a similar sterile line after from three to five backcrossings. This is easily accomplished since all that is necessary is to put a complete chromosome set from one type into the cytoplasm of the sterile type. No chromosome crossing over is involved as in the case where a single gene is being transferred and recombined into a new chromosome set.

The complete control of the chromogenes on size and maturity is illustrated in a striking manner in a cross and backcross of a sterile late southern field corn line with a fertile, very early, sweet corn line. The late field corn plants grew over ten feet tall in Connecticut and flowered about 90 days after planting. The early sweet corn inbred by which it was pollinated grew about four feet high and flowered in 60 days. After three backcrosses the sterile version of the early line is practically identical in size, structural details, and in time of flowering, to the early fertile inbred. Similar results have been obtained in other combinations by making the original cross and backcrosses reciprocally, except that the crosses on cytoplasmic fertile plants remain fertile.

In other combinations as well, backcrossed sterile lines are similar in every respect to their corresponding fertile lines except in pollen production. The two lots grow to the same height, flower at the same time, and have the same structural details in all parts of the plant, as shown in table 1. This is further proof that the cytoplasm has very little to do with the ordinary variations within species. This fact had been well established by the similarity in reciprocal crosses within the species. Both lines of evidence show that the ordinary varietal differences are brought about by chromogenic differences and that the cytoplasm is basically the same.

TABLE 1

A comparison of fertile and sterile maize plants having the same nuclear but differing in cytoplasmic constitution.

	FERTILE	STERILE	
5 Inbreds	72.3	70.1	Height of plant in inches
7 Single crosses	102.6	97.7	Height of plant in inches
7 Single crosses	58.5	58.3	Days to first silk
3 Crosses of three inbreds	111.7	108.9	Yield in bushels per acre
3 Crosses of four inbreds	123.9	119.0	Yield in bushels per acre

In general, the inbred lines that are weak in pollen production are easy to sterilize. The first generation crosses of such inbreds, on the plants used as sterilizers, are usually completely pollen-sterile and successive backcrossed generations also remain sterile. A few plants may extrude some anthers and a small amount of pollen may be produced. The inbred lines that are good pollen producers, the ones that are usually used as pollinators in the commercial production of hybrid seed corn, usually show much pollen production in the first crosses and successive backcrosses with the same sterilizer stocks.

When the anthers on these plants are stained with iodine solution some of the pollen grains appear to be fully formed and well stored with starch. GABELMAN (1949) has shown that the proportion of well filled and partially filled pollen grains varies widely in some progenies.

A few inbred lines and genetic stocks have the ability to restore almost complete normal pollen production to the first generation crosses of sterile by fertile. Pollen counts show up to 95 percent of normally well filled and dark staining pollen grains. For example, a sterile line of Illinois A inbred crossed by a linkage tester stock produced eleven plants of which four had 95 percent or more normal-appearing pollen grains, five plants with 30 to 50 percent normal-appearing pollen, and two plants with less than 5 percent. The same sterile inbred crossed by a dioecious male plant produced ten sterile and five fertile plants. Three of the fertile plants were self-fertilized and two produced well filled ears, and one was about one-fourth filled.

The pollen from partially fertile plants that is capable of producing seed when used alone was applied in competition with normal plants. To test the ability to function in competition with normal plants, pollen was collected from yellow-seeded, partially fertile plants, used alone to test its ability to function, and then put on fresh silks of a white-seeded plant. At the same time pollen from other white-seeded plants was placed on the same flowers. Approximately equal quantities were applied. On seven ears produced in this way six yellow seeds were found among 1085 white seeds where equal numbers would be expected if both kinds of pollen were equally effective. See table 2.

TABLE 2

The effectiveness of pollen from partially-fertile, yellow-seeded, plants in competition with normal pollen from white-seeded plants on white-seeded (C20) plants.

SOURCE OF POLLEN	NUMBER OF WHITE SEEDS	NUMBER OF YELLOW SEEDS
A^{s4}×Oh28	187	0
A^{s4}×Oh28	86	0
A^{s4}×Oh43	88	0
A^{s4}×B164	232	0
A^{s4}×B164	78	2
$C106^{s2}$×Oh41	319	3
(Wf^{s3}×Oh7)C107	95	1
	1085	6

Sterile C106 inbred crossed by normally fertile Ky21 produced two progenies of which all plants were normal in pollen production. A sterile single cross (Wf9 sterile ×38-11) pollinated by the same Ky21 line produced an all fertile progeny. The same C106 sterile plant cross-fertilized by a first generation hybrid (Ky39×Ky21) produced 45 completely sterile and 22 completely fertile plants in five progenies. All of the fertile plants that were self-fertilized produced a full set of seed and an examination of the pollen showed 95 percent or more of normal appearing grains. This pollen has not been tested in competition with normal pollen.

The fertile plants of the cross sterile A by dioecious male and the linkage tester segregated in the second selfed generation into completely sterile and partially fertile plants with varying amounts of pollen production. The fertile plants from the crosses with Ky21 have not yet been grown but presumably will segregate since the combination of (Ky39×Ky21) F_2 on the sterile inbred segregates.

The pollen restoring ability of Ky21 was indicated in results obtained by JOSEPHSON and JENKINS (1948). They found that Ky21 gives normal pollen production in hybrids where Indiana 33–16 and Ky27 are used as seed parents. These two inbreds in other combinations where Ky21 was not used as pollinators were highly pollen sterile. Other inbreds that have the ability to restore pollen production, either completely or partially, are Texas 127C, Illinois Hy and Minnesota B164. A number of other lines commonly used in the commercial production of hybrid seed corn have this ability to restore pollen in varying degrees.

All of the evidence shows clearly that there are chromogenes that have the ability to overcome the sterility usually exhibited in plants that have the sterile plasmagene. So far, no cytoplasmic sterile plant has been restored to complete fertility in all of the plants of the following generations. Whether the chromogenes in Ky21 and similar lines can do this when they are in the homozygous condition remains to be determined.

In a routine conversion of many commercial inbred lines to the sterile tassel condition it has been found that about half of these lines possess genes that restore pollen production to a moderate degree. Since the first outcrosses to these lines are usually variable in pollen production it shows that these lines, although highly inbred and carefully selected for uniformity, are nevertheless heterozygous with respect to these genes that normally have no visible effect and are not consciously selected for or against. This shows clearly that there is a considerable amount of enforced heterozygosity in maize inbreds as has been suspected from other evidence.

By testing individual plants of a normal fertile line for their inability to restore pollen production when crossed on to sterile plants, it is possible to select and establish sub-lines that maintain the sterile lines completely sterile. By a similar process it is possible to increase the ability of many lines to restore a high degree of pollen production in crosses with these sterile lines. It is easier to establish, and to maintain by continuous back crossing, completely sterile lines by using inbreds that are normally poor pollen producers and conversely the good pollen producers are more favorable material for restoring pollen production.

SUMMARY

1. Pollen abortion in maize and other plants is brought about by recessive genes in the chromosomes and also by extra-nuclear factors presumably propagated and transmitted in the cytoplasm.
2. These two kinds of inherited determiners are designated as chromogenes and plasmagenes.
3. Both plasmagenes and chromogenes are variable in their control of pollen production.
4. Other chromogenes have the ability to restore pollen production in variable amounts in plants that are completely cytoplasmically sterile in other combinations.
5. Plasmagenes that condition pollen sterility apparently have no other effect upon the growth and structural details of the plants.
6. Plasmagenes and chromogenes for pollen sterility when acting together in the same plants apparently have no effect on each other and are independent in their action.
7. Pollen produced on partially fertile plants with the plasmagene for sterility is less effective than normal pollen when in competition.

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