Reversion in Cytoplasmic Male Sterile Plants of Beta Vulgaris[1]

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Cytoplasmically inherited pollen sterility has been reported in an ever-increasing number of plant species over the past thirty years. Its utilization in the production of hybrid seed has increased similarly. Yet the carriers of cytosterility are still unknown. Two hypotheses dealing with the nature of the carrier of cytosterility have received special attention. According to the first, the determinants are particulate units, which are not transmissable from cell to cell except by division (2)[3]. The determination of cytoplasmic characters, such as "killer" in *Paramecium* (5) and certain types of chlorophyll variegation in *Epilobium* (3) have been ascribed to particulate, non-genic factors, too. According to the second hypothesis, cytosterility is determined by a non-particulate, diffusible substance, not necessarily synthesized at the site of its action. In *Petunia*, cytosterility has been reported to be transmitted through a graft (1). The observations on reversion of male sterile beet plants to partial fertility reported in this paper support the second hypothesis.

Materials and Methods

Cytosterile lines of sugar beets were received from Dr. F. V. Owen in 1949. Cytosterile lines of table beets were obtained by systematically backcrossing these steriles and their resulting progenies to pigmented table beets. Within these backcrossed progenies were many lines segregating for sterility and fertility. It was assumed, based on Owen's hypothesis (4), that these steriles were of the genotype S xx zz. The original source of cytosterility was maintained as a sterile non-pigmented line by backcrossing continuously to the non-restorer line SLC 03. These latter steriles, hereafter referred to as "original cytosteriles." were used as controls to study the behavior of certain of the "derived cytosteriles" which were obtained in the table beet material.

The entire plant population studied was subjected to long days and low temperatures while seedlings and was thereby induced to flower in the first year. Because of this forced development, vegetative growth prior to flowering was somewhat restricted.

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[3] Numbers in parentheses refer to literature cited.

Observations

Among the derived cytosteriles were numerous red plants which also had bright red anthers (6). Pollen of these derived pigmented cytosteriles did not differ in development from pollen of unpigmented cytosteriles. The endothecium was unspecialized. The microspores disintegrated soon after being released from tetrads; their surface was soft and undifferentiated.

In many of these pigmented plants, anthers of late-appearing flowers underwent a conspicuous change. These were the flowers at the tip of the main flowering stalk and on the late flowering lateral stalks. The red color in the anthers disappeared, the anthers turned yellow and opaque, became more plump, and resembled semi-sterile anthers in appearance. A similar change in anther appearance was subsequently found in plants devoid of red pigment, but the color contrast between old and young anthers was not as conspicuous. Microscopic examination revealed that in these changed anthers the typical spiral thickenings of the endothecium were present, as well as xylem vessels. These anthers and their pollen grains resembled the semi-sterile plants reported by Owen to carry S cytoplasm and one or two of the restorer genes.

The pollen of reverted anthers was always more completely developed than pollen of earlier developed red anthers and resembled the semi-sterile pollen in possessing a sculptured exine. Prior to reversion, microspores did not persist long after release from tetrads, and anthers were typically flabby and semi-transparent. At the onset of reversion, in flowers close to the tip of the flowering stalk, pollen grains developed to a more advanced stage. A soft exine began to appear, but maturity was not reached. In still later appearing flowers, well-formed pollen grains of very regular spherical shape developed with a hard exine covered with prominent buckles. These pollen grains resembled those found in normal plants immediately prior to anthesis. At this stage, the anthers were opaque and yellowish. At a still later stage, a varying percentage of fertile pollen grains was found. Except for this simultaneous occurrence of fertiles and steriles in the last stage, pollen grain samples, as obtained from any single anther population were very uniform at any given moment, and the change in pollen development was gradual. The change in anther appearance and in the somatic anther tissue sometimes preceded the shift in pollen development. Reversion apparently affected the somatic tissues directly and somewhat earlier than the gametic tissues.

The plants, as grown in the greenhouse, differed somewhat in flowering time. While some plants were at or past the peak of flowering, others bore immature flowers in the inflorescence. Even though all plants were subjected, as nearly as possible, to the same environmental conditions, reversion never occurred in plants in which flowering had been delayed, or in which flowering was just starting, but occurred only in plants which had flowered for a considerable length of time. This seems to indicate that reversion is not brought about by specific environmental factors, but the internal condition of the plant itself. Reversion occurred in plants which were flowered particularly early. These plants had considerably less vegetative mass at flowering time than plants which did not revert.

Reversion was never observed in the "original sterile" lines. In only one case was a change in the endothecium noted. This line was maintained by back-crossing to the normal non-restorer SLC 03, hence it may be concluded that SLC 03 does not transmit the ability or tendency to revert to its offspring. Most of the plants in which reversion occurred were in F_2 populations of the cross (4460h0 x SLC 03) x Perfected Detroit. Since the seed parent of this original cross did not revert, it is assumed that the reversion occurred under the influence of the Perfected Detroit genome. This would not exclude genetic recombination within these lines.

After reversion had been observed in cytosteriles, it was sought in semi-steriles, too. The percentage of fertile grains was expected to be higher in late appearing flowers, at the upper part of the flowering stalk. A single plant was chosen for this study: since all anthers on the plant were uniform in appearance, they were chosen by location only. Anthers from the upper part of the inflorescence had an average of 34 percent fertile appearing pollen grains, while anthers from the lower part of the inflorescence averaged 20 percent fertile appearing pollen grains. This difference was highly significant.

Discussion

Reversion of pollen sterile plants to pollen fertile plants has been noted by several workers, and the cause has usually been ascribed to environmental factors. The present observations reported here indicate, however, that the reversion of plants under nearly identical environmental conditions depends on some internal factor or factors. Reversion of the cytosterile plants to partial fertility occurred in those plants in which the vegetative-

generative balance had been shifted toward the generative side very early. The vegetative development of these reverted plants had been interrupted by early flowering. This relationship suggests that the vegetative plant parts produce some substance or substances which are necessary for the maintenance of the sterile state. Reversion appears to be dependent on the exhaustion of reserve substances which accumulated during vegetative growth. These hypothetical substances could not be cell limited, as they appear to be transmitted to several parts of the plant simultaneously.

These results do not preclude the possibility that reversion could be brought about by either an exhaustion of a "sterility substance" or by accumulation of "fertility substances" in certain cells through random segregation until a certain threshold is reached. Neither do they preclude the exhaustion or accumulation of a diffusible substance or substances which could activate or deactivate cell limited particulate units.

The cell-limited particulate hypothesis implies that reversion would be associated with a particular cell lineage, in which random segregation has led, by chance, to an accumulation of fertile particles or depletion of sterility particles. It does not explain the simultaneous occurrence of reversion at different sites on a plant, as observed in the beet. However, the simultaneous effect in different plant parts does fit the assumption of a diffusible substance. The association of reversion with changes in somatic anther tissue, and the concommitant changes in pollen development are also more easily explained by the assumption of control by a diffusible substance.

The reversion that takes place from "derived cytosteriles" to conditions of partial fertility is very similar morphologically to the differences that exist between the genotypes S xx *zz* and S Xx *zz*, or S xx Z*z*, or S X*z* Z*z*. This implies that within the plant physiological changes can take place of the same magnitude as known genic changes.

Although reversion has been observed in "derived cytosteriles" only, the mechanization probably has broader application pertinent to the nature of the cytoplasmic factor and thereby be applicable to cytosterility in general. The data also suggest possible mechanisms for the instability of certain steriles under different cultural and environmental conditions.

Summary

Cytoplasmic male sterile sugar beets were crossed by fertile table beets. Some segregants of these crosses started out as typical cytosteriles, but in their latest developed flowers, produced a varying percentage of fertile pollen (3%-19%). This reversion was not caused by environmental factors alone, but depended on some internal condition of the plant, related to the relationship between vegetative and generative development. An early shift of the normal balance toward generative development is associated with reversion. This indicates, that the maintenance of the sterile state requires some product of the vegetative growth, probably a diffusible material.

Reversion is gradual, and different stages of the process can be distinguished. Pollen grain populations in a plant at any given moment are very uniform. This, too, supports the assumption that reversion is due to an accumulation of a "fertility substance" or to exhaustion of a "sterility substance"; not to segregation of particulate sterility factors.

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