

# A Method of Determining Self-Fertility and Self-Sterility in Sugar Beets, Based Upon the Stage of Ovule Development Shortly After Flowering

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Sugar-beet-pollen grains germinate after selfing on the surface of the stigma, but in the tissues of the pistil the growth of the pollen tubes becomes slower or stops completely. For eight or nine days after selfing the pollen tubes are unable to reach the embryo sacs and for the most part the ovules remain non-fertilized. In three or four days after selfing, the synergids are not broken through, but their outlines become wavy and their shape irregular. In seven or eight days the protoplasm in non-fertilized embryo sacs, in synergids and egg-cells, is constructed into dark stained threads (Figure 1a). The central nucleus is covered with chromatin threads which protrude through the membrane. In 10 or 12 days only a dark clump remains from the egg-cell and synergids, the ovules become shrivelled and the embryo sac is tightened by the nucellus.

The slow growth of the pollen tubes and non-occurrence of self-fertilization is the most important cause of self-sterility. If the pollen tubes after selfing grow fast enough and if fertilization takes place, we see another factor which provokes self-sterility in beets. This factor is the destruction of the newly formed zygote. Sometimes the embryo sac degenerates before the embryo starts to develop. The central nucleus of the embryo sac divides and forms some nuclei of the endosperm, but the egg-cell begins to degenerate before dividing.

In the other cases the embryo starts to develop normally but at a certain stage the growth ceases and degeneration takes place. At the stage when the embryo is globe-shaped, the cells of the degenerating embryo differ from the normal embryo by their dark staining nuclei and by the disappearance of the nucleoli. The endosperm nuclei have an abnormal shape, they are shrivelled and covered by the chromatin threads. At the stage of the heart-shaped embryo the contents of the cells degenerate

**Figure 1. (See page 199) Ovule development in self-sterile sugar beets as contrasted with normal development, (a, upper right) Degenerating non-fertilized embryo sac. XI000; (b, upper left) degenerating embryo and endosperm after selfing. X330; (c, lower left) normally-developing embryo and endosperm after crossing. X330; (d, middle right) dark lump remained from non-fertilized ovule in 12 days after selfing. XI5; (e, lower right) degenerating shrivelled seed after self-fertilization. XI5.**

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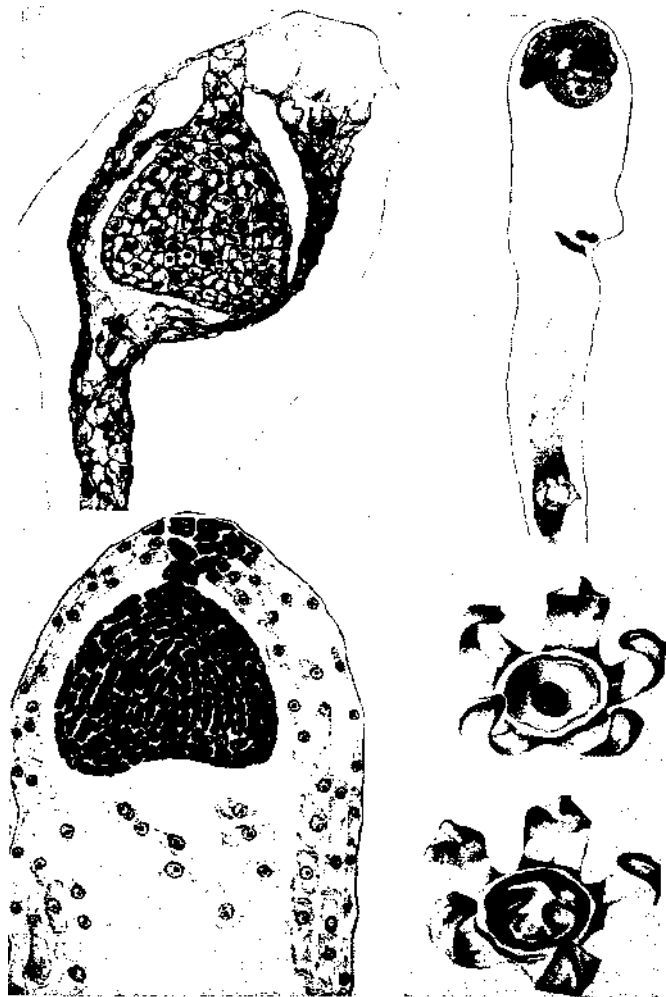


Figure 1.

(Figure 1b). The endosperm tissue is represented by a trace of demolished cells. In some ovules the embryo degenerates after having formed the seed-leaves. The seed leaves or cotyledons are curved and the root has a defective shape. The embryo cells are empty, in many places the cell membrane begins to degenerate. Simultaneously with the degeneration of the embryo sac, the ovule also degenerates. It becomes wrinkled and contracted and the seed coat is pressed in. The germination ability of selfed seeds is often very poor. This is caused by the death of the embryo and the absence of normal seeds in the developed seed-ball. Destruction of the embryo is the earliest manifestation of the degeneration process caused by genes which have a lethal action in the homozygote after selfing.

The rate of pollen-tube growth depends on the degree of self-fertility of the plant. The lower the percent of the fertilized ovules on the plant, the slower is the growth rate of pollen tubes, and the later the fertilization and the embryo development. We observed that in the highly self-fertile group nearly one-half of the plants (41.7%) were fertilized during the first day and one-half (50.1%) on the second day after selfing. Most of the partially self-fertile plants were fertilized on the second day after selfing (89.6%). In poorly self-fertile plants the growth of the pollen tubes is so slow that none of them were fertilized the first day and nearly half of the plants of this group (46.2%) were not fertilized for three days. Thus the rate of pollen-tube growth is associated with the degree of self-fertility. In some highly self-fertile plants the pollen tubes grow with the same rapidity by selfing as by crossing, but to produce a normal set of seeds the self-fertile plants must also possess the ability for normal development of the embryo (Figure 1c).

Highly self-fertile plants may arise in populations as a consequence of mutation of a gene which provokes a high rate of pollen-tube growth after selfing and a combination of these genes with such genotypes as can undergo self-fertilization without degeneration of embryos. Partially self-fertile races may manifest different degrees of both of these processes. A poor degree of self-fertility could be caused either by the lack of self-fertilization or by the death of the embryos. When self-fertile races are involved in breeding processes, it may often be necessary to determine the degree of self-fertility of a plant during the time of its flowering. A simple method, based upon the stage of ovule development shortly after flowering, can be used to determine the degree of self-fertility. Non-fertilized ovules appear in 10-12 days after opening of the flower as small, dark lumps on the bottom of the expanded cavity of the ovary (Figure 1d). The ovules which have started to develop after selfing, but in which the embryos have degenerated, appear as large but shrivelled seeds within the cavity of the ovary (Figure 1e).

The branches of a plant intended for investigation must be bagged. The buds must be investigated in the summertime 10 to 12 days after they have finished flowering. The ovary must be opened and determinations made of percentage of (a) small dark ovules, (b) normal developing seeds and (c) shrivelled seeds. The percent of small dark ovules on the bottom of the ovary shows the percent of non-fertilized ovules and the percent of self-sterility of the race. In highly self-sterile plants almost all ovules appear as small dark lumps. The percent of normally developed seeds indicates

the degree of self-fertility of the plant. In highly self-fertile races almost **all** ovules are large, non-shrivelled and **fill** the cavity of the ovary. The amount of large but shrivelled seeds shows the amount of fertilized but dead ovules. They must be added to the amount of normally developed seeds to determine the degree of self-fertilization of the plant. At the same time percentage of shrivelled seeds indicates the amount of degenerating embryos and the influence of the second factor which provokes self-sterility. The degree of self-fertility or self-sterility of a plant can be established quickly and easily on the basis of this analysis. Then the plant investigated in this manner can be used for crossing or selfing.

### **Summary**

**Self-sterility in beets is caused by: absence of self-fertilization because of slow rate of pollen-tube growth, and degeneration of embryos after selfing. Non-fertilized ovules appear in 10-12 days after opening of flower as small dark lumps on the bottom of the expanded cavity of the ovary. Determination of the degree of self-sterility or self-fertility is based upon examination of ovules during the time of flowering of the plants.**