Normal Gaspe Flint averages only 5 or 6 leaves on the main stalk. Tassels are visible in the leaf whorl two or three weeks after planting dry seed and pollen sheds in about one month. It has been suspected for some time that the growing point in the seed of Gaspe Flint has already been converted from the vegetative to the reproductive stage. Thus only the tassel would form after planting. Leaf number would be limited to those which have differentiated in the embryo of the seed while attached to the mother plant.

My working hypothesis to explain the early flowering main stalk and id-like tillers involves maternal genotype control of the growing point in the seed and autonomous genotype control in the tillers. That is, when id/id seed is produced on an +/id plant, the hormone pattern in the non-short day maternal plant may cause the embryo growing point to change from the vegetative to the reproductive stage as the seed matures. Growing points subsequently produced by the id/id plant in the form of tillers would be under the control of the plant's own genotype and would be indeterminate in growth.

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1. Further observations on the etched phenotype.

A. Lack of gene expression in the root. In order to determine whether or not the starchless sectors found in et/et/et endosperm were present in et/et roots, the following procedure was followed: ten 10 mm. roots (chosen because of strong etched expression in the endosperm) were hand sectioned and stained with iodine. If staining of the starch grains was initially poor, the section was washed with a drop of concentrated HCl and restained. No starchless sectors were found in any areas of the root exhibiting detectable starch concentrations. In addition measurements of starch grain number, size, and location in the roots of these etched plants were not significantly different from comparative measurements made on two different non-etched inbred types.

Since the effect of the et allele is manifested only early in the embryology of the endosperm and shoot (virescence) it was thought that the 10 mm. roots observed may have been "too old" for et expression. However, roots just emerging through the seed coat also were without detectable starch sectoring.

Observations on 9, 10, and 11 day old endosperms disclosed the same sectoring pattern seen previously in the mature kernel. These sectors of starchless cells are evident in the young endosperm as soon as enough starch develops to disclose their presence. This is interpreted to mean that starch is never formed in these sectors instead of being formed and then degraded after snythesis.

The etched allele, like sugary, affects nuclear control over the plastid in the endosperm tissue and is without corresponding effects on the same plastid in a different tissue--the root. It would be of interest to screen other known gene controlled plastid alterations (in the endosperm and shoot) to determine if any of these exhibit a comparable interaction with the root plastids.

B. Endosperm cultures. After finding cell lineages in the endosperm which do not store starch while other cells (the majority) do produce starch it was thought desirable to attempt an isolation of each type in tissue culture. The basic question we were interested in was whether the starch producing cells would give rise to starchless or vice versa. Pieces of endosperm were planted 9 and 10 days after pollination on unmodified Coe's media (MNL 1961). Serving as controls for starch snythesis patterns were other starchy inbred types (W22, W23, W8, 4Co63) and sugary Black Mexican sweet corn.

To date we have not been able to develop clones of callus which parallel the cell types in the organized endosperm.

We have found that the rapidly dividing cells at the periphery of the callus do not contain starch grains whereas the older cells (in from the clump surface and presumed not to be dividing) do show starch grains. The distribution of the starch containing cells in both etched and non-etched cultures appears scattered; that is, they are intermingled with the starchless cells. It is because of this lack of uniform starch synthesis that we are unable (as yet) to see any of the characteristic clearcut sectoring of the organized endosperm.

Unexpectedly the etched callus exhibits a growth rate that is greater than that of Black Mexican, a very fast grower. This contrasts strongly with the other starchy endosperm types which are growing very poorly--if at all. The relative growth responses by the different endosperms are as follows:

Very poor growth
W22 and W23
Poor to good growth
W8, 4Co63
Good growth
Black Mexican
Best growth
Etched

Inbreds W22 and W23 have a much higher proportion of flint type starch than W8 or 4Co63--these being more floury. It appears that in tissue cultures cells having a shift away from full starch synthesis can divide at a higher rate than those cells committed to full starch production.

Irwin M. Greenblatt
Ann L. Lewis

2. Corn root callus cultures.

For the past nine months we have continuously cultured root callus of different inbred lines employing an unpublished technique originally developed by Nickell (formerly of the Pfizer Co.) and communicated to us by Dr. Phinney of U.C.L.A. The media used is a modified basic White's with 2-4D as a growth stimulating component. (We will gladly supply the exact recipe and procedure upon request by any interested party.)

The cut surfaces of the primary root and/or secondary and adventitious roots serve as donating cells for the callus.

Sub-cultures have been made repeatedly so we now have clumps of cells, mitotically active, which do not contain any differentiated elements from the parent root system.

The growth characteristics of these root calluses are very different from those exhibited by a fast growing tobacco callus (for example). The most striking feature is that under constant conditions of light, heat, and humidity these clumps of cells show very erratic growth phases. We have recorded some pieces suddenly doubling in size in a six day period and then just as suddenly coming to an apparent complete stop for over a month. Some clumps were scored as being dead, left alone, and five weeks later scored as having new growth developing. The growth, which is definitely an increase in cell number, does not occur uniformly over the surface of the callus. Instead, sites of growth develop and it is these cells that continue to grow forming a "knob" of cells extending from the body of the callus.

When the callus goes into a sporadic "dormant" phase it is characteristic to see cell enlargment taking place all along the surface. Such a callus then appears very glossy.

Controlled variations in light and temperature seem not to affect the callus growth. We have been growing these cultures under high continuous light. Sample cultures kept under open room conditions seem not to grow any differently.