

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For the past nine months we have continuously cultured root callus of different inbred lines employing an unpublished technique originally developed by Nickell (formerly of the Pfizer Co.) and communicated to us by Dr.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 enlargement 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.

Raising or lowering the amount of 2-4D in the media is without gross effect except at the null level. Without 2-4D a large number of cultures have been observed to generate small organized roots.

Irwin M. Greenblatt  
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### 3. Gene expression in root tissue cultures.

Like the endosperm callus, the root callus exhibits pigmentation. To summarize our results to date:

| <u>Genotype</u>                               | <u>Callus Phenotype</u> |
|---|-------------------------|
| W22 A C R <sup>r</sup> b pl df                | Red                     |
| W22 A C R <sup>r</sup> b pl Df <sub>cl*</sub> | Colorless               |
| W22 A C R <sup>ch</sup> B Pl                  | Dark purple             |
| W23 A C R <sup>sc</sup> p <sup>WR</sup>       | Colorless               |
| W23 A c r <sup>G</sup> p <sup>RR</sup>        | Colorless               |
| W23 A C R <sup>ch</sup>                       | Purple                  |

\*The state of the Diffuse allele here used is a very stable and strong pigment inhibitor.

Each of these callus phenotypes corresponds to what is seen in the organized root. Note that the red pericarp allele does not produce a detectable effect. R<sup>r</sup>, R<sup>ch</sup>, B, Pl, Df<sub>cl</sub>, all known to affect root color, are similarly active in the callus.

Those cultures scored as colorless are not strictly so. All the cultures have amber color (characteristic of most plant callus). The intensity of this amber coloring increases considerably with aging.

When pigment develops it does so on a cell to cell basis. That is, a cell is either completely pigmented or not at all. The placement of these pigmented cells with respect to each other appears almost at random--given a specific area in the callus. The older the area (in terms of how long ago it stopped dividing) the higher the frequency of pigmented cells.

Pigment from all of the colored cultures diffuses out into the media, sometimes producing a dark ring of pigment surrounding the clumps of callus.

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