

In conclusion, the R-Lc region does not appear to be duplicated in the immediate vicinity of the R locus. The Lc-marked segment and either the P- or the S-marked segment would therefore constitute a direct but displaced duplication, just as the P- and the S-marked segments comprise a direct, tandem duplication.

Hugo K. Dooner

6. Induction and maintenance of maize callus tissue.

Yamada, *et al.* (Proc. Japan Acad. 43: 156, 1967) and Carter, *et al.* (Nature 214: 1029, 1967) demonstrated that callus could readily be induced in rice and wheat by germinating the seeds on a medium which contained greater than 5 mg/l 2,4-D. We have used this method for maize and have obtained nearly 100% success regardless of the strain used.

Maize seeds are surface sterilized by stirring in detergent and 5% Chlorox, rinsed, and soaked overnight in aerated water. The seeds are again sterilized with 5% Chlorox and the embryos with a portion of the scutellum are removed under sterile conditions. These are planted on Murashige and Skoog medium (Physiol. Plant. 15: 473, 1962) containing 10 mg/l IAA, 0.04 mg/l kinetin, 25 mg/l 2,4-D and 10 g/l agar. The cultures are grown under continuous low light at 25° to 30°C. On this medium the primary root of the germinated seedling thickens and grows only several millimeters. The shoot grows a few centimeters and dies. The mesocotyl swells and unorganized callus proliferates from this region. Under the best conditions a cubic centimeter of callus is formed in six weeks. This callus may be divided and subcultured on Murashige and Skoog solid medium without 2, 4-D. Callus induced last March is still growing after being subcultured several times on this medium. Particularly rapid growth of maize callus can be obtained in liquid shake culture. Again, Murashige and Skoog medium is used, but without agar. The cultures are shaken at 120 rpm at 30°C.

We have not obtained complete differentiation of the callus in culture. Under the levels of hormone employed, normal appearing roots are often initiated, but other than occasional green buds, no shoots are formed.

The rate of callus growth, but not its induction, appears to be strain dependent. Single cross hybrids grow faster than inbreds, and some inbreds do better than others. One inbred which has performed well in our hands is A632. Callus has also been induced in putative androgenic haploid seed obtained from Dr. J. L. Kermicle. These are particularly slow growing.

Corn callus from other than endosperm origin has also been reported by others (cited in Masteller and Holden, Pl. Phys. 45: 362, 1970). Dr. Ed Green is also conducting extensive studies on maize tissue in culture at the University of Minnesota.

Ben Burr
Oliver Nelson

7. A recently isolated mutant with an opaque phenotype.

The designation, opaque-6, is assigned to a recently isolated mutant with an opaque phenotype inherited as a Mendelian recessive. The mutant, which has good expressivity, is not allelic to opaque-1, opaque-2, opaque-4, opaque-5, or horny. The homozygous mutant plants die when about 2" tall. The only plants from mutant seeds surviving to maturity are heterozygotes resulting from heterofertilization. When compared to normal maize, there is no change in the amino acid profile of the collective endosperm proteins. The mutation was detected in a popcorn line by R. B. Ashman (his number, ASX 566).

It is my understanding that the mutant (floury-10) reported by McWhirter (MNL 45:184) last year will now be designated opaque-7.

Oliver Nelson

8. The location of lo₂.

The lethal ovule mutant, lo_x, reported in MNL 43: 145 as being 6 map units from wx on chromosome 9, is located distal to wx. The data leading to this conclusion are derived from the following cross:

$$\begin{array}{r} c \text{ sh} + wx \text{ gl}_{15} \\ \hline C \text{ Sh lo Wx Gl}_{15} \end{array} \quad \times \quad \begin{array}{r} c \text{ sh} + wx \text{ gl}_{15} \\ \hline c \text{ sh} + wx \text{ gl}_{15} \end{array}$$