be quite advantageous over the current sterility test methods.

RICHARD J. HICKEY

RESEARCH LABORATORIES,
COMMERCIAL SOLVENTS CORPORATION,
TERRE HAUTE, IND.

A METHOD FOR THE CULTURING OF EXCISED, IMMATURE CORN EMBRYOS IN VITRO[1]

THE culturing of excised, immature plant embryos *in vitro* is a very useful technique for the propagation of otherwise abortive embryos often encountered in hybridization work.[2, 3, 4] A method, adapted from that developed by van Overbeek *et al.*[4, 5] for *Datura*, was found applicable to the culturing of excised corn embryos[6] 8 to 10 days after pollination and 0.3 to 3 mm in length.

The ear of corn was carefully husked, dipped into 70 per cent. ethanol and washed with sterile distilled water. Then the corn kernels were cut from the cob aseptically into a sterile Petri dish. About 6 kernels were held between 2 microscope slides, which had been previously dipped in 70 per cent. ethanol and flamed, and cut with a sterile, thin razor blade. The embryos were lifted from the endosperm by means of sterile, spear-shaped dissecting needles and placed on the surface of the sterile culture medium contained in half-dram shell vials, fitted with cotton plugs. More rapid growth was obtained when the embryos were placed on the surface of the agar medium than when submerged. The vials were then incubated at 30° C.

Unlike *Datura*, 10-day-old proembryos over 0.3 mm in length did not require the addition of coconut milk to the medium described by van Overbeek *et al.*[4] for continued growth. For embryos smaller between 0.3 and 1 mm in length, however, a higher sucrose concentration of 5 per cent. must be used. Otherwise, no growth will result. Excised 10-day-old embryos below 0.25 mm in length did not grow even in the presence of coconut milk. Also, the growth of the embryos, particularly the epicotyl, was accelerated by the addition of 1.5 gm of asparagine per liter of culture medium. Thus, 10-day-old embryos with an initial length of 2 mm grew in the van Overbeek basic medium to a length of 13 mm in 10 days. With the addition of asparagine, comparable embryos in a parallel test grew to 27 mm in the same length of time.

[1] Work supported in part by Grant No. 720 of the American Philosophical Society, to which the authors are indebted.

[2] F. Laibach, *Jour. Hered.*, 20: 200, 1929.

[3] H. B. Tukey, *Proc. Am. Soc. Hort. Sci.*, 32: 313, 1934.

[4] J. van Overbeek, M. E. Conklin and A. F. Blakeslee, *Am. Jour. Bot.*, 29: 472, 1942.

[5] J. van Overbeek, R. Siu and A. J. Haagen-Smit, *Am. Jour. Bot*, 31: 219, 1944.

[6] We are indebted to Drs. J. L. Randolph and R. A. Brink for the suggestion of using corn embryos made at the recent Smith College embryo culture conference.

To give an idea of the rate of growth of corn embryos cultured *in vitro* at 30° C in van Overbeek's basic medium containing 5 per cent. sucrose, plus 1.5 mg asparagine and 0.001 gamma biotin per cc of culture medium, the average growth of 10-day-old corn embryos of different initial lengths is plotted in Fig. 1. Each initial size, with the exception of the largest, is represented by 30 to 60 embryos. The largest group represents the average of eight.

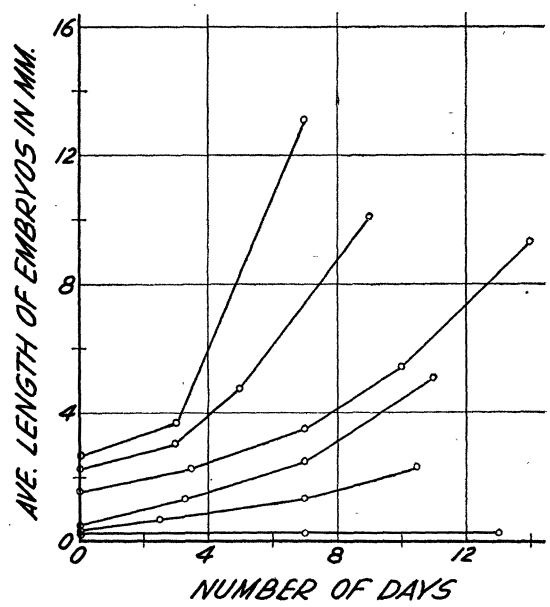

FIG. 1. Growth of excised 10-day-old corn embryos *in vitro*.

From the growth data presented above, it is apparent that the so-called "embryo factors" of coconut milk[4, 5] are not limiting for the survival of the corn embryo. Excised 10-day-old corn embryos above 0.3 mm in length do not require coconut milk for continued growth *in vitro*, while smaller embryos do not survive even with the addition of coconut milk to the medium. It seems likely, therefore, that the growth factors derived from the corn kernel, which are necessary for the growth of the corn embryo, are different from those in coconut milk, which are required by *Datura* proembryos.

A. J. HAAGEN-SMIT
R. SIU
GERTRUDE WILSON

CALIFORNIA INSTITUTE OF TECHNOLOGY

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