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Callus Formation and Differentiation in Tissue Cultures of Normal and Texas Cytoplasmic Male-Sterile Corn[1]

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PEARSON, CHRISTINE B., and HARRY T. HORNER, JR. (Department of Botany and Plant Pathology, Iowa State University, Ames, Iowa 50011). Callus formation and differentiation in tissue cultures of normal and Texas cytoplasmic male-sterile corn. *Proc. Iowa Acad. Sci.* 82(3-4): 163-165, 1976.

Callus cultures were established from 10 lines of *Zea mays* containing cytoplasms with normal and Texas cytoplasmic male-sterile factors. Calli from the different lines varied in their growth characteristics. One callus line was treated with *Helminthosporium maydis* race T toxin extract.

INDEX DESCRIPTORS: Callus Formation, Callus Differentiation.

The establishment of tissue cultures from agronomic plants, and more specifically corn (*Zea mays* L.), has been recently reviewed by Green, Phillips, and Kleese (1974). Sheridan (1972), Burr and Nelson (1972), and Green *et al.* (1974) have successfully initiated and maintained calli from excised embryos of a variety of normal corn lines using 2,4-dichlorophenoxyacetic acid (2,4-D) as the auxin. To our knowledge, there have been no reports to date of comparable studies on cytoplasmic male-sterile lines of corn.

Tissue cultures can provide a large population of similar undifferentiated cells which might be used to study differentiation and regeneration or to monitor host-pathogen responses of particular plant cell lines to determine susceptibility or resistance (Hildebrandt, 1965a, b). In the latter case, normal (N) cytoplasm and Texas cytoplasmic male-sterile (Tcms) corn could be studied by using *Helminthosporium maydis* race T toxin; *H. maydis* race T was the causative agent of the southern corn leaf blight epidemics of 1970-71 in the U.S. (Tatum, 1971; Ullstrup, 1972). Because of these possibilities, our objectives in this study were to establish and maintain callus cultures from both N and Tcms lines of corn, as attempted in an earlier study (Rogers, Gál, and Horner, 1974), to assess the response of the calli to toxin extracts from *H. maydis* race T, and to attempt to differentiate the calli into whole flowering plants.

MATERIALS AND METHODS

Seeds from the following inbred N and Tcms lines of *Zea mays* L. were used: Oh43, Wf9, CI31A, B14, B37, Mo17, A632, A635, A619, and C103.

Initiation and Maintenance of N and Tcms Cultures

Callus cultures were initiated by following procedures modified from Burr and Nelson (1972) and Sheridan (1972). The steps of our procedure were: 1. Corn kernels were surface sterilized for 10 minutes in a solution consisting of 1 g mercuric chloride and one drop of 'Tween 80 detergent in 100 ml water. 2. Kernels were rinsed four times in sterile water and left to soak overnight in the last rinse to soften the kernel for embryo dissection. 3. Embryos were aseptically removed from the kernels with forceps and surface sterilized for seven minutes in a solution of 10 ml Chlorox and one drop of 'Tween 80 detergent in 90 ml water. 4. Whole embryos were rinsed four times in sterile water, placed on potato dextrose agar (Difco) in petri plates, and incubated for two weeks at 28° C in the dark, during which time they expanded and grew, producing roots and elongated shoots. 5. Mesocotyl regions were aseptically excised with forceps and placed on 50 ml of modified Murashige and Skoog (1962) medium (sucrose concentration was lowered to 25 g per liter, agar was reduced to 6 g per liter, and kinetin and 2,4-D levels were adjusted to 1 mg per liter and 10 mg per liter respectively) for initiation of callus. 2,4-D levels of 1, 3, 5, 10, 15, 20, and 25 mg per liter were tested for initiation of callus. 6. For maintenance of calli the 2,4-D concentrations of 0, 1, 2, 3, 5, and 10 mg per liter were tested, and the calli were incubated at 28° C in the dark, with subculturing every 30 days.

Differentiation of N and Tcms Cultures

The modified Murashige and Skoog medium was used, but with the 2,4-D and indoleacetic acid replaced by 0-5 mg α-naphthaleneacetic acid per liter and 0-5 mg kinetin per liter. Differentiating calli were placed in a growth chamber with a 14 hour photoperiod (1,100 footcandles, fluorescent lights) at 27° C during the day and 16° C at night. After two weeks the calli were either subcultured on the same medium or transferred to sterile vermiculite in the greenhouse and watered with Hoagland's solution (Hoagland and Arnon, 1938).

Liquid Cultures

Liquid culturing was attempted by using calli initiated on the solid medium. The modified Murashige and Skoog medium was used without agar and with 0-10 mg 2,4-D per liter. After inoculation with 1/2 cm^3 chunks of calli, cultures were shaken continuously on a 60 cycle per minute reciprocal shaker under fluorescent lights.

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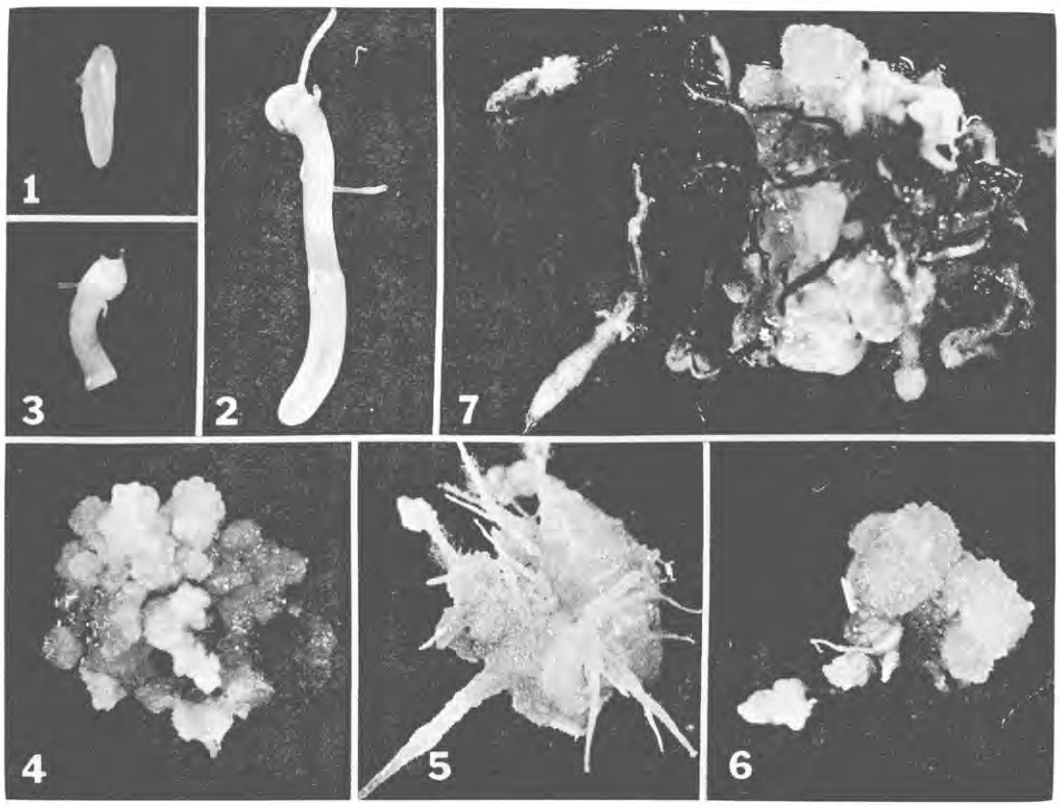

Figures 1-7. Stages depicting initiation, maintenance, and differentiation of B14 Tcms corn callus.
Figure 1. Plucked embryo on potato dextrose agar medium. X2.3.
Figure 2. Enlarged embryo after two weeks on potato dextrose agar medium. X2.4.
Figure 3. Excised mesocotyl region of embryo. X2.4.
Figure 4. Compact round clumps of callus on maintenance medium. X2.4.
Figure 5. Callus with roots and root hairs. X2.2.
Figure 6. Fluffy white callus growth. X2.2.
Figure 7. Callus on differentiating medium shows prolific root growth and a few green stubby protuberances. X2.4.

Toxin Treatment

Healthy calli from N and Tcms B14 corn were placed on the modified Murashige and Skoog medium containing 2 ml per liter *H. maydis* race T toxin extract and incubated as previously described for 30 days. The diluted toxin extract caused a 50 percent reduction in primary root elongation of germinated B37 Tcms seedlings after 24 hours' exposure.

RESULTS AND DISCUSSION

In early experiments, whole embryos or portions of embryos were placed directly on modified Murashige and Skoog medium. Placement of embryos on potato dextrose agar for the first two weeks to allow hydration and some growth promoted consistent and rapid callus formation. Figures 1-3 illustrate the growth of the embryo on potato dextrose agar (Figures 1, 2) and show the mesocotyl region excised for initiation of callus (Figure 3). Callus was obtained from all 10 lines in both the N and Tcms cytoplasms by this procedure.

The callus produced by all lines was yellowish, often grew in compact round clumps (Figure 4), and was friable and easily broken apart by forceps. Frequently, root-like structures complete with root hairs formed (Figure 5), and occasionally fluffy white callus grew (Figure 6). The fluffy white growth indicated rapidly growing callus. Compact yellowish callus occasionally turned brown and died after subculturing.

Genetic variability in callus initiation was examined in N and Tcms lines. Calli of different lines varied in compactness, color, amount of callus formed, proliferation of roots, and the ability to survive subculturing. For example, the callus of Wf9 (both N and Tcms) usually grew in compact balls and was yellowish; the callus of CI31A (both N and Tcms) was lighter in color, fluffier, and produced more roots. The ability of a line (both N and Tcms) to initiate callus was judged on the basis of the proportion of embryo mesocotyl chunks that formed at least 1/2 cm^3 of callus when placed on modified Murashige and Skoog medium. N and Tcms calli of each line were classified together because, in general, they were similar in appearance and in growth characteristics. Callus initiation of each line was classified as good (Oh43, Wf9, CI31A, B14; approximately 40 percent of the mesocotyl chunks formed callus), fair (B37, Mo17, A632, A635,

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A619; 10-30 percent formed callus), or poor (C103; less than 10 percent of the chunks formed callus). Classifications were based on more than 75 embryos from each line. CI31A subcultured poorly and no calli survived beyond three transfers. B14, Oh43, and Wf9 calli were still proliferating after seven or more transfers. Green *et al.* (1974) and Burr and Nelson (1972) found similar variability in the inbred and single-cross corn lines they tested for callus initiation.

Initiation of our calli was best accomplished at 10 mg of 2,4-D per liter where reasonably fast growth of callus occurred. At this concentration much of the root formation prevalent at lower levels of 2,4-D was prevented. Good subculturing of initiated calli occurred at 3 mg of 2,4-D per liter. At this concentration there was the best compromise between undue root formation and inhibition of growth.

Liquid culturing was attempted without success. Callus chunks turned brown and never grew. No explanation for the lack of growth is available because, using similar methods, Burr and Nelson (1972) and Sheridan (1972) obtained rapid growth in liquid shake cultures.

Calli placed on Murashige and Skoog medium modified for differentiation typically formed prolific roots and several green bud-like stubby protuberances (Figure 7), but no leaves or shoots ever formed. Calli with roots and stubby protuberances were maintained through three transfers on differentiating medium without significant change. Transfer of differentiating calli to sterile vermiculite watered with Hoagland's solution resulted in death of the calli within two weeks.

The addition of the toxin extract from *H. maydis* race T to the agar medium caused a reduction in growth and darkening of B14 Tcms calli. This was determined by visual comparison of the latter with Tcms B14 calli on the regular agar medium and N B14 calli treated with the same level of toxin extract. The inconsistent growth of calli made interpretation of toxin-treatment difficult and the plotting of a dosage-response curve impossible. Often as many as half the N and Tcms callus chunks died during each subculturing period for no apparent reason, while the remaining calli flourished.

Further experimentation with different lines, or perhaps other culturing techniques, might be fruitful in establishing more consistent growth of callus that would be amenable to experimentation with *H. maydis* race T toxin.

SUMMARY

Callus cultures wese established from embryo mesocotyl regions of selected lines of *Zea mays* L. containing cytoplasms with normal (N) and Texas cytoplasmic male-sterile (Tcms) factors. Undifferentiated calli and calli with roots and green protuberances were produced from both the N and Tcms lines of Oh43, Wf9, CI31A, B14, B37, Mo17, A632, A635, A619, and C103. Callus growth varied from good to poor among lines but was relatively consistent within the N and Tcms calli of the same line. Tcms B14 calli exposed to *Helminthosporium maydis* race T toxin extract showed less growth and darkening of the calli. Uniform callus growth was difficult to maintain in some of the controls and toxin treatments.

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