A germination procedure for corn seedlings

A procedure has been developed for the germination of large quantities of either etiolated or green corn seedlings. This procedure employs treatment of the seed with the fungicide Captan (N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide), two surface sterilizations in 0.5 sodium hypochlorite and germination in sterile polycarbonate boxes (Econo-cages, Maryland Plastics, Inc., 9 East 37th Street, New York, N.Y. 10016) on 2% bacteriological agar.

Dichloromethane (DCM) was proposed by H. Meyer and A. M. Meyer (1971, Science 171:583-584) as a solvent for introduction of chemicals into dry seed, since treatments for up to 24 hours with this chemical had no effect on germination or oxygen uptake. Seeds (approximately 400 g) were stirred with 2 g Captan per liter DCM for 24 hours. Upon removal, the seeds were allowed to dry thoroughly and were carried through to planting on the same day. Results were satisfactory, however, when they were held in the dried state for a week or more. Captan-treated seeds would germinate as well as seeds treated with DCM alone and slightly better than untreated seeds. In addition, there was far less contamination in the Captan-DCM treated seed.

Each 100-gram batch of Captan-DCM-treated seed was immersed in 200 ml of 0.5% sodium hypochlorite for 15 minutes and rinsed three times with approximately 300 ml per rinse of distilled water. The seeds were then immersed in 300 ml distilled water for 4 hours to induce growth of fungal spores that might have survived the sodium hypochlorite treatment. At the end of this period, the seeds were drained and treated a second time with 200 ml 0.5% sodium hypochlorite for five minutes. This second treatment was followed by three rinses with 200 ml distilled water and holding for 30 to 60 minutes in a final rinse of 200 ml distilled water containing 1% Captan.

Prior to the surface sterilization procedure, 20 grams of agar and 1 liter distilled water were placed in a clean polycarbonate box, which was then covered with aluminum foil, autoclaved at 121 C with 15 psi pressure for 20 minutes, and allowed to cool at least 4 hours prior to use. One 29.2 x 18.4 x 12.7 cm polycarbonate box was prepared for each 100-gram batch of seed.

After surface sterilization, the seeds were drained, spread in a monolayer on the agar, and then covered with approximately 500 ml dry vermiculite. For germination of etiolated seedlings, the polycarbonate boxes were re-covered with aluminum foil and placed in light-proof cardboard freezer boxes. They were subsequently placed in a growth chamber (28-30 C) and harvested 7-9 days after planting. For germination of green seedlings, ethanol-washed polyethylene film may be used to cover the polycarbonate boxes.

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Electrophoresis of shoot mitochondrial proteins from diverse cytoplasms

Six inbred lines (Ky21, Hy2, CI21E, N6, Tr and K55) were backcrossed into five cytoplasms (cms-T, cms-S, cms-C, Wn and 249) to produce a diallel consisting of 36 lines (see Table 1). As shown in Table 1, the six inbreds selected differ in their fertility-restoring capabilities for the cms-T, cms-S and cms-C cytoplasms.

Seedling shoot mitochondria were isolated from each of these lines and analyzed with two electrophoretic techniques. One technique employed the detergent sodium dodecyl sulfate (SDS). Proteins solubilized and electrophoresed in the presence of SDS are separated by differences in their molecular weights. The other technique, isoelectric focusing, allows a separation of proteins by their charge differences. In both techniques, slab acrylamide gels were used to facilitate visual comparisons of the banding patterns that resulted.

Four categories of protein bands were found. The first category included bands that were equally present in all 36 patterns and therefore yielded no genetic information. The second category consisted of bands that varied in staining intensity