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

Table 1. Fertility ratings of Maintainer, sterile and fertile cytoplasms in six inbred backgrounds.

Inbred	Cytoplasm	Backcrosses	Fertility Rating[a]	1975 Rating[b]
Ky21	Maintainer	continuous	-	5
	cms-T	15	4	5
	cms-S	15	4	5
	cms-C	13	4	5
	Wn	>6	-	5
	249	>5	-	5
CI21E	Maintainer	continuous	-	-
	cms-T	14	1	1
	cms-S	5	5	5
	cms-C	11	3	1
	Wn	9	-	5
	249	8	-	5
K55	Maintainer	continuous	-	5
	cms-T	14	5	5
	cms-S	4	1-2	2
	cms-C	11	5	5
	Wn	7	-	5
	249	8	-	5
N6	Maintainer	continuous	-	5
	cms-T	13	1	1
	cms-S	13	1	1-2
	cms-C	13	5	5
	Wn	9	-	5
	249	11	-	5
Hy2	Maintainer	continuous	-	5
	cms-T	>3-(originally	1	1
	cms-S	7 on type)	1-2	1-2
	cms-C	11	1-3	1
	Wn	8	-	5
	249	7	-	5
Tr	Maintainer	continuous	-	5
	cms-T	12	1	1
	cms-S	12	5	5
	cms-C	5	1	2-4
	Wn	9	-	1-4
	249	8	-	5

[a]Fertility Rating (Beckett, J. B., 1971, Crop Sci. 11:724-727): 1-male sterile; 2-sterile anthers exserted; 3-partially fertile; 4-slightly subnormal; 5-fully fertile.

[b]1975 Rating: 1-no anthers; 2-needle-like anthers, no pollen; 3-partially fertile; 4-slightly subnormal; 5-fully fertile.

between different inbred patterns, but not among cytoplasmic sources of the same inbred. This variation was taken as evidence for the involvement of nuclear genes that affect the concentrations of these bands. A third category included bands that were present in one or more, but not all, of the inbred lines. These bands were not found to vary between different cytoplasmic versions of the same inbred line. Therefore, it was concluded that the presence of these bands is determined by nuclear genes. However, none of these category-3 bands could be correlated with

the restoration of fertility for any of the male-sterile cytoplasms and, consequently, do not appear to be the products of nuclear restorer genes. Finally, in a few instances, particular bands were observed to vary in only one cytoplasmic version of one inbred line. These may be the result of specific nuclear-cytoplasmic interactions. Protein bands that consistently varied between the cytoplasmic versions of these inbreds were not observed.

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Electrophoresis of shoot and tassel mitochondrial proteins from diverse cytoplasms

Important considerations for the identification of specific gene action are the tissue and stage of development at which this action would be expected to occur. Rf3, the standard restorer for cms-S cytoplasm, is known to be expressed after the formation of the microspore (Buchert, J. G., Proc. Nat. Acad. Sci. USA 47:1436-1440, 1961). This fact suggests that Rf3 may produce a protein product just after microspore formation that is not present in microspores with rf3.

If mitochondria are the determinants of cytoplasmic male sterility and the Rf3 allele acts to correct this defect, then a consistent difference in mitochondrial isolates from post-meiotic tassels should be detectable between cms-S versions of two inbreds that possess different alleles (Rf3 and rf3) at this locus.

To investigate this hypothesis, mitochondria were isolated from shoot and tassel tissues of maintainer, cms-S, Wn and 249 cytoplasmic versions of K55 (non-restorer of cms-S) and Tr (restorer of cms-S) inbreds. The same two electrophoretic techniques were used to separate the mitochondrial proteins as in the preceding report.

A comparison of the banding patterns from shoot and tassel mitochondria isolated from the same line revealed developmental differences in protein constituents. Protein bands that were present in only shoot mitochondria and, reciprocally, bands that were present in only tassel mitochondria were identified. However, it was apparent that none of these developmental differences were the result of post-meiotic action by the Rf3 gene since a band present in only the tassel patterns of the various cytoplasmic versions of the inbred Tr (restorer of cms-S) was not identified.

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Renaming of B-A translocations

I propose three changes in designation of translocations between B chromosomes and members of the basic set:

1. Call them B-A translocations instead of A-B translocations, since the B is normally listed first (e.g., TB-1a). Listing the A chromosome first (T1-Ba) would tend to obscure the uniqueness of these translocations.

2. Incorporate L or R into the name of each B-A translocation to indicate the A chromosome arm involved. TB-1a would thus become TB-1La; TB-1b would become TB-1Sb. This change should increase convenience in use.

3. Rename the compound translocations of F. A. Rakha and D. S. Robertson (Genetics 65:223) and of Robertson (NL 49:79) to indicate the B-A translocation originally used to develop each. TB-4L, 1L(4692) would thus become TB-1La-4L4692. Since a portion of the long arm of chromosome 1 is uncovered by this compound translocation, it is important to include 1L in the name. And since a portion of 4L is attached to the B1L chromosome, the order should be 1L-4L instead of the reverse. Concurrence of E. H. Coe, Jr., J. L. Kermicle, R. J. Lambert, Bor-yaw Lin, and D. S. Robertson with the changes outlined above has been obtained.

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