and plotted against a standard curve (from purified DIMBOA). Concentrations were expressed as mg DIMBOA/g fresh weight. Seedlings were grown under the same greenhouse conditions to 60-76 cm height for inoculation with H. turcicum. Plants were placed in an inoculation chamber, inoculated and incubated for 18 hrs. at 68°F. and 100% humidity. The degree of infection was determined on the youngest expanding leaf of each plant five days after inoculation. Percent leaf infection was calculated with the use of a transparent grid that estimated area of leaf lesions relative to the total area of the leaf.

Results showed mean concentrations of DIMBOA to range from 0.66 mg to 0.06 mg with the exception of the bxbx genotype which contained a concentration of DIMBOA below the limits of detection by this method. Percent leaf infection varied from 8.24 to 19.04 percent. A significant negative correlation (r = -0.61) was obtained between these two traits indicating that inbred lines of maize with high concentrations of DIMBOA generally have improved resistance to H. turcicum.

We have recently developed a more rapid procedure for analysis of DIMBOA which should be useful in breeding programs.

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1. Water-insoluble protein electrophoresis: a potential tool for maize gene product analysis.

Most of the work that has been reported on genetic variation at the protein level has involved water-soluble proteins, especially isoenzyme systems. There exists, however, in plant and animal cells alike, a large fraction of protein which is water-insoluble. Because of the problems inherent in handling this material and in finding a suitable solvent system that can be maintained throughout separation and analysis, e.g., in a gel electrophoresis system, little work has been done with

these proteins. However, several systems are available for such analysis, and of these I have found one using phenol, acetic acid, urea and water, both as the sample solvent as well as the running solvent in the gel, to be very reliable and repeatable.

Sodium dodecyl sulfate (SDS) has, along with other detergents, come to be widely used as a dissociating and solubilizing agent for water-insoluble proteins. However, applications of this method to plant materials have been quite limited (Hoober, 1970, J. Biol. Chem. 245: 4327-34). The use of phenol, acetic acid and water (2:1:1, w/v/v) as a hydrophobic protein solvent was first suggested by Takayama, MacLennan, Tzagoloff and Stoner (1964, Arch. Biochem. Biophys. 114: 223-30). While this is a good solvent, acrylamide will not polymerize in the presence of phenol, so Takayama, et al. prepared their gels with 5 M urea and 35 per cent acetic acid. Both the SDS and the Takayama systems have proven unreliable with the plant materials used in our laboratory. Cotman and Mahler (1967, Arch. Biochem. Biophys. 120: 384-96) overcame the problem of two different solvent systems in the gel electrophoresis of mammalian neuronal membrane proteins by adding the phenol solvent to the gels after polymerization. I have adapted their technique for the electrophoresis of hydrophobic proteins from several sources in maize: chloroplasts, seeds and pollen.

In each case the sample was prepared so as to remove the water- and lipid-soluble components. Chloroplasts were isolated and disrupted by sonication (Shortess, 1974, J. Exp. Bot., in press), seeds were homogenized in a Sorval Omnimixer, and pollen was ground with an equal weight of sand in a mortar with a pestle. The residue in each case was washed twice in water, once in cold 90% acetone, once in cold 100% acetone and once in diethyl ether. This was allowed to air dry at room temperature and could be stored indefinitely at room temperature without adverse effects. The hydrophobic protein was dissolved in phenol, acetic acid and water (2:1:1, w/v/v) in 3 M urea (PAW/U). Half molar sucrose was added to increase the viscosity, which facilitated sample application to the gel. The chloroplast extract was dissolved at the rate of 7.0 to 10.0 mg/ml, while the seed and pollen extracts were dissolved at the rate of 50 to 100 mg/ml. The latter two extracts contained a great deal more

extraneous material, starch in the case of the seeds and sand in the case of the pollen, which was removed by centrifugation after at least an hour of constant agitation in the solvent.

The gels as polymerized contained 12% acrylamide, 0.3% N,N'-methylenebisacrylamide and 35% acetic acid. The catalysts were ammonium persulfate, 0.064 gm/16 ml of solution and N,N,N',N',-tetramethylethylenediamine 0.08 ml/16 ml of solution. They were cast in glass tubes, 5 x 70 mm I.D. After polymerization under a fluorescent lamp for two hours, the gels were pre-electrophoresed for at least eight hours at from one to two mA/gel. The baths contained 10% acetic acid. Then the gels, still in the glass casting tubes, were placed in PAW/U and allowed to equilibrate by diffusion. The solvent was changed after two, 24 and 48 hours, and the gels were ready for use after 72 hours from the start of the equilibration, being constantly agitated during the whole period. The equilibration was carried out at 20°C, since higher temperature causes bubbles to form between the gel and the tube.

From 50 to 100 ml of sample were placed on the gel. This was over-layered with PAW/U without sucrose. The gels were run at 3.0 mA/gel for 75 minutes, with 10% acetic acid in both baths. The upper electrode was the anode. After electrophoresis the gels were removed from their tubes and stained in 0.5% amido-Schwartz black stain, and destained electrophoretically. All gels were stored in 10% acetic acid with no detectable loss of banding pattern after a year.

All of the materials analyzed produced from ten to 20 separate, distinguishable bands. When seeds from 20 inbred maize lines were examined, a great deal of variation, presumed to be genetic, was observed among the water-insoluble proteins of the seeds (Shortess, 1973, Genetics 74: s253). Some lines showed a high degree of homogeneity while others revealed single band variants within the line. Variations have also been observed among the chloroplasts and pollen from various lines, but to a much lesser extent.

The exact function of these proteins is yet to be determined. I have some evidence to support the hypothesis that at least some of those hydrophobic proteins found in the seed, almost all of which are found in the embryo, simply serve a storage function, to be used by the seed

during germination. If this is the case, then this would account for the considerable variation among these proteins without any overt phenotypic variation. If they are merely storage molecules, they would serve only to provide amino acids for the seedling prior to the initiation of photosynthesis, a very nonspecific function. As far as the pollen and chloroplasts are concerned, one may also presume that at least some of these proteins are membrane components. There is no evidence to support any specific function.

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1. Comparison of mitochondrial DNA from hybrids with normal and Texas cytoplasms.

Texas cytoplasm (cms T) contains factors for male sterility and susceptibility to two leaf diseases, southern leaf blight, Helminthosporium maydis and yellow leaf blight, Phyllosticta zeae. These traits are known to be inherited in an extra chromosomal fashion. A recent study (1) has found a difference in the response of mitochondria from corn with normal and sterile cytoplasm when challenged by a pathotoxin from H. maydis race T. Since this study indicated the involvement of mitochondria, mito-chondrial DNA (mt DNA) must be given consideration as a possible site of factors responsible for the traits associated with cms T. Indeed, mt DNA from normal and T cytoplasms may be speculated to differ in base composi-tion by virtue of significant alterations in the mt DNA of the Texas cyto-plasm. In this connection, the mt DNA from normal and Texas cytoplasm has been isolated and characterized with respect to buoyant density and molar percent guanine and cytosine (molar % GC).

Mitochondrial DNA was isolated from two hybrids, NC232 x T204N (normal cytoplasm) and NC232 x T204 cms T. Roots and coleoptiles from 7-10 day old etiolated maize seedlings and leaves from 2-3 month old plants served as sources of mitochondria. Plant materials were ground in