Response of Mitochondria from Corn Cytoplasms to the Pathotoxin of Helminthosporium maydis Race T

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ABSTRACT

The susceptibility of 26 corn cytoplasms to Race T of *Helminthosporium maydis*, and the response of mitochondria from these cytoplasms to the pathotoxin produced by the fungus was determined. Field tests showed that cytoplasms T, HA, and Q were susceptible to *H. maydis* and laboratory tests showed that mitochondria from these same three cytoplasms were susceptible to respiratory control ratio (RCR) decline in the presence of the pathotoxin. This indicates that the mitochondria are a likely site of pathotoxin effect and that cytoplasms with mitochondria susceptible to the pathotoxin will be susceptible to *H. maydis*.

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Additional key words: southern corn leaf blight, male-sterility.

During 1970, southern corn leaf blight developed in near-epidemic proportions which seriously reduced corn *(Zea mays* L.) yields. The causal organism was identified as Race T of *Helminthosporium maydis* Nisikado and Miyake. Plants having the Texas (T) source of male-sterile cytoplasm (Tcms) were susceptible to the disease whereas plants with normal (N) cytoplasms were resistant, thus indicating that some cytoplasmic component determined resistance or susceptibility. Miller and Koeppe (5) showed that the oxidative phosphorylation and swelling-contraction functions of mitochondria from Tcms corn were disrupted by the pathotoxin of *H. maydis,* whereas mitochondria from normal cytoplasm corn were not affected. We investigated the pathotoxin response of mitochondria of other cytoplasms and compared these to field reactions to *H. maydis*. The results are presented here.

MATERIALS AND METHODS.—Inbred lines of corn with different male-sterile (cms) and normal (N) cytoplasms (Table 1) were planted in 25-plant rows in two replications. Except as noted, all cytoplasms were in inbred Ky 21. All plants were inoculated in the leaf whorl about 1 mo after planting with ground diseased leaves collected in 1970. Although evidence of flecking developed in a few days, the plants were inoculated a second time 9 days later. Overhead irrigation was provided to aid in disease development.

Ratings for leaf blight were made when maximum disease had developed, 18 and 52 days after the first inoculation. Ratings were made on a scale of 0 (no evidence of infection), to 5 (abundant lesions on all leaves and the plants prematurely killed).

Seeds having the different cytoplasms were germinated in the dark at 30 C in shallow baking pans on paper towels saturated with 10^{-4} M $CaCl_2$. Roots and shoots from 4-day-old seedlings were collected and rinsed with cold deionized water. All subsequent steps of mitochondria isolation were carried out in an ice bucket or refrigerated centrifuge using a procedure similar to that of Miller et al. (4). The plant tissue was homogenized with a mortar and

pestle in 80 ml of 0.4 M sucrose - 50 mM potassium phosphate buffer, pH 7.5 - 5 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was strained through nylon mesh and cell debris was cleared by centrifuging at 1,000 *g* for 5 min. The mitochondria were collected by centrifuging at 10,000 *g* for 10 min. The mitochondrial pellet was dispersed in 30 ml 0.5 M sucrose - 0.5 mM EDTA and 10 ml 0.6 M sucrose was carefully layered on the bottom of the tube. The mitochondria were collected at 9,500 *g* for 15 min and the supernatant aspirated. Mitochondria were suspended in 1.0 ml 0.5 M sucrose - 5 mM citrate, pH 7.5 for the respiration experiments.

Respiration was determined polarographically with a Clark oxygen electrode (Yellow Springs Instrument Company). Respiratory control ratios were determined by dividing the state-III respiration rate by the immediately following state-IV respiration rate. The respiration medium contained in 2.0 ml volume: 40 μmoles potassium phosphate buffer pH 7.5, 240 μmoles sucrose, 32 μmoles α-ketoglutarate, 0.2 μmoles $MgSO_4$, 320 μg NAD, 160 μg TPP, 80 μg coenzyme A, 4 mg bovine serum albumin, and 290 μg to 1,308 μg protein mitochondria. After one determination of respiratory control (addition of 0.4 μmoles ADP) either 40 or 50 μliters of pathotoxin were added to the reaction vessel, followed by one or more additions of 0.4 μmoles ADP. The respiratory control ratios were determined three or more times with the same preparation of mitochondria. Three or more isolations were made from each of the N and T cytoplasms of inbreds T222 and Ky21.

The pathotoxin was obtained from shake cultures of *Helminthosporium maydis* Race T growing on synthetic medium described by Hooker, et al. (1). Protein was determined by the method of Lowry et al. (3).

RESULTS AND DISCUSSION.—The leaf blight severity index ratings on two dates have been reported (2). These data, along with the respiratory control ratios determined in the presence and absence of the pathotoxin, are shown in Table 1. Cytoplasms T, HA, and Q were the only cytoplasms rated above 2.0 in the earlier leaf blight severity rating. The same three cytoplasms were the only ones rating above 3.0, and all were rated 5.0, in the later rating.

Respiratory control ratios determined for the various cytoplasms showed that only the three susceptible cytoplasms had RCR declines greater than 0.2 when pathotoxin was added to the respiration medium. The mitochondria from the susceptible mitochondria all had RCR declines of 0.6 or greater.

Selected traces from the different cytoplasm types are shown in Fig. 1. Results with N and T cytoplasms in inbred T222 confirm the report by Miller and Koeppe (5) that RCR declines in the presence of pathotoxin in T but not in N. Analysis of variance showed that treatments were significantly different at the 0.01 confidence level. Separation of the means using Duncan's multiple range test showed the only difference to be the decline in RCR in mitochondria from the T cytoplasm in the presence of toxin. Mitochondria from T cytoplasm in inbred Ky 21 also demonstrated declines in RCR in the presence of pathotoxin. Likewise HA and Q, the other cytoplasms susceptible in field tests, maintained respiratory control in the absence of pathotoxin but RCR declined rapidly when pathotoxin was introduced into the reaction medium.

Loss of respiratory control may be due to a decrease in respiration rate in the presence of a phosphate acceptor (state III) or to an increase in the respiration rate in the absence of an acceptor (state IV). Our data would indicate that both factors are operative when pathotoxin is present in the reaction medium of mitochondria from susceptible cytoplasms; that is, the state III rate is reduced and the state IV rate is increased. This is slightly different from the results of Miller and Koeppe. They used three different substrates and found different reactions to each. Perhaps these differences resulted from changes not directly related to the function being measured, as they suggested.

The close agreement found between the field tests for susceptibility of cytoplasms to *H. maydis* and the suppression of respiratory control of the mitochondria in the laboratory indicates that either measurement could be used to predict the other. That all susceptible cytoplasms contained mitochondria affected by the pathotoxin and conversely, that all resistant cytoplasms either were

TABLE 1. Reaction to southern corn leaf blight and respiratory control ratios of mitochondria from corn inbreds with different cytoplasms determined before and after the addition of *Helminthosporium maydis* race T toxin

	Leaf blight severity index		Respiratory control ratio	
Cytoplasms[a]	Rated July 9	Rated August 12	Without toxin	With toxin
C	1.0	2.5	1.6	1.6
CA	2.0	2.0	1.6	1.5
D	1.0	2.0	1.7	1.6
EK	1.0	2.0	1.6	1.8
EP	1.5	2.0	1.7	1.8
G	2.0	3.0	1.5	1.5
HA	4.0	5.0	1.8	1.2
I	1.0	2.0	1.8	1.8
IA	2.0	2.5	1.7	1.5
J	1.5	2.0	1.6	1.5
J (WF9)	1.8	3.0	1.7	1.8
K	2.0	2.5	1.5	1.6
M	2.0	2.5	1.4	1.4
ME	1.5	2.0	1.8	1.9
ML	2.0	2.5	1.4	1.4
MY	1.5	2.0	1.8	1.6
R	2.0	2.5	1.6	1.6
RB	2.0	2.5	1.5	1.6
S	1.5	2.5	1.6	1.6
SD	1.5	2.5	1.6	1.6
T	4.5	5.0	1.7	1.1
TA	1.0	2.0	1.5	1.4
VG	1.5	2.5	1.5	1.6
W	2.0	3.0	1.6	1.6
Q	4.0	5.0	1.8	1.1
Chapalote	1.5	2.0	1.7	1.7
N	1.4	2.4	1.7	1.8
N (T222)	2.5	3.0	1.7	1.8
T (T222)	5.0	5.0	1.9	1.2

[a]The cytoplasms were in the inbred line Ky 21, except where indicated in parentheses.

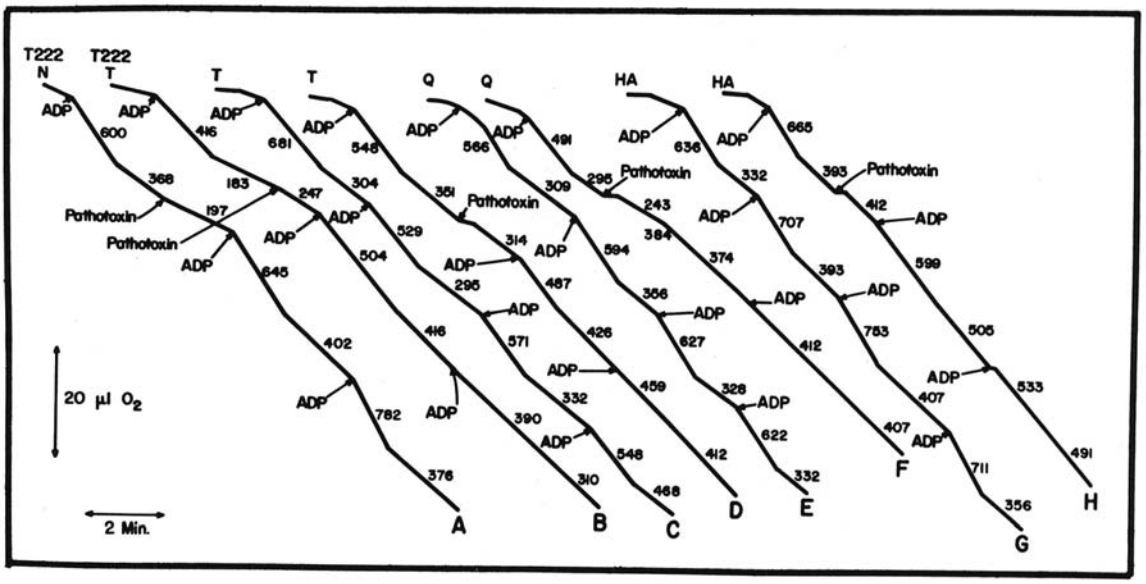

Fig. 1. Time-rates of oxygen utilization by isolated mitochondria with male-sterile or normal cytoplasms graphically plotted to illustrate the effects of 50 µliters of *Helminthosporium maydis* race T pathotoxin or 0.4 µmoles of adenosine diphosphate. Curves A and B illustrate the difference in response of mitochondria from N and T cytoplasms to pathotoxin. Curves C through H indicate the effects of pathotoxin to mitochondria from susceptible male sterile cytoplasms. The numbers are the respiratory rates in µliters oxygen consumed per h.

unaffected or only marginally affected by the pathotoxin, indicates an important difference in the mitochondria of resistant and susceptible cytoplasms.

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