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Influence of *Helminthosporium maydis*, Race T, Toxin on Potassium Uptake in Maize Roots

II. SENSITIVITY OF DEVELOPMENT OF THE AUGMENTED UPTAKE POTENTIAL TO TOXIN AND INHIBITORS OF PROTEIN SYNTHESIS[1]

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ABSTRACT

Basal K^+ uptake in the root midzone region (cm 2 + 3 + 4) of N and T cytoplasmic versions of each of four maize inbreds was equally sensitive to the toxin(s) of *Helminthosporium maydis*, race T. Basal K^+ uptake in the root apex (0–1 cm) and augmented K^+ uptake in the root midzone were more toxin-sensitive in inbreds W64A(T) and Mo17(T) than in inbreds W64A(N) and Mo17(N). This differential response of N and T cytoplasms to toxins was not found for corresponding cytoplasms of inbreds WF9 and B37.

Development of the augmented K^+ uptake rate in midzone segments of W64A(T) was blocked by a toxin concentration which did not affect augmentation development in W64A(N). Augmentation development was more toxin-sensitive in T than in N cytoplasmic versions of all inbreds tested. Fertility-restoring nuclear loci decreased but did not eliminate the toxin sensitivity of augmentation development as observed in root midzones of inbred A619(T). Chloramphenicol- and/or cycloheximide-sensitive protein synthesis was required for augmentation development, but not for expression of either basal or augmented K^+ uptake.

Texas cytoplasmic male-sterile (T) maize (*Zea mays* L.) is more susceptible to *Helminthosporium maydis* (Nisikado and Miyake), race T, than corresponding normal (N) cytoplasm genotypes (3, 8, 10, 14). With the exception of the leaf chlorosis bioassay (3, 9, 11, 15), the effects of the toxin(s) of *H. maydis*, race T, are not restricted to T cytoplasm. Thus, a qualitative distinction between plants with N and T cytoplasms is rarely observed since N plants are sensitive at high toxin concentrations (4, 6, 11, 16).

A complicated interaction exists between toxin and root in inbred W64A with respect to K^+ uptake (4). K^+ absorption rates vary along the root, and these rates are correlated with the toxin sensitivity of both basal and augmented uptake rates. Basal rates of K^+ uptake are measured in freshly excised root segments, whereas augmented rates are measured after aeration of the segments in water for 90 min. Basal and augmented K^+ uptake rates increase with increasing distance from the apex through 6 cm from the apex (4). Basal K^+ uptake in T versions is more toxin-sensitive than in N versions only in the apical root region of inbred W64A; in more mature regions (cm segments 2 + 3 + 4 from the apex), basal uptake is equally toxin-sensitive in N and T. Augmented K^+ uptake in these more mature regions of N and T roots is more toxin-sensitive than basal K^+ uptake, and the augmented increment in K^+ uptake rate is inhibited 20 to 25% by toxin in N and 70 to 80% in T. Thus, despite the likelihood that the mechanism of K^+ uptake is in all cases the same, differences exist in toxin sensitivity of K^+ uptake between basal and augmented rates for the same root region, N and T versions for the same root region, and apex and midzone regions of the same root (4).

The present report distinguishes the expression of basal and augmented K^+ uptake from the phenomenon of augmentation development using several maize inbreds. Only augmentation development in excised root segments of T versions of several inbreds is consistently more toxin-sensitive than in the corresponding N versions. In addition, toxin sensitivity is only partially decreased in T by restoration of fertility. Augmentation development in W64A is also sensitive to CAP[5] and CYC.

MATERIALS AND METHODS

N and T cytoplasmic versions of *Zea mays* L. inbreds W64A, Mo17, B37, and WF9 were used. Inbred A619 with N, T, or restored T(T_{Rf}) cytoplasm was provided by Dr. M. T. Turner, Funk Seeds International. Excised 1-cm root segments of 6- to 7-day-old seedlings grown above aerated 1 mM $CaSO_4$ were labeled with ^{86}Rb for 15 min in 2 mM KCl and 1 mM $CaSO_4$, and washed for 20 min in cold 1 mM $CaSO_4$ as described (4). K^+ uptake during short term exposure of low salt roots was estimated by ^{86}Rb uptake (4). Basal rates of K^+(^{86}Rb) uptake were measured in freshly excised root segments, whereas augmented rates were measured after aeration of the freshly cut segments in water or 1 mM tris-MES buffer at pH 6, for 90 min. The toxin sensitivity of expression of augmented K^+ uptake was assayed by labeling the aerated segments with ^{86}Rb in the presence of toxin. The toxin sensitivity of augmentation development was assayed by including the toxin during aeration in water but not during subsequent ^{86}Rb labeling.

The toxin extract of *H. maydis*, race T, was isolated as described (4), and exhibited qualitative selectivity between N and T cytoplasms of each inbred in the leaf chlorosis bioassay. A 1:1000 dilution of toxin stock (called 0.1%) caused a 65%

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[5] Abbreviations: CAP: *d*-threo-chloramphenicol; CYC: cycloheximide.

reduction in basal K^+ uptake in midzone segments (cm segments 2 + 3+ 4) of the roots of W64A(N) and W64A(T).

RESULTS

The toxin (0.05%) sensitivity of basal K^+ uptake in the tip and midzone regions of four different inbreds having N or T cytoplasm was examined (Table I). In two inbreds, W64A and Mo17, basal K^+ uptake in the tip was more sensitive in T than in N root segments. In WF9 and B37, basal K^+ uptake in the tip in N and T root segments was approximately equal in sensitivity.

In the midzone region (cm segments 2 + 3 + 4), basal K^+ uptake in N was as toxin-sensitive as in T in all inbreds with the exception of inbred WF9. Thus, differential toxin sensitivity of basal K^+ uptake in the apical as well as midzone regions is inbred-dependent and not cytoplasm-dependent.

Basal K^+ uptake in W64A, N and T, was equally toxin-sensitive over a range of toxin concentrations (Fig. 1a). Augmentation development in W64A(T) was prevented by 0.05% toxin but was not affected in W64A(N) (Fig. 1b). Therefore, 0.05% toxin was used to challenge augmentation development and expression of K^+ uptake in midzone root segments of both N and T versions of the four inbreds (Table II). Expression of augmented K^+ uptake was slightly more sensitive in W64A(T) and Mo17(T) than in W64A(N) and Mo17(N), but this difference was not observed for inbreds B37 and WF9. In WF9, expression of augmented K^+ uptake was actually more toxin-sensitive in N than in T.

Augmentation development was more toxin-sensitive in T than in N of each inbred (Table II). Toxin had no effect on the development of the augmented K^+ uptake rate in W64A(N) or Mo17(N), but it did inhibit augmentation development in B37(N) and WF9(N) by 50 to 60%. The only assay which consistently showed the T versions to be more toxin-sensitive than the N versions was the development of the augmented K^+ uptake potential.

Male-fertility in T cytoplasm plants can be restored (T_{Rf}) by nuclear genes. Several T_{Rf} inbreds showed a leaf response to toxin intermediate to that of N and T plants, and toxin-induced swelling of isolated mitochondria of T_{Rf} plants was also intermediate to that of N and T mitochondria (17). Restored T plants showed intermediate toxin sensitivity of mitochondrial enzymic activities (2). The toxin sensitivity of augmentation development

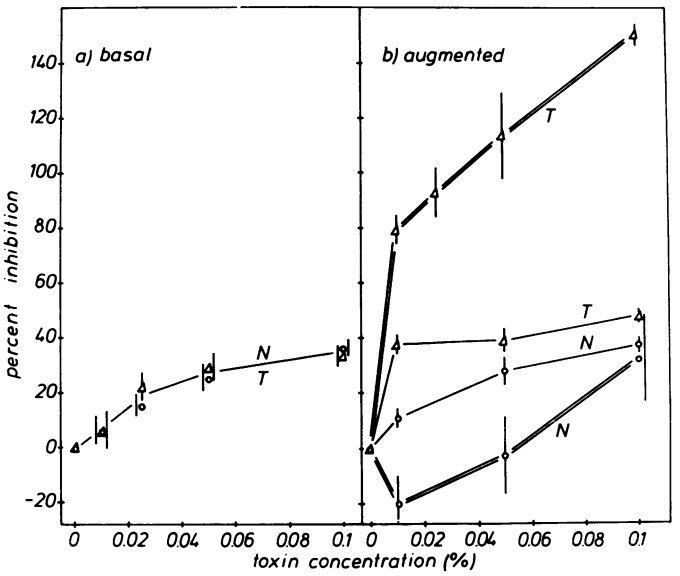

FIG. 1. Toxin inhibition of basal and augmented K^+ uptake, and of augmentation development, in midzone root segments of inbred W64A. 0: N cytoplasm, Δ T cytoplasm; a: basal K^+ uptake; b: augmented K^+ uptake; (——): inhibition of expression of uptake rate (toxin only present during ^{86}Rb labeling); (═══): inhibition of development of the augmented uptake rate (toxin only present during aeration of root segments). Standard error of the mean is shown at each point and displaced for visual clarity.

TABLE I. Toxin sensitivities of basal K^+ uptake in tip and midzone root segments of four maize inbreds.[1]

			N		T	
inbred	root region	treatment	nmoles K^+ g fw·15 min	% inhib.	nmoles K^+ g fw·15 min	% inhib.
W64A	tip	basal	278 ± 13		235 ± 7	
		basal + toxin	255 ± 14	8 ± 5	181 ± 10	23 ± 4
	midzone	basal	394 ± 12		352 ± 11	
		basal + toxin	300 ± 21	24 ± 6	254 ± 18	28 ± 5
Mo17	tip	basal	226 ± 6		262 ± 4	
		basal + toxin	190 ± 12	16 ± 5	186 ± 9	29 ± 3
	midzone	basal	138 ± 2		148 ± 8	
		basal + toxin	119 ± 6	14 ± 5	123 ± 3	17 ± 2
B37	tip	basal	287 ± 2		285 ± 8	
		basal + toxin	213 ± 1	26 ± 1	223 ± 14	22 ± 5
	midzone	basal	360 ± 39		358 ± 8	
		basal + toxin	216 ± 13	40 ± 4	200 ± 34	44 ± 9
WF9	tip	basal	585 ± 35		330 ± 5	
		basal + toxin	351 ± 33	40 ± 6	218 ± 3	34 ± 1
	midzone	basal	280 ± 14		224 ± 14	
		basal + toxin	238 ± 16	15 ± 6	161 ± 11	28 ± 5

[1] tip = apical 1 cm including terminal 5 mm, and midzone = cm 2 + 3 + 4 of 7 day seedlings, except for inbred W64A in which the tip was cm 1 + 2; 0.05% toxin used throughout. Values are presented as means ± S.E. for more than three replicates.

TABLE II. Toxin sensitivities of augmentation expression and development in midzone root segments.

		N		T	
		nmoles K^+	%	nmoles K^+	%
inbred	treatment[1]	g fw·15 min	inhib.	g fw·15 min	inhib.
W64A	basal	394 ± 12		352 ± 11	
	augmented	600 ± 30		660 ± 19	
	augmented, then toxin[2]	433 ± 31	28 ± 5	410 ± 20	38 ± 3
	augmented with toxin[3]	606 ± 35	-3 ± 14	318 ± 47	116 ± 24
Mo17	basal	138 ± 2		148 ± 8	
	augmented	266 ± 9		324 ± 35	
	augmented, then toxin	162 ± 7	39 ± 3	165 ± 8	49 ± 2
	augmented with toxin	271 ± 6	-4 ± 4	247 ± 15	44 ± 9
B37	basal	360 ± 39		358 ± 8	
	augmented	461 ± 26		457 ± 19	
	augmented, then toxin	286 ± 19	38 ± 4	264 ± 15	42 ± 3
	augmented with toxin	402 ± 16	58 ± 16	317 ± 19	141 ± 20
WF9	basal	280 ± 14		224 ± 14	
	augmented	470 ± 29		428 ± 18	
	augmented, then toxin	301 ± 21	36 ± 5	337 ± 19	21 ± 5
	augmented with toxin	370 ± 22	53 ± 12	251 ± 22	87 ± 11

[1] midzone root segments = cm 2 + 3 + 4 of 7 day seedlings; 0.05% toxin used throughout. Values presented as means ± S.E. for more than three replicates.

[2] toxin only present during ^{86}Rb labelling; this measures effect on expression of augmented uptake. Inhibition calculated with reference to augmented rate, as, for W64A(N): [(600-433)/600]·100.

[3] toxin only present during 90 min aeration in water; this measures effect on augmentation development. Inhibition calculated on the augmentation increment or increase above basal rate, as for W64A(N):

$$\frac{(600 - 394) - (606 - 394)}{(600 - 394)} \cdot 100.$$

TABLE III. Effect of toxin on augmentation development in N, T_{Rf}, and T versions of maize inbred A619.[1]

	N		T_{Rf}		T	
	nmoles K^+	%	nmoles K^+	%	nmoles K^+	%
treatment	g fw·15 min	inhib.	g fw·15 min	inhib.	g fw·15 min	inhib.
basal	140 ± 7		152 ± 11		129 ± 4	
augmented	206 ± 12		255 ± 16		222 ± 6	
augmented with toxin	192 ± 6	21 ± 9	223 ± 12	31 ± 12	178 ± 2	47 ± 2

[1] midzone root segments = cm 2 + 3 + 4 of 7 day seedlings; 0.1% toxin. Values are presented as means ± S.E. of 5 or more replicates.

in root midzones of inbred A619(T_{Rf}) was between that of A619(N) and A619(T) (Table III).

Protein synthesis is required for development of augmented K^+ uptake rates, but not for the expression of augmentation or the expression of basal K^+ uptake (Table IV). Under conditions where neither CAP (100 μg/ml) nor CYC (10 μg/ml) impaired expression of basal or augmented K^+ uptake in W64A root midzone segments, each inhibitor partially prevented augmentation development. CYC was more effective than CAP.

DISCUSSION

The means by which *H. maydis*, race T, toxin(s) selectively increase fungal virulence in T cytoplasm plants is not known. Because evidence suggests that membrane-associated functions are sensitive to the toxin (1, 5, 7, 12), we chose to study how toxin affects K^+ absorption in the root (4).

Basal and augmented K^+ uptake in the midzone region of roots having T cytoplasm were not consistently more toxin-sensitive than in roots having N cytoplasm. In fact, in certain inbreds, K^+ uptake in N cytoplasm roots was as toxin-sensitive as in T cytoplasm roots. The development of augmented K^+ uptake rates, however, was consistently more toxin-sensitive in roots having T than in those having N cytoplasm. It was also more sensitive in plants with T cytoplasm which were male-fertile (T_{Rf}) (Table III). Therefore, toxin sensitivity of T plants is not associated exclusively with male-sterility.

Augmentation development requires protein synthesis in both

TABLE IV. Effects of protein synthesis inhibitors and toxin on K^+ uptake in midzone root segments of inbred W64A.

	N		T	
treatment[1]	nmoles K^+ g fw·15 min	% inhib.	nmoles K^+ g fw·15 min	% inhib.
basal	394 ± 12		352 ± 10	
basal + toxin	300 ± 21	24 ± 6	254 ± 18	28 ± 5
basal + CAP	377 ± 20	4 ± 4	373 ± 19	-6 ± 4
basal + CYC	392 ± 22	0.5 ± 5	385 ± 20	-8 ± 6
augmented	600 ± 30		660 ± 19	
augmented, then toxin[2]	433 ± 31	28 ± 5	410 ± 20	38 ± 3
augmented, then CAP	670 ± 46	-10 ± 6	632 ± 35	4 ± 5
augmented, then CYC	622 ± 41	-4 ± 5	604 ± 43	8 ± 6
augmented with toxin[3]	606 ± 35	-3 ± 14	318 ± 47	116 ± 24
augmented with CAP	558 ± 36	20 ± 17	569 ± 35	30 ± 12
augmented with CYC	466 ± 24	65 ± 8	446 ± 24	69 ± 7

[1] midzone root segments = cm 2 + 3 + 4 of 7 day seedlings; 0.05% toxin, 100 μg/ml CAP, and 10 μg/ml CYC used throughout. Values presented as means ± S.E. for more than seven replications.

[2] inhibitor only present during ^{86}Rb labelling; this measures effect on expression of augmented uptake. Inhibition calculated with reference to augmented rate, as, for CAP on W64A(N): $[(600 - 670)/600] \cdot 100$.

[3] inhibitor only present during 90 min aeration in water or buffer at pH 6.0; this measures effect on augmentation development. Inhibition calculated on the augmentation increment or increase above basal rate, as, for CYC on W64A(N): $\frac{(600 - 394) - (466 - 394)}{(600 - 394)} \cdot 100$.

N and T plants. The ability of both CAP and CYC to inhibit augmentation development, but not the expression of either basal or augmented K^+ uptake (Table IV), suggests that protein synthesis utilizing both 70S (organellar) and 80S (cytoplasmic) ribosomal systems contributes to the augmentation phenomenon. The contribution of both 70S and 80S protein-synthesizing systems to augmentation development is also consistent with the observation that the toxin sensitivity of inbred A619(T_{Rf}) was intermediate to that of A619(T) and A619(N). This implies that both nuclear and cytoplasmic genetic elements contribute to augmentation development and is in accord with the observation that T cytoplasm inbreds vary widely in susceptibility to fungal infection and sensitivity to toxin (6, 10, 16, 17), which has been ascribed to nuclear genetic contribution in certain cases (10, 15).

We suggest that toxin sensitivity of maize inbreds having T cytoplasm is not a consequence simply of functional deficiencies of a given organelle, for instance, the mitochondrion (2, 5, 13), but represents the disturbance of a complex interaction between nucleus, cytoplasm, and plasma membrane. We measured this disturbance with respect to augmentation development of K^+ uptake in the root, which is selectively toxin-sensitive in T cytoplasm plants, and found that the involvement of both nuclear- and organelle-directed protein syntheses is implied.

The development of augmented K^+ uptake rates in maize roots provides an experimental system capable of describing a number of the events associated with toxin sensitivity. Both male-fertile and male-sterile plants are capable of augmentation development, which appears to result from a metabolic interaction of nucleus, cytoplasm, and plasma membrane. This interaction is disrupted more readily by toxin in the roots of T than in those of N cytoplasm plants.

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