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Influence of *Helminthosporium maydis*, Race T, Toxin on Potassium Uptake in Maize Roots[1,2]

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ABSTRACT

The effect of a toxin extract of *Helminthosporium maydis*, race T on K^+ (^{86}Rb) uptake by excised root segments of normal (*N*) and Texas cytoplasmic male-sterile (*T*) versions of corn inbred W64A was investigated. The uptake of K^+ was inhibited in both *N* and *T* roots by the toxin. This was true for both basal (freshly excised) and augmented (pretreated with aeration) K^+ uptake. Augmented uptake was more toxin-sensitive than basal uptake (irrespective of cytoplasm type), and the augmented uptake in *T* roots was seven to eight times more toxin-sensitive than in *N* roots.

Specific zones of roots differed in their basal and augmented K^+ uptake rates as well as their toxin sensitivities. The root apex of *T* was more sensitive to toxin than the apex of *N* roots when basal K^+ uptake was measured. In mature zones of the root, *T* was more sensitive than *N* when augmented rates were measured. During the development of the augmented K^+ uptake capacity in either *N* or *T* roots, the sensitivity to the toxin did not change; uptake in *N* roots was inhibited by 10 to 25% and uptake in *T* roots was inhibited by 70 to 80%.

The difference in toxin sensitivity of K^+ uptake between *N* and *T* roots may be due to *N* possessing a protective mechanism which is deficient in *T*.

The extreme susceptibility of Texas cytoplasmic male-sterile (*T*) maize to Southern Corn Leaf Blight is cytoplasmically conditioned and inherited in association with the non-Mendelian determinant of male sterility (12, 18). *T* versions of a given maize inbred are more susceptible to *Helminthosporium maydis*, race T than normal cytoplasmic (*N*) versions (7, 10). The fungal pathogen synthesizes a toxin complex which accounts for this difference in levels of susceptibility of the host cytoplasmic types (7, 8). Leaf chlorosis caused by the fungus can be duplicated by toxin preparations from infected leaves or culture filtrates of the fungus (14, 24) The basis for the differential toxin sensitivity between *N* and *T* plants is the objective of our work.

Maize plants with *T* cytoplasm have been shown to be more toxin-sensitive than *N* plants by criteria of leaf chlorosis (14, 27), root elongation (3, 13, 20), electrolyte leakage (3, 8, 9, 22), and swelling (5, 6, 15), phosphorylation (21), and enzyme activities (23) of mitochondria. Stomatal closure (2), root uptake of ^{86}Rb, membrane potentials of root cells (2, 6), and microsomal ATPase activity (25) have also been reported to be more toxin-sensitive in *T* plants. The differences between *N* and *T* plants may therefore be the result of differences between membrane systems. We have focussed attention on the effect of a toxin extract of *H. maydis*, race T on K^+ uptake in excised roots of *N* and *T* cytoplasmic versions of the maize inbred W64A.

MATERIALS AND METHODS

Kernels of maize (*Zea mays* L.) inbreds W64A, B37, and WF9, having either *N* or *T* cytoplasm, were surface-sterilized and germinated on stainless steel mesh above aerated 1 mM $CaSO_4$ (11). Excised roots of 6- to 7-day seedlings were cut into 1-cm segments, excluding the terminal 5 mm. In the following experiments, either randomized segments of the apical 6 cm, specific root zones (ordered segments) of the apical 10 cm, or cm-segments 2 + 3 + 4 (midzone) were used. Root segments were vigorously aerated for 15 min (absorption was linear over this period) at room temperature in 5-ml, volumes of ^{86}Rb solution containing 2 mM KCl and 1 mM $CaSO_4$. For the experiments shown in Table I and Figure 1, the absorption period was 60 min during which time some augmentation occurred. The segments were exposed to label and toxin simultaneously. Labeled segments were washed for 20 min in 40 ml of cold 1 mM $CaSO_4$, weighed, and assayed for ^{86} Rb content by liquid scintillation spectrometry. Transfer of segments between solutions was by filtration on a Büchner funnel. Influx of K^+ and ^{86}Rb were taken to be equivalent using tissue grown in the absence of exogenous K^+ (17).

Basal rates of K^+ uptake were measured in freshly cut root segments, whereas augmented rates were measured after aeration of segments in distilled H_2O. This augmented uptake potential has been referred to as in "induced" ion uptake (19), or as "aging" (1, 16) and may be an important feature of the transport process.

Helminthosporium maydis Nisikado and Miyake, race T, was grown for 8 to 10 days in shake culture (100 rpm, 24 C) on Fries medium (26), without micronutrients. The culture fluid was filtered through cheesecloth, concentrated to one-tenth volume by flash evaporation at 45 C, and precipitated (12 hr, 4 C) with two volumes of cold methyl alcohol. The precipitate was removed by centrifugation and the methyl alcohol was removed from the aqueous-methyl alcohol phase by flash evaporation. The remaining aqueous extract was partitioned with four successive extractions using equal volumes of water- saturated 1-butanol. The butanol was removed by flash evaporation, and the remaining aqueous extract was subjected to four sequential partial evaporations with 100-ml aliquots of H_2O yielding a concentrated stock solution of the toxin complex of *H. maydis*, race T. In this paper two toxin preparations (A and B) were used. The preparations varied in their potency; preparation B was about 10 times as effective as preparation A in inhibiting the basal K^+ uptake rate in roots. A 1:100 dilution of the toxin stock preparation (called 1% toxin) contained less than 0.3 pmole K^+/ml as determined by flame photometry.

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[2] This paper is dedicated to Dr. Leon Bernstein—a genuine professional and gentleman.

Calculations for Figures 3, 4, 5, and 7 were made as follows:

$$\% \text{ inhibition of basal } K^+ \text{ uptake rate} = [(A - B)/A]\,100 \quad (1)$$

$$\% \text{ inhibition augmented } K^+ \text{ uptake rate} = \left[\frac{(C - D) - (A - B)}{C - A}\right] 100 \quad (2)$$

where the measured K^+ uptake rates are A = basal, B = basal + toxin, C = augmented, D = augmented, then toxin.

RESULTS

The influence of toxin on K^+ uptake was first studied using random 1-cm segments of the apical 6 cm of roots of inbred W64A. The uptake by both *N* and *T* root segments was toxin-sensitive. Fifty per cent inhibition of basal K^+ uptake occurred in *N* and *T* root segments at 3.6 and 1% toxin concentrations, respectively (Fig. 1a).

Pretreatment of intact or excised roots by aeration in H_2O increased (augmented) the subsequent uptake of K^+ as compared to the basal uptake of K^+ (Table I). $CaSO_4$ was not essential for this effect, nor did wounding (excision) contribute to augmentation. Augmented K^+ uptake of W64A, *N* and *T*, was more toxin-sensitive than basal K^+ uptake (Fig. 1b). A 50% reduction in augmented K^+ uptake was obtained in *N* segments with about 2.2% toxin, and in *T* segments with 0.3% toxin. Augmented K^+ uptake by both *N* and *T* root segments was more toxin-sensitive than the basal K uptake, and augmented K^+ uptake by *T* segments was seven to eight times as toxin-sensitive as augmented K^+ uptake by *N* root segments.

Experiments with specific root zones (ordered segments) rather than random segments demonstrated that K^+ uptake rates, augmentation potential, and toxin sensitivity depended upon root maturity (Table II). Various aspects of these data are presented in Figures 2 to 5.

The topography of basal and augmented K^+ uptake is shown in Figure 2. In both *N* and *T* roots uptake rates were maximal in cm-segments 5 + 6. These rates varied as much as 80% between apex and cm-segments 5 + 6. The observed difference in basal absorption rates by *N* and *T* roots is probably not physiologically meaningful since these rates varied from experiment to experiment.

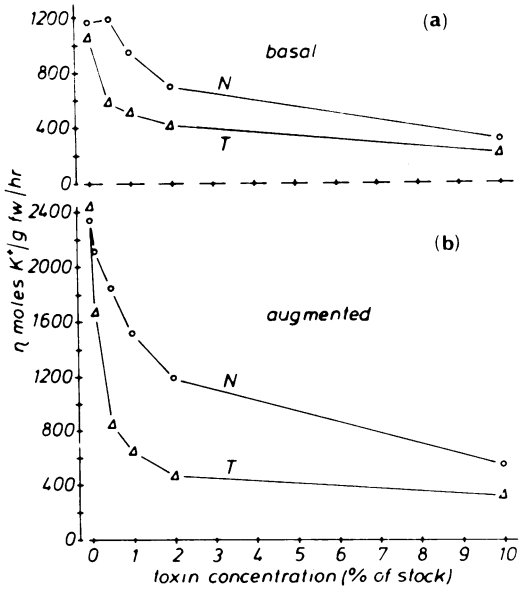

FIG. 1. Toxin inhibition of (a) basal and (b) augmented (aeration for 3 hr) K^+ uptake in 1-cm random segments of maize inbred W64A, *N* and *T* cytoplasm. Uptake period was for 60 min. Toxin preparation A. The average standard error of the mean was 7.7% of the mean.

Table I. *Effect of Excision, Aeration, and $CaSO_4$ on Development of Augmented K^+ Uptake in Roots of Maize Inbreds W64A, B37, and WF9*

K^+ uptake was for 60 min in 2 mM KCl, 1 mM $CaSO_4$ except as indicated..

Pretreatment	K^+ Uptake	
	nmoles/g fresh wt·hr	*% of basal*
Intact roots		
W64A none (basal)	792 ± 36	100
2 hr aeration in H_2O (augmented)	1436 ± 102	181
Excised cm-sections[1]		
B37[2] none (basal)	543 ± 118	100
3 hr aeration in 0.5 mM $CaSO_4$ (augmented)	1054 ± 196	194
3 hr aeration in H_2O (augmented)	1261 ± 124	232
WF9[3] none (basal)	1383 ± 66	100
3 hr aeration in H_2O (augmented)	3189 ± 242	246
no aeration, 3 hr in H_2O	991 ± 13	76

[1] One-cm root segments (randomized) of the terminal 6 cm of the root.
[2] External K^+ concentration was 1 mM.
[3] External K^+ concentration was 1.43 mM.

Table II. *Basal and augmented K^+ uptake and toxin sensitivity in ordered root segments of N and T cytoplasmic versions of the maize inbred W64A*

Cm-Segment	Toxin Treatment[1]		*N*	*T*
			nmoles K^+/g fresh wt.·15 min	
1 + 2	Basal	−	320 ± 24	310 ± 25
		+	219 ± 20	141 ± 23
	Augmented[2]	−	543 ± 43	421 ± 44
		+	277 ± 31	211 ± 37
3 + 4	Basal	−	520 ± 48	437 ± 31
		+	264 ± 29	205 ± 18
	Augmented	−	818 ± 76	642 ± 40
		+	446 ± 60	280 ± 12
5 + 6	Basal	−	580 ± 65	436 ± 39
		+	268 ± 26	239 ± 42
	Augmented	−	835 ± 59	783 ± 42
		+	442 ± 44	288 ± 8
7 + 8	Basal	−	415 ± 22	337 ± 42
		+	238 ± 6	199 ± 53
	Augmented	−	532 ± 36	540 ± 45
		+	361 ± 73	239 ± 31
9 + 10	Basal	−	338 ± 40	242 ± 26
		+	190 ± 7	149 ± 19
	Augmented	−	393 ± 42	368 ± 24
		+	233 ± 41	218 ± 26

[1] One per cent toxin (preparation A).
[2] Ninety min aeration in H_2O.

The toxin sensitivity of basal K^+ uptake rates varied along the root (Fig. 3a). *N* roots were least sensitive to toxin at the root tip, whereas *T* roots were most sensitive at the tip. As the roots elongated, *N* became more sensitive and *T* less sensitive.

The only zone where *N* and *T* roots could be distinguished with respect to the toxin sensitivity of basal K^+ uptake was at the apex. Figure 3b plots the basal K^+ uptake rate for each region against the inhibition by toxin, and shows that as basal K^+ uptake increased with root maturation in *N*, and possibly in *T* roots, the toxin sensitivity also increased.

The capacity of different regions of the root to augment is shown in Figure 4a. The data are presented as a percentage of the basal K^+ uptake rate. The augmentation of a given root region was different between *N* and *T* roots. *N* roots exhibited greatest augmentation near the tip, whereas *T* roots exhibited greatest augmentation in the 5 + 6 cm region. Figure 4b shows the percentage inhibition by toxin of the augmented K^+ uptake increment in *N* and *T* roots. The toxin sensitivity of the augmented K^+ uptake increment was calculated by assuming that the toxin-sensitive fraction of basal K^+ uptake was present after augmentation and represented a background within the augmented K^+ uptake potential. The toxin sensitivity of the augmented K^+ uptake increment (Fig. 4b) correlated visually with the changes in augmentation potential of both *N* and *T* roots (Fig. 4a), with *T* roots being much more toxin-sensitive in the mature regions of the root. This is in contrast to the inability of toxin to distinguish between *N* and *T* roots in the mature regions with respect to the basal K^+ uptake (Fig. 3a). Figure 5 is a plot of the augmented K^+ uptake increment against toxin sensitivity. The per cent inhibition increased in both *N* and *T* roots as the augmented increment increased, as was clearly the case for basal K^+ uptake in *N* roots (Fig. 3b).

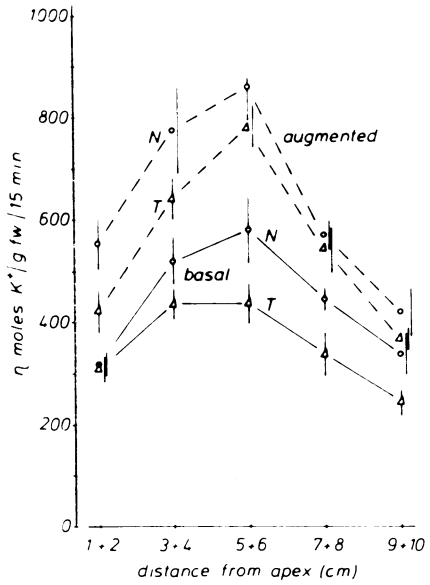

FIG. 2. Topography of basal and augmented K^+ uptake along the root of W64A, *N* and *T* cytoplasm (Table II). Standard error of the mean is shown for each point.

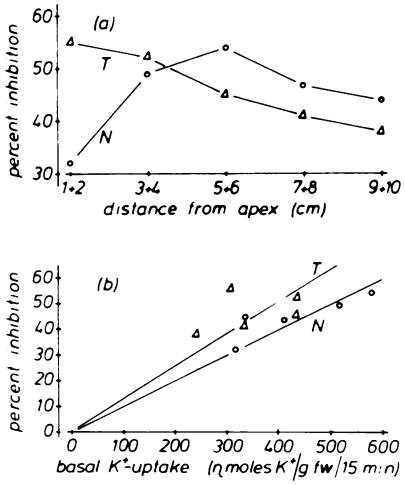

FIG. 3. a: Toxin sensitivity of basal K^+ uptake along the root of W64A, *N* and *T* cytoplasm (derived from Table II); b: toxin sensitivity of basal K^+ uptake as a function of basal rates of K^+ uptake (○), *T* (△). Curves fitted visually.

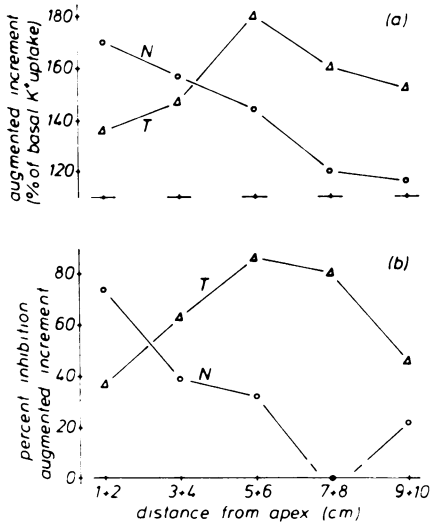

FIG. 4. a: Augmented K^+ uptake (as a percentage of the basal K^+ uptake) along the root of W64A, *N* and *T* cytoplasm (derived from Table II); b: toxin sensitivity of the augmented increment of K^+ uptake along the root.

Since toxin sensitivity correlated with the magnitude of the augmented increment during root development (Fig. 4b), it was of interest to determine whether toxin sensitivity of the augmented increment also increased during short term augmentation development. Figure 6 shows the timer course of augmentation development in cm-segments 2 + 3 + 4 (midzone regions) and its toxin sensitivity. The increase in K^+ uptake during aeration in H_2O commenced without a lag period, was nearly linear for 30 min, and was complete after 60 to 90 min (Fig. 6). After various intervals of aeration, the toxin sensitivity of the augmented K^+ uptake rate was assayed by labeling the segments in the presence of 0.1% toxin (preparation B). The toxin sensitivity of the augmented increment differed between *N* and *T* midzone root segments (Fig. 7), as it had in the case of ordered segments (Fig. 4b). The toxin sensitivity of this augmented increment did not change during the development of the augmented K^+ uptake potential. Throughout augmentation development, 10 to 25% of the augmented K^+ uptake potential was sensitive in *N* roots (with the exception of the 15-min pretreatment which showed about 50% inhibition), and between 70 and 80% was sensitive in *T* roots.

DISCUSSION

The uptake of K^+ in both *N* and *T* roots of maize inbred W64A was sensitive to a partially purified toxin complex from *H. maydis*, race T. Although it was possible to distinguish *N* and *T* roots of W64A with respect to K^+ uptake sensitivity (Fig. 1), we believe it is important that both cytoplasmic versions were inhibited by the toxin. Furthermore, the toxin sensitivity of both *N* and *T* roots was correlated with K^+ uptake rates (Figs. 3b and 5). These results are consistent with the observations that *N* versions of maize are partially toxin-sensitive in the root elongation bioassay (7, 20), as well as in other physiological phenomena (4, 8, 9, 22).

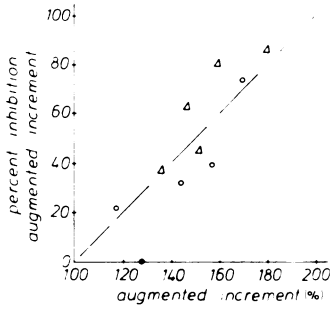

FIG. 5. Toxin sensitivity of the augmented increment of K^+ uptake as a function of the augmented increment (% above basal) of K^+ uptake. *N* (○), *T* (△). Curve fitted visually.

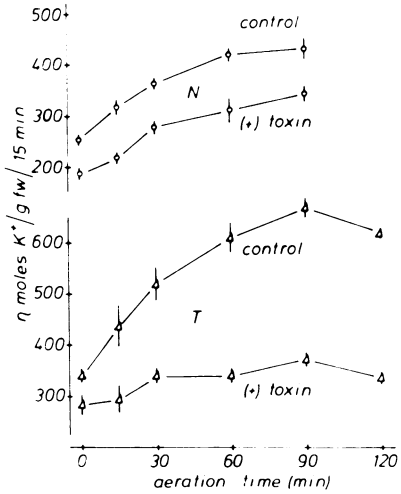

FIG. 6. K^+ uptake by root zones 2 + 3 + 4 (midzone) of W64A, *N* and *T* cytoplasm in the presence and absence of 0.1% toxin (preparation B) at various stages of augmentation development. Standard error of the mean is shown for each point.

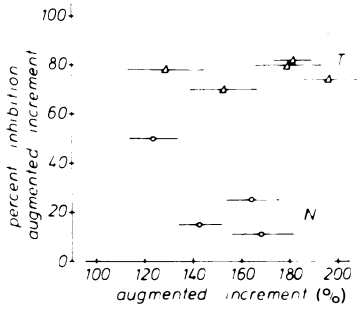

FIG. 7. Toxin sensitivity of the augmented increment of K^+ uptake as a function of the augmented increment (% above basal) of K^+ uptake (derived from data of Fig. 6). Standard error of the mean for the augmented increment is given for each point.

Root elongation, and thus the physiological age of cells, was found to be an important factor in demonstrating the sensitivity of maize roots to the toxin complex. This was found for both the basal (Fig. 3a) and augmented (Fig. 4b) K^+ uptake rates. *N* and *T* could be distinguished only in the young region of the root 1 + 2 cm from the root tip) when the basal K^+ uptake rates were measured. *N* and *T* could be distinguished in the more mature regions of the root when augmented K^+ uptake rates were measured. Thus the toxin sensitivity of basal and augmented uptake changes during root development, even though the mechanism of K^+ uptake is presumably the same.

Because K^+ uptake in both *N* and *T* roots was inhibited by the toxin complex, and since a correlation exists between toxin sensitivity and K^+ uptake rates, it is clear that some component of the K^+ transport process in both cytoplasms is sensitive to the toxin. It is also likely that the mechanism of K^+ transport into cells is similar in *N* and *T* roots. The several differences in toxin sensitivity between *N* and *T* roots suggest the presence of an adjunct system that confers a differential protection of the K^+ transport process. We postulate that a protection mechanism of the transport system exists in both *N* and *T* roots, but its' effectiveness (either in amount, rate of formation, or placement) is restricted in *T* roots.

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