DIFFERENTIAL LEAF ABSORPTION OF A HIGH-MOLECULAR-WEIGHT PHOSPHATE IN MAIZE (ZEA MAYS, L.) PLANTS OF DIFFERING CYTOPLASMS*

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SUMMARY: The absorption of foliar applied tripolyphosphate is significantly greater in cytoplasmic male-sterile (Tcms) plants than in plants having normal (N) cytoplasm. There is no difference in orthophosphate absorption between Tcms and N cytoplasm, nor is there any difference in the translocation of either source of phosphorus (P) inside the plant 12 days after application. The differential uptake of the large molecular P compound by the Tcms and N cytoplasm is believed related to membrane permeability.

INTRODUCTION

The cytoplasm associated with male sterility in Zea mays, L., Texas type (Tcms), is differentially sensitive to infection by the fungus, Helminthosporium maydis Nisikado and Miyake, race T (1,2). The disease selectively attacks inbreds and hybrids of maize with Tcms cytoplasm, but plants with normal (N) cytoplasm are relatively resistant.

Aside from the male-sterile phenotype, major differences distinguish Tcms and N cytoplasm in the presence of the pathotoxin of H. maydis. Tcms shows several effects not observed with N. After exposure of Tcms to the pathotoxin, several investigators have reported leaf chlorosis and necrosis (2,3), inhibition of seedling root elongation (1,4,5), swelling and other changes in mitochondria (6,7), ion leakage from roots and leaves (5,8,9,10), and uncoupling of oxidative phosphorylation (6,11). Arntzen (5,12) reported that the pathotoxin induced stomatal closure, followed by inhibition of photosynthesis and reduction of the ATP concentration. Mertz and Arntzen (13) found depolarization of the membrane electrical potential. Tipton et al. (14) measured an inhibition of K^+-dependent

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ATPase. Garraway (9) found leakage of peroxidase with Tcms cytoplasm after inoculation with race T. In none of these cases did the N cytoplasm show similar effects.

On the basis of these observations, several authors (5,10) concluded that membranes might be either the primary or secondary site of pathotoxin effects (8,10). Specific effects on mitochondrial membranes have been reported in an early step of the electron-transport chain, preceding entry of electrons from succinate dehydrogenase, which represents the first ATP-coupled site associated with the endogenous NADH dehydrogenase (11).

There is some evidence in the literature that the membranes of Tcms and N cytoplasm might differ in certain intrinsic properties, especially membrane permeability. Halloin (10) reported a rate of carbohydrate leakage in Tcms roots that was three times higher than in N roots in the absence of toxin. Tcms leaves had a 15% higher rate of carbohydrate leakage than did N leaves. In the absence of toxin, Arntzen et al. (5) found a slightly higher (nonsignificant) ^{32}P leakage in Tcms roots and a slightly higher ^{32}P uptake by untreated Tcms mitochondria.

The findings reported in this paper show differences unassociated with pathotoxin effect in the N and Tcms cytoplasms. A differential exists between the two cytoplasms and is exhibited by their capacity to absorb a high-molecular-weight phosphate.

MATERIALS AND METHODS

Leaf application of P

The two genotypes, Nrf_1rf_1 and $Tcmsrf_1rf_1$, of the maize inbred B37 used in this study are essentially isolines and differ only in their cytoplasm, N and Tcms. Seeds were planted in well-fertilized soil in No. 10 cans in the greenhouse on July 31, 1972. Quantities of 0 (control), 170.32 microgram P as orthophosphate and 218.32 microgram P as tripolyphosphate, were applied on Aug. 30 in a volume of 25 microliters of solution to a circular area of 1.2 cm diameter of the youngest mature leaf surface. The circular delineations were

made by means of a cork borer (No. 7), which had been dipped in a warm mixture of three parts paraffin and one part lanolin. Commercial sodium tripolyphosphate was purified by four recrystallizations according to a method described by Van Wazer (15) and converted into ammonium tripolyphosphate by means of ion exchange (16). All solutions contained a surfactant (0.1% Tween-80) to lower the surface tension and were neutralized to pH 7.0 with ammonium hydroxide. There were 20 replications per treatment, and the experimental design was a randomized complete-block design with 10 blocks.

Assay of P uptake and translocation

Half the replications were sampled by punching out the treated areas with a cork borer, slightly larger than the one used to make the waxy circles, 24 hr after the P applications. The leaf discs were submerged in 10 ml of 0.25% Tween-80 solution in a 60-ml bottle and were shaken vigorouly for 5 min. The leaf discs were then transferred to empty bottles, and both the leaf washings and the leaf discs were analyzed for total P by using a dry-ashing method with magnesium acetate and determination of P according to Watanabe and Olsen (17). The second sampling was done 12 days after application of the P solutions.

Unpublished research (first author) has proved that the absorption rate of foliar applied P by maize is a linear function of the quantity applied, at least up to the concentration beyond which leaf damage occurs. When different amounts of P of various P compounds are applied it is best to make a comparison of their absorption, expressed as a percentage of the applied quantity. By subtracting the amount of P in the leaf washing from the quantity of P applied and making a correction for the amount of P found in the leaf washing of the control treatment, it was possible to determine how much of the applied P was absorbed within a certain time. Subtracting the amount of P present in the leaf disc from the amount of absorbed P and correcting this for the P content of the control disc make it possible to calculate how much of the absorbed P was translocated from the treated area. The correction factors were determined by averaging the 10 replications of the control treatment.

Table 1

Absorption and translocation of ortho- and tripoly-phosphate by normal and Tcms cytoplasm of one maize inbred in 1-day and 12-day sampling times. Each value represents the average of 10 replications.

Cytoplasm	Length of experiment (days)	P in leaf washing (µg)	P in leaf disc (µg)	P absorbed as % of applied P	% T>N	P translocated as % of absorbed P
			Control (0.1% Tween-80)			
Normal	1	0.69	17.73			
Tcms	1	0.77	16.52			
Normal	12	0.75	15.93			
Tcms	12	0.93	15.59			
			Orthophosphate			
Normal	1	79.49	63.07	53.7		49.8
					-4.5	
Tcms	1	83.65	60.65	51.3		49.0
Normal	12	5.57	41.68	97.2		84.4
					0.3	
Tcms	12	5.13	45.39	97.5		82.1
			Tripolyphosphate			
Normal	1	211.03	23.49	3.7		28.0
					159.5	
Tcms	1	198.20	29.44	9.6		37.9
Normal	12	129.03	39.72	41.2		73.4
					54.9	
Tcms	12	80.02	50.38	63.8		74.7

RESULTS AND DISCUSSION

Table 1 shows the means of the P analyses of the leaf washings and discs and the calculated quantities of absorbed and translocated P.

Table 2 gives the analyses of variance for the percentage P absorbed and that translocated from the treated area.

P absorption

Statistically, the P absorption is significantly different for all three

Table 2

Analyses of variance for P absorption and translocation.

	Sources	d.f.	Mean squares of P absorption	Mean squares of P translocation
1	Blocks	9	34.24	59.42
2	Cytoplasms (A)	1	875.03**	78.25
3	P treatments (B)	1	41218.51**	3294.32**
4	Sampling times (C)	1	41121.47**	28106.80**
5	A x B	1	1164.92**	259.36
6	A x C	1	472.29**	129.00
7	B x C	1	5.55	266.79
8	A x B x C	1	241.98**	64.10
9	Error	63	31.13	79.27

**Indicate significant at 1% level.

main effects: cytoplasms, P treatment, and sampling time. Each cytoplasm has a greater P uptake of orthophosphate than of tripolyphosphate at the time of treatment--1 day and 12 days later. The P absorption is in all instances significantly greater after 12 days than after 1 day. Because of the statistically significant two-factor interactions, cytoplasm by P treatment and cytoplasm by sampling time, and the three-factor interaction, the simple effects with respect to differences in cytoplasms should be considered.

The simple effects are listed in Table 3 and indicate that the T cytoplasm has a significantly greater absorption of P from tripolyphosphate for both sampling times (159.5%, 1 day; 54.9%, 12 day) than does the N cytoplasm. No difference between the cytoplasm in absorption of P from orthophosphate is evident, however, indicating that membrane permeability is not limiting the P uptake mechanism (carrier) with the orthophosphate. The greater absorption of tripoly-phosphate (a larger molecule with a larger negative charge than that of ortho-

Table 3

Mean squares and F-values of the simple effects of the P absorption.

Treatment comparisons	Mean squares	F
Between cytoplasms within orthoP for 1 day	28.77	<1
Between cytoplasms within orthoP for 12 days	0.66	<1
Between cytoplasms within tripolyP for 1 day	174.55	5.61+
Between cytoplasms within tripolyP for 12 days	2550.25	81.92**

+,** Indicate significance at 2% and 1% levels, respectively.

phosphate) by the T cytoplasm suggests a difference in the intrinsic properties between the membranes of Tcms and N cytoplasms.

P translocation

Table 2 shows a significant difference in P translocation between the two phosphates and between sampling times, but not between the cytoplasms (Table 2, line 2).

The differences between the two compounds in P translocation seen at the 1-day sampling time disappear by 12 days. This is presumably because there is a greater original concentration gradient for the orthophosphate than for the tripolyphosphate for both cytoplasms, leading to a greater driving force for P translocation. This is consistent with unpublished research (first author) that, within a given variety of corn, the amount of P translocated from the treated area, expressed as a percentage of the absorbed P, was always the same, regardless of the form and concentration of P applied. Thus, the difference between the two phosphates between the cytoplasms does not reside in P translocation, but in differential absorption.

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