GROWTH AND RNA SYNTHESIS IN MAIZE SEEDLINGS TREATED WITH ETHYL METHANESULFONATE

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SUMMARY

Corn seeds were treated with the chemical mutagen ethyl methanesulfonate and the resulting seedlings were examined for growth and RNA synthesis. Ethyl methanesulfonate treatments reduced shoot elongation, seedling growth, and chromatin-directed RNA polymerase activity of shoot tissues. The chromatin RNA polymerase activity of control seedlings peaked at 24 h. This peak was delayed up to 12 h by the chemical mutagen treatment. Saturation of embryonic axis chromatin with E. coli RNA polymerase indicated that ethyl methanesulfonate increased the amount of DNA available as template. In both the treated and control axes, the available template decreased with tissue differentiation.

The alkylating agent ethyl methanesulfonate has been widely used as a chemical mutagen[1-3]. Aside from its mutagenic activity, however, this chemical has been observed to alter the growth pattern of treated corn. Lund[3] and Glover (unpublished data) measured several agronomic parameters of corn grown from ethyl methanesulfonate-treated seed. They found that treatment with this chemical delayed seedling emergence, reduced plant height, and severely affected the phenotype of treated plants. These data suggest that ethyl methanesulfonate results in physiological as well as genetic changes in treated tissue. We are now reporting some changes in the patterns of growth and RNA synthesis in corn seedlings at very early periods after treatment with ethyl methanesulfonate.

MATERIAL AND METHODS

Seed treatment: Corn seeds (Zea mays L. var. H49 x B37) were soaked in an aerated 30° water bath for 24 h and then put into solutions of ethyl methanesulfonate in 0.05 M phosphate buffer, pH 6.0, for 4 h at 30°. Control group seeds were treated similarly, but without ethyl methanesulfonate. After treatment,

seeds were rinsed with de-ionized water, placed embryo-down on moist Kimpak, and put into a germinator at 30° until the time of assay. Germinating seedlings were kept moist with 10^{-3} M calcium chloride throughout the growing period.

Growth measurements: Embryonic axes were removed from the seeds at various times after treatment. Samples of 25 axes were weighed, dried 24 h in an oven at 60°, and reweighed. At the 72 h stage of growth, shoots were removed from the axes and measured as well as weighed.

Chromatin isolation: Embryos were excised after seed treatment and cooled on ice. Samples (5 to 20 g, depending on the age of the tissue) were extracted at 2-4° according to the method of Huang and Bonner[4]. The chromatin pellet was suspended in 0.01 M Tris-HCl, pH 8.0. Aliquots taken from this suspension were used to assay chromatin RNA polymerase activity.

Chromatin RNA polymerase activity: The incorporation of labeled nucleoside triphosphate into trichloroacetic acid-insoluble material was used as a measure of RNA synthesis. The reaction mixture contained, in μ moles, the following: GTP, CTP, and ATP, 0.1 each; UTP, 0.005; $MgCl_2$, 0.5; $MnCl_2$, 0.125; Dithiothreitol, 0.5; Tris-HCl (pH 8.0), 15; and 7.5 μC of (^{3}H) UTP (12.1 mC/μmole) and chromatin equivalent to 10-20 μg of DNA in a final volume of 0.2 ml. The reaction was incubated 15 min. at 37° and stopped with the addition of cold 10% trichloroacetic acid. Precipitates were washed onto glass fiber filters, dried under infra-red lamps, and counted in a Tri-Carb liquid scintillation spectrometer[5].

DNA determination: Chromatin DNA was determined according to Burton[6] after hydrolysis in 0.5 N perchloric acid for 45 min. at 70°.

RESULTS

Growth of corn shoots 72 h after seed treatment with ethyl methanesulfonate is illustrated in Fig. 1. The reduction in both fresh weight and length of shoot tissue was linear with increasing concentrations of mutagen in the treatments; both growth parameters were affected to the same degree by the seed treatment. Fresh weight accumulation by the developing embryonic axis was reduced by ethyl methanesulfonate within 24 h after treatment, and the reduction became more pro-

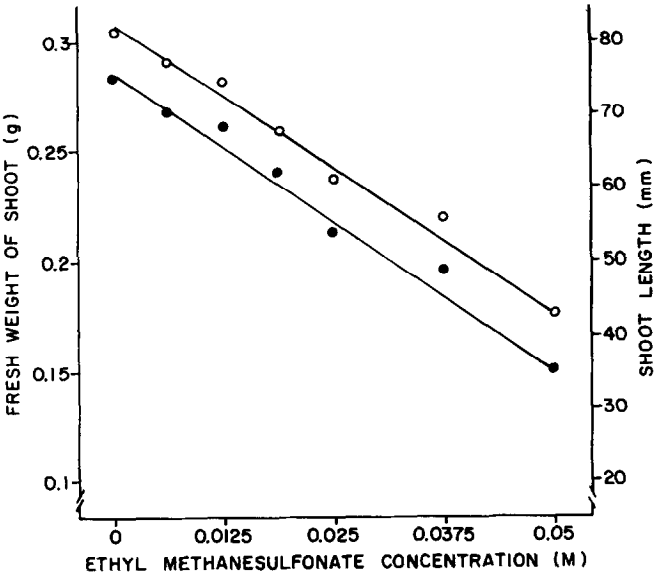

Figure 1. Growth of corn shoots 72 h after treatment period. Shoot length, O; fresh weight of shoots, ●.

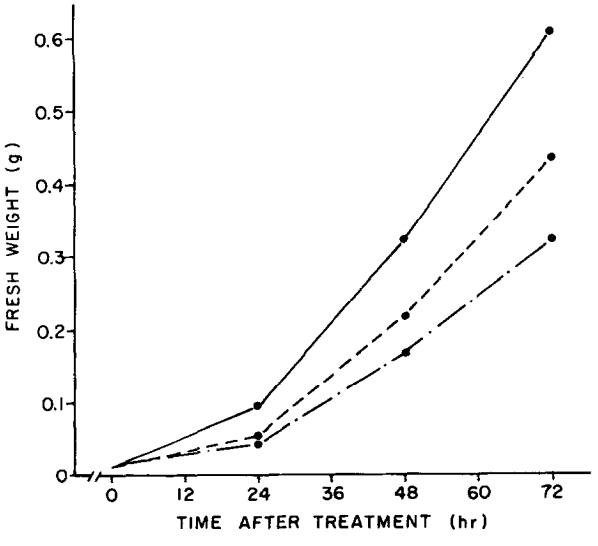

Figure 2. Growth of embryonic axes after treatment. Control, ———— ; 0.025 M ethyl methanesulfonate, - - - - -; 0.05 M ethyl methanesulfonate, —•—•

nounced at the later stages of growth (Fig. 2).

The peak of chromatin RNA polymerase activity in the embryonic axis was either decreased or delayed 12 h in appearance by the treatment with ethyl

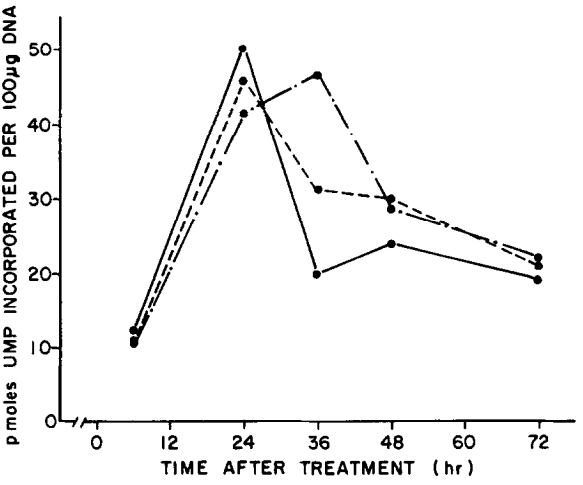

Figure 3. Chromatin RNA polymerase activity of embryonic axes after treatment. Symbols are the same as in Figure 2.

methanesulfonate (Fig. 3). By 72 h after initiation of the treatments, both the ethyl methanesulfonate-treated and control axes had comparable chromatin RNA polymerase activities. Shoot chromatin RNA polymerase activity increased markedly, however, between 48 and 72 h after treatment (Table 1). The increase in chromatin RNA polymerase activity was greater in control tissue than in ethyl

Table 1. RNA Synthesis in Corn Shoots after the Treatment Period.

Treatment	p moles (^{3}H) UMP incorporated[1] per 100 µg DNA		Specific Activity[2] cpm $^{32}P/A_{260}$ RNA
	time of assay (hours after treatment)		
	48 h	72 h	72 h
Control	19.7	50.4	1918
0.025 M EMS	16.0	39.3	1567
0.05 M EMS	14.4	26.4	1034

1 Chromatin RNA polymerase activity of shoot tissue at indicated times after the treatment period.

2 Specific activity of RNA extracted from shoot tissue after labeling with ^{32}P. Fifty shoots were incubated 5 h with 500 µC ^{32}P in .25 ml of 5 x 10^{-4} M ammonium citrate buffer, pH 6.0, containing 1% sucrose and 50 µg/ml chloramphenicol. RNA was extracted as previously described[7], plated and counted.

methanesulfonate-treated tissues. At 72 h, shoot chromatin RNA polymerase activity followed the same pattern as ^{32}P incorporation into RNA by excised shoot tissue (Table 1).

DNA template availability notably decreased with the age of the tissue. However, while treated seeds also showed a decreased DNA template availability, there was significantly more DNA available at the 24 h stage and throughout the aging seedlings. The proportion of DNA template remaining available during the differentiation process was dependent on the concentration of ethyl methanesulfonate in the treatment.

Table 2. Potential RNA Synthesis of Chromatin DNA from Control and Ethyl Methanesulfonate-Treated Corn Embryos.

Treatment	p moles (^3H) UMP incorporated per 100 µg DNA[1]			
	time of assay (hours after treatment)			
	24 h	36 h	48 h	72 h
Control	3577 (100%)	3200 (89%)	1988 (56%)	1264 (35%)
0.025 M EMS	4330 (121%)	2528 (71%)	1958 (55%)	1911 (53%)
0.05 M EMS	4911 (137%)	3273 (92%)	2940 (82%)	2793 (78%)

[1] Values in parenthesis indicate the percent of the 24 h control DNA template available present in each treatment.

DISCUSSION

The growth capacity of seedlings treated with 0.05 M ethyl methanesulfonate was about one-half that of control seedlings. There was less-pronounced inhibition of seedling growth with lower concentrations of the alkylating agent. Included in this growth reduction was the failure of treated shoot tissue to elongate at a rate comparable to that of untreated seedlings. The germination of treated seeds was normal, indicating that reduced shoot elongation was not attributable to delayed germination. This points to the conclusion that the metabolic events leading to germination had either preceded the ethyl methane-

sulfonate treatment or were unaffected by it.

It has been shown that a specific complement of RNA molecules is required for cellular elongation to occur[8,9]. Therefore, inhibition of the synthesis of RNA may be the rate-limiting step in the elongation of treated shoots. This viewpoint is supported by the close correlation found between the inhibition of growth and RNA synthesis as measured by ^{32}P incorporation by treated shoots. Furthermore, a pronounced decrease in chromatin-bound RNA polymerase activity was observed in treated shoots, emphasizing the reduced transcription rate in these treated shoot tissues.

While inhibition of growth in shoot tissues has been directly correlated with reduced RNA synthesis, whole embryos exhibited a different RNA synthesis profile. Ethyl methanesulfonate caused a small but definite reduction in chromatin RNA polymerase activity in the embryos 24 h after treatment. After this period, control axes lost chromatin-bound RNA polymerase activity quite rapidly, while treated axes either maintained capacity for RNA synthesis for an additional 12 h (0.05 M ethyl methanesulfonate) or lost RNA polymerase activity more slowly (0.025 M ethyl methanesulfonate). By the 72 h stage, all treatments had lost chromatin RNA polymerase activity to approximately the same level.

These data suggest that RNA synthesis between the first 24 and 30 h following treatment is essential to normal seedling growth. The effect of ethyl methanesulfonate on growth can be visualized in at least two ways: (a) prevention of the production of an essential population of RNA molecules at the DNA level and/or (b) the maintenance of RNA polymerase activity after a critical stage of development. The latter effect might then lead to the production of RNA molecules not required in the developmental process (or related to an earlier developmental process) and resulting in a retarded rate of differentiation.

Control embryos exhibit a marked reduction in DNA template availability during differentiation. This was shown by analysis of embryo chromatin RNA template availability at 24 h and later time periods. Such a reduction in template availability suggests a loss in genome transcription with differentiation.

However, in embryos which had been treated with ethyl methanesulfonate, a marked increase in DNA template availability was observed at the 24 h stage and was maintained through the 72 h stage, showing an inhibition of genome repression.

Two major effects of ethyl methanesulfonate on RNA synthesis have been observed: (a) the inhibition of repression of DNA, and (b) the alteration of endogenous RNA polymerase activity. It is suggested that both of these phenomena may be the result of modification of the DNA bases by ethyl methanesulfonate. In vivo treatment with ethyl methanesulfonate has previously been shown to result in ethylated purine bases of the DNA under similar conditions[10-12].

Reduced seedling growth, especially reduced shoot elongation, would explain the delayed seedling emergence found by Lund[3]. Reduced mature plant height and abnormal phenotype[3] indicate that the corn plant, once subjected to ethyl methanesulfonate, recovers neither normal growth nor differentiation capability.

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