*Cantaurus*, Vol. 2, 40-42, May 1994 © McPherson College Division of Science and Technology

# The Effects of Soybean Development with EMS Treatment

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**Abstract**

Soybean seeds were soaked in EMS, a mutigenic chemical. Both the parental and F1 plants from these seeds were observed for retarding effects. Comparing the parental and F1 generations showed evidence that the retarding effects may be carried from the parental plants to the F1s treatment; their heights were 4.53 cm and 3.43 cm respectively. When comparing the controls of both generations

## Introduction

Ethyl methane sulphonate (EMS) is a well-known mutagenic chemical used to produce genetic variation in legumes. In this experiment soybean seeds were soaked in different concentrations of EMS/distilled water solutions. One of the main effects observed in EMS treatment of this kind is retarded seedling growth. Retarding effects of this type can often be used as a criterion for judging the effectiveness of mutagenic agents (Dixit and Dubey 1987). M1 (first generation) data on seedling growth was taken in addition to M2 (second generation) measurements. The object to this study was to compare the M1 and M2 data and determine to what degree the retarding effects were carried over genetically and expressed in the second generation of seedlings.

## Materials and Methods

On June 20, 1993 EMS dilutions were made at 11:00 p.m. Five different EMS/distilled water dilutions were made: 0.0%, 0.001%, 0.01%, 0.1%, and 0.5%. The seed soaking process was done in covered containers at 26.6°C in the absence of light for six hours. The soybean variety used was Stine 2250, which is a mid-group II maturity. All seeds were completely submerged in the EMS/distilled water solutions. After the six-hour period, the EMS solution was drained from each soaking container and planting began (June 21, 1993). These seeds were planted in a 6.2 m x 18.8 m plot located in a field under a corn/soybean rotation in Adel, Iowa. On May 25, 1993, Trefland (pre-emergence herbicide) was applied approximately 6.5 cm deep to this plot. Planting was done with a push planter at a depth of 3 cm. One row of each concentration was planted except for 0.5% where two rows were done. The six rows were approximately 1.4 m apart with an approximate length of 17.2 m and a plant approximately every 17.2 cm. These plants were grown and then harvested on October 8, 1993. No M1 growth data was collected at this time.

Harvesting was done by clipping the top half of a randomly selected forty-five to fifty-five plants. These clippings were placed in mesh bags by concentration and allowed to ripen and dry before threshing. This was done because harvesting time was restricted and some of the higher concentration plants were not completely mature due to the EMS treatments. Each plant clipping was threshed with a portable thresher, and the seed from each clipping was packaged in a separate envelope. Each envelope was assigned a number. This seed was used for the M2 seedlings which were grown and compared with treated seeds which represented the M1 generation.

The M2 generation and second M1 generation were grown in a greenhouse. The seed soaking procedure was repeated for seeds that represented the second M1 generation.

Ten pots for each of the following were made: (M1: 0.0%, 0.001%, 0.01%, 0.1%, 0.5%); (M2: 0.0%, 0.001%, 0.01%, 0.1%, 0.5%, negative control, selected 0.5%). Packets one through ten of the M2 selections were used in planting. The negative control consisted of seeds which were not soaked in distilled water for the six-hour period. The selected 0.5% consisted of seed which was chosen from the 0.5% packets expressing poor physical appearance. Bleeding hylems and wrinkled seed coats were two main characteristics used during selection. Three seeds were planted in each pot except for the M1 0.5% pots which had five seeds. This was done to assure against the possibility of poor germination caused by the high EMS treatment. Each seed was placed three centimeters deep into a peat-lite potting soil mixture. After the seedlings emerged all but one plant was removed from each pot. The plant closest to the center of the pot was selected to remain. All plants were grown under growth lights which operated 14 hours a day.

Each plant was measured in centimeters for hypocotyl length and epicotyl length. Total plant height of each plant was calculated by adding these two measurements of each plant. The plants' growth stage was also recorded during the same time intervals as the other measurements were taken. The hypocotyl length was determined by measuring the distance on the stem between the soil surface and the cotyledonary node. The epicotyl length was determined by measuring the distance on the stem between the cotyledonary node and the start of the shoot apex. The growth stage was identified by the number of nodes on the main stem divided by two.

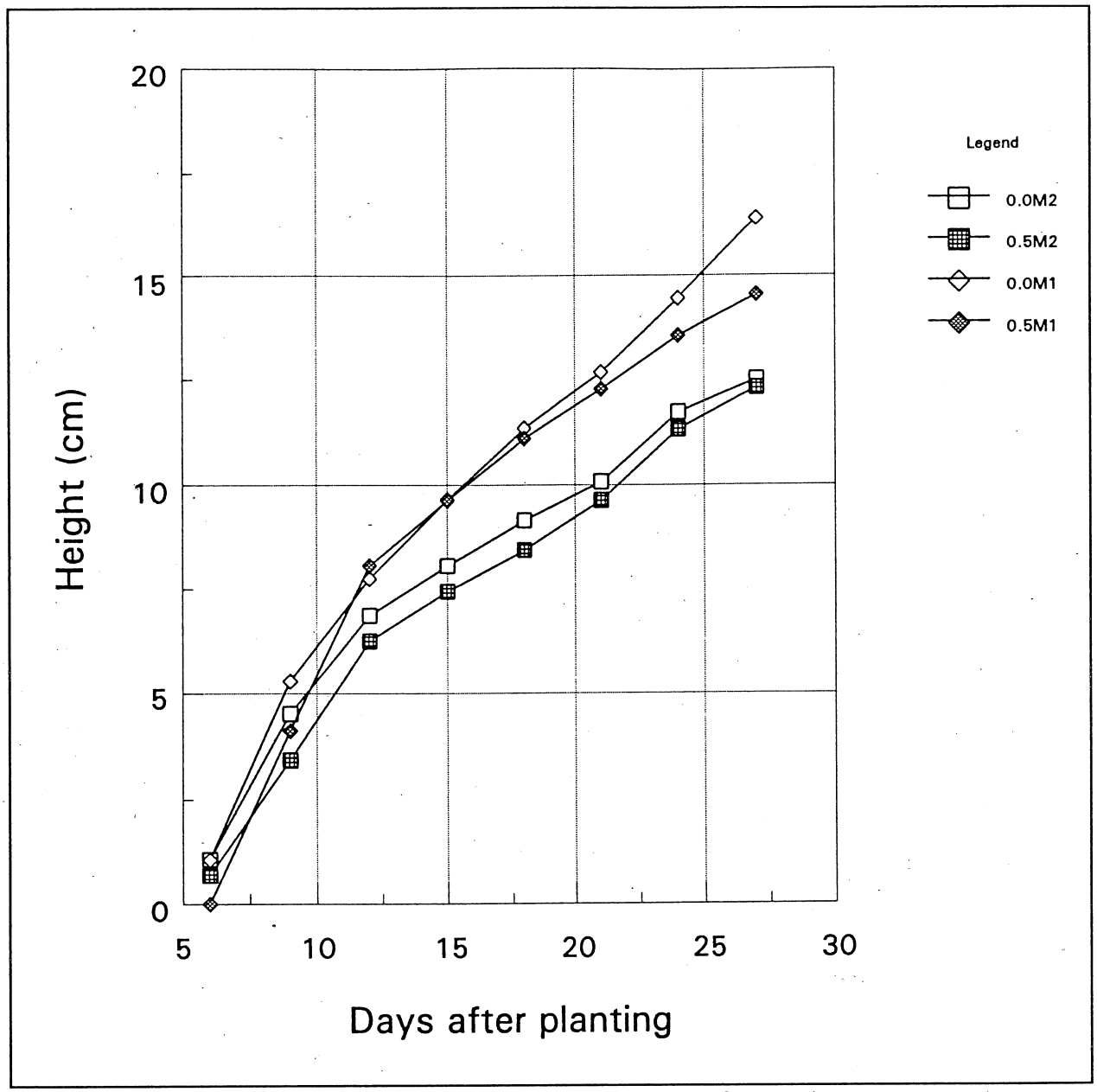


**Figure 1.** Height of control and the 0.5% treatment plants in M1 and M2 generations.

Measurements were spaced three days apart and taken eight separate times starting six days after planting.

**Results**

When comparing the plant heights of the 0.5% treatments of each generation to their controls, a significant difference was found; this difference was only found within the first and second measurement days. No other concentrations expressed a difference. On day one the M1 control averaged 1.08cm while the M1 0.5% treatment plants had not emerged. A T-test did not need to be conducted on this comparison. On the same day the M2 control was taller ($P < 0.018$) than the M2 0.5% treatment; their heights were 1.09 cm and 0.7 cm, respectively. On day two the M1 control was taller ($P < 0.033$) than the M1 0.5% treatment; their heights were 5.3 cm and 4.12 cm, respectively. The M2 control was taller ($P < 0.0001$) than the M2 0.5% treatment; their heights were 4.53 cm and 3.43 cm, respectively. When comparing the controls of both generations of measurement days one and two, no significant difference was observed.

**Discussion**

Plants (M1) grown from seeds treated with the 0.5% EMS solu tion expressed a significant reduction in height compared to the control. The plants grown from seeds derived from plants which had a similar EMS seed treatment expressed similar growth patterns (refer to days six and nine on Figure 1). This supports the concept that retardation effects, stimulated by EMS treatment, are carried over from the first generation to the second. This retardation effect seems to be associated with seed germination and early seedling height. The cause of the effect could be derived from a number of variables. It is possible mutigenic changes are a contributing factor, although a more sound explanation would associate the poor development of seeds from highly stressed plants which were grown from seed physically damaged by the EMS solutions. This physical damage would certainly affect germination in a negative way. This was evident in the 0.5% M1 generation and mildly represented in the 0.5% M2 generation. This concept would not associate genetic alterations to the retardation effects. According to Gunkel 1965, growth retardation may be caused by growth inhibition due to destruction of apical meristems or partial failure of internode elongation; auxin production may also be affected (Dixit and Dubey 1987).

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**Acknowledgements**

I would like to thank N.A. Dutrow for being my main advisor, J.P. Frye for analysis assistance, and M.A. Bretz and B.N. Rolston for data collection assistance.