indicate a similarity between the nature of the Rutherglen Bug attack and that of the wheat Bug in New Zealand wheat, in which the insect injects an enzyme which denatures the protein in the grain (Burnett, 1976).

Rutherglen Bug damage has been attributed to the bugs sucking the sap under high evaporative demand conditions and thus causing the leaves to wilt (Evans, 1943). Davidson and Peairs (1966) used a similar explanation with respect to *Nysius ericae*. But this does not appear to be the case in sunflower. The resistance to flow through sunflower petioles being attacked by the bug was 5550 bars $sec^{-1}$ $cm^{-3}$ compared with 250 bars $sec^{-1}$ $cm^{-3}$ in unattacked plants (Turner and Downes unpublished). This 22-fold increase in resistance suggests that the bugs are not merely removing sap, they are impeding sap flow, perhaps by the injection of enzymes.

The evidence above suggests that resistance to Rutherglen Bug is available in wild sunflowers. One form of resistance of value is the non-preference type in which the insects are repelled by resistant plants. If on the other hand the bugs attack but the plants are not damaged it is possible that some plant compound, perhaps a tannin, precipitates the protein in the enzyme injected before it damages the plant.


## ACKNOWLEDGEMENTS

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## LITERATURE CITED


BURNETT, P. 1967. Cereal crop pests. *In* New Zealand Insect Pests. *Ed.* D.N. Ferro, Lincoln University College of Agriculture Canterbury.

DAVIDSON, R.H. and PEAIRS, L.M. 1966. Insect pests of farm garden and orchard 6th edit. Wiley N.Y.

EVANS, J.W. 1943. Insect pests and their control. Government Printer, Hobart.

FLINT, W.P. 1921. Chinch bug resistance shown by certain varieties of corn. Journal Economic Entomology 14, 83 — 85.

GREAVES, T. and ROCHFORD, R.R. 1946. Observations on the relative susceptibility of potato varieties to attack by the Rutherglen Bug. CSIR Journal 19, 387 — 93.

JONES, E.T. 1937. Differential resistance to chinch bug attack in certain strains of wheat. Kansas Academy of Science Transactions 40, 135 — 42.

KEHAT, M. and WYNDHAM, M. 1972. The effect of food and water on development, longevity, and fecundity in the Rutherglen Bug. *Nysius vinitor* (Hemiptera: Lygaeidae). Australian Journal of Zoology 20, 119 — 30.

KEHAT, M. and WYNDHAM, M. 1973. Flight activity and displacement in the Rutherglen Bug *Nysius vinitor* (Hemiptera: Lygaeidae). Australian Journal of Zoology 21, 413 — 26.

MILLIKEN, F.B. 1918. *Nysius ericae*, the false chinch bug. Journal of Agricultural Research 13, 571 — 8.

OFFICIAL METHOD OF ANALYSIS OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 11th edition. 1970, 446. A.O.A.C. Washington.

PAINTER, R.H. 1951. Insect resistance in crop plants. New York. Macmillan.

SLATER, J.A. 1964. A catologue of Lygaeidae of the World. University of Connecticut, Storrs.

SNELLING, R.O., PAINTER, R.H., PARKER, J.H. and OBSORN, W.M. 1937. Resistance of sorghum to the chinch bug. USDA Technical Bulletin 585. 560.


T1982GEN24

# CHEMICALLY INDUCED MUTATIONS IN SUNFLOWER.


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## ABSTRACT

**Sunflower seeds of a well adapted, high general combining ability inbred line were treated at varying concentrations and times with the chemicals ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG). Germination tests were conducted on germination paper and in soil in the greenhouse to determine the optimum concentration and time of treatment. Field tests were used to confirm the results of the germination tests. The $M_2$ and $M_3$ generations gave individual plants which exhibited earlier bloom date and added resistance to sunflower rust (*Puccinia helianthi*, race 1).**


## INTRODUCTION

Chemically induced mutations, a supplement ot normal plant breeding methods, were rare in sunflower *(Helianthus annuus)* (Fick, 1978, Voskoboinick and Soldatov, 1974) but in other crop species considerable success was noted. Chemical mutagens affect genic changes as opposed to chromosomal changes from X-ray or gamma radiation (Simons, 1979). Breeding material of seed or flowering plants that was well adapted but lacking one or two desirable characteristics would be excellent source material since it offers the greatest opportunity to be useful in hybrid seed production (Simons, 1979). Several choices of chemical mutagens have been employed and the means of application has varied among researchers (Anonymous, 1977; Voskoboinick and Soldatov, 1974).

The objectives of this study were to develop methodology and test the feasibility of producing functional sunflower mutants by employing chemical mutagens.

## MATERIALS AND METHODS

Ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) solutions were prepared to give concentrations of 50, 250, 500, 2500, and 5000 $\mu$g/ml of each chemical. The pH of each mutagen at various concentrations was determined, Table 1. Achenes of the well adapted, high general combining ability inbred, Dahlgren H6B, were treated by vacuum infiltration for one hour under alternating tension of 0 to 1.36 atm. Each concentration of both chemicals and a distilled water check were included in two runs. Germination data were collected from treated seed on split trials by immediately placing on (1) germination paper (rolled towels) after seven days and (2) in greenhouse potting soil after 14 days. A portion of the achenes was force air-dried at 20C for 48 hours and subsamples were tested for germination by the aforementioned methods. Mutagen treated achenes were air-dried for field (sandy soil in Florida) studies.

**Table 1. pH of Ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) at various concentrations.**

| | pH* | |
|---|---|---|
| Concentration | EMS | NTG |
| 50 μg/ml | 6.91 | 6.12 |
| 250 μg/ml | 6.39 | 5.94 |
| 500 μg/ml | 5.16 | 6.11 |
| 2500 μg/ml | 4.05 | 5.82 |
| 5000 μg/ml | 3.93 | 5.72 |

* Corning pH meter model #125

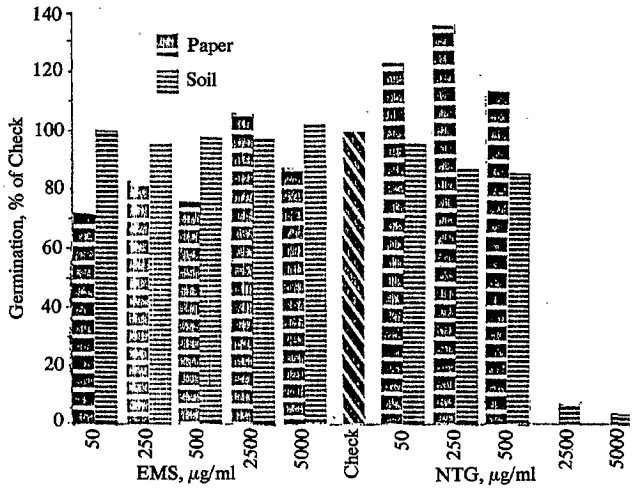
An experiment to determine the optimum time of infiltration was conducted by using 2500 μg/ml of both EMS and NTG for 10, 20, 30, 40, 50 ad 60 minutes. Achenes were air-dried and germination tests were conducted (1) in greenhouse soil and (2) in the field (sandy soil in Florida).

Achenes harvested from random selfed greenhouse and field plants were grown as head rows in the $M_2$ generation in the field (Fargo clay soil in Minnesota) at 36.3 M/ha in a 0.9 m row spacing. Data was collected for (1) morphological abnormalities, (2) reaction to race 1 of sunflower rust (*Puccinia helianthi)* under indirect artificial inoculation, (3) height of plants at 55 days from seeding, (4) bloom date of randomly selfed plants, (5) number of achenes produced on selfed plants and (6) oil content of achenes. Certain lines were advanced to the $M_3$ generation to confirm that the observed mutation was transmitted.

## RESULTS

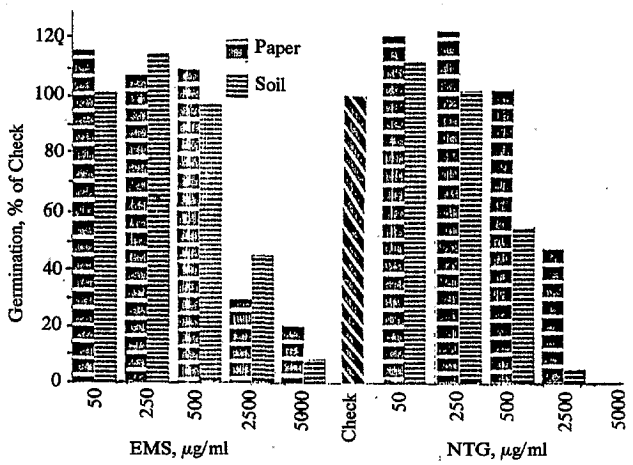
The germination percentage of achenes varied considerably between EMS and NTG at the five levels of concentration when placed on rolled paper towel or in potting soil immediately after treatment for one hour, Fig. 1. The germination differences between the EMS treatments on rolled paper towel were from 72 to 105 percent of the check whereas the differences were less pronounced in potting soil. Germination was considerably higher in NTG treated achenes than the check at the lowest concentrations 50, 250 and 500 μg/ml on rolled paper towels. NTG was lethal at concentrations of 2500 and 5000 μg/ml when tested on rolled paper towel. A similar trend was noted for germination in greenhouse potting soil. Germination was lower at 50, 250 and 500 μg/ml of NTG than that of check, Fig. 1., in potting soil.

**Figure 1. The effect of various concentrations of ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-nitrosoguanidine (NTG) on achene germination. Achenes were infiltrated with each chemical for 60 minutes under alternating tension of 0 to 1.36 atm. and immediately placed (1) on rolled paper towels and (2) in greenhouse potting soil.**



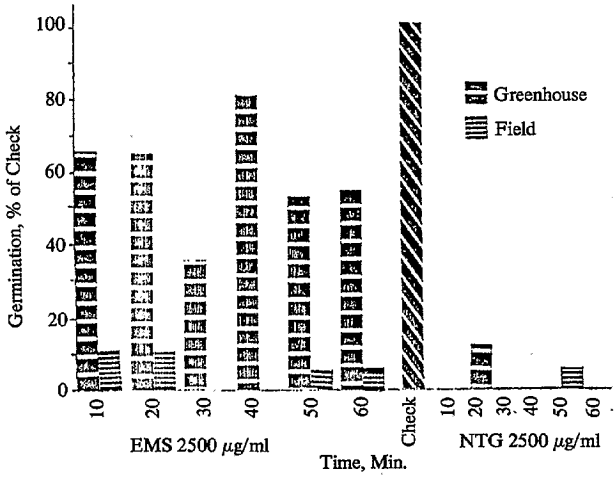

Once the seeds had been air-dried, germination trials were conducted on rolled paper towel and in potting soil, which gave similar results for the two mutagens, Fig. 2. There was an increase in germination in EMS and NTG treated achenes at 50 μg/ml irrespective of testing methods. EMS increased germination at the 250 and 500 μg/ml concentrations for both testing methods, whereas a significant increase in germination was observed in NTG treated achenes only at the 250 μg/ml concentration. There was a sharp decrease in germination in NTG treated achenes at 500 μg/ml concentration in potting soil. Both NTG and EMS were lethal at 2500 and 5000 μg/ml, Fig. 2.

**Figure 2. The effect of various concentrations of ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) on achene germination. Achenes were infiltrated with each chemical for 60 minutes under alternating tension of 0 to 1.36 atm. and air-dried before being placed (1) on rolled paper towels and (2) in greenhouse potting soil.**



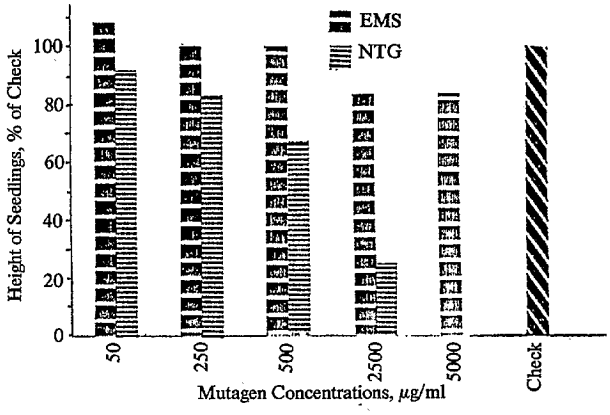
There was a significant difference in germination when the achenes were infiltrated at different time intervals and air-dried before testing either in rolled paper towels or in the field, Fig. 3. NTG inhibited germination irrespective of the testing method used. The germination of EMS treated achenes varied from 35 — 91 percent of the check in the rolled paper towel test, whereas 0 — 11 percent of the check in the field test was noted. There was only 11 percent germination in NTG treated achenes in rolled paper towel (infiltrated for 20 minutes) and five percent germination in field (infiltrated for 50 minutes).

**Figure 3. The effect of mutagen infiltration time in germination of sunflower achenes. The mutagens ethyl methanesulphonate (EMS) at 2500 μg/ml and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) at 2500 μg/ml were applied to achenes by infiltrating at alternating tensions of 0 to 1.36 atm. for 10, 20, 30, 40, 50 and 60 minutes, air-dried and placed (1) in greenhouse potting soil and (2) in field soil.**




Seedling height at 20 days showed that NTG was more damaging than EMS at all concentrations, Fig. 4. A slight stimulation of plant growth was noted for EMS at the 50 μg/ml concentration as compared to the check. At the two highest concentration levels the height was reduced to 83% of the check. Height of plants treated with NTG ranged from 92 to 25 percent of the check between the 50 and 2500 μg/ml concentrations. No plants survived at the 5000 μg/ml level of concentration.

**Figure 4. The influence on seedling height of various concentrations of ehtyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) infiltrated into sunflower achenes seeded in greenhouse potting soil.**




Plant height, measured at 55 days from seeding, Table 2, resulted in three treatment means, EMS 2500, EMS 5000 and NTG 2500 μg/ml, significantly shorter than the check. At 55 days of age, most plants had initiated floral buds. Those that had initiated floral buds were in a rapid stage of growth where up to 5 cm per day increased height was possible (Lofgren, unpublished data). Shorter plants were undoubtedly in the pre-bud stage and therefore growing more slowly. Twelve plants were significantly shorter than any check plant for a calculated mutation frequency of 0.90%. final plant heights were quite uniform.

Bloom date was significantly delayed for each of the highest treatments, EMS 500 μg/ml and NTG 2500 μg/ml, Table 3. These plants were later to initiate floral buds and therefore were shorter at 55 days of age as noted above. Significantly earlier blooming plants were noted in the treatments EMS 250 (1) and NTG 50 (2).

**Table 2. Mean plant height and standard error for 55 day old plants in the M2 generation grown in Fargo clay soil in Minnesota following treatment with various concentrations of Ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG).**

| Treatment, μg/ml | No. of Plants | Plant Height, cm $\bar{x}$ | $s_{\bar{x}}$ | Range |
|---|---|---|---|---|
| EMS 50 | 230 | 97 | 16 | 45 — 127 |
| EMS 250 | 125 | 101 | 14 | 30* — 122 |
| EMS 500 | 183 | 104 | 17 | 12* — 135 |
| EMS 2500 | 84 | 79* | 18 | 15* — 122 |
| EMS 5000 | 54 | 75* | 14 | 45 — 95 |
| check | 160 | 100 | 16 | 60 — 130 |
| NTG 50 | 193 | 97 | 13 | 40 — 122 |
| NTG 250 | 180 | 92 | 16 | 45 — 120 |
| NTG 500 | 232 | 89 | 20 | 12* — 125 |
| NTG 2500 | 52 | 78* | 20 | 22* — 109 |

* Significantly shorter than the check, the frequency of individual plants that were shorter for the various treatments were EMS 250 (2), EMS 500 (1), EMS 2500 (2), NTG 50 (1), NTG 500 (2) and NTG 2500 (4).

**Table 3. Mean bloom date and standard error for plants in M2 generation grown in Fargo clay soil in Minnesota treatment with various concentrations of Ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG).**

| Treatment, μg/ml | No. of Plants | Bloom date, days for seeding, May 25 $\bar{x}$ | $s_{\bar{x}}$ | Range |
|---|---|---|---|---|
| EMS 50 | 94 | 70.0 | 1.7 | 63 — 73 |
| EMS 250 | 60 | 69.5 | 2.7 | 58* — 74 |
| EMS 500 | 78 | 69.5 | 2.1 | 59 — 74 |
| EMS 2500 | 26 | 71.3 | 3.2 | 59 — 74 |
| EMS 5000 | 14 | 72.8* | 1.3 | 70 — 74 |
| Check | 50 | 69.5 | 2.3 | 61 — 74 |
| NTG 50 | 77 | 70.1 | 2.6 | 58* — 74 |
| NTG 250 | 67 | 70.2 | 1.6 | 67 — 74 |
| NTG 500 | 79 | 70.5 | 2.2 | 59 — 7a4 |
| NTG 2500 | 11 | 72.8* | 1.1 | 71 — 74 |

* Mean significantly different from check, EMS 250 had one and NTG 50 had two plants which bloomed significantly earlier than any check plant.

The mean number of achenes per selected plant was found to differ significantly from the check only for the NTG 2500 μg/ml treatment, Table 4. The number of plants used to arrive at this mean was quite small but the high individual was still below the highest in the test. No individual plant gave significantly higher achene number than the high check. Considerably higher number of potential sites for achene formation were present on the sunflower head but under selfing conditions the counts obtained here were considered average or above for inbred lines.

The EMS 5000 μg/ml treatment had a mean 100 achene weight which was significantly lower than the mean of the check, Table 5. This can be attributed to the association with later bloom and lower oil content, Table 6, of this particular treatment. Individual plants had achene weights over a wide range but no significant difference was noted. Dahlgren H6B had a heavy (and large) achene which differed from most oil-type achenes in (1) in air space between the hull and kernel and (2) an ovate, acute shape.

Five individual plants; EMS (1), EMS 5000 (1), and NTG 500 (3) μg/ml; had significantly lower oil content of achenes than the lowest check plant, Table 6. Unfortunately, achenes with significantly higher oil content were not observed.

**Table 4. The mean number of achenes, standard error, and range of achene numbers from selected plants for treatments of Ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguandine (NTG) in the M2 generation.**

| Treatment, μg/ml | No. of Plants | Achenes Per Plant Mean | $s_{\bar{x}}$ | Range |
|---|---|---|---|---|
| EMS 50 | 84 | 553 | 153 | 212 — 837 |
| EMS 250 | 48 | 594 | 158 | 292 — 926 |
| EMS 500 | 61 | 620 | 134 | 333 — 906 |
| EMS 2500 | 21 | 606 | 149 | 192 — 832 |
| EMS 5000 | 12 | 519 | 238 | 4 — 728 |
| Check | 40 | 585 | 126 | 366 — 852 |
| NTG 50 | 54 | 613 | 209 | 239 — 891 |
| NTG 250 | 53 | 631 | 170 | 19 — 955 |
| NTG 500 | 47 | 571 | 208 | 117 — 991 |
| NTG 2500 | 5 | 728* | 226 | 364 — 950 |

* Mean significantly higher than the check based on the standard error of the check.

**Table 5. Mean weight of 100 achenes, standard error, and range of 100 achene weights for Ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) treatments from plants in the M2 generation.**

| Treatment, μg/ml | No. of Plants | Weight of 100 Achenes, g $\bar{x}$ | $2_{\bar{x}}$ | Range |
|---|---|---|---|---|
| EMS 50 | 72 | 6.85 | 1.12 | 5.39 — 10.21 |
| EMS 250 | 48 | 6.89 | 0.92 | 4.69 — 9.06 |
| EMS 500 | 83 | 6.80 | 1.73 | 5.14 — 9.46 |
| EMS 2500 | 20 | 7.21 | 1.00 | 5.70 — 9.52 |
| EMS 5000 | 3 | 5.87* | 0.79 | 5.09 — 6.66 |
| Check | 45 | 7.26 | 1.28 | 4.92 — 9.70 |
| NTG 50 | 54 | 6.66 | 0.81 | 5.32 — 8.98 |
| NTG 250 | 52 | 7.29 | 0.85 | 5.58 — 9.10 |
| NTG 500 | 46 | 7.61 | 1.06 | 5.70 — 10.19 |
| NTG 2500 | 5 | 6.13 | 0.52 | 5.55 — 6.69 |

* Mean significantly lower than the check. No individuals found to differ significantly from the range of the check.

**Table 6. Mean oil content, standard error, and range of oil content for selected plants for treatments of Ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) in the M2 generation.**

| Treatment, μg/ml | No. of Plants | Oil Content, (%) $\bar{x}$ | $s_{\bar{x}}$ | Range |
|---|---|---|---|---|
| EMS 50 | 85 | 38.5 | 2.71 | 31.3 — 43.8 |
| EMS 250 | 48 | 38.4 | 2.21 | 31.4 — 43.3 |
| EMS 500 | 61 | 36.9 | 2.80 | 30.3* — 43.2 |
| EMS 2500 | 21 | 37.2 | 2.85 | 31.9 — 42.5 |
| EMS 5000 | 11 | 35.5* | 3.22 | 30.8* — 40.5 |
| Check | 39 | 38.1 | 2.39 | 33.3 — 41.6 |
| NTG 50 | 54 | 37.3 | 2.24 | 32.9 — 42.3 |
| NTG 250 | 52 | 37.6 | 1.93 | 33.5 — 40.2 |
| NTG 500 | 47 | 36.7 | 2.72 | 30.3* — 41.1 |
| NTG 2500 | 5 | 38.7 | 2.41 | 36.2 — 42.2 |

* Oil content of the mean or individual plant significantly lower than the check, the number of plants with lower oil content of the achenes were EMS 500 (1), EMS 5000 (1) and NTG 500 (3).

**Table 7. Frequency of mutations for several characteristics in the $M_2$ generation following treatment with various concentrations of Ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG).**

| Characteristic | Frequency (%) |
|---|---|
| Small, yellow and/or deformed lethal plants | 0.67 |
| Reduced plant height, 55 day old plants | 0.90 |
| Earlier bloom date | 0.59 |
| Improved resistance to race 1 *Puccinia helianthi* | 0.20 |
| Decreased resistance to race 1 *Puccinia helianthi* | 1.38 |
| Ageotropism of upper stems | 0.59 |
| Decreased oil content of achenes | 1.04 |

Calculated frequencies for seven characteristics; i.e., earlier bloom date and improved resistance to race 1 of *Puccinia helianthi;* would be desirable and the frequency of these was among the lowest observed. Progeny of earlier blooming and rust resistant plants performed similarly in the $M_3$ generation. No evaluation of hybrid performance of these lines had been made at the time of this writing.

## DISCUSSION

The results indicated that infiltration was a rapid method of treating sunflower achenes to induce mutations. The amount of air space can be reduced to nearly zero by infiltration in 60 minutes (Urs, unpublished data) and replaced by chemical solution to subject the kernel to the action of the mutagen.

Lethal doses of both EMS and NTG were revealed at 2500 and 5000 $\mu$g/ml. At 2500 $\mu$g/ml the dosage was potent enough to reduce survival of seedlings drastically even at a treatment time of 10 minutes. Greenhouse conditions were more favorable for the survival of treated seedlings because of controlled temperature and moisture conditions.

Mutations were observed for several characteristics, most of which were undesirable. The earlier blooming selections were of particular interest, since they would provide parental stock for earlier hybrids for double cropping, later seeding following disaster conditions, or allow the growing of sunflower in a shorter season. Undoubtedly many other mutations occurred which were phenotypically undetected, especially albino and male or female steriles. The frequency of mutation, in this study, although higher than those reported for other species, was still quite low. Phenotypically, except for the small yellow and/or deformed lethal plants, all mutant forms resembled the source material, H6B.

## LITERATURE CITED

ANON. 1977. Manual on mutation breeding. 2nd Edition, International Atomic Energy Agency, Vienna, Austria.

FICK, G.N. 1978. Breeding and genetics. *In Sunflower Science and Technology,* American Society of Agronomy, Madison, Wisconsin. p. 279 — 338.

SIMONS, M.D., 1979. Modification of host-parastie, interactions through artificial mutagenesis. Annual review of Phytopathology 17:75 — 96.

VOSKOBOINIK, L.K. & K.I. SOLDATOV, 1974. The research trends in the field of sunflower breeding for heterosis at the All-Union Research Institute for Oil Crops (VNIIMK). In Proceedings of the 6th International Sunflower Conference Bucharest, Romania. p. 383 — 369.

T1982GEN25

# GENETIC ADVANCES BY MASS SELECTION IN THE OIL PERCENTAGE OF SUNFLOWER (*HELIANTHUS ANNUUS* L.).

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## ABSTRACT

**In order to increase the oil content in the sunflower crop, in 1976 have been initiated a breeding programme by mass selection. It was used to open-pollinate variety Guayacan INTA, which has good performance in seed yield, but low oil content.**

**Three cycles of selection have been realized in 1977, 1979 and 1980. In 1978 it was impossible to obtain another cycle because of unfavorable environmental contitions. The plants were selectioned in the isolate plot and were analyzed individually for oil content by MNR method. The selection index was near 20%.**

**In the analysis of the results was observed a continuous genetics advance by selection. The regression coefficient for oil content was b = 13.18% of average improvement per cycle compared to the original poulation. Besides the coefficient of genetics variation were similar, what it would permit to continue the selection programme.**

**Complete paper not received at time of printing.**