# The Action of Methyl Methanesulfonate on *Triticale hexaploid*[1]

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ABSTRACT

The effects of MMS on *hexaploid triticale* seeds were analyzed evaluating several characters. $LD_{50}$ value was established in a laboratory test and calculated as 0.39%w/v 1 hour at 20°C. In the $M_1$ generation plants, practically no effects were induced by the mutagen, except for germination at the highest dose tested (0.5%). A selection of semidwarf mutants was realized in $M_2$ plants. Frequency of mutants increased with the dose and the highest value was obtained in the 0.4% w/v 1 hour at 20°C, which fits well with the $LD_{50}$ value reported here.

COMPENDIO

Se analizaron los efectos del MMS sobre semillas de *Triticale hexaploide* cv. Bacum evaluando diversos caracteres en la generación $M_1$. Basados en pruebas de laboratorio la $DL_{50}$ se calculó en 0.39%p/v 1 horas a 20°C.

Prácticamente no se detectaron efectos del mutágeno en plantas de la generación $M_1$, excepto por la germinación la cual disminuyó significativamente a las más altas dosis probada (0.5% ). En las plantas $M_2$ se efectuó una selección de mutantes semienanos. La frecuencia de mutantes se incrementó con la dosis y el valor más alto se obtuvo a 0.4% p/v 1 hora a 20°C, lo cual concuerda con el valor de la $DL_{50}$ informado en este trabajo.

## INTRODUCTION

It has been recommended that the use of mutation breeding methods should be only applied in those plants with a poor natural variability and if a specific problem has to be solved (9). Triticale is valuable crop, but with a short evolutionary history. Therefore, mutation induction has been suggested to overcome difficulties in the breeding of this plant (11). However, very few reports of mutation induction in triticale have been made (4, 17).

Methyl methanesulphonate (MMS) is an effective monofunctional alkylating agent (2), but it has not been used extensively because of its toxic action (12). Some authors report better results for mutation induction with MMS when compared with other alkylating agents (16, 19, 20).

As part of a mutation induction program involving an hexaploid triticale variety with good agronomic characteristics, but with some problems, we have collected performance data from $M_1$ and $M_2$ plants following seed treatment with MMS.

## MATERIALS AND METHODS

Visually selected seeds of the Bacom variety of hexaploid triticale were used. This variety was choosen because it presents good agronomic characteristics, but has a severe lodging problem (1). Batches of 100 seeds (with four replications) were washed in tap water for two hours.

Afterwards the seeds were treated with fresh solutions of MMS (Merck) prepared in demineralized water at the doses 0.10, 0.30, 0.60, 0.90 and

1 Received for publication in July 8, 1985.

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The author wishs to thank: CIMMYT for seed supply; Escuela de Agricultura del Estado de México for greenhouse and experimental field facilities; Dr. Rubén Chávez for field work supervision and Dr. Robert Bye for suggested revisions in the English text.

1.0% w/v 1 hour at 20°C, a water control was carried out together with the treated lots. Both the washing and treatments were conducted according to the method of Meneses and Rubluo (10). Seeds were germinated in a controlled growth chamber at 20°C and in darkness. Percent of germination was determined one month after planting and dates transformed and analyzed using the statistical analysis system in order to calculate the $LD_{50}$.

In another set of experiments, seeds were treated similarly at the MMS doses of 0.10, 0.20, 0.30, 0.40 and 0.50 w/v 1 hour at 20°C, also with a water control. After treatment, the seeds were immediately sown in a greenhouse under a totally randomized design with four replications. The percent of germination was determined a month after the sowing date. At the end of the growing season, the following characteristics were recorded: 1) plant height; 2) weight of the seeds per plant; and 3) number of tillers.

In order to sow the $M_2$ generation, a plant progeny selection was performed with the $M_1$ plants, taking into consideration the plant height and weight of the seeds per plant, selecting those with short plant length and with the the highest seed weight.

Three replications of each treatment (84 seeds each) were sown in the field to obtain the $M_2$ generation. Normal cultivating practice was carried out in the greenhouse and field experiments. The $M_2$ population was visually inspected for semidwarf plants and precosity (days to flower) and data were recorded.

## RESULTS AND DISCUSSION

The $LD_{50}$ for MMS in the Bacum variety of triticale was determined. Seedling survival at one month after planting was reduced to approximately 50% compared to the non-treated (control) population at the 0.39% w/v 1 hour at 20°C (Fig. 1). A dose within plus or minus 20% from that found by laboratory test as an average of the estimated desirable dose range has been suggested for use in mutation breeding experiments (9). For this reason, we used treatments of MMS at levels of 0.1, 0.2, 0.3, 0.4 and 0.5% w/v 1 hour at 20°C in order to determine the mutagenic action of MMS on triticale seeds.

A variance analysis of data showed that, except for germination, the action of MMS on $M_1$ parameters was not significant. Duncan's multiple range test for means of germination values (Table 1) shows that the strongest dose (0.5% ) differs from all the other treatments and that the other doses were not significantly different between them, with the exception of the control and that of 0.3%.

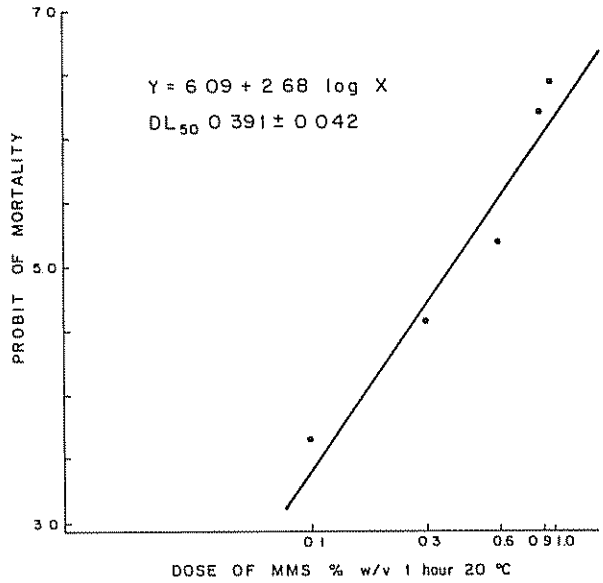


Fig. 1. Probit regression line for estimating the mortality induced in *Triticale hexaploide* Lart. (var Bacum) as exposed to different doses of MMS.

Table 1. Duncan test* for multiple rank of means of germinations** of $M_1$ seeds of *hexaploid triticale* var. Bacum treated with MMS.

| DOSE % w/v 1 hour 20°C | $\bar{X}$ | $S_{\bar{X}}$ |
|---|---|---|
| 0.0 | 65.20 a | |
| 0.1 | 63.45 ab | |
| 0.4 | 62.67 ab | 1.98 |
| 0.2 | 61.69 ab | |
| 0.3 | 57.11 b | |
| 0.5 | 35.52 c | |

* 5% significance.
** Germination in arc-sen values

These results suggest a slight sensitivity of triticale to the toxic action of MMS. This fact is probably due to the polyploid (hexaploid) nature of the triticale used in this work. These results are in agreement with those reported by Prassad and Goodward (13), who found a clear difference in the treatments between plants with different ploidy levels, reporting a greater sensitivity in the diploids as compared with tetraploid plants.

The proposed criterion (8) for semi-dwarf (reduced height) mutants is that the individuals be at least 20% less in height than the shortest control plants. In our study, the height of control population ranged between 95-120 cm. As a consequence,

Table 2. Characteristics of semidwarf mutants isolated from $M_2$ population of *hexaploid triticale* var. Bacum treated with MMS.

| MUTANT | Dose of MMS % w/v 1 hour 20°C | Height (cm) (cm) | Days-To Flower* | Number of Tillers | Weight/Seed |
|---|---|---|---|---|---|
| 1 | 0 1 | 67 | VR | 6 | 8 0 |
| 2 | 0 1 | 70 | L | 4 | 1.0 |
| 3 | 0.2 | 60 | L | 3 | 0.5 |
| 4 | 0 2 | 65 | N | 12 | 2 5 |
| 5 | 0.2 | 75 | R | 28 | 15.0 |
| 6 | 0.3 | 60 | R | 18 | 11 0 |
| 7 | 0.3 | 60 | R | 16 | 8 0 |
| 8 | 0 4 | 55 | N | 12 | 1 5 |
| 9 | 0 4 | 60 | R | 6 | 34 5 |
| 10 | 0 4 | 60 | R | 9 | 5 5 |
| 11 | 0 4 | 70 | N | 1 | 24 1 |
| 12 | 0 4 | 70 | N | 5 | 2 0 |
| 13 | 0 4 | 75 | VR | 3 | 3.0 |
| 14 | 0.4 | 75 | N | 21 | 4.0 |
| 15 | 0 4 | 75 | N | 10 | 14.0 |
| 16 | 0 5 | 60 | N | 17 | 7 0 |
| 17 | 0 5 | 65 | L | 3 | 22.0 |
| 18 | 0.5 | 65 | N | 2 | 2 0 |
| 19 | 0 5 | 75 | N | 13 | 9 5 |
| 20 | 0.5 | 75 | N | 5 | 4 0 |
| 21 | 0 5 | 75 | N | 3 | 1.0 |

* VR = Very Rapid (8 weeks) N = Normal (10-11 weeks)
R = Rapid (9 weeks) L = Late (12 or more weeks)

all those plants 75 cm tall or less were selected as semi-dwarf mutants. Table 2 shows the individual values for several characters of the semi-dwarf mutants (21 in total) selected in the $M_2$ population, and Fig. 2 shows the response of triticale to the different doses of MMS used here for the production of semi-dwarf mutants. In general, these responses increased with the dose. The highest frequency was at 0.4% , followed by a lower frequency at the highest dose (0.5% , Fig. 2).

Our results are in agreement with those reported by Rahaman and Soriano (15), who worked with these alkylating agents in rice and showed an increase of the mutation frequency with the dose; they also found a decrease in the mutation frequency at the higher doses. Furthermore, it has been known (2, 14) that a high mutation frequency is reached around $LD_{50}$. The results presented in Fig. 2 agree with this observation; the highest mutation frequency was obtained at the dose 0.4% and the $LD_{50}$ was calculated to be 0.39% (Fig. 1.).

The induction of dwarfness or semi-dwarfness is one of the characteristics more quickly obtained from mutation induction programs (18), and there

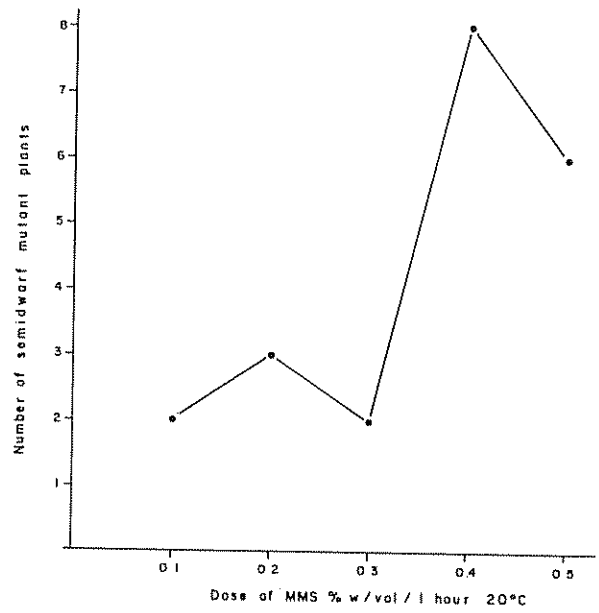


Fig 2. Effect of the doses of MMS on the induction of semidwarf mutants in *Triticale hexaploide* Lart var. Bacum.

are some reports of using mutagenic agents for the isolation of dwarf or semi-dwarf mutants (6, 21). Kiang and Halloran (7) treating soybean seeds with EMS, also isolated dwarf and earliness mutants, and suggested a relationship of height and maturity implying the height probable presence of pleiotropic behavior between these two characters. This may be the case in some of our results (Table 2, plants 1, 5, 6, 7, 9, 10 and 13)

Gaul *et al* (3) used the term "efficacy" to indicate the ability of a mutagen to produce beneficial mutations. According to our results (Table 2 and Fig. 2), the MMS seems to be a mutagen with high "efficacy". Avoiding its toxic colateral effects through manipulations in the treatments (5), it can be a useful mutagen for plant breeding purposes.

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