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# REDUCTION IN THE FREQUENCY OF N-METHYL-N-NITROSOUREA-(MNU)-INDUCED CHLOROPHYLL MUTATIONS IN BARLEY BY PRETREATMENT WITH LOW DOSES OF MNU

*Kadri JÄRVE, Jelena TSÕMBALOVA.* NITROOSOMETÜÜLKARBAMIIDI POOLT INDUTSEERITUD KLOROFÜLLMUTATSIOONIDE SAGEDUSE VÄHENEMINE ODRAL PÄRAST EELTÖÖTLUST NITROOSOMETÜÜLKARBAMIIDI MADALATE DOOSIDEGA

*Кадри ЯРВЕ, Елена ЦЫМБАЛОВА.* УМЕНЬШЕНИЕ ЧАСТОТЫ ИНДУЦИРОВАННЫХ *N*-НИТРОЗО-*N*-МЕТИЛМОЧЕВИНОЙ (НММ) ХЛОРОФИЛЬНЫХ МУТАЦИЙ В ЯЧМЕНЕ ПОСЛЕ ПРЕДВАРИТЕЛЬНОЙ ОБРАБОТКИ НИЗКИМИ ДОЗАМИ НММ

The biological effect of alkylating agents is due to the modification of DNA bases, the main attention being focused on the DNA lesion $O^6$-methylguanine and its persistence in cells. The repair of this modification by $O^6$-methylguanine-DNA-methyltransferase in *E. coli*, mammalian tissues and cells is enhanced by pretreatment with low adapting doses of indirect-acting alkylating agents and of some nonalkylating agents (Frosina, Abbondandolo, 1985).

In higher plants, the adaptive response manifesting itself as a reduction of mutagenic effects has been observed in *Vicia faba* (Rieger et al., 1982), *Tradescantia* (Veleminsky et al., 1983) and some other plants. No adaptive response has been detected in *Arabidopsis thaliana* by pretreatment with N-methyl-N'-nitro-N-nitrosoguanidine (Gichner, Veleminsky, 1982). Reduction in frequency of MNU-induced somatic mutations in *Tradescantia* has been obtained by pretreatment with direct-acting alkylating agents such as ethylnitrosourea, methylmethanesulphonate and MNU.

In this paper we shall deal with the effect of low doses of MNU on barley seed germination, survival of plants in $M_1$, and the number of chlorophyll mutations in $M_2$ induced by the mutagenic dose of MNU.

## Methods

Barley seeds (1000 seeds of the variety 'Miina') were treated with MNU in 0.1 M $Na_2HPO_4$-citric acid, pH 7 (300 ml) at room temperature for 24 hours. Low adapting doses (0.1 or 0.2 mM) of MNU were applied during the first 18 hours of treatment and replaced by challenging concentrations (2 or 3 mM) for the last six hours. MNU was dissolved in DMSO up to 1% final concentration. The buffer solution with DMSO was used as the control. After incubation the seeds were washed for 1 hour in running tap water.

## Results and discussion

The mutagenic doses of MNU (2 and 3 mM) were chosen according to I. Orav (Орав et al., 1976). In that work, treatment with 1.6 mM MNU at pH 7 had induced chlorophyll mutations in $M_2$ of the barley variety

Effect of pretreatment with adaptive doses of MNU on $M_1$ germination, $M_1$ survival and $M_2$ chlorophyll mutation frequency in MNU-treated barley

| Treatment | | $M_1$ germination, % | $M_1$ survival, % | $M_2$ plants (number) | $M_2$ chlorophyll mutants | |
|---|---|---|---|---|---|---|
| adaptive dose, mM | mutagenic dose, mM | | | | number | % |
| none | none | 84.0 | 84.0 | 4722 | 0 | 0 |
| 0.2 | none | 58.8 | 50.0 | 8506 | 4 | 0.05 |
| none | 2 | 40.3 | 24.3 | 1698 | 43 | 2.53 |
| none | 3 | 28.7 | 0.7 | 32 | 3 | 9.38 |
| 0.1 | 2 | 43.8 | 25.4 | 2260 | 64 | 2.83 |
| 0.1 | 3 | 37.0 | 15.8 | 855 | 41 | 4.80 |
| 0.2 | 2 | 36.5 | 40.5 | 4642 | 35 | 0.75 |
| 0.2 | 3 | 42.1 | 6.1 | 427 | 13 | 3.04 |

'Kharkovsky 306' with frequency of 2.58%. As under the same conditions 0.1—0.2 mM MNU did not induce any chlorophyll mutations, these doses were chosen as adaptive ones (Орав et al., 1976).

As seen in the Table, the higher adaptive dose of MNU (0.2 mM) if used alone, had a very low mutagenic effect in $M_2$ and caused a significant reduction in the survival of plants in $M_1$. The strong toxic effect of MNU is well known. Treatment of seeds with 1 mM MNU at pH 7 for 24 hours had resulted in the survival of only 62.2, 74.0 and 44.3% of plants of the varieties 'Ingrid', 'Kharkovsky 306' and 'Otra', respectively (Прийлинн, Орав, 1983).

No reduction of toxic effects by pretreatment of seeds with low doses of MNU could be detected (Fig. 1). Pretreatment of seeds with adaptive doses reduced the frequency of chlorophyll mutations induced by MNU, the maximum reduction being about three-fold (Fig. 2).

The adaptive response in *E. coli* protects cells, beyond mutagenic effects, also from lethal damage. The mutagenic and lethal adaptions depend on different repair pathways. Therefore it has been suggested that besides $O^6$-methylguanine-DNA-methyltransferase, another enzyme, 3-methyladenine-DNA-glycosylase II, must also be induced during the adap-

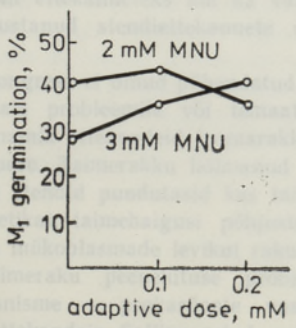


Fig. 1. Dependence of $M_1$ germination rate on the adaptive dose of MNU.

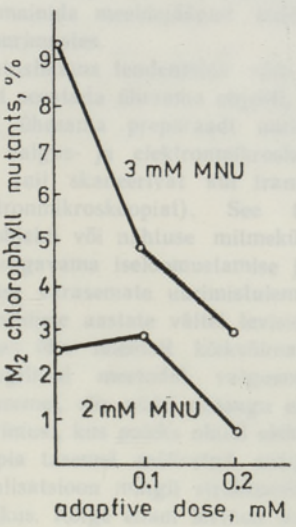


Fig. 2. Dependence of $M_2$ chlorophyll mutation frequency on the adaptive dose of MNU.

tion. P. Karran et al. (1982) have suggested that 3-methyladenine is the potentially lethal lesion whose repair is involved in the killing adaption.

Pretreatment of barley seeds with low adaptive doses of MNU reduced the frequency of MNU-induced chlorophyll mutations and did not have any effect on lethal damage. Further investigations will show whether the pretreatment with adaptive doses of MNU has any effect on removal of $O^6$-methylguanine from DNA MNU-treated barley seeds or not. Studies in the persistence of 3-methyladenine in modified barley seed DNA would also be informative.

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