SHORT COMMUNICATIONS

Mutagenicity of *N*-methyl-*N*-nitrosourea and *N*-ethyl-*N*-nitrosourea in durum wheat

Among the monofunctional alkylating mutagens, methylating agents are highly toxic and thus limited to use at lower concentrations\(^4,12\). Ethylating agents are less toxic so that relatively higher concentrations can be used which yield mutations in high frequencies\(^5,6,11,13\). The overall effect is that the ethylating agents show a high mutagenic efficiency\(^11,12,14\). Several reports have shown that at lower, equimolar concentrations the methylating mutagens are more effective than the ethylating ones (refs. 6, 10–12). Higher reactivity of the methyl group has also been reported in experiments with nucleic acids in vitro\(^9,19,21\). Methylation by 0.04 M MMS was, however, less effective in breaking the \(N\)-glucosidic bond and was less mutagenic than ethylation by 0.11 M EES in T4 phages\(^1\). EHRENBERG\(^3\) has pointed out that “the biological action pattern is determined not only by the intrinsic nature of the introduced alkyl but also by the manner in which it is introduced”.

The present experiments were initiated primarily in order to induce mutants of agronomic value in *durum* wheat. We found a significant difference in the frequency of mutations induced by MNU and ENU at equimolar concentrations. Furthermore, a literature survey indicated that most comparisons between ethylating and methylating agents have been made with alkanesulfonate or alkyl sulfate type mutagens and only a few have used alkyl-nitrosoureas at equimolar concentrations. Therefore, our results provide new information of interest in basic chemical mutagenesis.

**Material and methods**

Seeds of tetraploid wheat (*Triticum* *turgidum* (L) Thell. ssp. *turgidum* conv. *durum* (DESF)) cultivar Vijay, equilibrated to about 11% moisture content, were soaked for 18 h in 0.15 M citrate-phosphate buffer (pH 5). The soaked seeds were treated with 0.002 M MNU or ENU (Schuchardt) in the same buffer for 3 h. After treatment the seeds were thoroughly washed then soaked in deionized water for 2 h. During pre-soaking, mutagenic treatment and post-treatment soaking seeds were maintained at 24 ± 0.5° in a waterbath shaker. 100 seeds were treated in each of four replications. The concentrations of MNU and ENU used were determined by preliminary experiments in which the effect on seedling height of solutions ranging from 0.001 to 0.5 M were tested.

After post-soaking, the seeds were sown in the field. An equal number of controls was maintained at all stages. Survival of M1 plants and seed fertility were recorded at the time of harvest. The M1 plants were harvested individually and raised as plant progenies in the M2. The M2 population was scored for chlorophyll mutations at the seedling stage and for viable mutations at the time of heading and at harvest.

**Results and discussion**

MNU was more toxic than ENU, even at 0.002 M concentration, as shown by

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Abbreviations: EES, ethyl ethanesulfonate; EMS, ethyl methanesulfonate; ENU, \(N\)-ethyl-\(N\)-nitrosourea; MMS, methyl methanesulfonate; MNU, \(N\)-methyl-\(N\)-nitrosourea.
### TABLE I

**SURVIVAL AND SPIKE FERTILITY IN M₁**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant survival</th>
<th>Spike fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of plants</td>
<td>% of control</td>
</tr>
<tr>
<td>Control</td>
<td>350</td>
<td>100</td>
</tr>
<tr>
<td>MNU (0.002 M/3 h)</td>
<td>160</td>
<td>45.8</td>
</tr>
<tr>
<td>ENU (0.002 M/3 h)</td>
<td>207</td>
<td>59.1</td>
</tr>
</tbody>
</table>

*a From 350 sown seeds.

### TABLE II

**MUTAGENIC EFFICIENCY AND FREQUENCY OF CHLOROPHYLL AND VIVABLE MUTATIONS IN M₂**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of M₁ plant progenies</th>
<th>Number of M₁ plants scored</th>
<th>Number of M₁ plants segregating for chlorophyll + viable mutants</th>
<th>Mutation frequency % M₁ plants segregating Chlorophyll viable</th>
<th>Mutagenic efficiency (Mvp/1a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>350</td>
<td>35350</td>
<td>0</td>
<td>0</td>
<td>0.01850</td>
</tr>
<tr>
<td>MNU (0.002 M/3h)</td>
<td>160</td>
<td>41b</td>
<td>3160</td>
<td>109b</td>
<td>25.62 2.11 1.00</td>
</tr>
<tr>
<td>ENU (0.002 M/3h)</td>
<td>207</td>
<td>16b</td>
<td>19030</td>
<td>42b</td>
<td>7.72 0.22 0.18</td>
</tr>
<tr>
<td>χ² MNU vs. ENU</td>
<td>27.66</td>
<td></td>
<td>8109.92</td>
<td>8005.60</td>
<td></td>
</tr>
</tbody>
</table>

*a Mvp, Viable mutations per 100 M₁ plants; L, percentage lethality in M₁ (from survival data in Table I).

**comparisons of plant survival and of spike fertility (Table I). The frequencies of chlorophyll and viable mutations induced by MNU were significantly higher, based on the percentage of families segregating as well as per 100 M₁ plants, than those induced by ENU (Table II). Mutagenic efficiencies, as defined by KONZAK et al., were calculated by dividing the M₁ viable mutation frequency by percentage lethality in the M₁. The mutagenic efficiency of MNU was much higher than that of ENU.**

The mutagenicity of ENU on the gametophytic tissue of the moss *Pogonatum aloides* was reported to be higher than that of MNU, whereas the toxicity of the latter was greater in the range of 0.001 to 4 mM (ref.7). In barley also, the mutagenicity of 0.33 mM/24 h ENU treatment was higher than 0.12 mM/24 h MNU treatment. In Arabidopsis, concentrations of MNU and ENU required for inducing embryonic and chlorophyll lethals in 50% of siliquae were 0.16 and 0.72 mM respectively.

If the efficiency of a mutagen is measured as the highest mutation frequencies obtainable, ENU is certainly more efficient than MNU. However, at the low equimolar concentration used, both mutagenicity and mutagenic efficiency for viable mutations were higher for MNU than for ENU in *durum* wheat. Thus, MNU was found to be an efficient mutagen for wheat. In general, the results with these two ethylating and methylating nitrosoureas were similar to those reported using ethylating and methylating agents of the alkanesulfonate and alkyl sulfate classes, as discussed above.
Short Communications

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7 Franssen, M., and J. Moutschen, Mutagenicity of N-methyl nitrosoamides (NMU) and N-ethyl nitrosoamides (NEU) in the moss Pogonatum alloides (Hedw.) P. Beauv., Mutation Res., 16 (1972) 141–150.

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