ALTERATION OF THE MUTATION SPECTRUM IN BARLEY THROUGH TREATMENTS AT DIFFERENT PERIODS IN THE S PHASE OF DNA SYNTHESIS

M. S. SWAMINATHAN AND N. P. SARMA
Nuclear Research Laboratory, Indian Agricultural Research Institute, New Delhi

Changing the spectrum of mutations in a predictable manner and thereby achieving directed mutagenesis is an important goal of current mutation research.1-2-5-11-13 Nilan3 has reviewed the various reports of alteration in spectrum induced by specific mutagens or treatment conditions and has concluded that different mutagens and treatment procedures may induce some changes in the relative proportions of different types of mutations in higher plants. However, a precise control over mutation spectrum is yet to be achieved. Data are now available which indicate conclusively that when cells are treated with chemical mutagens such as Ethyl methane sulphonate (EMS) during the S phase of DNA synthesis, the mutation frequency is significantly higher.6-7-10 Recently, Creda-olmedo and Hanawalt13 found in Escherichia coli treated with N-methyl-N-nitro-N-nitrosoguanidine, that the maximum frequency of a given type of mutation occurs when the treatment is given at the time the gene is being replicated. It is also established now from studies in different test organisms, both plants and animals including man, that the DNA replication along a chromosome is asynchronous in time sequence. In some chromosomes the replication commences from the centromere and proceeds towards the telomere; in others the reverse happens. The possibility is thus open for affecting groups of loci preferentially by administering the treatment for short periods (pulse treatments) at different stages of S phase. In the present study, an experiment was undertaken to test the validity of this hypothesis.

In barley seeds germinated at 22°C, the S phase starts after about 16 hours of soaking in water as determined by the incorporation of 3H-thymidine and lasts for 4 hours.10 Based on this observation, seeds of the barley variety N.P. 104 were soaked in distilled water at 22°C for 16 hours. Starting from 16 hours of soaking when the S phase commences, the wet seeds were treated with 1% aqueous solution of EMS for 30 minutes. At the end of the treatment period, the seeds were washed in running water for 2 hours and then sown in a seedbed. Soaking in water also helps to create an anoxic condition and thereby a considerable degree of synchrony in division. Treatments were given in this way during 8 different stages of the S phase. Each M1 plant was selfed and seeds were collected from the main and secondary tillers separately. The M2 generation was raised as spike to row progeny and the population was scored carefully for chlorophyll mutations (Table I).

| Table I |

| Chlorophyll mutation spectrum in barley EMS : 1% for 30 minutes |

<table>
<thead>
<tr>
<th>Hours of soaking</th>
<th>No. of M1 spike progenies</th>
<th>No. of M2 seedlings scored</th>
<th>Total no. of chlorophyll mutants</th>
<th>Albina</th>
<th>Viridis</th>
<th>Chlorina</th>
<th>Xanthia</th>
<th>Stryicta</th>
<th>Tigrina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hrs.</td>
<td>Mins.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>00</td>
<td>423</td>
<td>13,011</td>
<td>86</td>
<td>22</td>
<td>22</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>30</td>
<td>341</td>
<td>8,916</td>
<td>58</td>
<td>28</td>
<td>17</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>00</td>
<td>287</td>
<td>8,594</td>
<td>85</td>
<td>40</td>
<td>13</td>
<td>17</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>30</td>
<td>363</td>
<td>11,845</td>
<td>140</td>
<td>51</td>
<td>19</td>
<td>4</td>
<td>47</td>
<td>2</td>
</tr>
<tr>
<td>18</td>
<td>00</td>
<td>262</td>
<td>8,869</td>
<td>134</td>
<td>60</td>
<td>22</td>
<td>15</td>
<td>27</td>
<td>4</td>
</tr>
<tr>
<td>18</td>
<td>30</td>
<td>362</td>
<td>10,399</td>
<td>88</td>
<td>33</td>
<td>13</td>
<td>14</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>19</td>
<td>00</td>
<td>276</td>
<td>8,323</td>
<td>44</td>
<td>12</td>
<td>16</td>
<td>14</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>30</td>
<td>200</td>
<td>5,624</td>
<td>34</td>
<td>16</td>
<td>14</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

The data reveal that xantha mutant begins to appear only in treatments made 60 minutes after the onset of the S phase. Tigrina appears in the next treatment phase. Albina, viridis, striicta and chlorina types occur in several treatments and the incidence of these is not confined to any particular fraction of the S phase. The complete absence of xantha in the first hour and its subsequent appearance in large numbers was the most conspicuous feature of this S phase fractionation experiment.

A major difficulty in the interpretation of such results is that a single phenotype may be governed by several loci. Thus, 250 to 300 loci are believed to be involved in chlorophyll synthesis in barley.8 Gustafsson4 suggested that 125 to 150 loci may be concerned with albina, 125 with viridis, 10 to 15 with xantha and 15 to 20 with rare types. Von Wettstein12 found that the three xantha loci studied by him were allelic. The genetics of xantha determination thus appears to be relatively more simple than that of albina, viridis, or chlorina. The specificity observed with regard
to the time dependence of its appearance may be due to the relatively few loci involved.

The effects of short duration treatments during the S phase are now being studied with simply inherited marker characters, both linked and unlinked. For this technique to be effectively used, bringing about a high degree of synchronisation of cell division, rapid diffusion of the mutagen and an effective evacuation of the mutagen at the end of the treatment period are essential. It is possible that loci which are not in the S phase may also be affected by the mutagen and hence only the relative frequencies of different classes of mutations can possibly be modified through this approach. The data of the present study are sufficiently promising to warrant a more detailed probe of the use of this technique in altering mutation spectrum.

ACKNOWLEDGEMENT

The junior author is grateful to the Indian Council of Agricultural Research for the award of Senior Research Fellowship during the tenure of which the present investigation is carried out.

5. —, Hereditas, 1963, 50, 211.

STUDIES ON GROWTH AND MUTATION FREQUENCY IN RICE IN TREATMENTS WITH DIMETHYL SULPHOXIDE AND ETHYL METHANE SULPHONATE

E. A. SIDDIQ, R. P. PURI AND V. P. SINGH

Nuclear Research Laboratory, Indian Agricultural Research Institute, New Delhi

THE introduction of dimethyl sulphoxide (DMSO), as a carrier in medicine and a solvent in biological works, has aroused much interest in the field of chemical mutagenesis. Earlier reports on DMSO emphasized its low toxicity and absence of any detectable side effect as a result of interaction with several drugs and aromatic compounds in particular. Probably this led Bhatia to assess its potentiating effect on chemical mutagens. The enhanced mutation frequency associated with a high percentage of survival in Arabidopsis thaliana realized by Bhatia led the present authors to make use of the penetrant-carrier in rice in which the husk hinders the easy and rapid uptake of chemical mutagens.

Swaminathan et al. concluded from preliminary studies that DMSO treatment was as toxic as any other chemical mutagen in rice. A more detailed study on the effect of DMSO alone and in combination with EMS carried out since then is presented in this paper.

The unhulled seeds of Tainan-3, a japonica variety of O. sativa presoaked in water for eight hours were treated with different concentrations of DMSO and DMSO + EMS. The effect of various treatments was measured as percentage reduction of germination, survival and seedling injury in the M sub 1 generation. The chlorophyll mutation frequency in the M sub 2 of a lot (seeds presoaked for 16 hours in water followed by treatment with EMS and EMS + DMSO for one hour) has been included so as to assess the effect of the penetrant-carrier on the mutation frequency.

The effect of DMSO alone as measured by the rate of germination, survival and growth rate indicated the carrier to possess local toxicity which followed a linear relationship with the concentration. At lower concentrations, the percentage of germination and survival was either on par with that of control or a little exceeded. However, data on growth rate showed proportionate decrease with increasing dose (Table I). Another feature of interest was that the percentage of seeds showing delayed germination increased with increasing concentration. It is known that higher concentration of DMSO causes high incidence of