Photoreactivation of Ultraviolet Irradiation-inactivated RNA from Tobacco Mosaic Virus on White Leaves of a Variegated Mutant of Xanthi Tobacco

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It had been observed by one of us (C. A. Knight, unpublished) that green, yellow, and white areas of variegated Xanthi tobacco plants (Nicotiana tabacum L. var. Xanthi nc) yield essentially the same numbers of lesions when inoculated with tobacco mosaic virus (TMV) or its RNA. Since the action spectrum for photoreactivation of viral RNA inactivated by ultraviolet radiation of wave length 254 nm. does not resemble the action spectrum for photosynthesis (Hidalgo-Salvatierra & McLaren, 1969), it seemed possible that photoreactivation (PR) can occur on leaves devoid of chlorophyll. We have tested this hypothesis on white leaves of a variegated mutant of Xanthi tobacco (Burk, Stewart & Dermen, 1964) and have observed a photorecovery of 30 to 35%.

Variegated Xanthi plants were grown from seeds kindly provided by Dr H. Dermen. The stems of variegated plants which had been grown to a height of 15 to 30 cm. were cut into 6 to 8 cm. pieces and grafted on to normal green stocks of Xanthi tobacco. Green leaves, variegated leaves, and all-white leaves issued from the grafts. Only the white leaves were used in the present experiments. When the white leaves were 8 to 14 cm. in length, they were randomly marked for assays with u.v.-inactivated TMV RNA for both dark survival and survival with photoreactivation (PR) (Bawden & Kleczkowski, 1959). The white leaves were dusted with 400-mesh carborundum and the plants were placed in a dark room illuminated only with non-photoreactivating red light (Hidalgo-Salvatierra & McLaren, 1969). The left half-leaf of each PR leaf, as viewed with the stem up, was inoculated with a suspension of unirradiated TMV-RNA at about 1.8 µg./ml. in 0.1 M-phosphate buffer (pH 7) containing bentonite at 1 mg./ml. Each corresponding right half-leaf was inoculated with a suspension containing bentonite at 1 mg./ml. and TMV-RNA at about 18 µg./ml. which had been irradiated with stirring at 254 nm. for 100 sec. at 31.6 x 10^-9 einsteins/ml./min. (0.024 J/ml.) in a quartz cuvette of 1 cm. path length; the survival of infectious TMV RNA under these irradiation conditions is about 10%. The dilutions at which the various irradiated and unirradiated samples of RNA were applied to plants were chosen so as to yield similar numbers of lesions on the opposite half-leaves of the test plants. Each PR leaf was washed free of its carborundum and the plants were placed in darkness overnight and next morning transferred to the glasshouse in daylight. The following day the leaves showed lesions which were counted; the chlorophyll content of the white leaves was only about 2% of that in normal green leaves.

The results of three experiments are summarized in Table 1 in terms of percentage survivals and average deviations from the means, as measured in the dark and under ‘black light’, together with the ‘percentage photoreactivation’ (Werbin et al. 1966). Generally half-leaves showed between 0 and 150 lesions. Only half-leaves with more than 10 local lesions are
included in the averages. The results are not changed significantly if leaves with less than 10 lesions are included. The application of 'Student's' t-test to the results of the three experiments showed the differences between TMV-RNA irradiation survivals in darkness and under PR conditions to be highly significant (probability < 1%).

Table 1. Survival of inactivated RNA on white leaves with and without photoreactivation

<table>
<thead>
<tr>
<th>TMV-RNA irradiated (µg./ml.)</th>
<th>Number of half-leaves</th>
<th>% survival*</th>
<th>% photoreactivation†</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>6</td>
<td>13 ± 4</td>
<td>27 ± 13</td>
</tr>
<tr>
<td>44</td>
<td>36</td>
<td>13 ± 9</td>
<td>24 ± 11</td>
</tr>
<tr>
<td>38</td>
<td>24</td>
<td>9 ± 5</td>
<td>20 ± 8</td>
</tr>
</tbody>
</table>

* % survival: number of lesions per half-leaf per µg. u.v.-irradiated RNA x 100

† % photoreactivation: \((1 - \frac{\log \text{surviving fraction in light}}{\log \text{surviving fraction in dark}}) \times 100\).

The observed photorecovery of from 30 to 35% on white leaves is within the range expected with normal tobacco (Evans & McLaren, 1969) and we conclude that photosynthesis is not required for photorecovery of u.v.-inactivated TMV-RNA.

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Department of Soils and Plant Nutrition  A. D. McLaren

J. Kirwan

O. Hidalgo-Salvatierra

E. D. Owen

Department of Molecular Biology  C. A. Knight

University of California

Berkeley

California 94720

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