1. Correlation of enzymatic activity with Wx dosage.

Recent studies on the waxy locus in our laboratory indicate that this locus probably is structural rather than regulatory in nature. One of the most important findings is that starch granule preparations from both diploid and tetraploid stocks show increased enzymatic activity with increasing numbers of Wx alleles.

Self pollinations and reciprocal crosses between wxco and Wx were made in both diploid and tetraploid stocks. The starch granules were prepared from developing seed frozen 16 days after pollination in the diploid series while those of the tetraploid series were prepared from endosperm collected 22 days after pollination.

The enzymatic activity is based on the measurement of the release of the ADP from ADP-glucose. It is clear from Table 1 that the enzymatic activity is related in a nearly linear manner with the number of Wx alleles. The enzyme preparations from the diploid series included the embryo which contains the same level of active ADP-G transferase in both wxco and Wx and its activity contributes about 1.5 μM ADP per Mg. of preparation. A correction has been made in the diploid series in order to get a hypothetical value for the enzymatic activity of endosperms.

The protein content of the tetraploid series was measured by the Lowry method. As shown in Table 2, the protein content increased about 0.2 μg per mg. of starch granules for each Wx allele added. It is obvious that the increase in enzymatic activity is almost proportional to the number of Wx alleles, and protein content above the base level, which might suggest that the Wx allele is responsible for the coding of the active enzyme protein while no protein is produced by the wxco allele.

Table 3 shows the percentage of amylose in starch of the diploid and tetraploid series; the percentage is measured on the basis of the Blue Value method (M. Ullmann and S. Augustat). In the case of Wx/Wx/Wx endosperms, the percentage of amylose increases with age and reaches a maximum of about 25% at maturity. As we know that the ADP-glucose transferase is responsible for amylose synthesis, it is not surprising that in both diploid and
tetraploid with two doses of \( Wx \) alleles the same percentage of amylose is found. The percentage of amylose increases with the increase in \( Wx \) alleles. However, the increase is not linearly proportional.

We have reported that \( Wx \) endosperm gives a measurable level of enzymatic activity and that this activity might be entirely due to the contamination from the closely adherent maternal tissue. Now we have been able to prepare the starch granules from \( Wx \) pollen grains where no question of contamination from maternal tissue exists. We still find low but measurable activity as shown in Table 4. Enzymatic activities are enhanced by the addition of a primer, maltodextrin. Three mutants, \( Wx^c \), \( Wx^B \), and \( Wx^{90} \), were studied in this experiment. They show the same \( K_m \) value, \( 5 \times 10^{-4} M \), and the same increase in activity with temperature within a certain range and are also similar in thermostability etc.

Starch granules also have been prepared from \( Wx \) pollen grains. This preparation is quite similar to the \( Wx/Wx/Wx \) endosperm preparation by all criteria employed.

### Table 1
Enzymatic activities of ADP-glucose transferase in diploid and tetraploid \( Wx \) dosage series

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Diploid</th>
<th>activities (( \mu M ) ADP/mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ( Wx )</td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>1 ( Wx )</td>
<td></td>
<td>6.9</td>
</tr>
<tr>
<td>2 ( Wx )</td>
<td></td>
<td>19.3</td>
</tr>
<tr>
<td>3 ( Wx )</td>
<td></td>
<td>27.3</td>
</tr>
<tr>
<td>Tetraploid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ( Wx )</td>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td>2 ( Wx )</td>
<td></td>
<td>15.2</td>
</tr>
<tr>
<td>4 ( Wx )</td>
<td></td>
<td>34.8</td>
</tr>
<tr>
<td>6 ( Wx )</td>
<td></td>
<td>46.6</td>
</tr>
</tbody>
</table>
Table 2
Protein content* of starch granules in tetraploid Wx dosage series

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Protein content (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Wx</td>
<td>1.1</td>
</tr>
<tr>
<td>2 Wx</td>
<td>1.6</td>
</tr>
<tr>
<td>4 Wx</td>
<td>2.0</td>
</tr>
<tr>
<td>6 Wx</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*Protein content was measured by Lowry method with bovine serum albumin as standard.

Table 3
The percentage of amylose of starch granules in both diploid and tetraploid series with regard to the number of Wx alleles

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Percentage of amylose*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diploid</td>
<td></td>
</tr>
<tr>
<td>0 Wx</td>
<td>2</td>
</tr>
<tr>
<td>1 Wx</td>
<td>6.5</td>
</tr>
<tr>
<td>2 Wx</td>
<td>14.0</td>
</tr>
<tr>
<td>3 Wx</td>
<td>17.5</td>
</tr>
<tr>
<td>Tetraploid</td>
<td>0.5</td>
</tr>
<tr>
<td>2 Wx</td>
<td>15.0</td>
</tr>
<tr>
<td>4 Wx</td>
<td>20.0</td>
</tr>
<tr>
<td>6 Wx</td>
<td>21.5</td>
</tr>
</tbody>
</table>

*The percentage of amylose was measured by the Blue Value method.
Table 4
The release of ADP mM/mg from ADP-glucose in preparations of starch granules from pollen grains of \(\text{WX}^c\), \(\text{WX}^b\), \(\text{WX}^9\) and \(\text{WX}\)

<table>
<thead>
<tr>
<th>Preparations</th>
<th>- maltodextrin</th>
<th>+ maltodextrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{WX}^c)</td>
<td>1.3</td>
<td>5.6</td>
</tr>
<tr>
<td>(\text{WX}^b)</td>
<td>1.4</td>
<td>4.6</td>
</tr>
<tr>
<td>(\text{WX}^9)</td>
<td>3.2</td>
<td>7.8</td>
</tr>
<tr>
<td>(\text{WX})</td>
<td>24.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Chia-Yin Tsai

2. **The use of \(\text{WX}, \text{ae}\) stocks in genetic investigations of the \(\text{WX}\) locus.**

For several years we have been using \(\text{WX}, \text{ae}\) stocks in our investigations of the \(\text{WX}\) locus. The interaction between \(\text{WX}\) and \(\text{ae}\) is such that the double mutant seeds have defective endosperms reminiscent of the sugary mutant. Seeds that are \(\text{WX}/\text{WX}/\text{WX}; \text{ae}/\text{ae}/\text{ae}\) seem to be distinguishable from \(\text{WX}/\text{WX}/\text{WX}; \text{ae}/\text{ae}/\text{ae}\) or \(\text{WX}/\text{WX}/\text{WX}; \text{ae}/\text{ae}/\text{ae}\) seeds. Thus if all stocks are made double mutant \(\text{WX}; \text{ae}\), in conventional analyses of crosses between 2 different \(\text{WX}\) alleles, the distinctive phenotypes can be used to detect the \(\text{WX}; \text{ae}\) recombinants as well as \(\text{WX}, \text{ae}\) contaminants.

Such a system has been used to repeat the conventional analysis of the cross between \(\text{WX}^9\) and \(\text{WX}^\text{Co}\). The F1 \(\text{Bz WX}^9\text{V} / \text{Bz WX}^\text{Co}\text{V} ; \text{ae}/\text{ae}\) was used to pollinate the tester stock \(\text{Bz WX}^\text{Co}\text{V} ; \text{ae}\). The reciprocal pollinations were also made. Of 36 plants from suspected \(\text{WX}/\text{WX}/\text{WX}; \text{ae}/\text{ae}/\text{ae}\) kernels on which test crosses by \(\text{Bz WX}^\text{Co}\text{V} ; \text{ae}\) were obtained, 31 were \(\text{WX}/\text{WX}; \text{ae}/\text{ae}\) as originally identified; 2 were \(\text{WX}/\text{WX}; \text{ae}/\text{ae}\) contaminants; 3 were \(\text{WX}/\text{WX}; \text{ae}/\text{ae}\) and were either misclassified or due to heterofertilization. Of 5 plants from kernels originally identified as \(\text{WX}/\text{WX}/\text{WX}; \text{ae}/\text{ae}/\text{ae}\) (contaminants), all were \(\text{WX}/\text{WX}; \text{ae}/\text{ae}\).

Of the 29 \(\text{WX}\) recombinants coming from the pollinations in which the \(\text{WX}^9/\text{WX}^\text{Co}\) heterozygote was the male parent, 18 were \(\text{Bz v} , 9 \text{ Bz v} , 1 \text{ Bz v} , \text{ and } 1 \text{ bz v}\). Table 1 compares these data to those gathered in 1960. The ratio of \(\text{Bz v}\) to \(\text{bz v}\) gametes in both tests is quite similar. However, in the 1963 test where