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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 wx^c 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 wx^c and Wx and its activity contributes about 1.5 mμM 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 wx^c 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. Ulmann 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, maltodextim. Three mutants, wx^{C}, wx^{B}, and wx^{90}, were studied in this experiment. They show the same Km value, 5×10^{-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

Preparations	activities(mμM ADP/mg.)
Diploid	
0 Wx	2.5
1 Wx	6.9
2 Wx	19.3
3 Wx	27.3
Tetraploid	
0 Wx	2.4
2 Wx	15.2
4 Wx	34.8
6 Wx	46.6

Table 2
Protein content* of starch granules in tetraploid Wx dosage series

Preparations	Protein content(μg/mg.)
0 Wx	1.1
2 Wx	1.6
4 Wx	2.0
6 Wx	2.4

*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

Preparations	Percentage of amylose*
Diploid	
0 Wx	2
1 Wx	6.5
2 Wx	14.0
3 Wx	17.5
Tetraploid	
0 Wx	0.5
2 Wx	15.0
4 Wx	20.0
6 Wx	21.5

*The percentage of amylose was measured by the Blue Value method.

Table 4
The release of ADP mμM/mg from ADP-glucose in preparations of starch granules from pollen grains of wx^C, wx^B, wx^{90} and Wx

Preparations	- maltodextim	+ maltodextim
wx^C	1.3	5.6
wx^B	1.4	4.6
wx^{90}	3.2	7.8
Wx	24.0	50.0

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2. The use of wx, ae stocks in genetic investigations of the wx locus.

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

Such a system has been used to repeat the conventional analysis of the cross between wx^{90} and wx^{Coe}. The F_1 Bz wx^{90} V / bz wx^{Coe} v; ae/ae was used to pollinate the tester stock bz wx^{Coe} v; ae. The reciprocal pollinations were also made. Of 36 plants from suspected Wx/wx/wx; ae/ae/ae kernels on which test crosses by bz wx^{Coe} v; ae were obtained, 31 were Wx/wx; ae/ae as originally identified; 2 were Wx/wx; Ae/ae contaminants; 3 were wx/wx; ae/ae and were either misclassified or due to heterofertilization. Of 5 plants from kernels originally identified as Wx/wx/wx; Ae/ae/ae (contaminants), all were Wx/wx; Ae/ae.

Of the 29 Wx recombinants coming from the pollinations in which the wx^{90}/wx^{Coe} heterozygote was the male parent, 18 were Bz v, 9 bz v, 1 Bz V, and 1 bz V. Table 1 compares these data to those gathered in 1960. The ratio of Bz V to bz v gametes in both tests is quite similar. However, in the 1963 test where

contaminants (which would be Bz V) could be detected, the percentage of Bz V gametes was much lower. This suggests that some of the Bz V recombinants detected in 1960 were due to contamination.

Table 1
The assortment of outside markers in Wx recombinants from the cross $\frac{Bz\ wx^{90}\ V}{bz\ wx^{Coe}\ v}$ for 1960 and 1963

	1963		1960	
	No.	%	No.	%
Bz v	18	62	63	58
bz v	9	31	27	25
Bz V	1	3.4	15	14
bz V	1	3.4	3	2.7
	29		108	

Oliver Nelson

3. The location of the waxy mutant H21.

One of the waxy alleles with which we originally worked was wx^{H21}. On the basis of recombinational frequencies (Wx) in intercrosses with C, 90, B, and a, it was felt that the most probable order was C, 90, H21. It has since been shown by conventional genetic analyses that C(Coe) is located distally to 90 as (Bz) C 90 (V).

A similar analysis has now been made for H21. Pollen from plants of the F_1 $\frac{Bz\ wx^{H21}\ V}{bz\ wx^{coe}\ v}$; $\frac{ae}{ae}$ was used to pollinate the tester stock bz wx^{Coe} v ae. Tassel collections were also made for estimates of Wx frequency by our standard pollen scoring techniques.

In a total population of 1,571,000 pollen grains from 9 plants, 776 Wx were detected or 49 x 10^{-5}. This compares with 46 x 10^{-5} estimated for the cross between C and H21 in our original experiments.