A PRELIMINARY NOTE ON THE FINE STRUCTURE ANALYSIS OF GLUTINOUS GENE IN RICE[1]

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In the past, even in the early nineteen fifties, the gene was considered as a functional entity within which genetical recombination did not occur. However, recent genetic analysis has increased enormously its resolving power owing to the development of microbial genetics and utilization of recombination as an important process of genetic analysis. Many studies have been done on the fine structure analysis of one gene. The results of Benzer (1955) with bacteriophage, of Pritchard (1955) with *Aspergillus*, and of Demerec, Blomstrand and Demerec (1955) with *Salmonella* and of others; all came to the same conclusion that there were several mutational sites within a single functional region, i. e. that one site would mutate in more than one ways from the wild type.

Nelson (1957) suggested that similar studies could also be made with higher organisms such as maize but these studies would be limited to the study of a gene with a goodsized population. With waxy gene, there existed the phenomenon of dimorphism, *Wx* pollen would be stained black and *wx* brown with I_2-KI solution. This was coupled with the fact that large population could be obtained for investigation when pollen grains were used as the unit of observation. The differential staining could be accounted for by the fact that the standard type *Wx* pollen grains consisted of amylose and amylopectin (1:3) in the starch granules, while the *wx* mutant contained amylopectin only. By crossing five independently occurring spontaneous *wx* mutant strains and examined the F_1 pollen grains, Nelson (1959) found recombination occurred between different sites within a single "cistron". According to Pontecorvo (1958), the ensemble of mutational sites at which the mutants were non-complementary to each other and showed *cis-trans* effect was the "cistron" which was first named by Benzer (1957). Still later, Nelson (1962), observed that high frequencies of *Wx* pollen grains which were higher than either of the parental stocks were obtained

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by both pollen analysis (102×10^{-5}) and by conventional backcross (99×10^{-5}). It was noticed that heterozygosity for mutant alleles at the *waxy* locus was a prerequisite for *Wx* frequencies above the parental stocks.

Glutinous rice is a cultivated variety for many generations in many parts of the world where rice is grown. The genetics of this character is similar to that waxy in maize (see literature citation of Chao, 1928). Instead of being designated as *Wx wx* in maize, the gene symbol is *Gl gl* in rice.

It occurred to us that by using Nelson's method of study, we might be able to find out the existence of genic sites of this locus if we could make crosses of different varieties of glutinous rice as they were found in different parts of the world after being isolated by space for so many generations of cultivation in the course of evolution. Accordingly, 31 varieties of glutinous rice (15 japonica and 16 indica) were selected and crossed in different combinations. If it could be arranged the male parent used must possess dominant genetic marker(s), so as to enable us to identify the authenticity of the hybrids later. Altogether, there were 56 corsses from which F_1 plants were obtained and the hybridity was authentic. Hybrids between indica and japonica with high sterility were discarded, because it would complicate the issue. Accordingly, most of the counts were obtained from hybrids of indica with indica and japonica with japonica. However, sterility was rather high even in some of the intra-sub-specific crosses.

The results are shown in table 1. The number of pollen granis of each floret would vary from 2,000 to 6,000 depending on the fertility of the hybrids.

Table 1. *Estimates of Gl frequencies of 56 crosses between 31 glutinous varieties in different combinations.*

Crosses (i=japonica, i=indica)	No. of pollen grains estimated	*Gl.* frequency ($\times10^{-5}$)
5301(j) × 5305(j)	200,140	1.99
5301(j) × 5307(j)	35,040	0
5302(j) × 5307(j)	69,100	0
5303(j) × 5122(i)	66,180	0
5303(j) × 5023(i)	66,770	4.49
5303(j) × 5808(i)	82,920	4.82
5307(j) × 5305(j)	68,430	16.07
5307(j) × 5504(j)	294,170	3.05
6002(j) × 5122(i)	37,210	5.37
6002(j) × 5123(i)	152,290	8.53
6003(j) × 5808(i)	143,790	5.56
6004(j) × 5808(i)	188,970	8.46
5504(j) × 5307(j)	136,320	19.07

5505(j) × 5307(j)	92,520	7.56
5506(j) × 5122(i)	79,390	12.59
5506(j) × 5029(i)	59,420	28.61
5506(j) × 5808(i)	153,940	23.38
5601(j) × 5305(j)	20,080	0
5103(i) × 5805(i)	36,530	0
5103(i) × 5809(i)	143,850	27.80
5109(i) × 5023(i)	106,520	3.75
5109(i) × 5808(i)	61,680	3.24
5109(i) × 5809(i)	24,070	4.15
5109(i) × 6001(j)	61,300	9.78
5109(i) × 6002(j)	91,050	15.37
5109(i) × 5401(j)	84,420	11.84
5109(i) × 5506(j)	228,300	52.12
5110(i) × 5023(i)	183,270	15.82
5110(i) × 5029(i)	147,190	5.43
5110(i) × 5803(i)	89,240	0
5110(i) × 5805(i)	126,140	11.09
5110(i) × 5809(i)	132,270	15.87
5110(i) × 6001(j)	135,540	2.21
5110(i) × 6003(j)	103,810	16.37
5110(i) × 6006(j)	94,480	13.75
5110(i) × 5401(j)	110,400	6.34
5110(i) × 5505(j)	26,550	26.36
5110(i) × 5506(j)	118,070	35.57
5110(i) × 5601(j)	99,130	13.11
5122(i) × 6001(j)	352,840	16.15
5122(i) × 6002(j)	65,950	28.81
5122(i) × 6003(j)	54,770	20.08
5134(i) × 5803(i)	211,560	15.59
5134(i) × 5809(i)	172,180	0.58
5009(i) × 6003(j)	120,580	4.14
5023(i) × 6001(j)	152,190	1.97
5023(i) × 6002(j)	108,550	0
5023(i) × 6003(j)	78,490	0
5023(i) × 6006(j)	99,200	3.02
5028(i) × 5805(i)	113,300	25.59
5028(i) × 5809(i)	151,140	3.30
5028(i) × 南260(i)	64,090	0
5029(i) × 6001(j)	147,200	3.39
5029(i) × 6002(j)	62,660	22.34
5029(i) × 6004(j)	18,450	0
南274(i) × 5803(i)	49,410	20.23

From table I, it can be seen that the majority of the hybrids have high *Gl* frequencies. It could be assumed, therefore, that intra-locus recombination would have taken place. From these preliminary results, 6 indica varieties were chosen based on the high frequency of *Gl* when they were in combination with other varieties. For comparison, the *Gl* frequencies of these chosen varieties are presented in table 2.

Table 2. *Estimates of Gl frequencies of the chosen indica glutinous stocks.*

Variety	No. of microspores estimated	*Gl* frequency ($\times 10^{-5}$)
5028	86,150	3.5
5029	91,980	4.3
5109	86,540	6.9
5110	84,640	7.1
5122	84,580	0
5805	83,720	0

There is variation of *Gl* frequency among these varieties. However, they are much lower than the hybrids in which they are involved. The occurrence of *Gl* in these parental stocks may be explained by spontaneous mutation. Diallel crosses are to be made so as to work out the complementation of these sites later.

Summary

Fifty-six different crosses were made by using 31 different glutinous varieties selected from stocks with distant origins. Nelson's technique was followed in the fine structure analysis of this gene locus. The *Gl* frequencies varied from different crosses. Some had no *Gl*. The combination with highest *Gl* frequency had 52.12×10^{-5}. For further complementation studies, 6 indica varieties were chosen based on their high frequencies of *Gl* in the hybrids they were involved. The *Gl* frequencies of these parental varieties were very much lower than that of their hybrids.

水稻糯性因子 (Glutinous gene) 的初步分析

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在此初步試驗中，將31種不同血緣的糯稻作雜交，得到56種雜種。照 Nelson 氏的方法：花粉經 I_2-KI 溶液染色，糯性 (*gl*) 花粉呈紅棕色，非糯性 (*Gl*) 花粉呈深藍色，進行糯性因子之分析。結果所得各個雜種的 *Gl* 頻度 (frequency) 自 0 到 52. 12 $\times 10^{-5}$ 各不相同。

在 *Gl* 頻度較高的雜種中，選取六種 indica 親本栽培。將作完全互交(diallel crosses)以進一步做 Complementation 試驗。

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