

To determine whether a component of the instability was due to the different growth factors in the media (2,4-D; 2,4,5-T; or 2,4,5-TP), all counts were pooled for this assessment according to media type (Table 2). Both diploid and haploid cultures did not appear to have been affected by the different growth factors in the media.

Whether complete chromosome stability would be realized upon continued growth of these cultures cannot be as yet determined. However, finding such a high proportion of cells with the stable karyotype after nine months of culturing suggests that they serve as the primary progenitors in future callus growth. Otherwise their relative frequency should decline with time.

The data reported here show that studies of somatic mutability would be profitably undertaken under such a technical regime of tissue culture. In the case of haploid callus it would seem at this time to be workable material for a study of induced mutation rates. Since a completely defined media is available, mutations affecting basic metabolic pathways could be selected for. It does not now seem out of place to suggest that this technology of tissue culturing could yield genetic information of the order found in microbial genetics of today with the exception of not having a high resolution recombination system.

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1. Linkage relationship between $\underline{Y-y}$ and $\underline{Rf_2-rf_2}$ on chromosome 6.

From tests involving $\underline{Rf_2}$ and a series of chromosomal translocations, it appears that $\underline{Rf_2}$ is located on the short arm of chromosome 6 (Beckett, Maize Genetics Coop. News Letter 36:31, 1962).

The data which follow were obtained from crosses involving $\underline{Y-y}$ and $\underline{Rf_2-rf_2}$. Since $\underline{Y-y}$ is reported to be located approximately 13 crossover units from the distal end of the short arm of chromosome 6, it was expected that $\underline{Y-y}$ would be closely linked with $\underline{Rf_2-rf_2}$.

From the following linkage data it appears that these two genes are not linked and therefore Rf₂-rf₂ is not on the short arm of chromosome 6.

Cross #1	Parent A		x	Parent B	
	<u>Rf₁Rf₁rf₂rf₂YY^T</u>			<u>Rf₁Rf₁Rf₂Rf₂YY</u>	
		Expected F ₂ Ratio		Actual F ₂ Ratio	
χ^2 for <u>Y-y</u> Segregation	3.43	(9)		69	Yellow Fertile
χ^2 for <u>Rf-rf</u> Segregation	3.43	(3)		35	Yellow Sterile
χ^2 for Linkage	1.37	(3)		35	White Fertile
χ^2 for Total	8.24	(1)		<u>14</u>	White Sterile
				<u>153</u>	
<u>P = .50 - .20 for Linkage χ^2</u>					

Cross #2	Parent C		x	Parent D	
	<u>rf₁rf₁Rf₂Rf₂YY</u>			<u>Rf₁Rf₁rf₂rf₂YY</u>	
		Expected F ₂ Ratio		Actual F ₂ Ratio	
χ^2 for <u>Y-y</u> Segregation	0.04	(27)		164	Yellow Fertile
χ^2 for <u>Rf-rf</u> Segregation	2.97	(21)		97	Yellow Sterile
χ^2 for Linkage	1.63	(9)		49	White Fertile
χ^2 for Total	4.64	(7)		<u>40</u>	White Sterile
				<u>350</u>	
<u>P = 0.20 for Linkage χ^2</u>					

Backcross #1

Parent C Parent D
rf₁rf₁Rf₂Rf₂YY x Rf₁Rf₁rf₂rf₂YY Parent D

		Expected BC Ratio	Actual Backcross Ratio
X^2 for <u>Y-y</u> Segregation	4.32	(1)	160 Yellow Fertile
X^2 for <u>Rf-rf</u> Segregation	4.32	(1)	150 Yellow Sterile
X^2 for Linkage	3.11	(1)	151 White Fertile
X^2 for Total	11.75	(1)	$\frac{213}{674}$ White Sterile
P = .10 - .05 for Linkage X^2			

Cross #3

Parent C Parent D
rf₁rf₁Rf₂Rf₂YY x Rf₁Rf₁rf₂rf₂YY
 (F₁) Rf₁rf₁Rf₂rf₂Yy x Rf₁Rf₁Rf₂rf₂yy Parent E

		Expected Ratio	Actual Ratio
X^2 for <u>Y-y</u> Segregation	0.14	(3)	141 Yellow Fertile
X^2 for <u>Rf-rf</u> Segregation	0.74	(1)	38 Yellow Sterile
X^2 for Linkage	0.74	(3)	127 White Fertile
X^2 for Total	1.62	(1)	$\frac{42}{118}$ White Sterile
P = .50 - .20 for Linkage X^2			

Cross #4	Parent A		x	Parent F	
	<u>Rf₁</u> <u>Rf₁</u> <u>rf₂</u> <u>rf₂</u> <u>YY</u>			<u>rf₁</u> <u>rf₁</u> <u>Rf₂</u> <u>Rf₂</u> <u>VY</u>	
		Expected F ₂ Ratio		Actual F ₂ Ratio	
x ² for <u>Y-y</u> Segregation	1.04	(27)	125	Yellow Fertile	
x ² for <u>Rf-rf</u> Segregation	1.24	(21)	114	Yellow Sterile	
x ² for Linkage	0.50	(9)	50	White Fertile	
x ² for Total	1.78	(7)	<u>40</u>	White Sterile	
			<u>329</u>		
<u>P = .50 for Linkage X²</u>					

Cross #5	Parent A		x	Parent G	
	<u>Rf₁</u> <u>Rf₁</u> <u>rf₂</u> <u>rf₂</u> <u>YY</u>			<u>rf₁</u> <u>rf₁</u> <u>Rf₂</u> <u>Rf₂</u> <u>VY</u>	
		Expected F ₂ Ratio		Actual F ₂ Ratio	
x ² for <u>Y-y</u> Segregation	.06	(27)	68	Yellow Fertile	
x ² for <u>Rf-rf</u> Segregation	.00	(21)	46	Yellow Sterile	
x ² for Linkage	.92	(9)	18	White Fertile	
x ² for Total	0.98	(7)	<u>22</u>	White Sterile	
			<u>154</u>		
<u>P = .50 - .20 for Linkage X²</u>					

The parents involved in each cross were test crossed to lines of know Rf genotypes in order to verify parental Rf genotypes.

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