In the above matings all recognized states of the Idf allele were utilized, i.e. mutable (high), mutable (very low), stable (but highly active phenotypically).

Thus Idf does not seem to substitute for two known transposable elements, Ac and Spm. A test to determine if Idf can substitute for Dt induced a_1-mutability will be made. However, another negative result is expected in this test for the following reasons: Two main features of transposable elements are lacking from the Idf spectrum of mutations: (1) No regular stable class (either phenotypically active or inactive) occurs among the Idf mutant types. (2) Non-diffuse segregants from Diffuse heterozygotes do not carry any modifiers of the diffuse phenotype as might be expected were transposable elements involved.

It is currently believed that the cause of Idf mutability is most likely not a transposable element but some other gene action control type mechanism.

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3. Karyotype stability of haploid and diploid maize root tissue cultures.

In our first attempts to determine karyotype stability in maize root tissue cultures, chromosomes were counted nine months after callus initiation and again after twelve months. Counts made at nine months showed all cultures to be diploid except one (8 cultures out of 9) which was a chimera of 2n/4n. The second round of counts made at 12 months showed all to be diploid (the culture with a chimera was not recounted due to poor growth) (MNL 1963).

On the basis of these results it was considered important to inquire into the relative stability of haploids. Haploids were obtained from the mating 22 A C r^g x 22 A C R^{sc} by selecting the resultant kernels having purple aleurone and colorless scutellum.

Such presumed haploids (with diploid controls) were germinated sterilely on agar media. When seedlings were transferred to modified White's media, root tips were removed and fixed in acetic alcohol for later confirmation of presumed chromosome numbers. The assessment of chromosome numbers in a cell was made of late prophase, metaphase, and early anaphase periods of cell division. Only rapidly growing tissue could provide the various periods for examination.

Three different growth factors were used in the media: 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, and 2,4,5-trichlorophenoxypropionic acid. Samples of callus tissue taken for chromosome counts were fixed in 3:1 acetic alcohol, stained and squashed in aceto carmine. Counts were made from temporary slides. Sampling of cultures for counts began three months after germination.

These initial assessments revealed that both haploid and diploid cultures had unstable chromosome complements, diploid cultures giving 2n, 4n and 8n counts and haploid cultures giving n, 2n, 4n and 8n counts. In addition, both sets of cultures gave occasional counts of 15 or 16 and 25 to 31. The odd counts obtained (15, 25, etc.) were not the result of chromatids falling apart -- in those cells in which odd numbers of chromosomes were counted, all chromosomes consisted of two chromatids. There was also a variance in "normal" chromosome counts -- haploid roots and callus tissue gave counts of 9, 10 and 11 (the mean value was 10); diploid cells gave 19, 20 and 21 chromosomes (the mean value was 20) per nucleus. In these cases also, two chromatids were seen for each chromosome. In none of the cells examined were chromosome bridges or fragments found.

This variability in chromosome number led to the suggestion that, since the previous counts had been made nine months after initiation, perhaps these cultures would also be stable after that length of time. Further counts of haploid and diploid cultures were then postponed until nine months after initiation.

To determine whether karyotype instability was dependent on time, counts were pooled according to the time the sample was taken after callus initiation (Table 1). The later samples of diploid cultures showed chromosome numbers to be stabilized primarily at the 2n level (two cultures were 2n/4n chimeras). Haploid cultures, even after nine months, showed an unstable chromosome number; however, the proportion of haploid to diploid cells increased with time. Since all sampled cultures did not grow at the same rate, relative time (first sample, second sample, etc.) was compared with absolute time (three months, four months, etc.). When this was done, diploids seemed to be stable at the diploid level generally by the second sample; haploids were unstable throughout.

Table 1

Chromosome counts (in percent) compiled by age of culture

	3 Months					4 Months					5 Months				
	n	2n	4n	8n	odd	n	2n	4n	8n	odd	n	2n	4n	8n	odd
Diploid Cultures*		67.38	19.56		13.05		52.23	27.3	5.65	14.81		75.01	24.99		
Number of Samples**	-	3	1	-	1	-	14	8	3	4	-	4	1	-	-

	6 Months					9 Months				
	n	2n	4n	8n	odd	n	2n	4n	8n	odd
Diploid Cultures*		50	50			6.84	63.28	29.87		
Number of Samples**	-	1	1	-	-	1[1]	13	2	-	-

	3 Months					4 Months					5 Months				
	n	2n	4n	8n	odd	n	2n	4n	8n	odd	n	2n	4n	8n	odd
Haploid Cultures*	16.37	55.56	13.45		14.62	28.58	46.02	21.74	3.65		35.6	35.42	28.97		
Number of Samples**	1	4	2	-	2	6	8	4	1	-	4	5	2	-	-

	6 Months					9 Months				
	n	2n	4n	8n	odd	n	2n	4n	8n	odd
Haploid Cultures*	47.37	38.14	12.46	2.02		66.47	30.09	1.65		1.79
Number of Samples**	1	6	2	1	-	6	6	1	-	1

*, ** See Table 2. [1] One cell in one sample

Table 2
Chromosome counts (in percent) compiled on the basis of media used

	2, 4-D					2, 4, 5-T					2, 4, 5-TP				
	n	2n	4n	8n	odd	n	2n	4n	8n	odd	n	2n	4n	8n	odd
Diploid Cultures*	-	53.64	25.28	7.44	13.63		51.16	48.84			5.52	52.56	25.12	4.23	12.57
Number of Samples**	-	14	6	1	2	-	7	1	-	-	1	14	6	2	3
Haploid Cultures*	34.06	38.83	16.42	3.05	7.65	46.01	32.41	21.58			54.06	45.94			
Number of Samples**	11	19	9	2	3	5	4	2	-	-	2	6	-	-	-

* Percentage of values adjusted to a sample size of 100

** Number of samples used in each category

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 Y-y and Rf_2-rf_2 on chromosome 6.

From tests involving Rf_2 and a series of chromosomal translocations, it appears that 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 Y-y and Rf_2-rf_2. Since 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 Y-y would be closely linked with Rf_2-rf_2.