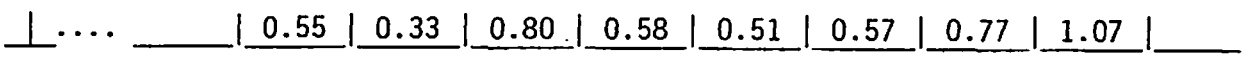
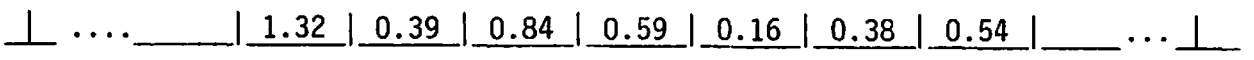
Strong negative interference and consequent high coincidence values were obtained in the majority of the three-point tests. This may be explained by a high degree of effective pairing in this region due to homology of the component segments. It can be postulated that various members of the gene cluster at the R region in R-ch complexes might have originated as tandem repeats through unequal crossing over. Two or more genes thus assembled together would acquire altered functions through mutation or position effect. On the basis of three-point data, tentative maps for the R region in Ecuador R-ch and New Mexican R-ch1 isolates are constructed as below:



Ecuador R-ch



New Mexican R-ch1

K. R. Sarkar, J. K. S. Sachan and Gita Guha

Characterization of the Spf and Dil factors of the spotted-dilute R system —

To explain the aleurone spotting behavior in the unstable R alleles designated as "spotted-dilutes" (R-sd), Sastry and Kurmi (MNL 44:101) postulated the presence of two dominant modifiers, Dil (diluting factor) and Spf (spotting factor). Our studies involving two isolates, R-sd2 and R-sd4, confirm these postulations. Homozygous R-sd plants on selfing occasionally yield intense, spotted and dilute kernels in addition to the spotted-dilute kernels. Data from selfing and testcrossing heterozygous R-sd/r plants involving isolates R-sd-4 are presented in Tables 1 and 2.

Segregation of Dil was regular in the majority of ears, showing 3:1 ratios on selfing and 1:1 on testcrossing. The other factor, Spf, however, did not exhibit regular Mendelian behavior and consideration of its linkage with R does not fully explain the erratic segregation pattern. Segregation of both Dil and Spf in R-sd2 stocks was more irregular, not conforming to the 1:1 ratio in most of the ears obtained in the testcrosses.

Selfing of heterozygous dilutes of the R-sd4 isolate (R Dil/R dil) yielded dilute and non-dilute (intense) kernels in a 3:1 ratio, confirming that the diluting factor is a dominant independent modifier of the S component of the R locus and is not concerned with the spotting phenomenon. Linkage data obtained by crossing R-sd stocks with the waxy translocation series and selfing or testcrossing

Table 1. Segregation on selfing of R-sd4/r plants.

Pedigree	Types of kernels							χ^2 3:1 for color	χ^2 3:1 for spotted: non-spotted	χ^2 3:1 for dilute: non-dilute
		Colored								
	Spotted-dilute	Spotted	Dilute	Intense	Total	Color-less	Total			
129.2-1	87	15	3	10	115	39	154	0.008	11.49	0.65
129.2-2	150	48	120	42	360	105	465	1.45	76.80	0.00
130.2-1	130	2	44	58	234	85	319	0.46	43.12	0.04
130.2-2	120	15	10	28	173	63	236	0.36	0.84	0.002
131.2-1	179	9	68	81	337	99	436	1.22	66.34	0.52
131.2-4	150	42	111	34	337	121	458	0.49	58.40	1.07
134.1-1	114	33	89	35	271	98	369	0.47	62.24	0.001
135.1-2	140	38	110	32	320	117	437	0.73	64.04	1.66
Total	1070	202	555	320	2147	727	2874	0.13	284.20	0.54

Table 2. Segregation in R-sd4/r x rr test crosses.

Pedigree	Types of kernels							χ^2 1:1 for color	χ^2 1:1 for spotted: non-spotted	χ^2 1:1 for dilute: non dilute
	Spotted-dilute	Colored				Color-less	Total			
		Spotted	Dilute	Intense	Total					
129.2-6 x 100 B.1	104	85	28	6	223	227	450	0.03	107.72	7.52
129.2-7 x 100 B.1	60	40	30	35	165	140	305	2.05	7.42	1.36
129.2-8 x 100 B.1	103	151	23	31	308	315	623	0.79	129.86	10.18
132-1 x 100 B.2	40	35	20	15	110	98	208	0.69	14.54	0.90
132-3 x 100 C	58	41	33	38	170	168	338	0.01	4.60	0.84
132-5 x 100 C	38	51	85	15	189	194	383	0.06	0.64	17.18
132-6 x 100 C	100	138	15	25	278	301	579	0.91	141.02	8.28
132-7 x 100 C	87	132	16	19	254	249	503	0.05	133.28	9.06
133.2-3 x 100 A	119	115	23	35	292	299	591	0.08	106.08	0.22
133.2-4 x 100 A	68	93	26	5	192	210	402	0.81	88.02	0.08
Total	777	881	299	224	2181	2201	4382	0.09	5.90	0.38

the translocation heterozygotes indicated that Dil is most probably located on chromosome 10. A relative estimation of anthocyanin content for kernels in segregating ears showed that dilute kernels contain 4-10 times less anthocyanin than the normally pigmented ones.

Spf failed to produce any response in standard R. It may be suggested that the R allele in R-sd isolates carries a controlling element conditioning it for Spf response. In crosses of R-sd4 with 15 geographical R isolates, only two produced any response in F_1. In the rest of the crosses solid-colored kernels were obtained. In F_2, nine of these crosses yielded a 13:3 (non-spotted:spotted) ratio, indicating no response of these R alleles to Spf. Six isolates, however, showed a 3:1 (spotted:non-spotted) segregation, suggesting that these alleles are responsive to Spf action.

Spotting patterns in the isolates are distinct and can be easily identified. Spotted-dilute phenotypes could be reconstituted by crossing "spotted only" (Spf, no Dil) derivatives from R-sd4 with "dilute only" (Dil, no Spf) individuals from R-sd2, R-sd6 and R-ch. Spf and Dil seem to be autonomous and the two phenotypes are superimposed in spotted-dilute kernels. However, spotting patterns in these reconstituted types were not the same as in R-sd4, indicating that Spf is the general regulator and that distinguishable phenotypes are due to different "states" of the R locus in these responsive alleles.

Spf and Spm show close parallelism in behavior. However, when Spf was crossed with c2-m2 (no Spm) to observe whether it can induce Spm, only spotted-dilute phenotype was recovered. Crosses between R-sd and a Dt produced overlapping patterns of the two phenotypes in F_1. Testcross with a a gave a 1:1:1:1 segregation for intense, spotted, dotted and colorless kernels, suggesting that these systems operate independently of each other.

A dosage effect study of Spf revealed that the number of spots (mutations) per kernel increases on increasing Spf dose from one to three, the relationship being almost linear. Average numbers of spots per kernel with 1, 2, and 3 doses were 39, 227 and 300 respectively. The size of the spots also showed an increase with increased dosage, although their shape and pigmentation intensity were relatively unaltered.

In crosses between R-mb and R-sd, it was further observed that the R-sd allele is not paramutable. The number of spots on R-sd kernels and their shape, size and intensity of pigment remained unaltered following association with the paramutagenic R-mb allele. The heterochromatic knob, K, on chromosome 10 was found to repress the action of Spf to some extent, reducing the number of spotted

kernels. In the testcross ears from r K/R-sd k and r k/R-sd k heterozygotes, the percentage of spotted kernels among colored kernels in the former was 46.8% and 81.0% in the latter.

In order to establish linkage relationships of Spf and Dil, the two spotted-dilute isolates were crossed with golden plant, colorless seed testers (g r-g). From the testcross segregations the following linkage values were estimated:

	R-sd2	R-sd4
g-R	12.83 ± 0.99	14.16 ± 0.98
g-Spf	35.86 ± 1.42	27.84 ± 1.26
g-Dil	33.51 ± 1.39	49.32 ± 1.41
R-Spf	41.62 ± 1.46	32.61 ± 1.32
R-Dil	34.29 ± 1.40	48.53 ± 1.41
Spf-Dil	46.07 ± 1.47	51.07 ± 1.41

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Induction of mutations with tassel and pollen treatments — In a search for a suitable technique for induction of mutations, a number of chemicals were tried for seed, tassel and pollen treatments in an inbred, stock 2. Seed treatments were not very effective, as the concentrations used were rather low. The chemical mutagens were EMS (ethyl methane sulfonate), DES (diethyl sulfate), NMU (nitroso methyl urea), NEU (nitroso ethyl urea), BMS (butyl methane sulfonate) and NG (nitroso guanidine). Tassel treatments involved dipping the cut end of

Table 1. Mutation frequencies in tassel and pollen treatments.

Treatment	Percentage of M2 families segregating		
	Chlorophyll mutants	Endosperm mutants	Floral & ear Abnormalities
EMS 0.03% T	1.7	16.5	23.1
0.05% T	3.9	16.5	9.3
DES 0.03% T	0.0	3.8	21.8
0.05% T	4.0	10.6	14.1
NMU 0.005% T	0.9	5.4	20.5
0.0075% T	2.1	8.4	17.6
NEU 0.005% T	0.06	12.3	6.1
0.0075% T	0.0	12.0	9.0
BMS 0.03% P	12.5	0.0	25.0
NG 0.35% P	7.3	20.2	7.13

T = tassel treatment; P = pollen treatment

a tassel for 48 hours in the mutagen solution, which was replaced periodically (6 hours interval), and collecting pollen from these tassels for pollination. For pollen treatment mineral oil was used for suspension of the chemicals. Concentrations and mutation frequencies are presented in Table 1 (see preceding page). NG appears to be the best treatment in these studies.

J.K.S. Sachan and K.R. Sarkar

Interesting chlorophyll mutants — In the course of our mutagenesis studies, we came across two interesting chlorophyll mutants. The first one was luteus type, having fine green spots on shining lemon yellow leaves. It originated from 15 Kr gamma ray seed treatment. The spots were sharp and present in all leaves, and the mutants survived up to the 4 to 5 leaf stage only. Number of green spots per leaf varied from 5 to 15. The mutant segregated in a 3:1 ratio in M2 (116 normal:34 mutant seedlings) and was maintained in heterozygous condition. In M3 and M4 families this mutant also appeared, but in addition to the spotted-luteus (l*-sp) types, luteus (no spots) and albino seedlings were also obtained. This may be a case of an unstable gene under the influence of controlling elements. In an attempt to locate this mutant, crosses with waxy translocation lines were made. Linkage indications with Wx were obtained in crosses involving T4-9g, T5-9a and T6-9 4505.

We called the second mutant "yellow virescent," as it differed from yellow-green and virescent. In M2, it was very weak with narrow leaves, unbranched tassels and no silk. There was 50% mortality before flowering, but the appearance and performance improved in M3 and M4. This mutant was crossed with the waxy translocation series and linkage data showed significance in crosses with T4-9c, T4-9b and T9-10b.

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Pollen grain diameter in maize — Studies on the pollen grain size in a number of inbred lines of maize revealed some interesting findings. Fully mature anthers which would shed pollen in an hour's time were collected from the main axis of the tassel on the second day of pollen shedding and preserved in 70% alcohol. Pollen samples from ten anthers from a tassel were stained in acetocarmine, and 25 random grains were measured. Thus, means of 50 observations from two plants constituted the pollen grain size of each inbred. Mean pollen diameter in two separate sets of inbreds, the first set comprising 41 lines grown in the summer at Delhi and the second set of 73 inbreds grown in the winter at Hyderabad, showed wide and significant line-to-line differences. Pollen diameter in these lines ranged from 81.9 to 114.1 micra. The frequency distribution was quite normal.