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RECESSIVE ALLELES FOUND AT *R* AND *C* LOCI IN MAIZE STOCKS SHOWING ABERRANT RATIO AT THE *A* LOCUS[1]

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ABSTRACT

Corn stocks showing virus-induced aberrant ratio (AR) at the "*A*" locus were found to have recessive alleles at the *R* and/or *C* loci. Since by the known pedigree these loci should be homozygous dominant, the results suggest an inactivation of maize genes by a mechanism as yet unknown. The presence of recessive alleles at these additional loci can explain the segregation ratios obtained in these particular stocks.

H. H. McKINNEY was the first to observe a mutation in a virus and interpret it as such (McKINNEY 1929, 1935). Throughout his career, he remained interested in mutants and strains of plant viruses and in possible genetic interactions between viruses and plants. Eventually, he and G. F. SPRAGUE found evidence of such an interaction between maize and three different plant viruses, barley stripe mosaic, wheat streak mosaic, and corn lily fleck viruses (SPRAGUE, McKINNEY and GREELEY, 1963; SPRAGUE and McKINNEY 1966, 1971). The most thoroughly investigated interaction is the aberrant-ratio (AR) phenomenon, which is a marked deviation from expected segregation ratios of kernel phenotypes in F_2 and subsequent progeny of a cross between a virus-infected male parent and an uninfected female parent. SPRAGUE and McKINNEY (1966) reported AR at four different loci. At the loci of interest to this report, the female parent used by McKINNEY and SPRAGUE was *aa A2A2 CC C2C2 RR*, the male parent was *AA A2A2 CC C2C2 RR*, (SPRAGUE, personal communication) and the virus was the Argentine Mild strain of barley stripe mosaic virus. At least one dominant allele is needed at each of these five loci for expression of aleurone color. In progeny of this particular cross, the expression of aleurone color should reflect the genotype at the *A* locus and should segregate as a single gene. However, the results were not as expected. Approximately 1 of 200 ears of the F_2 generation (from selfed F_1 plants) did not have the expected 3:1 ratio of colored to colorless kernels. (Upper case "A" represents colored, lower case "a" colorless in the fol-

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lowing.) Aberrant-ratio stocks were initiated from these ears and maintained by reciprocal crosses between A and a plants. A large proportion of the progeny from such crosses exhibited and continue to exhibit AR, that is, a ratio of A:a significantly different from 1:1 (*e.g.*, Table 1).

As a result of an earlier investigation on AR stocks received from G. F. SPRAGUE, we hypothesized that some stocks exhibiting AR at the "*A*" locus had recessive or masked alleles at the other loci required for aleurone color (SAMSON, BRAKKE and COMPTON 1979). A subsequent investigation has revealed recessive alleles at the *R* and *C* loci.

Three separate stocks showing AR at the "*A*" locus were received from G. F. SPRAGUE. As received, a small percentage of the colored seeds were not uniformly colored, but showed a variety of mottling, sectoring and other patterns. Seed produced at Lincoln has been similarly varied in pattern.. Seed for the present investigation was from the second increase of these stocks at Lincoln, each increase being by reciprocal sib crosses between A and a plants. Infection with maize dwarf mosaic virus strain B is common in the nursery at Lincoln, where these plants were grown, frequently reaching 100% late in the season, though only a small fraction of infected plants show mosaic. Infection with wheat streak mosaic virus in the nursery has been rare. No natural infection with barley stripe mosaic virus was observed. Since maize dwarf mosaic virus reportedly does not cause AR (SPRAGUE and MCKINNEY 1971; DOLLINGER, FINDLEY and WILLIAMS 1966), there is little reason to believe that the AR observed in these stocks was induced while they were maintained at Lincoln.

Crosses were made between AR plants and those of tester stocks obtained from the Maize Genetics Cooperation Stock Center, the University of Illinois. Each tester stock was homozygous recessive at one of the loci required for aleurone color, *i.e.*, *a*, *a2*, *c*, *c2* and *r*. Control crosses among the testers ($a \times r$; $a2 \times r$; $a \times c$; $a \times c2$; $c2 \times c$) gave all colored kernels as expected.

Part of the results obtained in crosses with one AR stock (labeled 874–30 by SPRAGUE) are given in Table 1. The data show the expression of AR and reveal the presence of recessive *a*, *r* and *c* alleles. Results with plant 2(A) may be explained by heterofertilization (SPRAGUE, 1932). Similar tests with another stock (67:946–43 of SPRAGUE or 67:120 of SAMSON, BRAKKE and COMPTON 1979) also revealed the presence of recessive *a*, *r* and *c* alleles (data not shown). Limited tests with a third stock (SPRAGUE's 67–801 × 800) revealed the presence of recessive *a* and *r* (data not shown). No successful cross with *c* was obtained with this last line. No evidence of recessive alleles at *a2* or *c2* loci were obtained in these tests. Few successful crosses were obtained with the *a2* tester.

The presence of recessive alleles at two or three loci when only one was expected does much to explain the aberrant ratio phenomenon. Crosses between plants from colored and colorless kernels of all possible genotypes with recessive alleles at two loci could give 0, 25, 50, 62.5 and 75% colorless kernels. If recessive alleles were present at three loci, the possible percentages include 0, 25, 44, 50, 62.5, 72, 75, 81 and 87.5. When experimental error is considered, these percentages cover most of the range from 0–100%. The presence of recessive alleles

TABLE 1

*Results of crosses between plants of A (colored) and a (colorless) phenotype of an AR stock and between these plants and those of tester stocks with homozygous recessive alleles at known loci**

Crosses with AR plants					Crosses with testers				
Parents		Progeny			Parents		Progeny		
Plant no. and (phenotype)		No. of colorless (a) and colored (A) kernels			Tester genotype	AR plant no. and (phenotype)	No. of colorless (a) and colored (A) kernels		
M	F	*a*†	*A*†	%*a*			a	A	%a
35(a)	2(A)	139	88	61	*a a*	2(A)	72	56	56
22(a)	2(A)	238	0	100	*c c*	22(a)	179	0	100
2(A)	22(a)	162	0	100	*c2 c2*	22(a)	0	341	0
2(A)	36(a)	213	0	100					
22(a)	5(A)	217	65	77	*a a*	22(a)	0	154	0
32(a)	7(A)	188	117	62					
7(A)	21(a)	28	11	72					
					r r	21(a)	22	0	100
36(a)	4(A)	46	36	57	*c c*	36(a)	210	0	100
25(a)	10(A)	152	122	55	*a a*	10(A)	0	73	0
9(A)	25(a)	158	78	67	*c c*	25(a)	93	97	49
25(a)	17(A)	36	24	60	*c2 c2*	25(a)	0	245	0
					a a	25(a)	1	195	0
27(a)	16(A)	215	236	48					
27(a)	14(A)	87	44	66	*a a*	27(a)	1	229	0
					c c	27(a)	180	165	52
34(a)	3(A)	137	42	77					
					a a	3(A)	160	152	51
38(a)	11(A)	74	35	68	*a a*	38(a)	85	112	43
38(a)	13(A)	97	30	76	*c2 c2*	38(a)	0	51	0
					c2 c2	11(A)	1	266	0
					a a	11(A)	73	78	48
23(a)	1(A)	128	75	63					
5(A)	59(a)	247	84	75					
46(a)	4(A)	295	117	72	*r r*	36(a)	26	26	50
					a a	24(a)	44	37	54
28(a)	6(A)	248	80	76					
					r r	11(A)	74	66	53
					r r	28(a)	176	...	100
8(A)	35(a)	125	74	63					
4(A)	94(a)	213	74	74					
10(A)	28(a)	116	72	62					
11(A)	31(a)	201	76	73					

* The AR corn line used in this experiment was originally received from G. F. SPRAGUE as a stock showing AR at the *A* locus induced by barley stripe mosaic virus. The seed was increased in Lincoln in 1975 and 1978 and in both years showed an excess of colorless kernels (58:178 A:a kernels in 1975 and 24:247 A:a kernels in 1978). In both years, increases were made by sib cross between plants of a and A phenotype.

† Single letters refer to phenotype and double letters in italics to genotype, *i.e.*, "a" indicates a kernel with colorless aleurone or a plant from such a kernel, and "A" indicates a kernel with a colored aluerone or a plant from such a kernel. Thus 15(A) is plant number 15 from a colored kernel. By pedigree, a and A plants should have genotypes *aa* and *Aa*, respectively, but since present results indicate they do not, the symbols A and a are used only to indicate phenotype, with no intended implication of genotype.

at additional loci would lead to results consistent with most properties of AR as given by SPRAGUE and MCKINNEY (1971), *i.e.*, equal male and female transmission in reciprocal crosses, reversal in phase from excess colored to excess colorless kernels, absence of linkage to other genes, reversion to normal segregation in low frequency and locus specificity. The last named property would be more accurately termed phenotype specificity.

Some reported properties of AR are not completely explained by presence of additional recessive alleles. SPRAGUE and MCKINNEY (1971) stated that plants of AR stocks gave normal segregation ratios when crossed to non-AR testers, a property not entirely consistent with presence of recessive alleles at other loci. SPRAGUE and MCKINNEY's reported data on this point are limited and do show some variation from expected ratios, which they interpreted as evidence for gene inactivation. We (SAMSON, BRAKKE and COMPTON 1979) obtained more unexpected segregation ratios in crosses to tester stocks than is apparent in results reported by SPRAGUE and MCKINNEY (1971). The persistence of AR stocks with a consistent excess of the dominant phenotype (SPRAGUE and MCKINNEY 1971) is also not readily explained by the presence of additional recessive alleles.

In their first publication on the mutagenic effect of plant viruses, SPRAGUE *et al.* (1963) reported mutations for vivipary, aleurone color, and white and virescent seedlings in the F_1 generation. Observations of F_2 and a limited number of F_3 progeny were consistent with the hypothesis that these mutations were monogenic, and no further investigations of them have been reported. The F_1 seeds also showed entire and sectored or fractional losses of dominant marker genes, such as *A1*. These mutations and the absence of expression of the dominant marker genes in the F_1 generation are all consistent with the hypothesis that virus infection resulted in inactivation of genes.

SPRAGUE and MCKINNEY (1966, 1971) first proposed inactivation of a plant gene by a virus as a possible explanation of aberrant ratio. The conclusion that the recessive *c* and *r* genes found in the present experiments resulted from virus inactivation of dominant genes remains tentative because of the length of time since the original cross. Genetic demonstration of inactivation would best be made in the immediate generations after treatment. Since SPRAGUE, MCKINNEY and GREELEY (1963) gave evidence for mutagenic effects other than AR in the F_1 generation after infection, it is not unreasonable to consider that mutation of *R* and *C* may have been induced at time of infection. Whether instability of these loci was also induced, leading to delayed mutation, cannot be determined from present information.

Two mechanisms may be proposed to explain virus-induced mutations in maize. These are not mutually exclusive mechanisms, nor are they the only possible ones. First, part or all of the virus genome, or a DNA copy thereof, might persist in the corn nuclei, perhaps integrated in the host DNA, and act as a controlling element (MCCLINTOCK 1956). It is unlikely that the complete viral genome persists unintegrated because no virus has been recovered from AR corn stocks, even though this corn is susceptible to virus infection (BRAKKE and SAMSON 1981). Second, virus infection of differentiating tassel meristems might interfere with

host DNA repair or proof-reading enzymes, thereby increasing the apparent rate of mutation.

These two mechanisms lead to different expectations for the timing of the mutations. If the first mechanism is correct, mutations could occur in the virus-infected progenitor plant and could continue to occur in succeeding generations of progeny. If the second mechanism is correct, all virus-induced mutations should occur in the virus-infected progenitor plant. The mutation rate in succeeding generations should be normal, unless the original mutation occurred in a mutator gene.

SPRAGUE and MCKINNEY (1966, 1971) reported that the AR effect occasionally appeared at a new locus in AR stocks. They postulated movement and/or duplication of the putative AR-inducing element to explain these apparent new mutations. However, in the absence of detailed pedigrees and of crosses with appropriate testers, it is impossible to know if the appearance of AR at a new locus was the result of a new mutation, or the delayed expression of an old one. Although the available data are insufficient to distinguish between the two mechanisms, it should be possible to obtain such data in the future.

Results similar to those reported here have been observed in concurrent and independent investigations by NELSON (1981a, b).

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