BROOKHAVEN NATIONAL LABORATORY*
Upton, New York 11973
Biology Department

1. Colored scutellum stocks to detect haploids and determine the distribution of a recessive gene in maize.

Maize geneticists have been interested for many years in inducing and detecting haploids. Haploids are of interest mainly because a completely homozygous diploid is produced when their chromosome complement is doubled, thereby obviating the several generations of inbreeding normally required to achieve uniformity. A considerable amount of the early studies on the genetics and application of haploid induction was done by Dr. S. S. Chase. Some of his first work dealt with the use of seedling markers to detect haploids. Some of this work is published in Genetics 34: 328-332, 1949 and in Heterosis, Chap. 25, pp. 389-399, 1952 and has recently been reviewed by Briggs (J. Heredity, in press).

The recent work of Coe and Sarkar (J. Heredity 55: 231-233, 1964) has shown that it is practical to detect haploids by scoring the dormant kernels. Their method uses stocks which are CC and which have colored scutella for the female parent. The male parent was a stock with a $C^{I}C^{I}$ genotype. The diploid F_1 of such a cross should give a colorless endosperm and no scutellum color while the putative haploids should show color in the scutellum since the sperm nucleus, carrying C^{I}, does not fertilize the egg cell which would give rise to the embryo. In growing the kernels with colored scutella they reported 97% haploids from several types of crosses.

Chase and Nanda (Am. Soc. Agron. Abstr., p. 17, 1965) recently reported a similar procedure to detect haploids. Their method involved the use of a stock with the genotype $\underline{b}\ \underline{pl}\ \underline{A}\ \underline{C}\ R^{nj}$:Cudu $\underline{pr}\ \underline{P}^{wr}$ which imparts a purple embryo, visible in the dormant kernel. This stock is used as the male parent and can be crossed to any material as the female parent. The F_1 seeds that lack purple color in the embryo are selected and sown as putative haploids. By this technique approximately 90% of the kernels can be discarded before germination. This method, in contrast to that of Coe and Sarkar, can be used to extract haploids from commercial material.

As reported by Emerson, Beadle and Fraser (Cornell Univ. Agr. Expt. Sta. Mem. 180: 1-83, 1935) and Sprague (U.S. Dept. Agr. Tech. Bull. 292: 1-43, 1932) many genes are

---

*Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

needed to produce pigmentation in the scutellum. Five of these--S_1, S_2, S_3, S_4, and S_5--are concerned with the extension of aleurone pigment to the scutellum. If purple or red aleurone is present (in the phenotype A_1 A_2 C R (I) $\overline{I}$) then scutellum color appears in the presence of: (1) the dominant allele S_1; (2) the recessive allele s_5; and (3) the dominant alleles of any two of the genes S_2, S_3, and S_4. The genes Pr and pr differentiate purple and red pigment in the scutellum as in the aleurone. It should be further noted that colored aleurone is required to produce scutellum pigmentation.

Chase has proposed that colored scutellum stocks be used to detect haploids in the dormant kernel and work has been initiated at Brookhaven to obtain data using such a method. This procedure of using colored scutellum stocks to detect haploids, as is true of the purple embryo marker technique, can be used to detect haploids in commercial material. Sib pollinations among the females can generally be detected since sibs will not have aleurone color, and in the stocks such as su_1 (sugary-1) and sh_2 (shrunken-2) they are readily detectable. To obtain scutellum color the genes A C R S_1 s_5 and at least two of the genes S_2, S_3, and S_4 are needed. Therefore, to produce scutellum color in a hybrid and to detect haploids by using colored scutellum stocks the recessive gene s_5 would need to be in the male as well as in the female. The critical gene is s_5 since the other genes needed to give scutellum color are dominant. Chase stated that scutellum stocks make excellent markers in certain stocks and that preliminary tests are necessary to determine their suitability in any given cross. One reason that preliminary crosses were necessary may have been because all of the female material that Chase used may not have been s_5/s_5. Therefore, it appears that by using the scutellum stocks as the male a survey of the frequency and distribution of a recessive gene could be made of various maize types, in addition to its use in detecting haploids.

Rhoades (see Principles of Genetics, 4th ed., Sinnott, Dunn and Dobzhansky, pp. 322-323, 1950) has self pollinated several varieties of open pollinated corn varieties. Many mutant genes were detected in this manner. Some of the genes found (white and yellow seedlings, defective endosperm, and germless and viviparous seeds) are lethal when homozygous, while others (virescent, pale green, and dwarf seedlings) may be classified as semi-lethals.

Bianchi and coworkers have carried on extensive studies, observing mutant genes in self pollinated progenies of Italian maize (MNL 38: 89-91, 1964 and earlier volumes). They have done allelism tests on some of the mutants obtained and have also classified the genes into seed,

seedling, and plant traits. Many different types have been discovered, some of which have been lethal and semilethal.

This work of Rhoades and Bianchi on inbreeding open-pollinated varieties of maize detects visible mutants directly and therefore some of the mutants may have been selected both naturally and artificially by man. In such studies the lethal genes are selected against naturally as albino seedlings, defective endosperm, germless and viviparous seed mutants. If any selection was made by man on mature plants certain morphological characters, e.g., stature mutants, may have been selected against. With the $\underline{s}_5$ gene such natural and artificial selection has probably not taken place. The reason for this is that this gene is not detectable by inbreeding and might not be considered as a visible in the usual sense. Therefore, the $\underline{s}_5$ gene may not have been selected naturally and quite certainly has not been selected for or against by man. That is, it probably has not been selected for or against in a conscious manner by man because the gene is detectable only by a special test cross. There is always the possibility that it may be linked with gametophyte factors which could bring about selection. Assuming that no gametophyte factors are involved it is quite probable that natural selection pressure would not be nearly as great on $\underline{s}_5$ as on an albino mutant; i.e., $\underline{s}_5$ is undoubtedly not a lethal. Also, $\underline{s}_5$ is probably not a detrimental gene. It therefore, seemed of interest to begin an assay of some maize material to obtain some information on the frequency and distribution of the $\underline{s}_5$ gene.

If the gene pr (in place of Pr) and the genes to give aleurone and scutellum color are present in a stock, red aleurone and scutellum color would be produced. By using such a stock as the male the frequency and distribution of the pr gene could be determined at the same time that the assay was being made for the $\underline{s}_5$ gene. That is, if the red scutellum stock was used as the male and the hybrid seed had red aleurone and red scutellum color the female stock would be pr. However, if the hybrid seed had a purple aleurone and purple scutellum the female would be Pr.

In order to obtain scutellum color the standard aleurone genes A C R are needed plus other factors discussed previously. If the inhibitor allele (C^I) of the C locus is present in the female no aleurone or scutellum color will be produced in the F_1. Therefore, this system of using scutellum stocks as the male to detect haploids and to assay a population for $\underline{s}_5$ will not be possible if C^I or any other aleurone color inhibitor gene(s) is present in the female material. However, by using the scutellum stocks as the male parent information on the distribution of such inhibitor genes can be obtained from the female material. Also, if aleurone color inhibitors are present, the purple embryo marker system of Chase and Nanda will not be usable.

For convenience the aleurone color inhibitor gene or genes will merely be considered as being a single gene throughout the remainder of this paper.

Scutellum color in maize has been known for many years (Sprague, U.S. Dept. Agr. Tech. Bull. 292: 1-43, 1932). Therefore, stocks with colored scutella are probably in various types of maize that represent various genetic backgrounds. It may therefore be worthwhile to survey these stocks for their ability to induce haploids. Coe (Am. Naturalist 93: 381-382, 1959) discovered a line of maize that produces 3.23% haploids. Prior to Coe's investigations, the highest frequency of haploids was reported by Chase (Genetics 34: 328-332, 1949) as 0.688%, with an average frequency of 0.111%. In order to perform such a survey colored scutellum stocks could be used as the male parent and crossed to a female parent that had been confirmed to be homozygous for the $\underline{s}_c$ gene. Also, the female parent cannot have any aleurone color inhibitor genes. By scoring the kernels from such a cross a survey could be made of various colored scutellum stocks to determine their ability to induce haploids.

The results of using colored scutellum stocks as the male on various types of maize are shown in Table 1. The third column, "No. seeds with colored aleurone and colorless scutella," gives the putative haploids in most cases. That is, the entries in this column should be haploid if the female parent is $\underline{s}_c/\underline{s}_c$ and if the female does not have an aleurone color inhibitor gene. The indication is that M14 has the required gene ($\underline{s}_c$) because most of the seeds have scutellum color. It therefore apparently is possible to detect haploids by using the scutellum stocks as the male in this cross.

In the Hayes White material, which is an open pollinated variety, one ear appears to be segregating for colored and colorless scutella. However, the number with colorless scutella are at such a frequency that they probably are not all haploids. This is confirmed since four of the seeds produced haploids (based on morphological criteria of the seedlings). The data do not fit a 3:1 ratio, but do fit a 13:3 ratio rather well. Various possibilities are being considered to explain such a ratio. The fact that the data fit a 13:3 ratio may not necessarily mean that two genes are segregating. That is, only one gene may be segregating, but the data deviate considerably from a 3:1 (12:4) ratio and appear to fit a 13:3 ratio. However, more data will be needed before a definitive conclusion can be made. Tentatively, it might be stated that $\underline{s}_c$ is segregating. Also, there is the possibility that some factor(s) may be segregating in the male. However, after harvest the kernels from the male parent were examined and all of them had scutellum color. Also kernels from another ear appear to be segregating for a color inhibitor gene in the female parent, because

Table 1
Results of Using Colored Scutellum Stocks as the Male and Crossing them with Various Females. Each Horizontal Line Represents an Individual Ear Except Where Noted.

Entries & (type)	No. seeds with colored aleurone & scutella	No. seeds with colored aleurone & colorless scutella	No. seeds with colorless aleurone & scutella	P value*	Haploids
M14 (dent)	318	3			
	257	20			
	319	13			
	300	6			
	251	7			
	290	4			
	404	18			1
	346	15			2
	202	5			
Hayes White (sweet, su)	314	6			2
	479	28			2
	278	66		.01 (3:1)	4
				.70 (13:3)	
	153	0			
	156	0	149	.70	
Illinichief† (sweet, sh_2)			3219		
Early Triumph (sweet, su)	368	33			2
	365	10			1
	271	4			
	37	0			
	28	1			
Tendercrisp (sweet, su)	205	29	158	.01	
	227	17	163	.01	
	170	11	190	.30	
	165	5	104	.01	
	137	17	120	.30	
	210	14	188	.30	1
	127	12	114	.50	
Minhybrid 250 (pop)			298		
			112		
			268		
			84		
			106		
Strawberry (pop)			154		
			55		
			69		
			83		

*Hypothesis of 1:1 ratio unless noted.
†Bulk of 9 ears.

a 1:1 ratio of seeds with colored aleurone and scutella to seeds with colorless aleurone and scutella was obtained.

Illinichief appears to carry an aleurone color inhibitor gene. This is a single cross hybrid and apparently both parents have the inhibitor. This material has recently been developed by a breeding program to replace the $\underline{su}_1$ gene with $\underline{sh}_2$. Therefore, it would be of interest to test the related single cross, i.e., Iochief, to determine if it carries an inhibitor or whether the inhibitor gene was added in addition to the $\underline{sh}_2$ gene. Early Triumph seems to carry the $\underline{s}_5$ gene in the homozygous condition, and therefore haploids should be obtainable from this material.

If one attempts to extract haploids from Tendercrisp only half of the material can be scored for haploids, i.e., the half with colored aleurone. The other half of the material has a colorless aleurone and therefore colorless scutella. This apparently is due to having an aleurone color inhibitor gene. Tendercrisp is presumably a single cross and one of the parents has an aleurone color inhibitor.

Minhybrid 250 apparently has an inhibitor gene since no aleurone color and hence no scutellum color was produced. The F_1 seed was segregating yellow ($\underline{Y}$) and white ($\underline{y}$) indicating that a cross rather than a sib was made. This is important to determine since the two lines that make Minhybrid 250 were derived from Japanese hulless. Japanese hulless has been reported to have a gametophyte factor by Nelson (Genetics 37: 101-124, 1952). The gametophyte factor may have been lost during the development of the lines; however, a low seed set was obtained in the cross.

Strawberry popcorn is not a very desirable source material from which to extract haploids or to survey for $\underline{s}_5$ since the seed has a red pericarp. However, by removing the pericarp no aleurone or scutellum color could be detected. This indicates that this material also has an aleurone color inhibitor gene. Strawberry popcorn, which is an open-pollinated variety, has a gametophyte factor and a low seed set was obtained in this cross also.

Based on morphological criteria of the seedlings, 15 haploids were obtained in this study (Table 1). By considering the seeds with colored aleurone this is a haploid frequency of 0.235%. To detect haploids by using colored scutellum stocks as the male on the various female stocks shown in Table 1 and considering only the kernels with colored aleurone, approximately 95% of the kernels can be discarded before germination.

This research has indicated that it may be feasible to use maize stocks with colored scutella to detect haploids. Also, stocks with colored scutella may be used to survey for the distribution of a recessive gene that is visible only by a particular cross.

Acknowledgement is made to William VonBergen of Seed Research Specialists, Inc., Ontario, Oregon for providing the Tendercrisp and Early Triumph seed.

Robert W. Briggs

2. Energy requirements and RBE for producing a cytogenetic phenomenon in maize by irradiating seeds with x rays and monoenergetic neutrons.

The frequency of occurrence of yellow-green (yg_2) sectors in seedling leaves that develop from irradiated Yg_2/yg_2 maize seeds was used as a criterion of radiation effect. The yg_2 phenomenon is due mainly to a break in chromosome 9 between the centromere and the Yg_2 locus, with loss of the Yg_2-containing segment. The dose-response curves for 250 kVp x rays (1420 to 14,250 rads) and for monoenergetic neutrons (0.43, 1.25, 1.80 and 14.7 MeV) were linear (or indistinguishable from linearity) and were independent of dose rate (with x rays from 10.3 to 1758 rads/min) thus indicating that breakage of the chromosome, with loss of Yg_2, may be due to a single charged particle. X-ray-induced yg_2 "mutation" rates were 16.4×10^{-7} and 8.3×10^{-7} per rad for cells of leaves 4 and 5, respectively. The "mutation" rates per rad for neutrons were dependent on the leaf scored and the neutron energies employed. For leaf 5 the range was from 3.9×10^{-5} (1.80 MeV) to 6.8×10^{-5} (0.43 MeV). The "effective volume" was assumed to be a sphere and, based on microdosimetric concepts, was computed to have a diameter of 1.35 μ in leaf 4 and 1.10 μ in leaf 5. The corresponding estimates arrived at by cytological methods were 1.52 μ and 1.38 μ, respectively. The results can be accounted for both relatively and absolutely on the assumption that the interphase chromosome is broken, to cause the occurrence of a yg_2 sector, when a single charged particle delivers an energy of approximately 93 KeV or more to a spherical region of the seed embryo cell nucleus that is approximately one micron in diameter but proportional to nuclear diameter.

The relative biological effectiveness of the neutron irradiations used, compared to 250 kVp x rays, ranged from 47 to 102.

Harold H. Smith
Harald H. Rossi