IOWA STATE UNIVERSITY
Departments of Agronomy and Genetics
Ames, Iowa 50011

1) Screening progeny of mutagen-treated soybean seeds for nonfluorescent root mutants.

Delannay and Palmer (1982) reported four nonallelic mutants, three recessive and one dominant, that controlled root fluorescence in soybean. It was during this investigation that we became interested in looking for mutagen-induced nonfluorescent mutants.

In the fall of 1980, we wrote to various soybean researchers who were engaged in mutagenesis programs. The results of screening progeny of mutagen-treated seeds for nonfluorescent root mutants are given in Tables 1-5.

Seeds either were planted in a sandbench or germinated as for mitotic chromosome preparation (Palmer and Heer, 1973) and seedlings were examined with a UV light. All seedlings that were suspected to be nonfluorescent were transplanted to pots and grown in the greenhouse. The self-pollinated progeny were examined with a UV light. If all progeny of a plant had nonfluorescent roots, the original plant was called a "confirmed" nonfluorescent root mutant. In several cases, all progeny gave fluorescent roots, which indicated that an error had been made and the original plant had fluorescent roots.

Table 1 presents results from mutagen-treated seeds obtained from the University of Illinois. A total of 12 confirmed nonfluorescent mutants was found. Two suspected nonfluorescent mutant plants are growing and progeny will be examined for root fluorescence when the plants are mature. Two suspected nonfluorescent plants gave no progeny. One plant was yellow and died as a seedling; another plant was sterile and produced no progeny. Confirmed mutants were obtained from fission neutrons, gamma rays, and nitrosomethyl urea treatments. A total of 65,554 seedlings were examined.

Table 2 presents results from sodium azide-treated seeds obtained from Purdue University. No nonfluorescent mutants were obtained among the 3,217 seedlings examined.

Table 3 presents results from sodium azide and ethylmethane sulfonate-treated seeds obtained from Iowa State University. No nonfluorescent mutants were obtained among the 51,670 seedlings examined.

Table 1. Mutagen-treated seeds - Illinois

Treatment	Cultivar	Generation	No. of seedlings	Root fluorescence results
1.6kR (fission neutrons)	Harosoy $rj_1\ rj_1$	M2	5222	5220 fluorescent 2 confirmed nonfluorescent
1.6kR (fission neutrons)	Williams	M2	5014	All fluorescent
2.2kR (fission neutrons)	Harosoy $rj_1\ rj_1$	M2	4955	All fluorescent
2.2kR (fission neutrons)	Williams	M2	6794	All fluorescent
20kR (gamma rays)	Harosoy $rj_1\ rj_1$	M2	3975	All fluorescent
20kR (gamma rays)	Williams	M2	5262	5260 fluorescent 2 confirmed nonfluorescent
25kR (gamma rays)	Harosoy $rj_1\ rj_1$	M2	3084	All fluorescent
25kR (gamma rays)	Williams	M2	4296	All fluorescent
Ethylmethane sulfonate-1*	Williams	M2	6077	All fluorescent
Ethylmethane sulfonate-2**	Williams	M2	7321	All fluorescent
NMU-1+	Williams	M2	6535	6528 fluorescent 4 confirmed nonfluorescent; 2 suspected nonfluorescent; 1 suspected nonfluorescent, was yellow and died.
NMU-2‡	Williams	M2	7019	7014 fluorescent 4 confirmed nonfluorescent; 1 suspected nonfluorescent, was sterile, no self- or cross-pollinated seeds were obtained.

*50 mM ethylmethane sulfonate for 9 hr, 9 hr postwash.
**50 mM ethylmethane sulfonate for 9 hr, 5 hr postwash.
+2.5 mM nitrosomethyl urea for 5 hr, 9 hr postwash.
‡2.5 mM nitrosomethyl urea for 5 hr, 5 hr postwash.

Table 2. Mutagen-treated seeds - Indiana

Treatment	Cultivar	Generation	No. of seedlings	Root fluorescence results
Sodium azide*	Amsoy 71	M2	3217	All fluorescent

*10 mM for 2 hr.

Table 3. Mutagen-treated seeds - Iowa

Treatment	Cultivar	Generation	No. of seedlings	Root fluorescence results
Ethylmethane sulfonate*	Beeson	M3	4230	All fluorescent
Ethylmethane sulfonate	Corsoy	M3	7308	All fluorescent
Ethylmethane sulfonate	Hardin	M3	10,692	All fluorescent
Ethylmethane sulfonate	Pella	M3	3348	All fluorescent
Ethylmethane sulfonate	Weber	M3	2772	All fluorescent
Sodium azide**	Coles	M3	4417	All fluorescent
Sodium azide	Hardin	M3	8840	All fluorescent
Sodium azide	Pride B216	M3	2232	All fluorescent
Sodium azide	Weber	M3	7831	All fluorescent

*25 mM ethylmethane sulfonate for 9 hr.

**1 mM sodium azide for 2 hr.

Table 4. Mutagen-treated seeds - North Carolina

Treatment*	Cultivar	Generation	No. of seedlings	Root fluorescence results
Ethidium bromide	Jackson	M2	188	All fluorescent
Ethidium bromide	Lee	M2	168	All fluorescent
Ethidium bromide	Ransom	M2	198	All fluorescent
Ethidium bromide	Ransom	M3	450	All fluorescent
Ethidium bromide	Ransom	M5	276	All fluorescent

*Seeds were soaked for 3 hr in the dark in 0.25-0.50% ethidium bromide.

Table 5. Mutagen-treated seeds - Tennessee

Treatment	Cultivar or PI	Generation	No. of seedlings	Root fluorescence results
2.0kR (fission neutrons)	Essex	M5	1757	All fluorescent
2.0kR (fission neutrons)	Lee 74	M5	454	All fluorescent
2.0kR (fission neutrons)	Ogden	M5	638	All fluorescent
20kR (gamma rays)	Essex	M5	9356	All fluorescent
20kR (gamma rays)	Essex	M6	6291	All fluorescent
20kR (gamma rays)	Forrest	M6	175	All fluorescent
20kR (gamma rays)	Ogden	M5	180	All fluorescent
20kR (gamma rays)	Pickett 71	M6	536	All fluorescent
EMS*	Bedford	M3	557	All fluorescent
EMS	Bedford	M4	780	All fluorescent
EMS	Centennial	M3	572	All fluorescent
EMS	Centennial	M4	415	All fluorescent
EMS	Essex	M5	915	All fluorescent
EMS	Essex	M6	7525	All fluorescent
EMS	Forrest	M6	711	All fluorescent
EMS	Pickett 71	M6	368	All fluorescent
EMS	Ogden	M5	650	All fluorescent
EMS	PI 88788	M7	415	All fluorescent

*50 mM ethylmethane sulfonate for 8 hr.

Results from ethidium bromide-treated seeds from North Carolina State University are given in Table 4. No nonfluorescent mutants were obtained among the 1,280 seedlings examined.

Table 5 gives results from mutagen-treated seeds obtained from the University of Tennessee. No nonfluorescent mutants were obtained among the 32,295 seedlings examined.

A total of 154,016 seedlings were examined. Twelve confirmed and four suspected (includes the two that gave no seeds) nonfluorescent mutants were identified. Two confirmed mutants, designated IL 3-1 and IL 3-2, have been studied genetically and the results are given in the following article. The remaining 10 confirmed mutants will be studied genetically.

A summary of the number of seedlings examined according to mutagen is:

Treatment	
Ethylmethane sulfonate	54,656
Gamma rays	33,155
Sodium azide	26,537
Fission neutrons	24,834
Nitrosomethyl urea	13,554
Ethidium bromide	1,280
Total	154,016

Acknowledgments. We thank the following individuals for supplying the seeds: J. E. Harper, USDA ARS, University of Illinois; J. R. Wilcox, USDA ARS, Purdue University; W. R. Fehr, Iowa State University; J. Burton, North Carolina State University; L. N. Skold, University of Tennessee; S. A. Ryan, CSIRO, Canberra, Australia; and E. Hammond, Iowa State University.

References

Delannay, X. and R. G. Palmer. 1982. Four genes controlling root fluorescence in soybean. Crop Sci. 22:278-281.

Palmer, R. G. and H. Heer. 1973. A root tip squash technique for soybean chromosomes. Crop Sci. 13:389-391.

Reid G. Palmer - USDA

J. Douglas Schillinger
University of Iowa
Iowa City, Iowa

Tracey Howson
University of Manchester
Manchester, England

2) Genetic studies with two mutagen-induced nonfluorescent root mutants.

In the preceding article, we described several nonfluorescent root lines that had been obtained from induced mutagenesis. Seeds of the cultivar 'Williams' had been treated with 20kR gamma rays. The seeds given to us were a bulk harvest of many M2 plants. Only two nonfluorescent seedlings, designated IL 3-1 and IL 3-2, were found among the 5,262 seeds germinated. These two nonfluorescent lines were studied genetically and the results are given in this report.

The two nonfluorescent plants were crossed with each other and gave F_1 plants with fluorescent roots; the F_2 segregation fit a 9 fluorescent:7 nonfluorescent ratio (Table 1).

Table 1. Root fluorescence of F_1 plants and F_2 progenies from crosses between two unknown nonfluorescent soybean mutants, IL 3-1 and IL 3-2

Unknown fluorescent line	F_1 †	IL 3-2 F_2 segregation Number of plants Fluorescent	Nonfluorescent	χ^2* (9:7)
IL 3-1	F	143	106	0.14

†F means fluorescent roots.

*χ^2 of 3.84 is significant at the 0.05 probability level.

When the same two nonfluorescent plants were crossed to lines with fluorescent roots, the F_1 plants invariably had fluorescent roots, and the F_2 segregation fit a 3 fluorescent:1 nonfluorescent ratio (Table 2).

The two nonfluorescent plants both were crossed to the four standard nonfluorescent lines described by Delannay and Palmer (1982). With crosses involving IL 3-1, F_1 and F_2 data indicate that a locus different from that of the four standard nonfluorescent lines was responsible for nonfluorescence (Table 3). This new mutant nonfluorescent line (IL 3-1) was assigned Genetic Type Collection Number T280 and the gene symbol fr_5 by the Soybean Genetics Committee.

With crosses involving IL 3-2 and PI 290136, F_1 and F_2 data gave all nonfluorescent plants, indicating that IL 3-2 and PI 290136 possess the same

Table 2. Root fluorescence of F_1 plants and F_2 progenies from crosses between two unknown nonfluorescent soybean mutants, IL 3-1 and IL 3-2, and two fluorescent lines

	IL 3-1				IL 3-2			
		F_2 segregation				F_2 segregation		
		Number of plants				Number of plants		
Fluorescent lines	F_1 †	Fluorescent	Non-fluorescent	χ^2 (3:1)*	F_1 †	Fluorescent	Non-fluorescent	χ^2 (3:1)*
T272H	F	200	71	0.21	F	155	49	0.01
Hark	F	325	107	0.01				

†F means fluorescent roots.

*χ^2 of 3.84 is significant at the 0.05 probability level.

Table 3. Root fluorescence of F_1 plants and F_2 progenies from crosses between two unknown nonfluorescent soybean mutants (IL 3-1 and IL 3-2) and the four standard nonfluorescent lines

Non-fluorescent lines	IL 3-1				IL 3-2			
		F_2 segregation				F_2 segregation		
		Number of plants				Number of plants		
	F_1 †	Fluorescent	Non-fluorescent	χ^2 (9:7)* or (3:13)	F_1 †	Fluorescent	Non-fluorescent	χ^2 (9:7)* or (3:13)
Minsoy (fr_1 fr_1)	F	219	168	0.02	F	170	138	0.14
PI 290136 (fr_2 fr_2)	F	207	157	0.06	NF	0	230	
PI 404165 (fr_4 fr_4)	F	192	151	0.01	F	235	179	0.04
PI 424078 (Fr_3 Fr_3)	NF	68	266	0.57	NF	32	158	0.46

† F means fluorescent roots; NF means nonfluorescent roots.

*χ^2 of 3.84 is significant at the 0.05 probability level.

gene for lack of root fluorescence (Table 3). Both parents are white flowered and have tawny pubescence, but PI 290136 has black seed coat, whereas IL 3-2 has yellow seed coat. The hybridity of the original cross was confirmed by observation of seed coat color of F_3 seeds on different F_2 plants. Crosses of IL 3-2 with the other three nonfluorescent standard lines gave segregation for fluorescent and nonfluorescent roots (Table 3).

F_2 field-grown plants of the cross Hark x IL 3-1 were single-plant threshed. Twenty seeds from each of the 200 plants were germinated and tested for root fluorescence. Data indicated that 48 plants were true breeding nonfluorescent and, thus, were genotype $fr_5\ fr_5$. A total of 53 plants were true breeding fluorescent (genotype $Fr_5\ Fr_5$) and 99 plants segregated about 3:1 for fluorescence:nonfluorescence ($\chi^2 = 0.92$), confirming the heterozygous genotype $Fr_5\ fr_5$. The F_2 genotypic ratio was a close fit to the expected 1:2:1 ($\chi^2 = 0.27$), which confirmed that nonfluorescence of IL 3-1 is conditioned by a single-gene recessive.

F_2 linkage tests were conducted between fr_5 and w_1 (Table 4). Percentage recombination was obtained from the ratio of products method (Immer and Henderson, 1943). Data indicated no linkage between fr_5 and w_1.

Table 4. F2 linkage test between IL 3-1 ($w_1\ w_1\ fr_5\ fr_5$) and soybean cultivar Hark ($W_1\ W_1\ Fr_5\ Fr_5$)

Genes	a	b	c	d	Sum	% R ± SE*	Linkage phase**
$W_1\ w_1\ Fr_5\ fr_5$	269	92	92	31	484	50.2 ± 3.4	C

*% R ± SE = percent recombination ± standard error.

**C = coupling.

Acknowledgments. We thank Dr. J. E. Harper, USDA ARS, University of Illinois, Urbana, and Dr. S. A. Ryan, formerly postdoctoral research associate, University of Ilinois, Urbana, and currently with CSIRO, Canberra, Australia, for supplying the seeds.

References

Immer, F. R. and M. T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-440.

Delannay, X. and R. G. Palmer. 1982. Four genes controlling root fluorescence in soybean. Crop Sci. 22:278-281.

Reid G. Palmer - USDA
Mary O. McFerson - USDA
Susan Yost - USDA

3) Nucleolus distribution in quartets from diploid and triploid soybean.

There are few studies on inheritance and behavior of nucleoli in soybean. Yamaha and Sinoto (1925) reported the behavior of nucleoli in somatic mitosis of 30 species of higher plants including *Glycine max* [*Soja*]. Palmer and Heer (1976) observed one large nucleolus and six small nucleoli in a 40-chromosome plant from the st_4 st_4 synaptic mutant. Folsom and Peterson (1984), in ultrastructural studies of soybean embryo sacs, noted that a micronucleolus often was associated with the nucleolus of an egg cell. In our study, observations on nucleoli distribution at tetrad stage of meiosis were conducted on meiocytes of male-fertile and male-sterile diploids derived from male-sterile ms_1 ms_1 progeny and of male-fertile and male-sterile triploids.

In diploids, only one nucleolus generally was found in the meiocytes at early stages of meiosis. As shown in Table 1, nucleoli distribution in the quartet microspores in both male-fertile and male-sterile diploid plants fell into three major classes: 1-1-1-1, 1-1-1-2, and 1-1-2-2. Frequency among these three classes differed from each other between male-fertile and male-sterile diploid plants. Furthermore, about 16% of the quartets in male-sterile diploids had more than two nucleoli in one microspore or more than two microspores in one quartet with two nucleoli, compared with only 1.2% in male-fertile diploids. Frequently, the nucleoli varied in size.

In triploids, the meiocytes generally have one nucleolus. Infrequently, one large and one small nucleolus were observed. Nucleoli distribution in quartet microspores of both male-fertile and male-sterile triploid plants followed the same pattern as that of diploid male-sterile plants, rather than that of diploid male-fertile plants (Table 1). Although the above three main patterns of nucleoli distribution in quartet microspores were observed in both

Table 1. Frequency and distribution of nucleoli in tetrad stage of meiosis in triploid and diploid soybeans

Type of distribution	Triploid plants				Diploid plants			
	Male fertile		Male sterile		Male fertile		Male sterile	
	No. of quartets	%	No. of quartets	%	No. of quartets	%	No. of quartets	%
1-1-1-1	143	39.2	75	39.9	192	71.1	123	39.7
1-1-1-2	85	23.3	44	23.4	57	21.1	85	27.4
1-1-2-2	88	24.1	37	19.7	18	6.6	52	16.8
1-1-1-3	9	2.5	5	2.6	1	0.4	7	2.3
1-1-2-3	9	2.5	1	0.5	1	0.4	8	2.6
1-1-2-4	1	0.3	2	1.1	-	-	-	-
1-1-3-3	1	0.3	-	-	-	-	1	0.3
1-1-3-4	1	0.3	-	-	-	-	-	-
1-2-2-2	13	3.5	7	3.7	1	0.4	15	4.8
1-2-2-3	5	1.3	6	3.2	-	-	13	4.2
1-2-3-3	-	-	2	1.1	-	-	-	-
1-3-3-3	-	-	3	1.6	-	-	-	-
1-2-3-4	-	-	1	0.5	-	-	-	-
2-2-2-2	8	2.2	3	1.6	--	-	3	1.0
2-2-2-3	2	0.5	2	1.1	-	-	1	0.3
2-2-3-3	-	-	-	-	-	-	2	0.6
Total	365	100.0	188	100.0	270	100.0	310	100.0

male-fertile and male-sterile diploid and triploid plants, frequency of meiocytes with only one nucleolus in each member quartet (1-1-1-1) in male-fertile diploid plants seems much higher than those of male-sterile diploids, and male-fertile and male-sterile triploids (71.1% vs. 39.7%, 39.2%, and 39.9%, respectively), while the frequency of two members with one nucleolus and the other two members with two nucleoli in each quartet (1-1-2-2) in male-fertile diploids seems much lower than those of male-sterile diploids, male-fertile and male-sterile triploids (6.6% vs. 16.8%, 24.1%, and 19.7%, respectively) (Table 1). The occurrence of more than one nucleolus in members of quartets from diploid meiocytes indicates that there might be more than

one genome or perhaps more than one locus involved in formation of the nucleolus. Sybenga (1972) noted that, in principle, many loci are capable of organizing nucleoli, but that under normal conditions only the major nucleolar organizer performs this function and suppresses all other loci. Sybenga (1972) also pointed out that suppression of nucleolar organization may be observed when genomes of different species are combined. Cultivar soybean (*Glycine max*), which behaves cytogenetically and genetically as a diploid, has been suggested to be a tetraploid (Hadley and Hymowitz, 1973). Therefore, if soybeans were of polyploid origin, two or more genomes might be involved in formation of the nucleolus. The failure of suppression of nucleolus formation in the other genome by the major genome might result in two or more nucleoli in one microspore.

Genomic unbalanced gametes are expected from the triploid meiocytes and the existence of four nuclei in coenocytic microspores might also have some effect on the normal formation of the nucleolus. These might lead to the variation in frequency of nucleoli distribution among classes between the male-fertile diploids and the other three sources (male-sterile diploids, male-fertile and male-sterile triploids). Frequently, small scattered nucleoli also were observed in some coenocytic microspores of both diploid and triploid male-sterile plants. Nevertheless, nucleoli in each member of most quartets tended to fuse before pollen development.

McClintock (1934) first reported that the development of the nucleolus is associated with the nucleolar organizing element in the satellite chromosome of maize and that the number of nucleoli in the resting nucleus generally is in proportion to the number of normal satellite chromosomes. Givens and Phillips (1976) used partial triploids and tetraploids of the nucleolar organizer region (NOR) to study the nucleolar distribution throughout meiosis in maize. They found that the site giving rise to the secondary constriction can organize a nucleolus and that duplication of this segment can result in formation of two nucleoli. In diploid soybean (2n=40), only one pair of satellite chromosomes was identified in our laboratory. Pillai (1976) noted a 2n=40 large seed variety with four satellite chromosomes in soybean (*Glycine max*). However, meiosis study was not done in their study. Zheng et al. (1984) also reported four satellite chromosomes in a diploid strain of wild soybean (*Glycine soja*). Previously, Biswas and Bhattacharyya (1972) reported that there are four pairs of chromosomes bearing secondary constrictions in

diploid soybean. Whether all these four pairs of chromosomes with secondary constrictions are associated with nucleolus formation is not known. Our study indicated that there might be more than one pair of chromosomes involved in formation of the nucleolus. Since the plants used in this study all derived from male-sterile ms_1 ms_1 progeny, further investigation on normal diploid genotypes is necessary to preclude material specificity or the effect of male-sterile ms_1 locus on nucleolus formation.

References

Biswas, A. K. and N. K. Bhattacharyya. 1972. Induced polyploidy in legumes: II. *Glycine max* (L.) Merr. Cytologia 37:605-617.

Folsom, M. W. and C. M. Peterson. 1984. Ultrastructural aspects of the mature embryo sac of soybean, *Glycine max* (L.) Merr. Bot. Gaz. 145: 1-10.

Givens, J. F. and R. L. Phillips. 1976. The nucleolus organizer region of maize (*Zea mays* L.). Ribosomal RNA gene distribution and nucleolar interactions. Chromosoma (Berl.) 57:103-117.

Hadley, H. H. and T. Hymowitz. 1973. Speciation and cytogenetics. pp. 97-116. *In* B. E. Caldwell, ed. Soybeans: Improvement, production, and uses. American Soc. Agron. Madison, WI.

McClintock, B. 1934. The relationship of particular chromosomal element to the development of the nucleoli in *Zea mays*. Z. Zellforsch. Mikrosk. Anat. 21:294-328.

Palmer, R. G. and H. Heer. 1976. Aneuploids from a desynaptic mutant in soybeans [*Glycine max* (L.) Merr.]. Cytologia 41:417-427.

Pillai, R. V. R. 1976. Diploids among the cultivars of soybean (*Glycine max* Linn.) in Manipur. Sci. Cult. 42:519-521.

Sybenga, J. 1972. General cytogenetics. Am. Elsevier Pub. Co., Inc. pp. 155-159.

Yamaha, G. and Y. Sinoto. 1925. On the behavior of the nucleolus in the somatic mitosis of higher plants, with microchemical notes. Bot. Mag. (Tokyo) 39:205-219.

Zheng, H. Y., R. Y. Chen and H. Sun. 1984. A diploid strain of wild soybean (*Glycine soja*) with four satellited chromosomes. World Soybean Res. Conf. III. Iowa State University. Ames, Iowa. Abstr. No. 438. p. 96.

Long-Fang Chen
Reid G. Palmer - USDA