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1) Intra-plant variation in mutation frequency and spectrum in soybean.

Upadhyaya (1976) observed that the number of mutant plants was exceedingly low in segregating M2 progenies in soybean. In M3 progenies of normal M2 plants, the number of segregating progenies was also not very high as compared with nonsegregating progenies. But, all the segregating progenies in M3 generation showed an excellent fit to the 3 normal:1 mutant ratio, indicating mutant as a monogenic recessive trait. Such a situation was encountered in many cases of albino, yellow leaf, crinkled leaf, and sterile mutants.

In most of the sexually propagated crops with short life cycle, like soybean, mutation induction for plant breeding purposes is preferably done by seed treatment. It is essentially a treatment of embryo meristems. In such crops, diplontic selection may result in the differences with respect to the frequency of mutated sectors in different plant parts or branches. Further, there may be more than one initial cell for the formation of branches and any of them may be mutated. Therefore, it was considered desirable to study the differences, if any, between different primary branches of each M1 plant with respect to mutation frequency and spectrum.

Materials and methods. Samples of 200 seeds of Bragg and Type-49 soybeans were irradiated with 15 krad of gamma-rays. Irradiated seeds along with controls were planted and observed throughout the season. The branches of each M1 plant in both the varieties were threshed separately and kept in separate packets. Progeny rows of each branch were sown and mutations were recorded for different qualitative characters. Observations on pollen fertility were recorded. The lines segregating for sterility could be easily identified at maturity, since the sterile plants remained green, whereas the normal ones matured normally. The ratios of normal versus mutants were determined for each of the segregating branch progenies. Chi-square test was employed to test the goodness of fit of genetic ratios.

Results and discussion. Four types of qualitative mutants (i.e., albino or yellow leaf, curved leaf, crinkled leaf, and sterile) were observed in M2 generation. The different branches of each of the M1 plants showed the differential behavior in releasing the mutant loci. In Bragg 15 krad a total of 82 branches out of 298 planted showed the mutations for leaf color (albino or yellow), leaf shape (curved or crinkled) and sterility. Of the total, sterile mutants were most frequent (79.27%) followed by chlorophyll deficient (12.34%), crinkled (6.17%) and curved leaves, which were lowest in frequency (2.43%). Of the branches mutating, first branch showed the maximum mutation (31.70%), followed by second branch (23.17%), third branch (19.51%) and fourth branch (14.63%). The seventh branch showed the lowest frequency of mutations of only 2.43% and that too only sterile mutants could be recovered (Table 1).

In the first branch, sterile mutants were most frequent and about 28.05% of sterile mutants were recovered as compared to crinkled leaf (2.43%) and chlorophyll deficient mutants (1.21%). Similarly, second branch also showed predominance of sterile mutants (15.85%) followed by 4.87% chlorophyll deficient mutants and 1.21% each curved and crinkled leaf mutants. The sixth and seventh branches showed only sterile mutants, 3.65 and 2.43%, respectively, and no mutants for leaf deformities or chlorophyll deficiency in Bragg.

In Type-49, the pattern was more or less similar and 27.12% of the branches showed segregation for different types of mutants. Out of a total 83 lines segregating, 80.72% were segregating for sterile mutants. The branchwise breakup of this was 22.89% in first, 12.05% in second, 13.25% in third, 12.05% in fourth, 8.43% in fifth, 6.02% in sixth, 3.61% in seventh, and 1.20% each for eighth and ninth branches. The mutants for leaf deformities such as crinkled and curved leaves were observed in first (2.40%) and second (1.20%) branches only. Albino and yellow leaf mutants were found in the progenies of first to fifth branches with relative frequencies of 3.61, 4.82, 2.40, 1.20 and 3.61%, respectively. Overall frequency of all types of mutants was highest for first branch (28.92%) followed by second (18.02%) and third (15.66%) branches. Eighth and ninth branches again showed lower number of mutants with a frequency of 1.20% each. The inheritance pattern of these mutants was studied in M2 generation and confirmed in M3 generation. All of them were monogenic recessive and a good fit to 3 normal:1 mutant segregation with high probability was observed.

Table 1. Branchwise frequency of different qualitative mutants in Ml plants of Bragg and Type-49 varieties of soybean induced by 15 krad of gamma-rays

Variety	Segregating for	Number of branches									Total frequency
		I	II	III	IV	V	VI	VII	VIII	IV	
Bragg	Yellow leaf and albino	1 (1.21)+	4 (4.87)	2 (2.43)	2 (2.43)	1 (1.21)	--	--	--	--	10 (12.34)
	Curved and crinkled leaf	2 (2.42)	2 (2.42)	1 (1.21)	2 (2.42)	--	--	--	--	--	7 (8.60)
	Sterility	23 (28.05)	13 (15.85)	13 (15.85)	8 (9.76)	3 (3.65)	3 (3.65)	2 (2.42)	--	--	65 (79.27)
											82‡ (27.52)
Type-49	Yellow leaf and albino	3 (3.61)	4 (4.82)	2 (2.40)	1 (1.20)	3 (3.61)	--	--	--	--	13 (15.66)
	Curved and crinkled leaf	2 (2.40)	1 (1.20)	--	--	--	--	--	--	--	3 (3.60)
	Sterility	19 (22.89)	10 (12.05)	11 (13.25)	10 (12.05)	7 (8.43)	5 (6.02)	3 (3.61)	1 (1.20)	1 (1.20)	67 (80.72)
	Total	50 (30.30)	34 (20.61)	29 (17.58)	23 (13.94)	14 (8.48)	8 (4.85)	5 (3.03)	1 (0.61)	1 (0.61)	83‡ (27.12)

+Values in parentheses are percentages.

‡Total frequency of mutants was tabulated over total number of lines planted (298 for Bragg and 306 for Type-49).

In this investigation, the results clearly revealed that mutations are generally produced in one or more early branches and the later formed branches seldom had mutations. A close perusal of the data reveals that the first two or three branches showed higher mutation frequency while the rest of the branches were completely normal. Thus, if an M1 plant is threshed as a whole and M2 progeny rows are raised from the bulk seed, then in the M2 generation the number of mutant plants is expected to decrease considerably and there may not be a good fit to the 3 normal:1 mutant ratio, as observed by Upadhyaya (1976). Subsequently, in the M3 generation, the expected ratio of 2 segregating:1 nonsegregating lines out of normal M2 plants sown may not be observed and results may not be in the expected pattern based on a particular hypothesis. Therefore, if M2 generation is raised by bulking the seeds of each M1 plant, the conclusions regarding genetic ratio should be delayed till the segregation pattern in M3 progenies is studied.

This situation seems to be analogous with ones observed in barley (Gaul, 1961), wheat (Goud, 1967) and rice (Yamaguchi, 1962). In wheat, there are five or six ear initials and if one of the initials is affected, we get the mutant individuals in the M2 population (Frydenberg, 1963). Further, Gaul (1916) reported that tillers in barley that developed later during the ontogeny of M1 plants showed a lower mutation frequency than those that developed early. In our investigation, we also observed similar pattern in soybean, except that branches substitute for tillers of cereals. The early-developed branches showed maximum mutation frequency and the late ones the lowest frequency of mutations.

In mutation breeding experiments, the sampling of branches of M1 plants is, therefore, recommended rather than the M2 seeds to obtain wide range of mutations and for employment of effective selection program subsequently. As is evident from the results of this study, the first four branches show above 80% of total mutations frequency (Table 2); therefore, while sampling these should be invariably included. In wheat, Goud (1967) has suggested the separation of first formed five or six tillers to recover higher mutation frequency.

Regarding the origin of branches in soybean, two possibilities are suggested on the basis of results obtained in the present study: (i) there may be two initial cells which may give rise to the alternate branches, or (ii) there may be different initials for different branches. Thus, any of the

Table 2. Total mutation frequency of different qualitative mutants in soybean

Variety	First two branches	Firt three branches	First four branches
Bragg	54.82	74.31	88.93
Type-49	46.97	62.62	75.87
Average	50.90	68.47	82.40

initials may be affected by the mutagenic treatment and the chances of simultaneous mutations affecting the same locus in all the initials are extremely rare. The latter possibility seems to be more likely. Existing literature also supports the hypothesis of existence of the different initials for different branches as in pea (Blixt et al., 1958). Monti (1965) concluded that five was the highest number of cell initials in an apical meristem of a primary stem and four for a secondary stem.

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References

Blixt, S., L. Ehrenberg and O. Gelin. 1958. Quantitative studies of induced mutations in peas I, Methodological investigations. Hort. Genetica 16: 238-250.

Frydenberg, C. 1963. Some theoretical aspects of the scoring of mutation frequencies after mutagenic treatment of barley seeds. Radiat. Bot. 3:135-143.

Gaul, H. 1961. Studies on diplontic selection after irradiation of barley. Pp. 115-128. *In* Effects of ionizing radiations on seeds. IAEA, Vienna.

Goud, J. V. 1967. Handling irradiated material in segregating generations. Curr. Sci. 36:557-558.

Monti, L. M. 1965. Chimera formation in pea plants raised from mutagen treated seeds. Symp. Mutational Process, Prahe, Agosto. pp. 217-222.

Upadhyaya, H. D. 1976. Induced genetic variability for quantitative and qualitative characters in different varieties of soybean. Unpublished M.Sc. thesis submitted to G.B.P.U.A.T. Pantnagar, India.

Yamaguchi, H. 1962. The chimeric formation in the X-1 panicle after irradiation of dormant rice seed. Radiat. Bot. 2:71-77.

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2) Induced variability for quantitative characters.

Gamma rays were used to induce the genetic variability for different quantitative characters in Bragg and Type-49 varieties of soybean.

Materials and methods. Samples of 200 seeds of Bragg and Type-49 soybeans were irradiated with 10 krad, 15 krad and 20 krad doses of gamma rays, a week before planting time. These irradiated seeds, along with the unirradiated controls, were planted in split-plot design with three replications. Eight seeds from each plant of M1 generation were taken and treatment-wise bulks were prepared. Planting was done in split-plot design using Bragg and Type-49 as main plots and doses as sub plots with two replications. Each plot consisted of 5 rows 6 m long and 60 cm apart. Twenty-five plants were tagged at about 15 days after germination to record the observations on different quantitative characters. Analysis of variance was conducted and, in order to test whether treated populations had significantly more variability than their respective controls in the M2 generation, the "F" test was used. It was assumed that the control populations could provide an estimate of environmental variability and the treated populations would include environmental as well as induced genetic variability. Heritability in broad sense was estimated only in those populations that had significant increase in variance over control.

Results and discussion

Days to flowering. Type-49 took more days to flower than Bragg (Table 1). The irradiated populations of Bragg and Type-49 did not differ significantly from their respective controls in respect to mean for this character. The extent of variability in the irradiated populations of both varieties was more than their control populations, but differences were significant only in the case of Type-49 15 krad and 20 krad with heritability of 65.5% and 65.6%, respectively.

Days to maturity. Varieties differed significantly with respect to number of days taken to maturity. Bragg matured earlier than Type-49. The effects of doses were significant only in Bragg. In case of Bragg 10 krad as well as Bragg 15 krad, the maturity was delayed by 4 and 3 days, respectively (Table 1). The estimated variance of the different treated populations was consistently higher than their respective controls; however, significant increase was observed only in case of Type-49 10 krad, 15 krad and 20 krad populations. None of the populations of Bragg that showed delayed maturity could show any significant increase in variance, indicating very little or no chance of selection. In Type-49 10 krad, 15 krad, and 20 krad populations, the broad sense heritability estimates were 51.8, 55.1 and 69.1%, respectively.

Plant height. Both the varieties differed significantly from each other with respect to plant height. Type-49 was taller than Bragg (Table 1). The overall effect of 10 krad was to shorten the plant height in both the varieties. However, differences were nonsignificant in the case of Bragg. Treatments with 10 krad and 15 krad doses of gamma rays decreased the plant height of Type-49 significantly.

Even though the average plant height of treated Bragg populations did not differ significantly from their control, the increase in the estimated intrapopulation variance was observed for 10 and 20 krad doses as compared to 0 krad. The broad sense heritability estimates were 38.1 and 41.9%, respectively, in Bragg 10 krad and 20 krad populations.

Primary branches per plant. The effect of different doses of gamma-rays was insignificant and the increasing or decreasing effects were not consistent. The intrapopulation variance of treated populations was not significantly different than the control, indicating virtually no mutations for the genes controlling this character (Table 1).

Pods per plant. Only the dose of 20 krad in Bragg (69.7 pods/plant) induced significantly higher number of pods per plant as compared to Bragg 0 krad (50.3 pods/plant). Highly increased intrapopulation variances were observed only in Bragg 10 krad, 15 krad, and 20 krad populations, as compared with Bragg

Table 1. Extent of variability for different quantitative characters in irradiated populations of soybean

	Bragg				Type-49				
Characters	0 krad	10 krad	15 krad	20 krad	0 krad	10 krad	15 krad	20 krad	C.D.
Days to Flowering									
Mean	40	40	39	40	61	61	62	62	1.3
Variance	0.88	0.94	1.12	1.21	1.01	1.25	2.93**	3.02**	--
Heritability (b)	--	--	--	--	--	--	65.5	66.6	--
Days to Maturity									
Mean	109	113*	112*	110	120	118	120	121	2.3
Variance	2.01	2.00	2.85	2.83	0.93	1.93**	2.07*	3.01*	--
Heritability (b)	--	--	--	--	--	51.8	55.1	69.1	--
Plant Height									
Mean	42.7	38.5	47.5	37.1	110.0	102.5*	103.6*	112.3	9.2
Variance	38.1	61.6*	57.3	65.6*	382.8	468.1	537.3	596.1	--
Heritability (b)	--	38.1	--	41.9	--	--	--	--	--
Branches per plant									
Mean	4.3	5.4	5.7	5.3	5.6	5.3	6.0	5.8	1.6
Variance	2.9	3.3	2.5	3.9	3.6	4.6	3.7	4.5	--
Heritability (b)	--	--	--	--	--	--	--	--	--
Pods per plant									
Mean	50.3	65.1	53.1	69.7*	68.3	79.2	68.9	64.8	18.7
Variance	400.9	647.3*	838.8**	781.4**	2043.0	2532.8	1923.8	2067.0	--
Heritability (b)	--	38.1	52.2	48.7	--	--	--	--	--
Seeds per pod									
Mean	2.10	2.21*	2.07	2.11	1.99	1.93	1.86*	1.86	0.07
Variance	0.04	0.06	0.06	0.06	0.05	0.06	0.08*	0.09*	--
Heritability (b)	--	--	--	--	--	--	37.5	44.4	--

continued

Table 1. Continued

Characters	Bragg 0 krad	Bragg 10 krad	Bragg 15 krad	Bragg 20 krad	Type-49 0 krad	Type-49 10 krad	Type-49 15 krad	Type-49 20 krad	C.D.
100-seed Weight									
Mean	15.1	15.3	17.2	16.5	9.7	10.3	10.7	10.7	2.3
Variance	6.4	5.3	26.6**	10.5*	1.7	1.6	2.7*	3.4**	--
Heritability (b)	--	--	75.8	38.8	--	--	37.3	50.0	--
Yield per plant									
Mean	15.9	20.0	16.8	20.1	11.4	11.3	11.5	11.7	4.6
Variance	59.1	82.6	101.1*	91.7	56.1	45.3	63.1	108.0*	--
Heritability (b)	--	--	41.5	--	--	--	--	48.1	--

*Significant at 5 percent level of significance.

**Significant at 1 percent level of significance.

control. The broad sense heritability estimates were 38.1% for Bragg 10 krad, 52.7% for Bragg 15 krad, and 48.7% for Bragg 20 krad (Table 1). The increase in mean number of pods per plant of Bragg 20 krad accompanied by higher estimates of variability and heritability suggests definite possibility of effective selection in this population.

Seeds per pod. The different doses of gamma rays had decreasing effect on seeds per pod in Type-49, but the number of seeds per pod was significantly higher in Bragg 10 krad only as compared with Bragg control. The estimates of intrapopulation variances were significantly greater than their control only in Type-49 15 krad and Type-49 20 krad, with broad sense heritability of 37.5 and 44.4%, respectively (Table 1).

100-seed weight. Bragg had bold seeds (16 g per 100 seeds) as compared to Type-49, which had 100-seed weight of 10 g. The mean weight of 100 seeds of different treated populations did not differ significantly from their controls, but the presence of higher intrapopulation variances in irradiated populations was observed in 15 krad and 20 krad treatments of both the varieties. In Bragg 15 krad, the variance was 26.6 as against 6.4 of Bragg 0 krad (Table 1). The range of 100-seed weight in the irradiated populations was very high and in Bragg it was as low as 10 g per 100 seeds to as high as 24 g, justifying the higher variance accompanied by no difference in mean seed weight of population as such. Heritability estimates in broad sense were 75.8% for Bragg 15 krad, 38.8% for Bragg 20 krad, and 50.0% for Type-49 20 krad, indicating that plants with bold and small seeds can be selected.

Yield per plant. Type-49 was poor yielder as compared with Bragg, which, due to its high yielding ability, is used as check variety in soybean experiments at country level. When averaged over both the varieties, treatment with gamma rays did bring some increasing effect, but these differences were not statistically significant. Bragg 10 krad and Bragg 20 krad populations had per plant yield of 20 g each as against 16 g of Bragg 0 krad (Table 1). Within-population variances of the irradiated populations were higher in most of the cases, but significant differences were observed only in case of Bragg 15 krad and Type-49 20 krad, with 41.5% and 48.1% broad sense heritabilities, respectively.

Significant increase in variances of treated populations over controls for most of the quantitative characters and fairly high heritability for yield components offer a definite possibility of improving these characters by further selection. In most of the cases, the mean of the treated populations was either slightly better or similar to the untreated control, which indicated that micro mutations were positive as well as negative. In this investigation, the relative superiority of the mean of some of the treated populations further indicate that positive micro mutants were more frequent than the negative ones. Our earlier studies (Upadhyaya and Singh, 1979) also indicated the increased variance of treated populations. For number of primary branches per plant, the intrapopulation variance of treated populations was not significantly higher than the control. In our earlier studies (Upadhyaya and Singh, 1979) also, this character showed similar behavior. The possible reason for such behavior may be either the number of genes responsible for branches is quite little or they are quite resistant for mutagens as compared with other characters.

The dose of 20 krad gamma rays was found to be most effective in inducing genetic variability for yield and contributing characters.

References

Upadhyaya, H. D. and B. B. Singh. 1979. Induced variability in soybean. Indian J. Genet. 39:207-209.

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3) A narrow leaf type soybean variety - PK-308

'PK-308' has been developed at this station from a cross of T-31 x Hardee following the pedigree method of breeding. It has been released by the Central Varietal Release Committee, Government of India, in 1984, for general cultivation in the northern plains of India. This is the first narrow-leaf type cultivar released in this country. It has outyielded 'Bragg' on an average by 16.75% over 5 years of coordinated testing in the northern plains (Table 1). In the Soybean Preliminary Observation Trials (SPOT) of INTSOY, 1982, PK-308 yielded 2240 kg/ha and occupied ninth rank in a trial of 16 varieties.

Being a narrow leaf type variety, PK-308 is expected to be superior to Bragg (broad leaflet) under intercropping and this has been demonstrated in intercropping experiment with maize at Pantnagar, where PK-308 has outyielded Bragg by 29.27% (Table 2).

Table 1. Yield (kg/ha) of PK-308 in the northern plain zone

Variety	Delhi	Pantnagar	Ranchi	Kalyani	Behrampur	Mauranipur	Mean
			1978				
Bragg	1260	2820	2806	757	1961	1040	1774
PK-308	1382	2959	2194	939	1993	1064	1755
CD 5%	213	715	356	267	249	--	
CV %	11	20	11	26	8	--	

1979

	Delhi	Pantnagar	Ranchi	Hissar	Mean
Bragg	1561	1328	1792	2177	1715
PK-308	1354	1701	1667	2929	1913
CD 5%	132	560	389	--	
CV %	6	31	14	--	

1980

	Delhi	Pantnagar	Ranchi	Mean
Bragg	2106	1342	1939	1796
PK-308	1917	1817	2006	1913
CD 5%	223	363	403	
CV %	21	15	14	

1981

	Delhi	Pantnagar	Ranchi	Mean
Bragg	1141	1343	1590	1358
PK-308	1024	1929	2378	1777
CD 5%	273	297	186	
CV %	13	16	8	

1982

	Pantnagar	Haldwani	Jorhat	Mean
Bragg	2153	2178	2786	2372
PK-308	2283	2600	4618	3167

Overall average

Bragg	1803
PK-308	2105 (16.75% increase over Bragg)

NB:CD and CV rounded off to full figures.

Table 2. Yield of PK-308 (kg/ha) in comparison with Bragg under intercropping with maize at Pantnagar in 1983*

Treatment	Grain yield (kg/ha)	
	Maize	Soybean
Pure maize at 60 cm row spacing	3697	--
Paired row of maize at 30/90 cm spacing	3488	--
Maize at 60 cm + 1 row of soybean in between 2 rows of maize (Bragg)	3488	854
Maize at 60 cm + 1 row of soybean in between 2 rows of maize (PK-308)	3502	1104
Maize at 60 cm + 1 row of soybean in between 2 rows maize (Shilajeet)	3697	1095
Paired rows of maize 30/90 cm + 2 rows of soybean (Bragg)	3488	898
Paired rows of maize 30/90 cm + 2 rows of soybean (PK-308)	3605	1161
Paired rows of maize 30/90 cm + 2 rows of soybean (Shilajeet)	3627	870
Sole crop of soybean (Bragg)	--	1984
Sole crop of soybean (PK-308)	--	2532
Sole crop of soybean (Shilajeet)		2243
Pure maize at 60 cm (Dummy treatment)	3746	--
SEM	0.692	0.875
CD 5%	2.08	2.620
CV %		10.71

Yield superiority of PK-308 over Bragg (Treatment 3 vs. 4) 29.27%.
Yield superiority of PK-308 over Bragg (Treatment 6 vs. 7) 29.28%.

*Data taken from project coordinator's report and summary tables of experiments, 1983-84, G.B.P.U.A.T., Pantnagar.

PK-308 is moderately resistant to yellow mosaic (yellow spots remain small) as compared to Bragg, which is susceptible and suffers severely in the northern plains. PK-308 is resistant to bacterial pustules (*Xanthomonas phaseoli* var. *sojensis*) and Alternaria leaf spot.

PK-308 has about 20% oil and 40% protein (Table 3). It has grey pubescence and white flowers. Plant height is 50-65 cm. It matures in about 110 days and is a week earlier than Bragg. Seeds are attractive yellow and medium in size (12 g/100 seed).

Table 3. Oil and protein content in PK-308

	1981		1982		Mean	
	Oil %	Protein %	Oil %	Protein %	Oil %	Protein %
Bragg	20.94	40.05	21.61	39.73	21.27	39.89
PK-308	20.52	38.11	19.94	42.53	20.23	40.32

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