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Effect of ^{60}Co Gamma Irradiation on Storability of Soybean Seed

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Abstract: The following three soybean genotypes: Gmx 92-6-10, Gmx 92-5-4^{E} and TGX 87^{D}1303 were subjected to 0, 50, 100, 150, 200, 250 or 300 Gy of ^{60}Co gamma irradiation at the Ghana Atomic Energy Commission, Kwabenya and sown at the Arable Crops Research Farm of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana to study their dosage response. The 250 Gy dose (LD_{50}) reduced both percentage emergence and seedling height by about 50% relative to the 0 Gy dose which served as the control and was therefore used as the dosage appropriate for induced mutations for the genotypes. The bulk of the seeds from the three genotypes were irradiated using the 250 Gy dose and variants from the M_2 and M_3 generations screened by storing either threshed seeds (Category A) or unthreshed seeds (Category B) at a temperature range of 22-25°C and relative humidity of 30-35% on laboratory wooden shelves for 4 months. Variants which had 80% germination or above after the storage period were selected and considered as putative mutants. Improvement in storability created through mutagenesis was calculated as gain in selection expressed as the difference in the mean percentage germination of the M_3 and M_2 populations (M_3-M_2). Genotype Gmx 92-6-10 produced the largest proportion of variants with improved storability particularly at the M_2 generation whilst TGX 87^{D}-1303 produced the highest gain in selection at the M_3 generation. Variability associated with Category A was higher than that by Category B and this gave room for selecting many variants with improved storability from Category A.

Key words: Gamma irradiation, genetic variation, induced mutation, viability, improved storability, soybean seed

INTRODUCTION

Soybean (*Glycine max* L.) seed is structurally weak and inherently short-lived as compared to other crop species (Delouche *et al*., 1973). Seeds of the crop are extremely fragile and easily subject to damage. The only way to determine its seed viability is to have a germination test run usually, the germination rate should be 85% or greater (Hans *et al*., 1997). During storage, the seed deteriorates rapidly (Heydecker, 1972) and as a result substantial losses in vigour and germinability occur particularly when there is hot humid weather and when warm temperatures during harvest periods interact with incidence of fungal pathogens. The rapid rate of deterioration in storage of the crop leading to low viability is a major constraint to its production in the tropical and subtropical areas. According to Verma *et al*. (2001), the decline in germinability of soybean seed is related to its initial degree of deterioration. Snow (1961) reported that although soybean grows and does well in Ghana, large-scale cultivation of the crop is not recommended until the problem of occasional complete failure, due largely or wholly to loss of seed viability had been solved. In Sierra Leone, Funnah (1976) also reported that soybean could have high yields and that the crop has a place in agriculture but one problem that needs solution immediately is that relating to low seed viability and emergence. In an attempt to solve this problem, various approaches have been made but they have centred mainly on the control of the environment. For instance, Boakye-Boateng and Hume (1975) stated that seed moisture content and air temperature of soybean during storage should be reduced to 11.2% and 22°C, respectively. According to Thseng *et al*. (1997), storability of soybean seed differs significantly among cultivars and it is genetically determined.

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Induced mutation creates genetic variation, which serves as basis for selection. It has its most prominent place when an otherwise good cultivar is to be improved in only one easily recognisable character leaving the rest of the genome essentially untouched (Bhatnagar and Tewari, 1990). Induced mutations in soybeans have been used to improve quantitative traits such as plant height, days to flowering and yield (Choudhary and Haq, 1976); seed colour (Patil *et al.*, 1982) and shattering habit (Misra *et al*., 1981).

According to Klu *et al*. (1990), gamma irradiation ranging from 100 to 300 Gy in winged bean cultivars Kade 6/16 and 11PS 122 produced mutants which showed increases in protein content over their parents. Bhatnagar and Tewari (1990) also reported that the mutant $T_1$14 obtained from the soybean cultivar 'Bragg' matured earlier and produced higher yield than the original parent. It is in light with this background of the crop that the present study was conducted to create genetic variation in three genotypes of soybeans through mutagenesis using ^{60}Co gamma rays, so as to select variants or mutants with improved storability.

MATERIALS AND METHODS

Characteristics of the soybean genotypes used for the study

The three soybean genotypes: Gmx 92-6-10, Gmx 92-5-4^{E} and TGX. 87^{D}1303 were experimental lines obtained from the Crops Research Institute, Fumesua, Ghana in June 2000. From August to November, 2000 the seeds were multiplied to raise enough materials for the study.

Genotype Gmx 92-5-4^{E} is yellow seeded; it takes 44 days to flower and matures in 92 days when the plant is 51 cm tall. It has seed yield potential of 1.2 t ha^{-1}. Seeds of Gmx 92-6-10 are green. This genotype flowers in 41 days after planting and matures in 95 days when the average plant height is 44 cm. It has seed yield of 1 t ha^{-1}. Genotype TGX 87^{D}-1303 is black seeded; it takes 35 days to flower and matures in 92 days. The seed yield potential is 0.83 t ha^{-1} and has an average plant height of 78 cm (Annual Report, CRI, 1997).

Radiation dosage response studies: An amount of 250 g of seeds from each of the three soybean genotypes were dried to 10% moisture content and subjected to 0, 50, 100, 150, 200, 250 or 300 Gy of ^{60}Co gamma irradiation at the Ghana Atomic Energy Commission (GAEC), Kwabenya, Ghana. The irradiated seeds and their control were kept in brown paper envelopes and transported back to Kumasi the following day after irradiation and sown in 1×1.4 m sized seed boxes at the Arable Crops Research Farm of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana in April 2001. Twenty seeds from each treatment dosage per genotype were drilled in rows spaced at 20 cm. The treatment combinations were replicated 4 times in a Randomized Complete Block Design (RCBD) and percentage emergence and height of all seedlings recorded ten days after planting.

Field experimentation: An amount of 2 kg of seeds from each of the three genotypes was subjected to 250 Gy of gamma rays at Kwabenya, Ghana in May 2001, after which they were transported back to Kumasi in paper envelopes. The irradiated seeds were then planted on the field two days later by drilling in rows spaced at 75 cm at the Arable Crops Research Farm of KNUST. An amount of 200 g of unirradiated seeds from each genotype was also sown similarly to serve as check. Plot size per treatment was 4×5 m. Treatments were replicated 4 times in a randomized complete block design. Karate 2.5 EC at the rate of 0.6l ha^{-1} and Thiodan 35 EC at the rate of 2l ha^{-1} were sprayed to control pre-flowering and post-flowering insect pests respectively. Weeding was done using a hoe as and when it was necessary. All M_1 plants were harvested at maturity in August 2001 and the pods dried in the sun for one week until seed moisture content was found to average 10%. The soybean pods were kept in sacks and threshed by beating the content of the sacks with sticks. Seeds of the M_1 generation were bulked together and sown again in September 2001 on the field to raise the M_2 generation.

Screening for improved storability: A total of 5,000 single M_2 plants were harvested in December, 2001 and divided at random into two groups. Plants in group one (Category A) were threshed and a total of 1,364 seeds were obtained. In group two, plants were unthreshed (Category B) and consisted of 432 seeds. Seeds and pods from individual plants in group one and two respectively were kept in brown paper envelopes and stored on laboratory wooden shelves at a temperature range of 22-25^{o}C and relative humidity of 30-35 % for a period of four months from January to April, 2002. Similarly, a random sample of unirradiated plants comprising 175 threshed and 152

unthreshed seeds were stored under the same conditions. At the end of the storage period, seeds from the two groups and their control (unirradiated) were drilled in the field in rows spaced at 75 cm on individual plant basis. Ten days after planting, percentage of seeds that emerged out of the total number of seeds sown from each plant was determined. Those seed lots with 80% emergence or above from each category were allowed to grow to maturity as M_3 generation and were considered as putative mutants with improved storability while seedlings from seed lots with less than 80% emergence were eliminated. The percentage emergence of the control was similarly calculated. A total of 2,000 single M_3 plants were harvested at the end of July 2002 for screening using the same screening methodologies as in the M_2 generation.

Data analysis: Data on percentage emergence and height of seedlings from the dosage response studies were subjected to analysis of variance using the 6th Edition of Genstat Statistical Software. Treatment means were separated using the least significant difference method. Variability in storability of variants created through mutagenesis in the M_2 and M_3 generations was determined using coefficient of variation of germination. Improvement in storability of variants was calculated as gain in selection expressed as the difference in mean percentage germination of the M_3 and M_2 populations (M_3-M_2). Dividing the means of the M_3 or M_2 population by the means of their control (unirradiated seeds), or the mean of M_3 population by the mean of the M_2 also gave an indication of the amount of improvement in storability created by induced mutation.

RESULTS AND DISCUSSION

Percentage emergence and seedling height: Percentage emergence and seedling height of irradiated seeds varied significantly among the dosages and genotypes as shown in Table 1 and 2. These traits decreased as the radiation dosage increased. The 250 Gy dose caused about 50% reduction in emergence and seedling height (LD_{50}) and so this dosage was chosen to treat the bulk of the seeds.

Variability in storability created through mutagenesis: For each genotype the total number of variants that recorded germination percentage in the range of 81-100% were selected and considered as putative mutants with improved storability. This was expressed as a percentage of the total number of plants screened. Screening by storing threshed seeds (Category A) produced 109, 55 and 63 putative mutants from 491, 312 and 561 single plants in Gmx 92-6-10, Gmx 92-5-4^E and TGX 87^D-1303 respectively (Table 3). These values represented 22%, 17.6 and 11.2 respectively of the total M_2 plants screened from the genotypes. For Category B, 41, 30 and 15 putative mutants out of 162, 129 and 141 M_2 plants from Gmx 92–6-10, Gmx 92-5-4^E and TGX 87^D-1303, respectively were selected representing 25.3%, 23.3% and 10.6% of the total M_2 plants screened.

Genotype Gmx 92-6-10 produced the highest proportion of putative mutants in the M_2 generation. The differences observed with respect to the proportion of variants, which showed improved storability, among other factors could be attributable to differences in the genetic make-up of the genotypes. This means that storability of soybean seeds significantly varies among genotypes and is genetically determined. This finding is in agreement with Thseng *et al.* (1997) who made a similar observation. Out of 345, 335 and 326 single plants-screened from Category A, 175, 127 and 112 putative mutants were selected respectively from Gmx 92-6-10, Gmx 92-5-4^E and TGX 87^D-1303. This represented 51, 38 and 32% of the total M_3 plants screened (Table 4). Similarly in Category B,

Table 1: Percentage reduction in emergence of irradiated seeds

^{60}Co gamma irradiation dosage (Gy)	Gmx 92-5-4^E	Gmx 92-6-10	TGX 87^D-1303	Mean
50	8	7	6	7
100	14	15	21	17
150	21	20	26	22
200	45	41	45	44
250	49	48	47	48
300	53	53	51	52
Mean	32	31	33	

CV % = 5.0, LSD (0.05): Dosage means = 1.52; Genotype means = 1.08; Dosage x Genotype = 2.64

Table 2: Percentage reduction in height of irradiated seeds

^{60}Co gamma irradiation dosage (Gy)	Gmx 92-5-4^E	Gmx 92-6-10	TGX 87^D-1303	Mean
50	8	16	15	13
100	12	21	26	20
150	29	28	30	29
200	46	49	49	48
250	48	50	62	53
300	52	55	65	57
Mean	33	37	41	

CV % = 4.7, LSD (0.05): Dosage means = 1.31; Genotype means = 0.93; Dosage x Genotype = 2.27

Table 3: Response of variants of three soybean genotypes to screening in the M_2 generation

	Gmx 92-6-10				Gmx 92-5-4^E				TGX 87^D-1303			
	Category A		Category B		Category A		Category B		Category A		Category B	
Germination %	Rad.	Cont.	Rad.	Cont	Rad.	Cont.	Rad.	Cont.	Rad.	Cont.	Rad.	Cont.
0-20	92	1	27	0	49	2	21	1	175	0	28	0
21-40	64	13	29	16	64	4	22	2	135	0	39	0
41-60	91	34	22	31	74	41	32	24	115	12	33	15
61-80	135	53	43	8	70	5	24	8	73	60	26	47
81-100	109	0	41	0	55	0	30	0	63	0	15	0
Total	491	51	162	55	312	52	129	35	561	72	141	62
Mean	98	10	32	11	62	10	26	7	112	14	28	12

CV% = 5.9, LSD (0.05): Genotypes = 1.1; Screening Methods = 0.9; Genotype x Screening Method = 1.6, Rad = irradiated seeds, Cont = Control/unirradiated seeds

Table 4: Response of three soybean genotypes to screening in the M_3 generation

	Gmx 92-6-10				Gmx 92-5-4^E				TGX 87^D-1303			
	Category A		Category B		Category A		Category B		Category A		Category B	
Germination %	Rad.	Cont.	Rad.	Cont	Rad.	Cont.	Rad.	Cont.	Rad.	Cont.	Rad.	Cont.
0-20	0	0	0	0	0	0	0	0	0	0	0	0
21-40	0	11	0	4	0	16	0	6	1	1	0	1
41-60	8	47	5	47	27	33	26	39	78	45	57	47
61-80	162	0	123	7	181	0	169	3	155	23	144	21
81-100	175	0	214	0	127	0	150	0	112	0	14	50
Total	345	58	342	58	335	49	345	48	346	69	346	69
Mean	69	12	68	12	67	10	69	10	69	14	69	14

CV% = 8.3, Genotypes = 2.06; Screening Methods = 1.68; Genotypes x Screening Methods = 2.90, Rad = irradiated seeds, Cont = Control/unirradiated seeds

Table 5: Coefficient of variation (cv) of germination of soybean variants stored under two conditions

	Category A				Category B			
	M_2		M_3		M_2		M_3	
Genotypes	Rad	Cont	Rad	Cont	Rad	Cont	Rad	Cont
Gmx 92-6-10	56	25	14	17	51	27	12	17
Gmx 92-5-4^E	51	23	16	21	52	24	16	18
TGX 87^D-1303	68	11	21	17	57	13	19	17
Mean	58	20	17	18	53	21	16	17

Rad = irradiated seeds, Cont = Control/unirradiated seeds

Table 6: Improvement in storability of Putative Mutants

		M_2 mean		M_3 mean		Improvement of M_2 over over control	Improvement of M_3 over control (%)		Improvement of M^3 over M_2 (%)
Genotypes	Screening methods	Rad	Cont	Rad	Cont			M_3-M_2	
Gmx 92-6-10	Category A	51	46	80	47	11	70	29	57
	Category B	56	48	83	52	17	60	27	48
Gmx 92-5-4^E	Category A	52	49	77	44	6	75	25	48
	Category B	54	53	78	49	2	59	24	44
TGX 87^D 1303	Category A	40	67	72	57	-40	26	32	80
	Category B	45	66	76	56	-32	36	31	69

Rad = irradiated seeds, Cont = Control/unirradiated seeds

63, 43 and 42% of the total plants screened from Gmx 926-10, Gmx 92-5-4^E and TGX 87^D-1303 were selected.

Generally, the frequency distribution of storability of the irradiated seeds was skewed to the right whereas that of the control was largely skewed to the left and this implies that some putative mutants with improved storability had been produced through induced mutation.

Coefficient of variation: Calculating coefficient of variation of germination indicated the extent of variation

created by irradiation. Generally, the M_2 variability was higher than that of M_3 and those of the controls as shown in Table 5. The reduced variability in the M_3 generation could be attributed to the effectiveness of selection at the M_2 generation and also the fact that some putative mutants with improved storability had been produced and that plants in the M_3 population were beginning to approach uniformity with respect to the altered trait. Category A created higher variation than Category B. In selection, the greater the variability, the higher the chance for selecting any desirable trait. The high variation associated with Category A resulted in high gain in selection of variants screened by this method (Table 6). Thus, the highest gain in selection of variants from genotype TGX 87^D-1303 is due to the high variation created in this genotype in the M_2 generation.

Improvement in storability of mutants: Differences were observed among genotypes with respect to improvement in storability of mutants. Genotype Gmx 92-6-10 produced mutants with the highest improvement in storability particularly at the M_2 generation (Table 6) because it responded the greatest to irradiation. At the M_3 generation, however, TGX 87^D-1303 produced the highest gain in selection whilst Gmx 92-5-4^E gave the least. Genotype TGX 87^D-1303 in the M_2 generation, produced some variants whose storability were less than the unirradiated seeds (control) and this explains why the genotype had negative improvement in the M_2 generation.

CONCLUSIONS

Dosage response studies using relative reduction in emergence and seedling height revealed that the most appropriate dosage for inducing variation in the three genotypes of soybean is 250 Gy of gamma rays. Variabilitycreated in the M_2 generation was high compared to that in the M_3 generation and the control. The reduced variation in the M_3 generation could be attributed to the effectiveness of selection in the M_2 generation and the fact that some putative mutants with improved storability had been produced. Genotype Gmx 92-6-10 produced the largest proportion of putative mutants with improved storability in M_2 whilst TGX 87^D-1303 produced the highest gain in selection at the M_3 generation.

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