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MUTAGENIC INDUCTION OF AGRONOMICAL AND YIELD CONTRIBUTING TRAITS IN SOYBEAN (*GLYCINE MAX* (L.) MERRILL) WITH GAMMA IRRADIATION

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ABSTRACT

The effects of different doses of gamma rays (0, 15, 18, 21 kR) on mutagenic induction in soybean (G*lycine max* (L.) Merill) cv ĐVN6, ĐT12 and ĐT20 were investigated. The sensitivity response of soybean to gamma radiation was determined based on seed germination, plant survival and growth reduction in the M_1 generation. The mutation changes were observed on the morphological and agronomic characters and yield components, including leaf color and shape, maturity, plant height, branching habit, number of branches per plant, pods/plant, 1000 seed weight and yield/plant in the M2, M_3 and M_4 generations. In general, the reduction of phenotypic expression in M_1 generation is proportional with the dose of gamma irradiation. Although cultivars responded slightly different, the critical dose for inducing mutation was established at 21 kR. In M_2 generation high proportion of chlorophyll and agro-morphological mutations were observed, indicative of effectiveness of mutagenic treatment. With regard to quantitative traits, both negative and positive shifts in mean values as well as increased phenotypic variation were recorded in M_2, M_3 and M_4 generation as a result of mutagenic treatment. Moderate to high broad-sense heritabilities in the M_3 generation were found for plant height, number of branches, number of pods per plant and seed weight, but rather low for grain yield per plant, suggesting possibility for improvement through selection of yield components. A total of 15 M_4-lines possessing agronomic and yield contributing traits similar to or better than the parents were selected and they offer good scope for improvement of soybean.

Key words: Gamma irradiation, mutagenic effects, soybean, yield traits.

Cảm ứng đột biến các tính trạng nông học và các tính trạng đóng góp vào năng suất ở đậu tương (*Glycine max* (L.) Merrill) bằng tia gamma

TÓM TẮT

Thí nghiệm được tiến hành nhằm xác định ảnh hưởng của tia gamma với các liều lượng khác nhau (0, 15, 18, 21 kR) đến cảm ứng đột biến ở các giống đậu tương ĐVN6, ĐT12 và ĐT20. Độ mẫn cảm của đậu tương với tia gamma được xác định dựa vào tỉ lệ nảy mầm, tỉ lệ sống sót và mức suy giảm sinh trưởng ở thế hệ M_1. Những thay đổi đột biến được khảo sát trên các đặc điểm hình thái, đặc điểm nông học và các yếu tố cấu thành năng suất, gồm màu sắc và dạng lá, thời gian sinh trưởng, chiều cao cây, mức độ phân cành, số cành, số quả/cây, khối lượng hạt và năng suất cá thể ở các thế hệ M2, M_3 và M_4. Nhìn chung, sự suy giảm về biểu hiện kiểu hình ở thế hệ M_1 tỉ lệ thuận với liều lượng chiếu tia gamma. Mặc dù các giống phản ứng có khác biệt nhưng không đáng kể, liều lượng tới hạn để cảm ứng đột biến được xác định là 21 kR. Ở thế hệ M_2 tỉ lệ đột biến diệp lục và đột biến hình thái tương đoi cao chứng tỏ hiệu quả của xử lý đột biến.. Với các tính trạng số lượng, đã quan sát thấy sự thay đổi giá trị trung bình theo hai hướng so với giống gốc cũng như tăng biến động kiểu hình ở các thế hệ M_2, M_3 and M_4. Các tính trạng chiều cao cây, số cành, số quả/cây và khối lượng hạt có hệ số di truyền nghĩa rộng từ trung bình đến cao, nhưng hệ số di truyền của năng suất cá thể thấp, cho thấy chọn lọc các yếu tố cấu thành năng suất có thể cải tiến năng suất. Tổng số 15 dòng thế hệ M_4 có những đặc điểm nông học và các yếu tố cấu thành năng suất tương đương hoặc tốt hơn các giống gốc được chọn lọc làm vật liệu cho việc cải tiến đậu tương.

Từ khóa: Ảnh hưởng đột biến, đậu tương, tia gamma, tính trạng năng suất.

1. INTRODUCTION

Soybean (G*lycine max* (L.) Merill) is an important world oil seed crop and posses a very high nutritional value. It contains about 20% oil and 40% protein and ranks the first in the world production of oilseeds. For Vietnam, soybean is the most significant grain legume for feed, oil and soyfood products. Soybean is a self-pollinated legume and natural crossing rate is rather low, varying from <0.5 to about 1% (Carson and Lersten, 1987). During the last the decades, soybean breeding has been limited to hybridization within primary gene pool, particularly among commercial germplasm and selection was focused on high yielding genotypes and high seed protein and oil. In effect, this leads to narrow genetic base of soybean cultivars (Singh and Hymowitz, 1999).

In Vietnam, the area under soybean and its production in 2010 was estimated at 197.8 thousand hectares and 297,000 MT, respectively.. Statistical data show that national average soybean yield in 2010 was only 1.5 tones ha^{-1}. Soybean is not a native plant of Vietnam. Therefore, the genetic diversity of soybean grown in Vietnam is rather poor, with most direct introductions from China and breeding and selection from the introduced germplasm. The limited use of diverse cultivars presents major constraints for soybean improvement in general and enhancing yield in particular through conventional breeding methods. Hence, alternative methods of generating variability have gained greater importance in breeding of crop plants including soybean and may serve as supplements to conventional breeding methods. In this context, mutation breeding would supplement conventional plant breeding as a source of generating new genetic variability and could confer specific improvement without significantly altering its acceptable phenotype (Ojomo et al., 1979). Although mutation breeding in soybean was lagging behind other economically important crops, more than 100 new soybean cultivars have been developed and released for commercial production through the use of chemical or physical mutagens (Shu and Manjaya, 2007). Mutagenic induction in soybean has produced considerable genetic variation for both qualitative and quantitative traits (Rawling et al., 1958; Papa et al., 1961; Santos et al., 1970; Constantin et al., 1976; Imam, 1978; Maheshwari et al., 2003) from which desirable and useful mutants could be selected. The traits meliorated by mutation include high oleic acid content (Tagaki và Rahman, 1996), fatty acid composition (Hammond and Fehr, 1983; Fehr et al., 1991; Wilcox and Cavins, 1990), high protein content (Srisombunet al., 2009), increased pod numebr plant-1 (Tambe and Apparao, 2009), herbicide tolerance (Sebastian và Chaleff, 1987; Sebastian et al., 1989), plant type (Khan et al., 2005), early maturity (Neto and Alves, 1997), breaking linkage block (Hajika et al., 1995).

Developing new cultivars of crops requires base populations that possess high genetic variability through several means: hybridization, mutation and genetic transformation. This is completely true for soybean, a strictly self-pollinated crop to facilitate selection efforts for desirable traits, such as yield components and tolerance to environmental stresses.

Mutation induction with ionizing radiation, especially gamma ray, is currently the most frequently used method to develop mutant cultivars, where hybridization and selection appear to be laborious and time consuming. The present investigation attempted to use gamma irradiation to induce changes in agronomic and yield contributing traits of existing cultivars, ĐN6, ĐT12 and ĐT20 for selection towards improving yield.

2. MATERIALS AND METHODS

Seeds of three soybean cultivars ĐN6, ĐT12 and ĐT20 were obtained from Center for National Testing of Plant Products and Fertilizers, Ministry of Agriculture and Rural Development. These cultivars are of determinate growth habit. The seeds were treated with gamma rays at 0, 12, 15, 18 and 21 kR at Hanoi Center for Irradiation. Each treatment consisted of 400 seeds. Both irradiated and control (0 kR) seeds were sown on field plots following a randomized block design in triplicate at a spacing of 40 x 10 cm (5 m^2 plots, 25 plants per square meter) to raise M_1 generation during 2010 spring season. Based on the effect and effectiveness of mutagen in terms of survival and growth reduction, all populations at dose of 21 kR were advanced for further evaluation and screening. The surviving M_1 plants were harvested individually and 50 plants were randomly taken to produce M_2 generation along with their original cultivars during summer-autumn 2010. Each M_2 progeny row consisted of 10 to 15 plants. Based on phenotypic appearance and in comparison with the control, putative variant plants were selected individually (pedigree method of selection) to establish M_3 generation progeny during 2011 spring season. Those M_3 lines showing good homogeneity were harvested and bulked to grow as M_4 lines in 2011 autumn-winter season. Individual selection was also practiced to establish M_4 in segregating families.

Treated and control plant progeny were carefully observed for seed emergence and plant survival (for M_1 generation), growth duration at the main stages, plant height, number of branches per plant, number of nodes per plant, number of pods per plants, 100 seed weight and individual plant yield (for M_2 to M_4 generation). For the M_3 generation, the quantitative characters associated with yield were statistically analyzed. Phenotypic, genotypic variance among M_3-families and broad sense heritabilities were calculated with the following formula: $h^2 = \frac{\sigma_G^2}{\sigma_P^2}$ wherein h^2 is the broad sense heritability, σ_P^2 is the total phenotypic variance of M_3-families, σ_G^2 is the genotypic variance. The genotypic variance was calculated as follow: $\sigma_G^2 = \sigma_P^2 - \sigma_e^2$ wherein σ_E^2 is the environmental variance which is the variance among individuals of the original variety.

3. RESULTS AND DISCUSSION

3.1. Effect of gamma rays on M_1 generation

Several abnormalities were observed in M_1 generation following gamma radiation. The undesirable changes resulting from chromosomal aberrations and toxicity are manifested as M1 damage such as lethality and injury, and these adversely expressed in germination, survival, seedling defects, plant growth and chlorophyll deficiency. Although there was slight difference among soybean cultivars in response to gamma irradiation, seed germination and survival of plants of all cultivars decreased progressively with increased dose of gamma rays (Table 1). The decrease in germination and survival has been attributed to the physiological disturbance or chromosomal damage caused to the cells of the plant by the physical mutagen. It was observed that reduction of survival was mainly due to malformed cotyledonary leaves or retarded primary leaf growth leading to the death of seedlings. These reduced germination and survival were also reported in several other studies with soybean (Constantin et al., 1976; Balakrishman, 1991; Karthika and Lakshmi, 2006; Pavadai et al., 2010), cowpea (Girija and Dhanavel, 2009), and yardlong bean (Manju and Gopimony (2009). In addition, irradiated seeds showed delayed germination compared to the control (Table 1). The duration from sowing to flowering have shortened with increased gamma dose but days to maturity delayed resulting in longer growth duration in all treated cultivars.

Observations on plant height, number of internodes and number of branches per plant showed that the rate of growth was apparently reduced by the mutagenic treatment (Table 2). At 21 kR, plant height was reduced by 35 to 47% compared to the control. (Hanafiah, 2010) found that plant height was reduced significantly in cultivar Argomulyo when treated with gamma ray at 200 Gy. Notably, the number of pod-bearing branches was suppressed by more than 50%. The reduction in all these agro-morphological characters could be due to adverse effect of gamma rays that cause chromosomal aberrations and therefore, physiological disturbances in metabolism.

Table 1. Effect of gamma rays on germination, survival and development stages of in M_1 generation of three soybean cultivars

Variety/ Gamma dose (kR)	Germination percentage	Survival percentage	Days to emergence	Days to flowering	Days to maturity	Growth duration
			ĐVN6			
0	93.7	89.3	6	36	47	89
12	90.7	82.7	7	35	49	91
15	88.0	72.7	8	34	50	92
18	84.7	60.3	9	32	52	93
21	81.3	46.0	9	31	54	94
			ĐT12			
0	96.7	91.3	5	35	39	79
12	93.0	83.7	6	34	42	82
15	89.0	74.3	6	32	46	84
18	85.3	62.7	7	30	48	85
21	81.3	58.0	8	29	49	86
			ĐT20			
0	94.7	89.7	6	35	47	88
12	93.0	83.3	6	34	50	90
15	88.7	74.7	7	32	52	91
18	85.0	63.3	8	31	54	93
21	80.7	48.3	9	29	56	94

Table 2. Effect of gamma rays on plant height, number of nodes and number of branches per plant in M_1 generation of three soybean cultivars

Variety/ Gamma dose (kR)	Plant height at flowering (cm)	Plant height at harvest (cm)	Number of internodes per plant	Number of primary branches per plant	Number of pod bearing branches per plant
			ĐVN6		
0	29.4	37	12.7	2.5	2.5
12	27.2	32.8	11.9	2.1	1.8
15	24.9	31.3	11.0	1.8	1.6
18	19.4	24.8	9.6	1.3	1.1
21	15.6	20.6	9.1	1.1	0.9
			ĐT12		
0	34.3	38.1	11.6	1.4	1.4
12	30.5	33.7	9.8	1.3	1.1
15	27.5	31.4	9.1	1.1	1.0
18	26.0	29.5	8.5	0.8	0.5
21	21.6	26.1	8.2	0.6	0.4
			ĐT20		
0	54.5	64.1	14.4	1.5	1.4
12	46.3	57.3	13.5	1.3	1.2
15	42.0	52.4	12.2	1.2	1.1
18	39.6	49.6	11.7	1.0	0.8
21	35.6	43	10.9	0.8	0.7

Similar to the growth characters, yield components and yield per plant significantly decreased with increased gamma dosage. The number of pods per plant and individual yields were reduced by 40 to 45% and 16 to 31 %, respectively in comparison with the control (Table 3). Moreover, the increased variation (s^2) observed among plant yields in the M_1 generation indicates direct mutagenic treatment effect.

LD_{50} is of great significance to determine the sensitivity of different genotypes to the critical dose of mutagens causing 50% lethality. Response analysis based on plant survival under field conditions in this study indicates that LD_{50} is around 21 kR although there was a slight variation between three cultivars. Previous studies revealed that the sensitivity or response to mutagen, including gamma rays is genotype dependent. The critical and optimum dose for mutagenic induction with gamma radiation in soybean varies between 12 and 25 kR (Valeva;1967) or between 200-250 Gray (Manjaya and Nandanwar, 2007, Hanafiah et al., 2010).

3.2. Effect of gamma rays on M_2 generation

Based on LD_{50} value and direct effect of gamma rays determined in the M_1 generation on plant growth, M_2 and later generations were advanced from those populations treated with 21 kR for further evaluation and screening. One of the important indicators to show the effectiveness of mutation is chlorophyll mutation. In the M_2 generation, both chlorophyll and viable mutants affecting morphological characters were identified based on the count of change over total number of plants across M_2 progenies. Chlorophyll mutations include chlorina (variegated yellow and green) and albina (whitish) (Table 4, Fig. 1). The percentage of chlorophyll mutations for ĐT20, ĐT12, and ĐVN6 was found as 0.38, 0.59 and 1.05, respectively. Atak et al. (2004), when three soybean cultivars, Amsoy-71, Coles and 1937 irradiated with 200 Gy of gamma rays, found that the frequency of chlorophyll mutants was around 1.0 - 1.5%. Changes in the trifoliate leaves include leaflet number, size, shape (Table 4, Fig. 2). Other changes include stem color, branching habit, reduced plant height (Fig. 3), flower color, sterility (Fig. 4), early or late maturity (Fig. 5) and seed color (Fig. 6).

Table 3. Effect of gamma rays on grain yield components in M_1 generation of three soybean cultivars

Cultivar	Dose of gamma rays	Number of pods per plant	1000 seed Weight (g)	Individual yield (g/plant)	Variation in individual yield (s^2)
ĐVN 6	0	35.5	185	8.6	0.24
	12	32.6	180	8.1	0.28
	15	28.7	174	7.5	0.31
	18	25.5	171	7.1	0.37
	21	21.0	167	6.0	0.43
ĐT 12	0	23.7	178	7.8	0.21
	12	23.1	173	7.3	0.23
	15	21.0	171	7.0	0.27
	18	17.3	168	6.7	0.33
	21	13.2	160	6.3	0.41
ĐT20	0	41.1	175	9.4	0.35
	12	35.3	171	9.0	0.46
	15	33.3	167	8.6	0.53
	18	31.7	163	8.1	0.61
	21	24.7	159	7.9	0.75

Table 4. Frequency and spectrum of mutations in the M_2 generation

Cultivar	Gamma rays	Chlorophyll	Leaf size and shape	Stem color	Shortened plant height with early branching	No branching	Seed shape and color
ĐT12	Control	0	0	0	0	0	0
	21 kR	1.05	1.26	0.42	1.05	2.11	1.68
ĐT20	Control	0	0	0	0	0	0
	21 kR	0.38	0.57	0	0.19	1.15	0.95
ĐVN6	Control	0	0	0	0	0	0
	21 kR	0.59	0.20	0.59	1.37	1.76	1.36

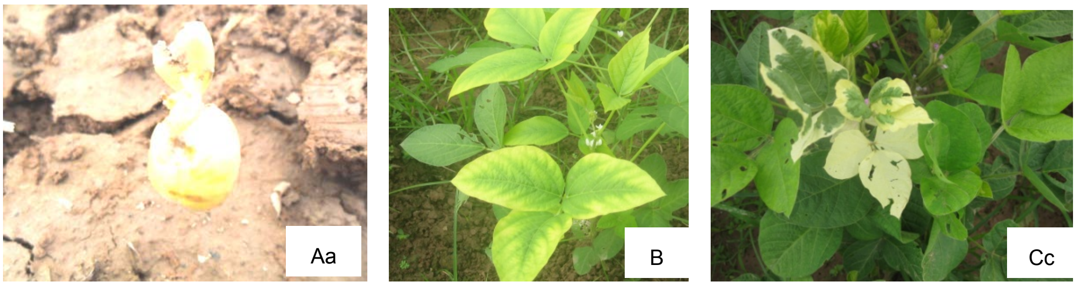

Fig. 1. Chlorophyll mutation: A (albina) and B (chlorina) in cultivar DT12; C (albina) in cultivar DVN6

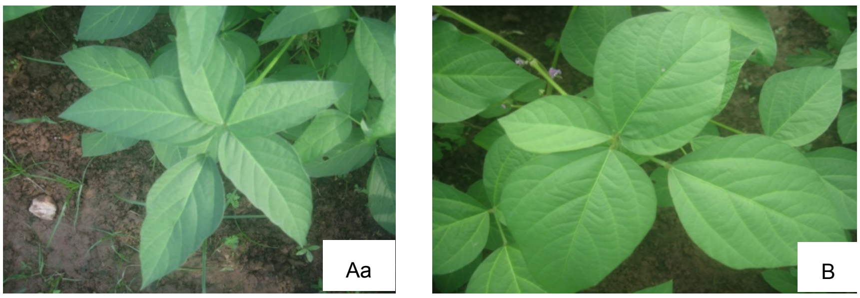

Fig. 2. Changes in leaves: A. petafoliate in ĐT12; B. tetra foliate in ĐT20

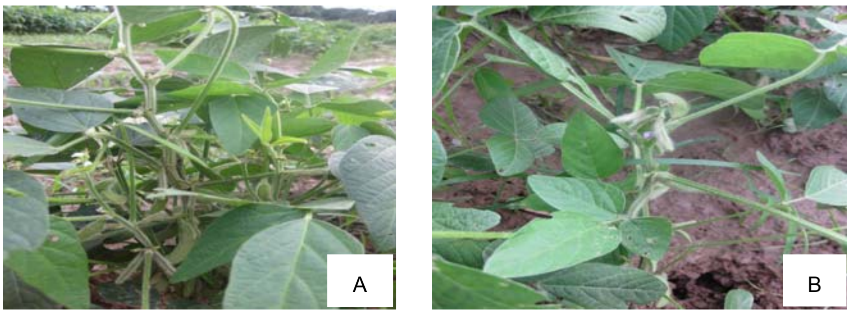

Fig. 3. Changes in plant height and branching habit. A. shortened plant height with early branching in ĐT12; B. no branching in ĐVN6

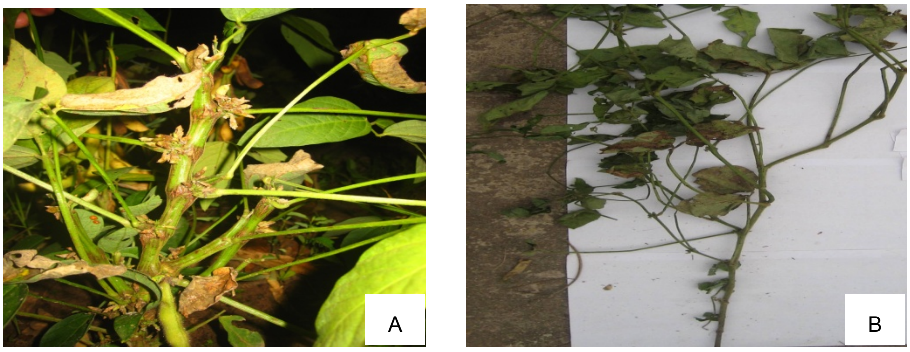

Fig. 4. Sterility mutation. A. semi-sterility in ĐVN6, B. complete sterility (no flower) in ĐT20

Fig. 5. Changes in maturity in ĐT20. Left: early mature mutant, Right original cultivar

Fig. 6. Change in seed color in ĐVN6

3.3. Evaluation of mutants in M_3 and M_4 generation

From M_2 generation, desirable mutants were identified and selected individually to establish M_3 families for further evaluation. Value range for agronomic and yield contributing traits of the M_3-families are presented together with their controls (Table 5). It is apparent that irradiation produced positive and negative shift for the values of morphological and yield components around the value of the original parent. It is notable that several families show early maturity, reduced plant height, higher number of pods per plant, larger seed and higher individual yield.

Table 5. Morphological characters and yield contributing traits in M_3-families in comparison with their control

Cultivar M_3-families	Days to maturity	Plant height (cm)	No. of branches	No. of pods/ plant	1000 seed weight (g)	Yield/plant (g)
ĐVN 6						
Control	101	36.8	7.1	33.4	175.6	10.4
M3-families	98 - 108	33.5 - 47.7	5.1 - 11.1	25.1-38.8	135.5-214.0	8.1 - 10.9
ĐT 12						
Control	99	33.4	3.5	35.2	167.8	9.2
M3-families	92 - 108	28.5 - 39.0	3.0 - 4.6	14.6 - 37.6	102.7 - 199.6	4.9 -10.5
ĐT 20						
Control	114	120.5	4.0	37.9	172.3	9.9
M3-families	97-114	82.7 - 131.8	2.8 - 4.7	23.0 - 45.7	101.5-228.9	7.4 - 11.8

The variances among M_3 lines and broad sense heritabilities for some quantitative traits, especially those associated with grain yield were calculated as indicator parameters for making decision on selection (Table 6). The heritability estimates were moderate to high for plant height, number of branches, number of pods per plant and seed weight, but rather low for yield per plant. Previous studies have reported the hereditary changes in desirable characters of crop plants using gamma irradiation, which contributed to 64% of radiation-induced mutant varieties (Ahloowalia et al., 2004).

Table 6. Genetic variation and heritability of yield contributing traits of M3-families

Original cultivar	Characters	Mean value	σ^2_P	σ^2_G	h^2
ĐVN6	Plant height (cm)	38.9	47.33	34.87	0.73
	Number of primary branches	6.43	1.00	0.38	0.38
	Number of pods per plant	32.84	38.44	25.70	0.66
	1000 seed weight (g)	172.02	404.81	249.56	0.61
	Yield per plant (g)	9.32	0.71	0.22	0.30
ĐT12	Plant height (cm)	33.6	39.96	25.98	0.70
	Number of primary branches	3.37	0.51	0.26	0.50
	Number of pods per plant	29.32	19.00	11.10	0.58
	1000 seed weight (g)	148.19	513.92	355.67	0.69
	Yield per plant (g)	7.69	1.36	0.55	0.40
ĐT20	Plant height (cm)	111.2	887.89	718.63	0.80
	Number of primary branches	3.28	0.35	0.19	0.54
	Number of pods per plant	34.84	38.81	26.87	0.76
	1000 seed weight (g)	162.51	539.63	406.47	0.75
	Yield per plant (g)	9.11	0.98	0.34	0.34

Table 7. Agronomic characters, yield components and grain yield of selected M4 lines

Cultivar/M 4- lines	Days to maturity	Plant height (cm)	No. of branches	No. of pods per plant	No. of filled pods per plant	Percent filled pods	1000 seed weight (g)	Yield/ plant (g)
				ĐVN 6				
ĐC	100	32.4	4.5	46.7	45.1	96.5	197.1	12.8
1-40	101	29.0	4.7	48.9	47.8	97.8	195.3	13.3
2-25	98	33.8	4.1	47.6	46.4	97.4	199.7	11.9
3-27	98	31.3	4.7	51.3	49.3	96.2	198.3	12.8
4-33	101	29.0	4.1	48.5	47.3	97.5	194.8	13.0
5-36	100	30.1	3.6	54.0	53.1	98.2	185.0	12.2
				ĐT 12				
ĐC	81	19.1	3.3	25.8	24.4	94.5	192.8	9.01
1-6	78	21.9	3.7	23.2	22.4	96.5	198.3	11.5
8-2	78	26.5	3.2	25.5	24.5	96.1	199.0	12.8
10-2	81	22.8	4.0	24.6	23.6	95.9	196.0	12.5
12-2	79	29.6	2.0	21.2	20.6	97.2	195.0	12.5
22-1	77	23.8	4.8	38.2	35.6	93.3	200.5	12.5
(26-1)	79	25.6	3.2	25.4	24.0	94.5	197.0	11.4
				ĐT 20				
ĐC	99	68.4	3.0	44.7	40.3	90.7	188.2	15.2
3-21	100	69.1	3.1	53.4	48.3	91.3	200.6	14.6
4-26	99	67.8	3.4	51.3	47.6	92.8	203.9	15.3
28-2	100	68.9	2.1	40.2	35.9	89.6	205.9	14.9
32-1	98	67.1	2.8	43.2	38.6	89.6	195.5	14.5

From three original populations a total of 15 M_4-lines were selected mainly based on individual plant yield, which was similar to, or higher than the original parent. The mean values for important agronomic characters and yield components are summarized in Table 7. In all mutants, the growth duration showed maturity similar to or slightly earlier (2-4 days) than the parent cultivar. Among three cultivars, the mean plant height was improved in shorter plant cultivar ĐT12. In comparison with the parent, the number of primary branches was higher in some M4 lines (ĐVN6-1-40, ĐVN6-3-27; ĐT12-10-2, ĐT12-22-1; ĐT20-4-26). Number of pods per plant and seed weight were higher in the majority of M4 lines than the original parent, especially in cultivars ĐT12 and ĐT20..

4. CONCLUSION

Gamma irradiation is an effective means of inducing agro-morphological and yield contributing traits in soybean. The critical dose is 21 kR. Increased phenotypic and genetic variation resulting from gamma irradiation facilitates selection of desirable mutants combining agro-morphological with yield components.

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