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Full Length Research Paper

Effects of electron beam radiation on trait mutation in azuki bean (*Vigna angularis*i)

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Dry seeds of azuki bean (*Vigna angularis*i), Jingnong 6 and Hebei 801 varieties were irradiated by electron beam of 100, 300, 600, 700 and 900 Gy, respectively. Mutations of leaf shape and color, seed size and shape, trailing, more branching, dwarfing, early or late flowering time and high yield were created in M_2 and M_3 generations. There were richest variations in Jinnong 6 treated with 600 Gy. Heibei 801 was more sensitive to electron beam radiation than Jingnong 6; more mutation types were produced at 100, 300 and 600 Gy. The pod number per plant, seed number and yield per plant of Jinnong 6 displayed a strikingly negative correlation to radiation dose, while the pod length, pod width, and 100-seeds weight of progenies from Hebei 801 had a significantly negative correlation with the radiation dose, but pod number per plants showed significantly positive correlation. Few of the normal phenotype plant in M_2 generation derived mutants of new leaf yellowing, narrow leaf, clustering flower and leaf, kidney or sword leaf in M_3 generation. Mutants of kidney and sword leaf, early flowering time from M_2 generation could be stably inherited in M_3 generation.

Key words: Azuki bean (*Vigna angularis*i), electron beam radiation, trait mutation.

INTRODUCTION

Azuki bean (*Vigna vulgaris* Ohwi and Ohashi) originated in China (Vavilov, 1935). It had been cultivated more than two thousand years. Azuki bean is one of the important food legumes in China. Its area is 2.5 to 3.0 million hm^2 and total yield is 3 to 4 million ton per year, ranking first in the world. Radiation breeding induce plant mutation; by X-ray, γ-ray, ion beam, laser beam, neutron and electron beam, which result in gene mutation and chromosome aberration, and then gain new variety (Chen, 2002). Calaldecatt (1955) firstly treated barleys using 2 MeV electrons beam and showed that electron radiation induced high mutation rate and wide mutation spectrum. Most of research reports of electron beam radiation breeding were published in China; the earliest is in the 1980s. Some researches revealed that electron beam radiation holds small physiological damage in M_1 generation and wide mutation frequency in M_2 generation.

5 MeV electrons and ^{60}Co-gamma-radiation were used to irradiate dry seeds of rice. The results showed that electron beam possess lower damage, higher mutagen frequency, and wider mutagen spectrum than ^{60}Co-gamma-radiation (Guo et al., 1982). The optimum doses for germinating seeds and dry seeds of rice were 50 and 150 Gy, respectively. Indica was more sensitive to electron beam than *Japonica* (Shu et al., 1996). In barley, the half lethal dose (LD_{50}) of electron beam radiation was 2.5×10^4 to 3.5×10^4 rad, lethal dose (LD_{100}) of 6.3×10^4 rad, and 1.0×10^4 to 2.5×10^4 rad was the appropriate inducing dose (Xu et al., 1983).

The dry seeds of 4 barley cultivars were irradiated by electron beam with doses from 100 to 300 Gy. The variation lines of high yield, dwarf and large 1000-grain weight were gained (Rui et al., 1995). The mutants of chlorophyll and growth period were created in M_2 generation of soybean treated by electron beam, and the appropriate radiation dose was 2.7×10^4 to 4.4×10^4 rad.

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(Li et al., 1988). LD_{50} was 40 Gy in M_1 generation of sorghum radiated by electron beam, different color sorghum seeds had different sensitivity on electron beam radiation, and the appropriate dose of white seed was from 30 to 50 Gy, while it was from 100 to150 Gy in red seed (Lu et al., 1995). 22 seedless mutants were selected from electron beam-induced orange bud. The suitable dose of treating sweet orange bud was around 5.0×10^3 rad and mandarin oranges was about 3.0×10^3 rad (Zhou et al., 1995). Electron beam radiation was also used in ornamental plants breeding. The mutants of flower color, flower petal, and flowering time were produced in tissue culture seedlings of chrysanthemum treated with 30 to 50 Gy electron beam (Lin et al., 2000). The percentages of bud formation of 100 Gy electron beam-irradiating Mauve and Indikon lines were 3.7 and 11.3%, respectively in African violet (*Saintpaulia ionahta*), and doses from 40 to 60 Gy were suitable for leaf tissue (Zhou et al., 2006). Electron beam inhibited growth and development of plants and resulted in flower mutation in cockscomb (*Celosia cristata* L). The mutation rate was between 0.5 to 2%. 150 Gy was appropriate dose of treating dry seed of cockscomb (Wang et al., 2006). LD_{50} and LD_{100} of electron beam-radiating scarlet sage were 55 and 85 Gy, respectively (Huang et al., 2007).

Electron beam radiation could significantly inhibit the growth and development of M_1 generation plants of Gladiolus in the seedling and initially flowering period; LD_{50} of treating corm of Super rose cultivar was 240 Gy, but LD_{50} of Beauty queen was greater than 240 Gy (Zhang and Wang 2008). Until now, the report of electron beam mutagenesis in azuki bean is still not published. In this research, azuki bean varieties Jinnong 6 and Hebei 801 were treated with different dose electron beam. The effects of mutation were analyzed for exploring an optimum inducing dose in azuki bean and creating more mutants. It is very useful in acquiring mutants for gene mapping, cloning and breeding of azuki bean.

MATERIALS AND METHODS

Azuki bean Jinnong 6 and Hebei 801 varieties were supplied by College of Plant Science and Technology in Beijing University of Agriculture. Jinnong 6 was bred by Beijing University of Agriculture. Whole growth period of Jingnong 6 was from 90 to 95 days. Its average plant height is 50 cm (Jin et al., 2000). Hebei 801 with big seed was bred by Hebei Province, and its average 100-seed weight is more than 20 g.

Electron beam radiation treatment

In 2007, dry seeds of Jingnong 6 and Hebei 801 were irradiated with electron beam of 100, 300, 600, 700 and 900 Gy dose, respectively (5 MeV, BF-5 electron linear acceleratorelectric current intensity 0.2 mA, 4 Gy/min) in Institute of Low-Energy Nuclear Physics of Beijing Normal University. 1800 seeds of Jinnong 6 were treated with 600 Gy dosage, and the rest doses treated 220 dry seeds of Jinnong 6, respectively. Each of HB801 220 dry seeds was radiated by 100, 300, 600, 700 and 900 Gy, respectively. The controls were non treated Jinnong 6 or HB801 dry seeds.

Planting

The electron beam-treated Jingnong 6 and Hebei 801 seeds were planted in the experimental field of Beijing University of Agriculture on June 13th 2007. Germinating rate of M_1 generation was investigated and calculated. Every plant was separately harvested in the autumn of 2007. On June 16th 2008, all seeds from M_1 generation were planted according to the individual plant. The row length was 3 m, and 35 seeds were sown in each row, and a row of control was planted per 10 rows. During the whole growing stage, the traits of plant architecture, leaf shape, leaf color, flowering time, pod color resistant and susceptible disease, and growth period in M_2 generation were investigated. Every single plant of trait mutation was recorded and tagged, respectively. All tagged morphological mutation plants were harvested individually in mature period, and then their branch number on main stem, plant height, pod length and width, seed color and shape, 100-seeds weight, seed number and yield per plant were tested. The data was analyzed statistically. On June 13, 2009, the seeds of tagged each mutant and a part of seeds from the non variational trait plants in M_2 generation were planted. One row contrast was grown at every 20 rows. The phenotype traits and growth period were extensively surveyed and tracked during the whole growing period in M_3 generation. Mutants were further identified. The data was statistically analyzed.

Statistical analysis

Average is $x\hat{} = \sum x / N$, in which, x^ delegates mean value, x the observed value, and N is the number of observed value. Coefficient variation (CV) = $\sigma / x\hat{}$, in which σ stands for standard difference, and CV is the statistics for elevating variation degree of all observed values. Correlation analysis is conducted using DPS analysis soft.

RESULTS

Impacts of radiation doses on germination rate of M_1 generation

Germination rate of Jingnong 6 and Hebei 801 radiated by electron beam decreased with the increase of radiation dosage (Table 1). No one seed of Jingnong 6 germinated at doses of 700 and 900 Gy. Hebei 801 had relatively higher germination rate than Jingnong 6, indicating that Hebei801 is more tolerant to electron beam radiation. It is evident that sensitivity of different azuki bean variety is different under the electron beam radiation. LD_{50} of electron beam radiating azuki bean is approximately 132 Gy.

Mutation types and frequency of M_2 generation

Jingnong 6 has phenotype of ovate leaf of deep green color and determined growth. The mutants of kidney leaf, sword leaf, lanceolate leaf, small heart-shaped leaf, light

Table 1. Germination rate of M_1 generation induced by electron beam in azuki bean.

Treatment	Dosage (Gy)	Number of seed	Number of seedlings (%)	Germination rate (%)	Relative germination rate[a] (%)
Jingnong 6 control	0	360	242	67.20	100.00
Jingnong 6	100	220	43	19.55	29.09
	300	220	19	8.63	12.84
	600	1800	81	4.50	6.70
	700	220	0	0.00	0.00
	900	220	0	0.00	0.00
Hebei 801 control	0	70	48	68.60	100.00
Hebei 801	100	220	108	49.09	71.56
	300	220	66	30.00	43.73
	600	220	11	5.00	7.29
	700	220	7	3.18	4.64
	900	220	8	3.64	5.31

Relative seedling rate = seedling rate of induced plants/ seedling rate of control plants × 100%.

Table 2. Mutation frequency of M_2 generation induced by electron beam in azuki bean.

Mutant trait	Jingnong 6 (%)			Hebei 801 (%)				
	100 Gy	300 Gy	600 Gy	100 Gy	300 Gy	600 Gy	700 Gy	900 Gy
Dwarf	-	3.23	0.58	0.27	0.75	1.67	-	-
Kidney leaf	-	-	0.58	1.64	3.01	1.67	-	-
Sword leaf	-	-	0.49	-	-	-	-	-
Small leaf	2.94	-	7.51	-	1.5	1.67	6.25	4.17
Small heart-shaped leaf	-	-	0.58	-	-	-	-	-
Early flowering	-	-	-	0.27	0.38	-	-	-
Late flowering	-	-	-	1.09	0.38	1.67	-	-
Light green leaf	-	-	9.83	2.73	3.38	6.67	-	-
Dark green leaf	-	-	-	-	-	-	6.25	-
Yellowing leaf	-	-	0.58	0.27	0.75	-	-	-
More branches	-	-	1.16	0.82	1.13	6.67	-	-
Trailing	-	-	-	0.82	1.13	6.67	-	-
susceptible mosaic virus	5.88	-	-	0.82	0.75	-	-	-
High yield	-	-	1.73	-	-	-	-	-

green and yellowing leaf, trailing, multi-branch, susceptible mosaic virus, dwarf and high yield were produced in M_2 generation (Table 2, Figures 1 and 2). Hebei 801 showed the phenotype of heart-shaped leaf and determined growth. Variations of dwarf, kidney leaf, small leaf, early or late maturing, light and dark green leaf and trailing in M_2 generation were created (Table 2, Figures 1 and 3). Electron beam radiation had better efficiency to Hebei 801 than to Jingnong 6. The most mutation types of Jingnong 6 were obtained at 600 Gy doses, while more variation types of HB801 were gained at 100, 300 and 600 Gy.

Impacts of electron beam radiation on agronomic traits of M_2 generation

Plant height, 100-seed weight and average node number of main stem increased in M_2 generation compared to Jingnong 6 control. Pod number per plant, seed number per plant and yield of single plant at low radiation dose increased and decreased at high radiation dose; both the

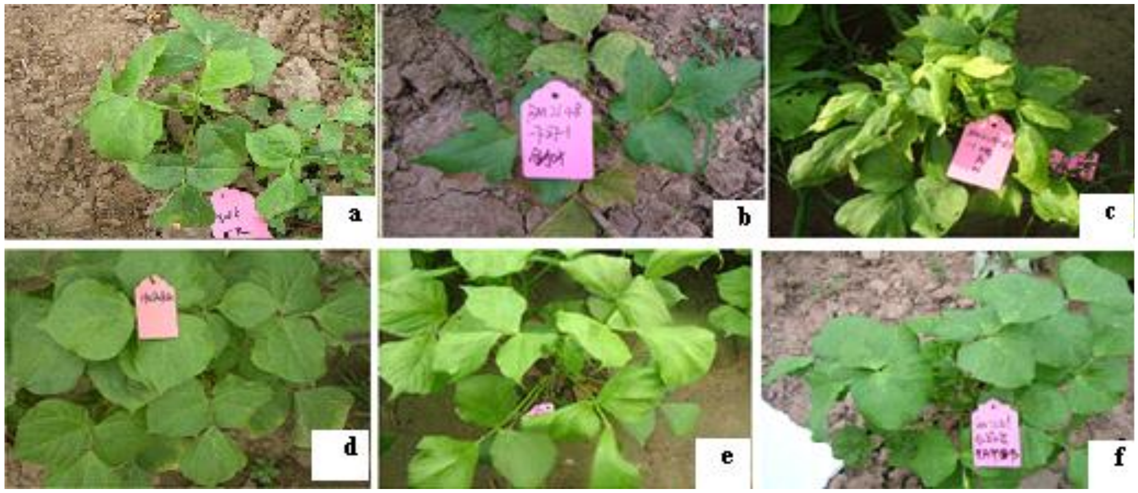

Figure 1. Mutants of leaf shape induced by electron beam. (a) Jingnong 6 control. (b) Sword leaf (600 Gy from Jingnong 6). (c) Lanceolate leaf (600 Gy from Jingnong 6). (d) Hebei 801 control. (e) Kidney leaf (100 Gy from Hebei 801). (f) Oval leaf (600 Gy from Hebei 801).

Figure 2. Mutant of yellowing leaf in Jingnong 6 treated by electron beam. (a) Jingnong 6 control. (b) Mutant of yellowing leaf (600 Gy).

Figure 3. Mutant of plant shape in Hebei 801 treated by electron beam. (a) Hebei 801 control. (b) Mutant of plant architecture and compound leaf (100Gy).

Table 3. The difference comparison of main agronomic traits in M_2 generation treated by electron beam with controls.

Trait	Jingnong 6			Hebei 801				
	100 Gy	300 Gy	600 Gy	100 Gy	300 Gy	600 Gy	700 Gy	900 Gy
	M-CK	M-CK	M-CK	M-CK	M-CK	M-CK	M-CK	M-CK
Plant height (cm)	6.14	1	1.6	2.31	-0.86	21.54	-3.54	-7.28
Length of pod (cm)	-0.23	-0.27	-0.11	0.16	-0.43	-3.07	-1.19	0.44
Width of pod (cm)	0.05	0.05	0.03	-0.04	-0.06	-0.16	-0.09	-0.05
Pod number per plant	4.46	0.75	-5.91	2.88	1.79	6.24	5.55	7.02
Seed number per plant	12.8	-9.6	-37.1	16.07	8.27	2.23	3.88	30.92
Yield per plant (g)	4.39	0.82	-4.95	0.77	-2.18	-5.72	-3.64	3.89
100 seed weight	2.35	2.85	3.59	-2.57	-4.21	-8.33	-6.16	-2.58
Mode number of main stem	2.01	2.36	0.76	1.28	0.45	2.78	0.22	0.82

M-CK, the average of mutants subtract the average of control.

Table 4. Coefficient of variation of main agronomic traits in M_2 generation induced by electron beam.

Trait	Jingnong 6				Hebei 801					
	Control	100 Gy	300 Gy	600 Gy	Control	100 Gy	300 Gy	600 Gy	700 Gy	900 Gy
Plant height (cm)	27.51	20.07	23.18	22.61	23.58	28.25	36.06	22.71	37.16	15
Node number of main stem	22.91	16.07	16.75	21.21	13.52	47.72	17.75	18.07	19.4	12.63
Pod length (cm)	10.87	23.13	15.68	18.01	12.35	58.36	17.13	28.69	28.53	12.28
Pod width (cm)	7.69	9.43	7.514	9.87	8.033	10.95	11.78	9.8	11.06	10.35
Pod number per plant	54.37	71.57	58.08	85.24	49.24	75.8	82.02	91.51	96.9	52.51
Seed number per plant	49.76	80.83	57.66	87.26	54.11	79.53	86.94	98.1	89.09	48.29
Yield per plant (g)	55.15	81.99	60.44	87.21	54.88	76.21	79.44	94.92	81.84	51.68
100-seed weight (g)	8.29	14.41	15.37	94.11	4.65	31.12	29.4	21.96	27.84	25.51

pod length and pod width were proximate to the contrast (Table 3). Plant height, 100-seed weight, node number of main stem, pod width were close to the contrast Hebei 801's in M_2 generation induced with different dose electron beam, while pod length and yield per plant increased. It is clear that same character of different azuki bean variety had differential sensitivity at same radiation dose.

Analysis on the coefficient of variation of agronomic characters in M_2 generation

On the whole, Jingnong 6 treated with 600 Gy dose had the max coefficient of variation (CV) in, pod number per plant, seed number per plant, yield per plant and 100-seed weight. Hebei 801 treated with 700 Gy recorded the max CV in plant height and pod number per plant, the most CV of node number of main stem, pod length and 100-seed weight at 100 Gy, as well as the most CV of seed number per plant and yield per plant at 600 Gy (Tables 4 and 5). The correlation between main agronomic characters of Jingnong 6, Hebei 801 and electron beam radiation dose was analyzed (Table 6). Pod number per plant, seed number per plant and 100-seed weight of Jingnong 6 had significantly negative correlation to the radiation dose; the higher the dose was, the higher the negative impact on these characters was. The pod length, pod width and yield per plant of Hebei 801 showed significantly negative correlation to radiation dose, indicating that pod length, pod width and per 100-seed weight decreased significantly under high radiation dose, while the pod number per plant increased obviously. It is evident that same character of different cultivars had different correlation to the radiation dosage, while the different character of same variety had different correlation to the radiation dosage.

Mutation and heredity in M_3 generation mutagenized by electron beam

The phenotypes of kidney leaf, sword leaf and early or late flowering mutants from M_2 generation can be stably inherited in M3 generation (Figures 4 and 5). However, some variation traits of the leaf color and susceptible mosaic virus could not stably be inherited or segregated in M_3 generation, presumably these characters are

Table 5. The difference of variation coefficient of the main agronomic traits between progenies of M_2 and controls.

Trait	Jingnong 6			Hebei 801				
	100 Gy	300 Gy	600 Gy	100 Gy	300 Gy	600 Gy	700 Gy	900 Gy
Plant height (cm)	-7.44	-4.33	-4.9	4.67	12.48	-0.87	13.58	-8.58
Node number of main stem	-6.84	-6.16	-1.7	34.2	4.23	4.55	5.88	-0.89
Pod length (cm)	12.26	4.81	7.14	46.01	4.78	16.34	16.18	-0.07
Pod width (cm)	1.74	-0.18	2.18	2.92	3.75	1.77	3.03	2.32
Pod number per plant	17.2	3.71	30.87	26.56	32.78	42.27	47.66	3.27
Seed number per plant	31.07	7.9	37.5	25.42	32.83	43.99	34.98	-5.82
Yield per plant (g)	26.84	5.29	32.06	21.33	24.56	40.04	26.96	-3.2
100-seed weight (g)	6.12	7.08	85.82	26.47	24.75	17.31	23.19	20.86

Table 6. Correlation analysis between main agronomic traits of electron beam radiating M_2 generation and radiation doses.

Agronomic trait	Rediation dosage (Jingnong 6)	Rediation dosage (Hebei 801)
Plant height (cm)	-0.06	0.07
Node number of main stem	-0.09	0.01
Pod length (cm)	0.03	-0.15**
Pod width (cm)	-0.01	-0.27**
Pod number per plant	-0.23**	0.08*
Seed number per plant	-0.28**	0.01
Yield per plant (g)	0.04	-0.21**
100-seed weight (g)	-0.25**	-0.06

*$p < 0.05$, **$p < 0.01$.

Figure 4. The heredity of kidney leaf mutant. (a) Phenotyepe of kidney leaf mutant BM2015 in M_2. (b) Phenotype of kidney leaf mutant BM2015 in M_3.

sensitive to environmental effects. Several mutants of kidney leaf, sword leaf, new leaf yellowing, plant yellowing in M_3 generation were separated from normal morphologic plants of M_2 generation (Figures 6, 7 and 8). Variational types of crimping leaf, clustering leaf or flower, poor fertility and less pod number from normal plant of M_2 generation were derived in M_3 generation (Figure 9). More mutants were segregated from normal

Figure 5. The heredity of sword leaf mutant. (a) Phenotyepe of sword leaf mutant BM2148 in M_2. (b) Phenotype of sword leaf mutant BM2148. in M_3.

Figure 6. Segregative mutants of M_3 generation from M_2 wild phenotyepe plant of Hebei 801 induced by electron beam. (a) Control Hebei 801. (b) Kidney leaf (100 Gy), (c) Sword leaf (100 Gy); (d) New leaf yellowing (100 Gy), (e) Narrow leaf (100 Gy). (f) Heart-shape leaf (600 Gy).

Figure 7. Segregative mutants of M_3 generation from M_2 wild phenotyepe plant of Jingnong 6 induced by electron beam. (a) Control Jingnong 6. (b) Sword leaf mutant (600 Gy). (c) New leaf yellowing mutant (100 Gy).

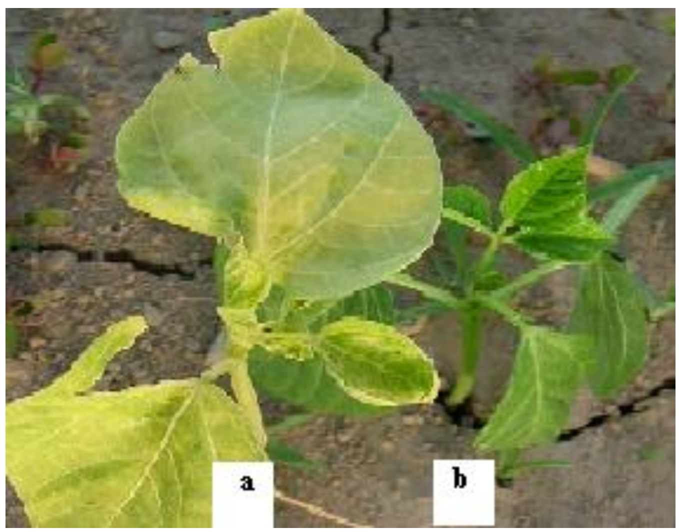

Figure 8. Segregated yellowing and leaf mutant of M_3 from M_2 wild phenotyepe progeny of HB801 induced by electron beam. (a) Yellowing and compound leaf-free mutant (300 Gy). (b) Normal phenotype plant.

phenotype plants of Hebei 801 than Jingnong 6. Continuous investigation will be done whether this mutant phenotype could stably be inherited.

Seed size and shape mutants in M_3 generation

Seed size and shape mutants were gained in M_3 generation. The average 100-seed weight of Jingnong was around 16 g; big and small seeds with average 100-seed weight of 24.0, 15.0, 9.2 and 5.6 g, respectively in M_3 generation (Figure 10). Jingnong 6 seed is big and elliptical; the round and short cylinder seeds were obtained in M_3 generation (Figures 11 and 12). Hebei 801's 100-seed weight was above 20 g. The mutants of medium and small seed size were got in M_3 generation. 100-seed weight of medium or small seed mutants was 15.0 and 9.5 g, respectively (Figure 13). Hebei 801 seed was long and cylindrical, and round and short-cylinder seeds were produced in M_3 generation (Figures 14 and 15).

DISCUSSION

Creating variation is the prerequisite of breeding new cultivars, mapping gene, and map-based cloning. Azuki bean is a cleistogamous plant with extremely low crossing and variation rate in natural environment. Electron beam radiation has little influence on the function of plasma membrane and protein, while it results in gene mutation through inducing much DNA damage of single strand breaks (SSB) and double strand breaks (DSB). The *G*-value for DSB formation of electron beam

Figure 9. New mutant from M_3 progeny of Hebei 801. (a) Clustering plant mutant (300 Gy); (b) Clustering flower mutant (300 Gy); (c) Control Hebei 801; (d) Flower of control Hebei 801.

Figure 10. Mutants of seed size from Jingnong 6; (a) big seed (300 Gy); (b) control Jingnong 6; (c) middle seed (100 Gy); (d) small and short cylinder seed (100 Gy); (e) smallest seed (600 Gy).

radiation in aqueous solution was 5.7 times higher than that caused by 60 Co-gamma rays (Zhu et al., 2008). Electron beam radiation has higher efficiency variation, low cost, safety and smaller radiation damage. Weng et al. (1974) thought that more mutants were segregated in electron beam-irradiated M_2 generation of soybean. This research indicates that electron beam irradiation result in many types of mutations in M_2 and M_3 generations of

Figure 11. Round seed mutant of Jingnong 6. (a) Control Jingnong 6. (b) Round seed mutant (600 Gy).

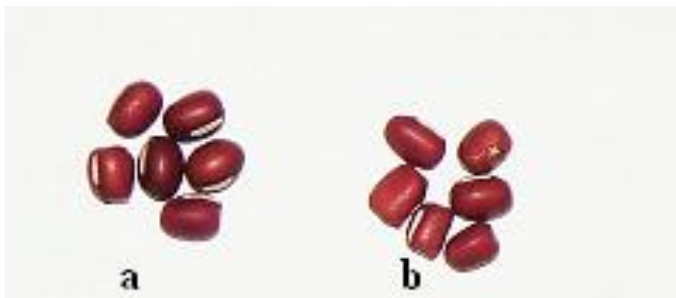

Figure 12. Cylinder seed mutant from Jingnong 6. (a) Control Jingnong 6. (b) Columnar seed mutant (100 Gy).

Figure 13. Mutant of seed size from Hebei 801. (a) Control Hebei 801; (b) Middle seed mutant (100 Gy); (c) Small seed mutant (100 Gy).

Figure 14. Round seed mutant from Hebei801. (a) Control Hebei 801; (b) Round seed mutant (300 Gy).

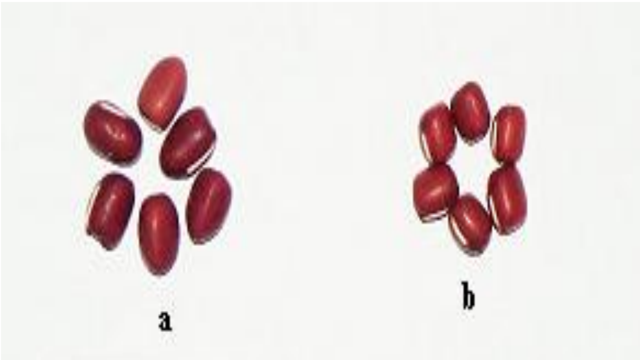

Figure 15. Short cylinder seed mutant of Hebei 801. (a) Control Hebei801; (b) Short cylinder seed mutant (100 Gy).

azuki bean.

Variation in M_2 generation originates from radiation and environmental effect. The CV of contrast is the reaction of environmental effect, while the difference between CV of M_2 generation and contrast reflects to radiation effect (Jin et al., 2000); because different varieties has significant difference in electron beam irradiation, therefore different azuki bean variety should be treated with its appropriate radiation dose for gaining the best mutation. Phenotype of sword leaf, kidney leaf, early and late flowering mutants can be stably inherited. Some variational characters of leaf color, susceptible mosaic virus in M_2 generation segregate failed to be inherited in M_3 generation; maybe these characters are controlled by recessive genes or are susceptible to environment. A few of normal phenotype plants in M_2 generation segregate out variations of narrow leaf, new leaf yellowing, clustering flower and leaf, kidney leaf, sword leaf in M_3 generation. These segregated mutants will be further identified in later generations. Mutation frequency and variational types induced by electron beam are overall lower than ethyl methane sulfonate (EMS) mutagenesis in azuki bean (Tong et al., 2010), but more mutants of seed size and shape are obtained. Electron beam mutagenesis is very useful for breeding, gene mapping, gene cloning and functional analysis in azuki bean.

Conclusion

Electron beam mutagenesis are effective in azuki bean and can create mutations of leaf shape and color, seed size and shape, plant architecture, plant height, early and late flowering time, trailing and high yield etc., especially to induce more mutants of seed size and shape. LD_{50} is about 132 Gy in azuki bean. Different azuki bean variety has different sensibility to electron beam radiation. There are most variation types in 600 Gy irradiating Jingnong 6, and 300 Gy treating Hebei 801. The mutants of kidney leaf and sword leaf, early or late flowering time from M_2 generation, can be stably inherited in M_3 generation.

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